

## DROSERACEAE GLAND AND GERMINATION PATTERNS REVISITED: SUPPORT FOR RECENT MOLECULAR PHYLOGENETIC STUDIES

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### Abstract

Droseraceae germination and leaf gland and microgland character state patterns were re-examined in the light of new molecular phylogenetic relationships. Phanerocotylar germination is basal in the family, with cryptocotylar germination having evolved at least twice; once in *Aldrovanda*, and again in *Drosera* within the *Bryastrum/Ergaleium* clade. Gland patterns also support major clades; with the *Bryastrum* clade taxa having marginal and *Rorella*-type glands whereas the terminal branch of the *Drosera* clade had marginal glands and most of the clade possessed biseriate type 3 glands. The gland and germination patterns are supported by growth habit features, suggesting that the family and the main clades within *Drosera* in particular have undergone major adaptive radiations for these characters.

### Introduction

Relationships between the genera and species of Droseraceae have been the subject of numerous studies, with a range of morphology-based systems produced, mainly using traditional characters such as habit, leaf-associated features and specialised propagation techniques (e.g. Planchon 1848; Diels 1906). Character evolution of traps has also been considered important in carnivorous plants (Juniper *et al.* 1989; Jobson & Albert 2002) and glandular patterns (Seine & Barthlott 1992, 1993; Länger *et al.* 1995) were considered important at the sectional level, as well as providing possible evidence of relationships and/or patterns with related families such as Plumbaginaceae (Wilson 1890; Schlauer 1997).

In addition, studies of characters such as cytology (e.g. Kondo 1976; Kondo & Lavarack 1984), germination (Conran *et al.* 1997), pollen structure (Takahashi & Sohma 1982) and phytochemistry (Culham & Gornall 1994) have added to the overall understanding of relationships in the family, contributing to several proposed classification realignments (Marchant *et al.* 1982; Seine & Barthlott 1994; Schlauer 1996).

Molecular sequence data were useful for showing the evolution of trap complexity in Lentibulariaceae (Jobson & Albert 2002) and relationships within Nepenthaceae where convergent morphology had made phylogenetic interpretation difficult (Meimberg *et al.* 2001). Molecular phylogenetic information also allowed the exploration of snap trap evolution in Droseraceae through character mapping of trapping morphology and mechanisms onto clades (Cameron *et al.* 2002), as well as for exploring the development predisposition for, and possible loss of carnivory in the Caryophyllidae (Meimberg *et al.* 2000). The development of new molecular phylogenies within Droseraceae has helped to resolve some of the ongoing controversies, such as the position of *Drosophyllum* (and support for its removal from the family) and the position of morphologically unusual or “isolated” *Drosera* species (Albert *et al.* 1992; Williams *et al.* 1994; Rivadavia *et al.* 2003). However, one of the biggest advantages of these molecular phylogenies is to assist in the study of character evolution, as well as the evaluation of different character set patterns for their usefulness in supporting classification within the family and exploring evolution and biogeography. For example, the Droseraceae molecular data are supported in part by chromosome number and biogeographic patterns, suggesting support for a possible southern hemisphere African-Australian origin of the family (Rivadavia *et al.* 2003).

Accordingly, this study re-examines germination data for the family and combines them with sig-

nificantly expanded leaf gland and microgland data to test the hypothesis that these characters show phylogenetically useful patterns under the revised molecular phylogeny for Droseraceae of Rivadavia *et al.* (2003).

### Materials and Methods

Gland diversity was investigated using both light and scanning electron microscopy. Specimens for whole-mount examination were immersed in 26% vol/vol lactic acid until cleared, then mounted in lactic acid and examined under a transmission light microscope. Material for scanning electron microscopy was fixed in 5% glutaraldehyde for 30 minutes, dehydrated through a graded ethanol series, critical point dried and coated using and Eiko IB. 5 ion sputter coater. Specimens were observed with a Hitachi S-570 scanning electron microscope at 5-15 kV, and the location and structure of any glands was classified relative to those reported by Seine & Barthlott (1992; 1993) and Langer *et al.* (1995).

Germination data from Conran *et al.* (1997), gland and microgland data from Seine & Barthlott (1993), Langer *et al.* (1995), and our own unpublished gland/microgland data for 180 species, subspecies, varieties, cultivars and morphotypes of *Drosera*, *Dionaea* and *Aldrovanda* were assembled for each of the different character states at the sectional level. However, as the previous two studies did not code gland types in the same manner, direct comparison between them was difficult. In an attempt to reconcile their respective leaf microgland classifications, the data for their studies and ours are summarised at sectional level with the respective gland classifications indicated in Table 1. These character state distributions were then mapped onto a condensed sectional-level cladogram derived from the clades obtained by Rivadavia *et al.* (2003), using the sectional classification of Schlauer (1996) and the clade-exploration program MacClade 3.06 (Maddison & Maddison 1996). Phylogenetically useful patterns of character states were then discussed in terms of evolution within the family.

Taxon	No. of taxa examined	St	Ma	T1 B, F, bi	T1 E, G, H, bi	T2 bi	T3 C, bi	T4-5 D, mu	T6 mu	T7-9 I, mu	T10-12 mu	Ge	GC	NC
<i>Aldrovanda</i>	1	0	0	F1	G1	a	0	0	0	0	0	CR	0	1
<i>Dionaea</i>	1	0	0	0	H1	a	0	0	0	0	0	HE	0	0
<i>Drosera</i>														
Subgenus <i>Arcturia</i>	1	1	0	0	0	0	0	0	0	1	0	PH	0	0
Subgenus <i>Bryastrum</i>														
Section <i>Bryastrum</i>	1	1	1	0	0	1	0	0	0	0	0	CR	0	0
Section <i>Lamprolepis</i>	43	43	34	B4	E2	0	0	43	0	1	4	CR	0	0
Subgenus <i>Coelophylla</i>	1	1	1	0	E1	0	0	1	0	0	0	CR	0	0
Subgenus <i>Drosera</i>														
Section <i>Arachnopus</i>	1	1	0	0	0	0	0	0	1	0	0	PH	0	0
Section <i>Drosera</i>	26	26	21	B8	E3	1	15	0	0	1	3	PH	16	0
Section <i>Oösperma</i>	16	16	13	B5	E4	0	10	0	0	2	0	PH	9	0
Section <i>Prolifera</i>	3	3	0	B2	E1	0	0	0	1	0	0	HE	0	0
Section <i>Psychophila</i>	2	2	0	B2	0	0	0	0	0	0	0	PH	0	0
Section <i>Ptycnostigma</i>	7	7	7	0	E4	0	1	0	0	2	1	PH	1	0
Subgenus <i>Ergaleium</i>														
Section <i>Ergaleium</i>	35	35	0	B3	E28	0	0	0	0	10	2	CR	0	0
Section <i>Stolonifera</i>	8	8	0	0	E6	0	0	0	0	4	0	CR	0	0
Section <i>Erythrorhiza</i>	17	17	0	0	E9	0	0	0	0	9	1	CR	0	0
Subgenus <i>Lasiocephala</i>	9	9	4	B8	E1	0	0	0	0	0	0	CR	0	0
Subgenus <i>Meristocaulis</i>	1	1	1	0	0	1	0	0	0	0	1	?	?	?
Subgenus <i>Phycopsis</i>	1	1	1	0	E1	0	0	0	0	1	0	PH	0	0
Subgenus <i>Regiae</i>	1	1	0	0	0	0	0	0	0	1	0	PH	0	0
Subgenus <i>Stelogyne</i>	1	1	1	0	0	0	1	0	0	0	0	PH	0	0
Subgenus <i>Thelocalyx</i>	2	2	2	0	E2	0	0	2	1	1	0	PH	2	0

Table 1: Numbers of taxa exhibiting gland and germination features for Droseraceae, summarised by genus, subgenus and section following Schlauer (1996) unless a section or species group was separated in Rivadavia *et al.* (2003): St = stalked central glands; Ma = stalked marginal unifacial glands; T1-T11 = sessile leaf microgland types as classified by Langer *et al.* (1995); B-1 = sessile microgland types as classified by Seine & Barthlott (1993); a = a related gland type not differentiated by Seine & Barthlott (1993); bi = biseriolate microglands; mu = multiseriolate microglands; Ge = germination type (P = phanerocotylar, C = cryptocotylar, H = hemicycotylar); GC = glandular cotyledons; NC = first leaves non-carnivorous. Gland data for subgenus *Meristocaulis* from Seine & Barthlott (1993) and Langer *et al.* (1995).

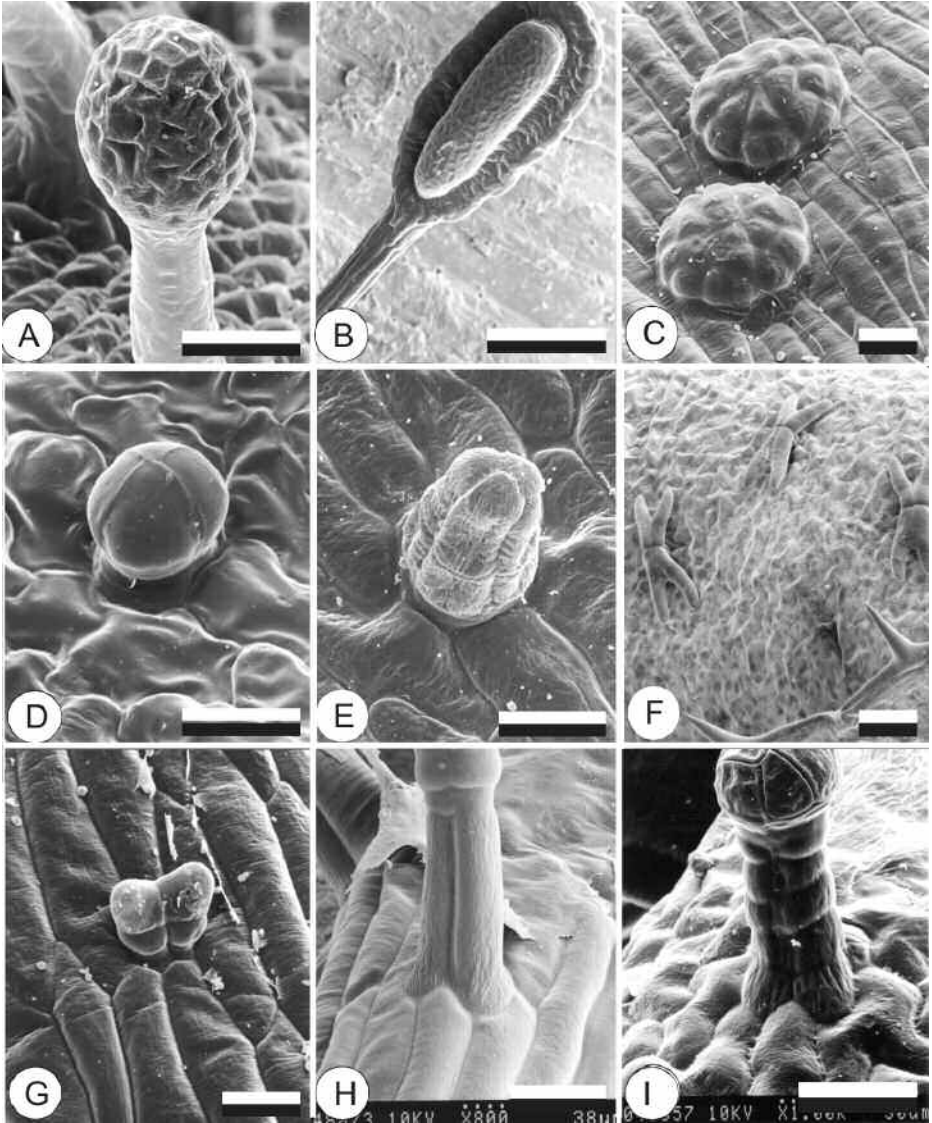


Figure 1: Droseraceae taxonomically informative gland types. A: *Drosera trinervia* Spreng. stalked gland; B: *D. capillaris* Poir. marginal unifacial gland; C: *Dionaea muscipula* Soland ex Ellis sessile multicellular glands; D: *Drosera menziesii* R.Br. ex DC. type 1B multiseriate gland; E: *Drosera erythrorhiza* Lindl. type 1E sessile multiseriate gland; F: *Aldrovanda vesiculosa* L. elongated sessile multiseriate glands; G: *Drosera capillaris* type 3 biseriate gland; H: *Drosera sewelliae* Diels type 4-5 sessile biseriate (*Rorella*-type) gland; I: *Drosera andersoniana* W.Fitzg. ex Ewart. & White type 11 inflorescence gland. Scale bars A, B = 50  $\mu$ m, C-I = 25  $\mu$ m.

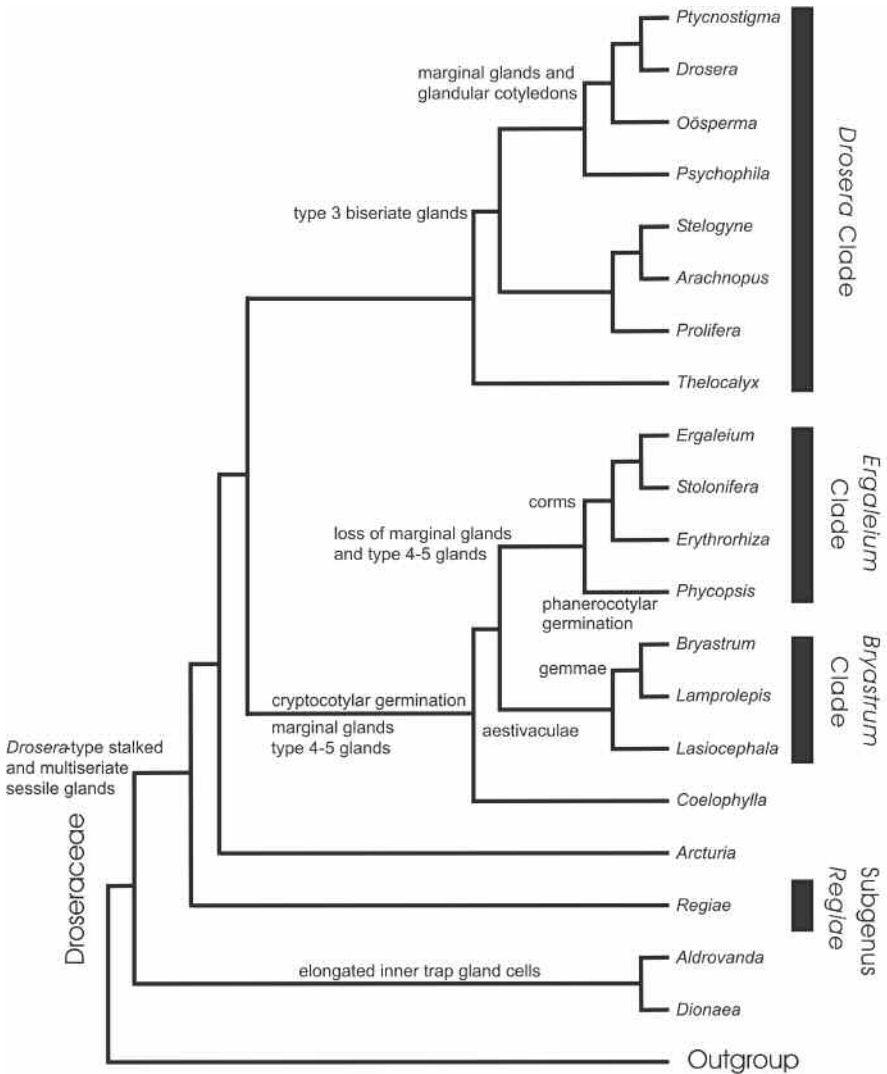


Figure 2. Condensed cladogram showing major groups derived from the molecular analysis of Rivadavia *et al.* (2003) and showing the relationship of the clades to various gland, germination and asexual reproductive features.

## Results

Gland patterns showed that *Drosera* was distinguished from its relatives by the presence of multiseriate sessile glands in addition to the distinctive *Drosera*-type stalked glands. All taxa possessing Type 3 biseriate glands (*sensu* Länger *et al.* (1995)) were located within the *Drosera* clade, and those with marginal glands were in a terminal clade containing sections *Drosera*, *Oösperma* and *Ptycnostigma*, as well as in the taxa now comprising the *Bryastrum* clade. The biseriate glands with elongated basal cells classed as Types 4 and 5 by Länger *et al.* (1995) were listed as *Rorella*-type glands by Seine & Barthlott (1993) because they were seen in subgenus *Bryastrum* (= *Rorella* auct. non Hall. ex All.: N. Marchant). The expanded data confirm this, with all taxa possessing these glands being members of the *Bryastrum* clade *sensu* Schlauer (1996) possessing these glands, as well as subgenus *Coelophylla* (*D. glanduligera* Lehm.) which was basal to the *Bryastrum*/*Ergaleium* clade pair.

When mapped onto the revised cladogram of Rivadavia *et al.* (2003), cryptocotylar germination becomes a synapomorphy for the clade representing the subgenera *Bryastrum*, *Coelophylla*, and *Ergaleium* (see Figure 2). With the exception of section *Phycopsis* (*D. binata* Labill.) all species in the clade so far germinated are cryptocotylar. The basal *Drosera* taxa are phanerocotylar with eglandular cotyledons, whereas those species that have glands on the cotyledons are restricted to the terminal clade containing sections *Drosera*, *Oösperma* and *Ptycnostigma*. These patterns are also supported by other features such as growth form (e.g. the tuberous habit of the *Ergaleium* clade taxa), distinctive petiolate leaves and the formation of summer-dormant aestivaculæ (Gaff 1981) shared between sections *Lamprolepis*, *Bryastrum* and *Lasiocephala*. There is also a propensity of cryptocotylar germination in dry-land habitat species (Pate & Dixon 1982) and those with asexual reproduction such as gemmae and tubers (Karlsson & Pate 1992) as seen in members of the cryptocotylar *Ergaleium* and *Bryastrum* clades.

## Discussion

Although some of the taxa studied here for gland data were also reported by Seine & Barthlott (1993) and Länger *et al.* (1995), our study provides data for a further 107 species, varieties, cultivars and morphotypes. In particular, we included many more taxa from sections *Lamprolepis* and *Lasiocephala*, so that between these three studies, most taxa within the family have now been surveyed. Our gland survey results for the additional taxa largely agree with those of the earlier studies. The *Ergaleium* clade was uniform in lacking marginal unifacial or spoon glands, as well as only possessing multiseriate microglands. There was considerable variability in inter-taxon gland types in the *Drosera* and *Bryastrum* clades. As with the earlier studies, there were both taxa with and without, marginal unifacial glands, but the dichotomy into taxa with multiseriate versus biseriate microglands was also observed.

Schlauer (1997) noted that gland studies in angiosperms related to Droseraceae were not new. *Dionaea*, *Aldrovanda*, *Drosophyllum*, *Polygonum* (Lersten & Curtis 1992), Nepenthaceae (Fahn 1979), Frankeniaceae, Plumbaginaceae and Tamaricaceae (Wilson 1890) all possess flat modified multicellular glands of sub-epidermal origin, which in Plumbaginaceae at least are thought to be derived from calcium carbonate-secreting Mettenian glands and developed into mucilage glands via intermediate forms. There are also symmetrical multicellular stalked glands, both vascularised and non-vascularised in Plumbaginaceae, Ancistrocladaceae and Dioncophyllaceae that are similar to those in *Drosera* and *Drosophyllum*. This indicates that the precursors for the trapping and glandular structures seen in Droseraceae are widespread in nearby outgroup Carophyllid families, possibly helping to explain the multiple development of carnivory within the group, or as suggested by Meimberg *et al.* (2000), the loss of carnivory in Ancistrocladaceae and most Dioncophyllaceae. This development is further seen within Droseraceae, where the basal taxa (section *Arcturia* and subgenus *Regiae*) are those with multicellular glands and unspecialised stalked glands, whereas the more derived *Drosera* and *Bryastrum* clades are where biseriate glands developed. Unifacial marginal glands seem to have developed several times, but also appear to have been lost secondarily in subgenus *Ergaleium*. Similarly, glandular cotyledons although found in the terminal *Drosera* clade, are also widespread in the outgroup families suggesting that this feature is ancestral and that its absence in various Droseraceae taxa possibly represents multiple loss.

Subgenus *Meristocaulis* was not included in the molecular phylogeny and its germination pattern is not known. The gland patterns (Table 1) are ambiguous, with marginal glands and Type T2 biseriate

and T11-12 multiseriate sessile glands. This pattern is seen in members of both the *Drosera* and *Bryastrum* clades, so that in the absence of germination data, the position of *Meristocaulis* is still uncertain.

Germination patterns show that cryptocotily has apparently evolved several times in the family (Fig. 2). In comparison with outgroup families; although Drosophyllaceae are cryptocotylar, the other related families (Tamaricaceae, Nepenthaceae, Polygonaceae, Plumbaginaceae and Limoniaceae) are all phanerocotylar, which is also the most common germination condition in the Caryophyllales (Clifford 1981; Clifford 1991). Conran *et al.* (1997) also described the germination pattern of hemicryptocotily, where the cotyledons emerge partially from the testa and are photosynthetic, but the apices remain enclosed in the seed. This pattern, seen in both *Dionaea* and *D. adelae* F.Muell. seedlings, seems to be an intermediate stage between crypto- and phanerocotily. Cryptocotily is a condition generally associated with large-seeded taxa, often from rainforests, and its occurrence in Droseraceae is considered unusual (Clifford 1984). The fact that it occurs in *Drosophyllum*, *Aldrovanda* and the *Ergaleium/Bryastrum* clade in *Drosera* suggests that there is an ecological association, as these are the taxa where there is often a need for long-term seed dormancy. This may be due to fire- (e.g. Ortega Olivencia *et al.* 1995 for *Drosophyllum*) or inundation-triggered germination (e.g. Kozschinski 1886 in the case of *Aldrovanda*). This applies especially in SW Western Australia where both summer fire or smoke exposure and then cool moist winter conditions are needed to stimulate germination in many angiosperm taxa including both Byblidaceae and some Droseraceae (e.g. Bell *et al.* 1993; Roche *et al.* 1998; Wills & Read 2002). Accordingly, the evolution of a larger seed reserve with a smaller embryo may be an advantage for long-term survival of these small seeds until environmental conditions are right for germination.

Seine & Barthlott (1994) and Conran *et al.* (1997) noted that there were gland and germination patterns supporting classification changes in Droseraceae, and the molecular data support the placement of *Ptycnostigma* and *Stelogyne* with *Drosera* and *Oöesperma* (along with sections *Arachnopus*, *Prolifera*, *Psychophila*, and subgenus *Thelocalyx*) in the phanerocotylar *Drosera* clade (Rivadavia *et al.* (2003)). Similarly, the taxa representing the *Bryastrum* clade are also united by *Rorella*-type glands and cryptocotylar germination, agreeing with many of the changes suggested by Conran *et al.* (1997), as well as some of the realignments of Schlauer (1996). Nevertheless, a number of the latter's subgenera could be returned to sectional level within expanded subgenera representing the *Drosera*, *Bryastrum* and *Ergaleium* clades. These data show that the molecular clades have an underlying basis in both anatomy and trap evolution, but also in ancestral ecological choices – the linkage in *Drosera* of dry-land-growing, asexually reproductive taxa with cryptocotylar germination, for example, shows that ecological adaptation seems to have been a major driving force for evolution within Droseraceae.

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