

Chapter 4. Experimental pollinations within and between members of the *Drosera peltata* complex (Droseraceae)

4.1 Introduction

The study of plant breeding systems has taxonomic and evolutionary implications. Inter-taxon compatibility, measured by seed set following cross pollinations, has been used to define taxon limits. Individuals that are cross-compatible and produce fertile offspring are considered to be conspecific, or belong to the same species, whereas those that do not are considered separate species. This is known as the biological species concept (Mayr and Ashlock 1991; Mayr 1993). The phylogenetic species concept approach also defines taxa by their gene flow, and estimates evolutionary relationships between entities. It is considered that unimpeded gene flow occurs within species, but that reproductive barriers prevent gene flow between species (Mayr 1993). The nature and extent of these reproductive barriers are considered to be commensurate in scale to the evolutionary divergence between the taxa of interest (Nixon and Wheeler 1990).

Reproductive isolation mechanisms may exist that prevent gene flow between individual plants or plant populations. These mechanisms may operate prior to pollination, between pollination and fertilization or post-fertilisation (Levin 1971). For example, otherwise reproductively compatible taxa may grow allopatrically, or may be sympatric but with non-overlapping flowering seasons, which prevents gene flow. Sympatric congeners may also have floral morphologies that allows access by some pollinators but not others, preventing heterospecific pollen transfer. Prezygotic reproductive barriers prevent heterospecific pollen from germinating on the stigmas, or chemically inhibit pollen tube growth in the style. Postzygotic reproductive barriers may occur so that hybrid zygotes fail to develop. Finally, hybrids may be sterile, or produce sterile offspring (Levin 1971; Charlesworth and Charlesworth 1987; Silvertown and Lovette-Doust 1993; Wendt *et al.* 2002).

Self-compatibility may evolve within a plant population or taxon depending on the balance between the transmission of advantageous selfing and the disadvantage of inbreeding depression (Charlesworth and Charlesworth 1987; Affre and Thompson 1999). The evolution of selfing may also be driven by pollinator limitation, or a lack

of mating partners in isolated populations arising due to dispersal or vicariance events (Wendt *et al.* 2002). Once self-compatibility has evolved autonomous self-pollination may develop as a way of ensuring for reproductive assurance, particularly where pollinator availability may be variable or the environment is marginal. Autonomous self-pollination can be an effective barrier to gene flow, especially where flowers become small and unattractive to pollinators, or are not visited by pollinators (that is, cleistogamous flowers) (Lloyd 1979). Autonomous self-pollination may therefore lead to rapid speciation.

Chance dispersal events of the propagules of self-compatible taxa has the potential to establish new populations; this is known as Baker's Law (Baker 1955; Baker 1967). Populations founded by such events contain a subset of the gene pool of the parent population. Further, if populations remain small, genetic drift, via chance mutation events, may change allele frequencies substantially from the original parental population (Silvertown and Lovette-Doust 1993). In addition, allele frequencies may change within isolated populations as they adapt to local environmental conditions. This process of adaptation will occur more rapidly and completely for self-fertilizing taxa (Charlesworth and Charlesworth 1987; Parker 1992). When plants from isolated populations are brought back into contact with parental stock and hybridized, the resulting progeny may be less fit than those produced within each population: this is known as outbreeding depression. Several studies, e.g. Waser and Price (1994), Affre and Thompson (1999), and Kelleher *et al.* (2005) have demonstrated that the level of outbreeding depression increases with distance between populations, indicating that such populations may be evolving independently along a cline (Schluter 2001).

Chance cross-fertilisation events between taxa may lead to the formation of hybrid progeny. In many cases such hybrids may be sterile or otherwise less fit than their parents, and do not persist, but this is not always the case (Stebbins 1959; Arnold 1992). Once formed, these crosses may be short lived and they may be assimilated into one parental line through introgression (Levin 1971). However, despite the odds, hybridization is a significant step in the formation of new taxa (Darwin 1859; Stebbins 1959; Potts and Reid 1988; Rieseberg 1995; Rieseberg 1997; Barton 2001;

Lau *et al.* 2005). Therefore, knowledge about the cross-compatibility of congeners provides important clues about the stability and taxonomic status of taxa.

Few studies have been conducted on the reproductive biology of members of the Droseraceae and the closely related *Drosophyllum lusitanicum* (Dioncophyllaceae: see Meimberg *et al.* 2000; Rivadavia *et al.* 2003). These studies have found different breeding systems within the species studied. Chen *et al.* (1997) found that 17 out of 20 Australian *Drosera* were self-incompatible, but plants could often reproduce clonally. They also found that in all cases the self-incompatibility mechanism in all self-incompatible taxa was pre-zygotic arrest of pollen tube growth within the style. In contrast, Murza and Davis (2005) found *D. anglica* in Canada to be both self-compatible and facultatively autogamous with seeds being produced in the absence of pollinators. Wilson (1994; 1995) examined *D. tracyii* in Florida and he found that flowers of this self-compatible species were pollinated by several species of bee. This species set no seed in the absence of pollinators.

Natural *Drosera* hybrids are known and artificial crosses have been made, but the resulting progeny are typically sterile (Cheek 1993). This suggests that genetic barriers exist within the genus to limit gene flow to members of the same species. Nevertheless some hybridisation events have led to the production of new species of *Drosera*, such as *D. tokaiensis* in Japan, (= *D. spatulata* × *D. rotundifolia* (Nakano *et al.* 2004), and *D. anglica* (= *D. linearis* × *D. rotundifolia*) in North America, Europe and Asia (Schnell 1995; Murza and Davis 2003).

The aims of this chapter are: (1) to examine the floral biology and breeding system of members of the *Drosera peltata* complex and (2) to test the taxonomic recognition of putative taxa (see Chapter 1). Experimental hand pollinations were conducted to determine the extent of autofertility, self-fertility, variation in fertility over geographic distance and interspecific fertility.

4.2 Materials and methods

4.2.1 Biology of study group

All members of the *Drosera peltata* complex share a common floral biology of short-lived flowers that open acropetally on a raceme. Each flower has five erect stamens that surround the ovary and styles (Planchon 1848; Marchant and George

1982; Lowrie 1987; Lowrie 1998) (see Figure 31). Towards the end of anthesis, the flowers of all species of the *Drosera peltata* complex, and indeed all members of the Droseraceae, undergo the same floral behaviour. Floral parts are not shed; instead the calyx and corolla inflex to cover the gynoecium and stamens. The petals wither and dry as the ovary swells if pollination has occurred. At maturity, the ovary splits along the sutures between each locule of the ovary, and the small ripe seed are shed through the gap between the base of the dry petals and sepals (Darwin 1875; Schnell 1974).

4.2.2 Plant material

A selection of members of the *Drosera peltata* complex were cultivated: *D. auriculata*, *D. peltata* 'Black Mountain, A.C.T.', *D. peltata* 'Isla Gorge, Qld.', *D. peltata* 'Red Rosette', *D. peltata* 'Western Australian Form' and *D. peltata* 'Western Sydney'. Study plants were collected from wild populations in eastern New South Wales, or were donated by colleagues from known provenance.

Vouchers of the plants studied will be lodged with NSW and NE.

The selection was biased towards those entities that grew to flowering in sufficient number in cultivation conditions provided. Individual plants were assigned a unique alpha-numeric code, based on putative taxon, provenance and an individual plant number.

4.2.3 General growing conditions

The study plants were grown in a shade house over one, two or three growing seasons. The shade house was covered in shade tan brown shade cloth, of approximately 1 mm² mesh, sufficient to exclude hover flies (Family Syrphidae, Order Diptera) (Naumann 1991). I have previously observed them visiting flowers of *Drosera peltata* in the wild. Hover flies may be the main pollinating agent for these plants. The mesh used was not fine enough to exclude thrips (Order Thysanoptera) (Naumann 1991), which are I have rarely observed on plants of *Drosera*, but which may occasionally visit their flowers. Plants of *Drosera peltata* were too slender and weak to support the weight of the bagging of individual inflorescences by fine mesh,

and applying mesh over individual pots appeared overly onerous (Keans and Inouye 1993).

Plants were grown in a substrate of approximately equal parts peat moss and sand. They were either grown in individual square pots that measured 4 cm wide by 10cm deep, or plants were grown communally in larger round plastic pots at least 8 cm diameter and 10 cm deep. In all cases plants were individually labelled. The pots were placed in plastic water trays so that the mix was kept moist to wet during the growing season, and the plants were exposed to direct sunlight for most of the day. No fertilizer was applied to the plants, however, plants did rarely capture, and digest, small flying insects (mainly fungus gnats: Families Scaridae and Mycetophilidae, Order Diptera (Naumann 1991)) during each growing season. Such prey could have increased the amount of flowers and seeds produced by such plants (Darwin 1875; Krafft and Handel 1991), and all plants had equal chance of obtaining such prey.

4.2.4 Floral biology

Plants were observed daily through the flowering period; floral colour, and floral longevity, and floral rewards were recorded. The movement of the anthers of several flowers were documented photographically to examine the effect this may have on autonomous selfing. The number of style segments per flower per putative taxon was counted and compared using an analysis of one-way variance (ANOVA). This was done because style segment number is a character used in previous taxonomic treatments of the genus (Planchon 1848; Diels 1906; Marchant and George 1982).

4.2.5 Breeding system of *Drosera peltata* ‘Black Mountain, A.C.T.’

Field and herbarium observations indicated that the putative taxon, *D. peltata* ‘Black Mountain, A.C.T.’ is the most widespread entity of the complex in eastern Australia. This taxon was therefore chosen to use as a model for the other entities in this complex. Plants of *D. peltata* ‘Black Mountain, A.C.T.’ that flowered were noted and flowers were pollinated using a range of treatments:

- Autogamy. No floral manipulation was performed to test for autonomous selfing. That is, untreated flowers, from which pollinators were excluded, were examined to see if seed was set. Seed set would then have resulted from autonomous selfing and self-fertilization. The absence of seed production would indicate either no autonomous selfing, or autonomous selfing but insufficient self-compatibility to produce mature seeds. Plants used in this study came from the following populations: Oasis, Cumboogle, Elong Elong, Premer and Bendemeer—all in N.S.W. This was the control experiment against which the percent of seed set of the following manipulations was compared.
- Self-pollination. Shortly following anthesis and the ripening of floral parts, stamens were removed one at a time from the target flower using a pair of fine forceps; grasping the filament below the anthers. The detached stamens were held with forceps and inverted, enabling the anthers to be brushed over the stigmas of the same flower. A 10x hand lens was used to check if pollen had been applied to the stigmas, and when this was confirmed, the stamens were discarded. Plants used in this study came from the same populations used in the control.
- Cross-pollination. In similar fashion to the self-pollination experiments, the anthers of synchronously open flowers from different plants of the same entity, and from the same population, were removed by forceps, inverted, and the anthers were rubbed over the stigmas of selected flowers. The styles were observed by a 10x hand lens to confirm that pollen had been applied. Plants used in this study came from the same populations used in the control.

In the self- and cross-pollination treatments all stamens were removed from each flower to prevent autonomous self-pollination. This was done by using a pair of fine forceps and grasping each filament below the anther, and tearing each one away from the styles in the centre of the flower, one at a time. The petals of each treated flower were also removed to reduce the attractiveness of manipulated flowers to any pollinators that may have entered the shadehouse (Kudoh and Whigham 1998). The base of each petal was grasped with a pair of fine forceps, and gently pulled away from the flower centre, until it became detached. This was repeated for each flower

until all petals were removed. Between 10 and 23 plants per population and between an average of 5.8 ± 0.7 (mean \pm SE) flowers were used for each pollination treatment, in each population.

To test for agamospermy, stamens and styles were removed, using fine forceps, from six flowers on four plants. None of these flowers set seed, indicating that agamospermy does not occur. This suggests that pollination is required to facilitate seed set (Richards 1997).

Each fruit was harvested between two and five weeks after pollination. Fruits were placed in individual paper envelopes. Each envelope was then labelled with the plant and flower number and date of harvest. The contents of each fruit were placed on a sheet of grid paper within a shallow container, and examined under low power magnification of a binocular microscope. Seed and ovules were counted using a handy tally counter. Ripe seeds and aborted seeds or unfertilized ovules were counted separately. Ripe seeds were plump and filled. They were opaque and generally black to dark brown in colour. Unfertilized ovules and aborted seeds were often indistinguishable from each other and were generally short, flat and translucent brown, black or cream in colour.

For each fruit, percent seed set was calculated as the number of ripe seeds divided by the number of ripe seeds plus the number of aborted and unfertilised ovules per fruit. Individual fruits were found to have an average of 139 ± 61 (mean \pm SD, $n = 536$) ovules (potential seeds) each.

A preliminary mixed-model two-way ANOVA was used to analyse all data for *D. peltata* 'Black Mountain, A.C.T.' to see if the effect of pollination treatment differed between populations. Pollination treatment was a fixed factor and population was a random factor. The pollination \times population interaction was not significant ($F_{8,72} = 1.28$, $P = 0.266$) and it was pooled with the error term for more powerful tests of the main factors.

4.2.6 Variation in fertility over geographic distance

Interpopulation cross-pollinations were undertaken using 46 flowering plants from 15 different populations of *D. peltata* 'Black Mountain, A.C.T.' Fresh pollen from flowers of the same entity, but from different populations, was applied to the stigmas of flowers of targeted plants. Percent seed set was calculated the same way as described in 4.2.5. Plants used in this study came from the following populations: Bendemeer, Premer, Gilgandra, Elong Elong, Beni, Cumboogle, Goolma, Oasis, Wilmatha Hill, Ilford, Wallaby Swamp, Mt. Ovens, Rockley Mountain, Castlereagh State Forest, and Mulgoa—all in N.S.W (Figure 30).

Distances between populations were calculated in ArcView 3.3 (Environmental Systems Research Institute 1999). Least squares regression analysis was used to examine the relationship between geographic distance and seed set. Reductions in seed set over geographic distance were used as evidence for outbreeding depression.

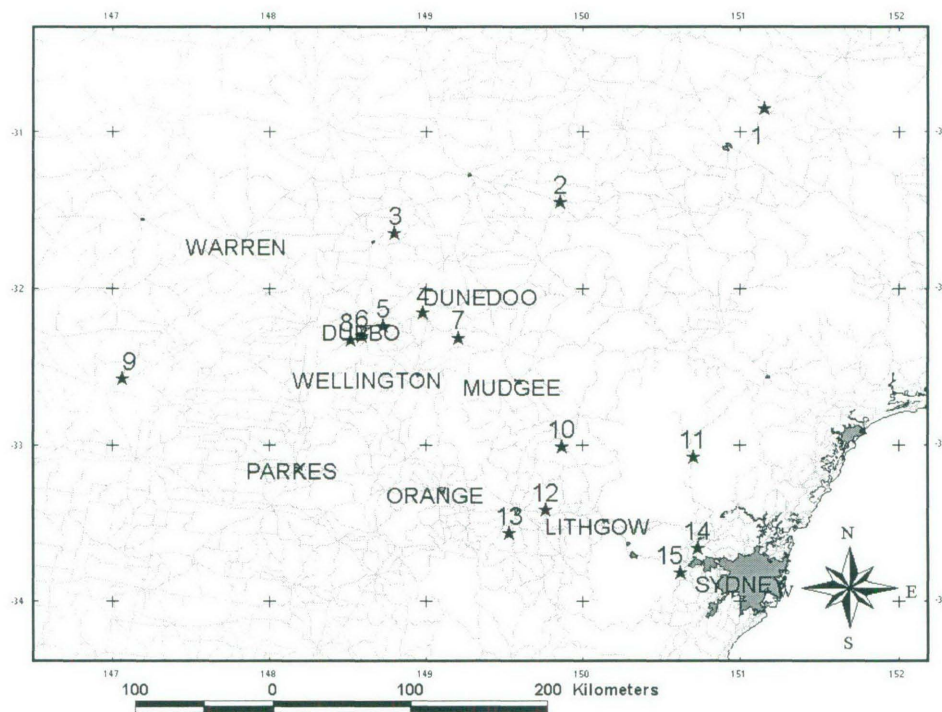


Figure 30. Location map of populations of *D. peltata* 'Black Mountain, A.C.T.' used in intra-entity pollinations. Location codes: (1) Bendemeer, (2) Premer, (3) Gilgandra, (4) Elong Elong, (5) Beni, (6) Cumboogle, (7) Goolma, (8) Oasis, (9) Wilmatha Hill, (10) Ilford, (11) Wallaby Swamp, (12) Mt. Ovens, (13) Rockley Mountain, (14) Castlereagh State Forest, and (15) Mulgoa.

4.2.7 Variation in fertility of between-entity crosses

Fifty-two flowering plants of *D. peltata* 'Black Mountain, A.C.T.' were cross-pollinated with other members of the complex to test for cross-compatibility of the different entities. The other entities used in this treatment were *D. auriculata*, *D. peltata* 'Red Rosette' and *D. peltata* 'Western Sydney'. The flowers were manipulated in the same way as described above (Section 4.2.5) except that pollen from other putative entities within the complex was applied to the stigmas of flowers of *D. peltata* 'Black Mountain, A.C.T.'. Within entity *D. peltata* 'Black Mountain, A.C.T.' pollinations were used as a control. Percent seed set was calculated in the same way as described above. A one-way ANOVA was used to assess whether seed set differed following within and between entity pollinations.

4.2.8 Variation in auto-fertility between entities

The most morphologically distinct members of the complex were compared in order to explore the variation in auto-fertility within the *D. peltata* complex (i.e. *D. peltata* 'Black Mountain, A.C.T.' versus *D. auriculata*). Examination of auto-fertility is justified because the analysis of the breeding system showed that seed set following autonomous self-pollination did not differ from the other treatments (see Results). Autofertility was assessed in five populations of *Drosera peltata* 'Black Mountain, A.C.T.' and two populations of *D. auriculata* as described above. A preliminary nested ANOVA with the populations nested within entities was used to examine differences between populations. No significant differences in percent seed set between populations of each entity was detected ($F_{5,27} = 2.00$, $P = 0.110$). Accordingly, data for each entity were pooled and autofertility was compared using a one-way ANOVA.

4.2.9 Statistical analysis

Analyses were performed using the statistical package Minitab (v. 13.1, Minitab Inc., 2000). The data were arcsine transformed and tested for normality by the Ryan-Joiner test, and tested for homogeneity by the Levene's test (Fry 1993).

4.3 Results

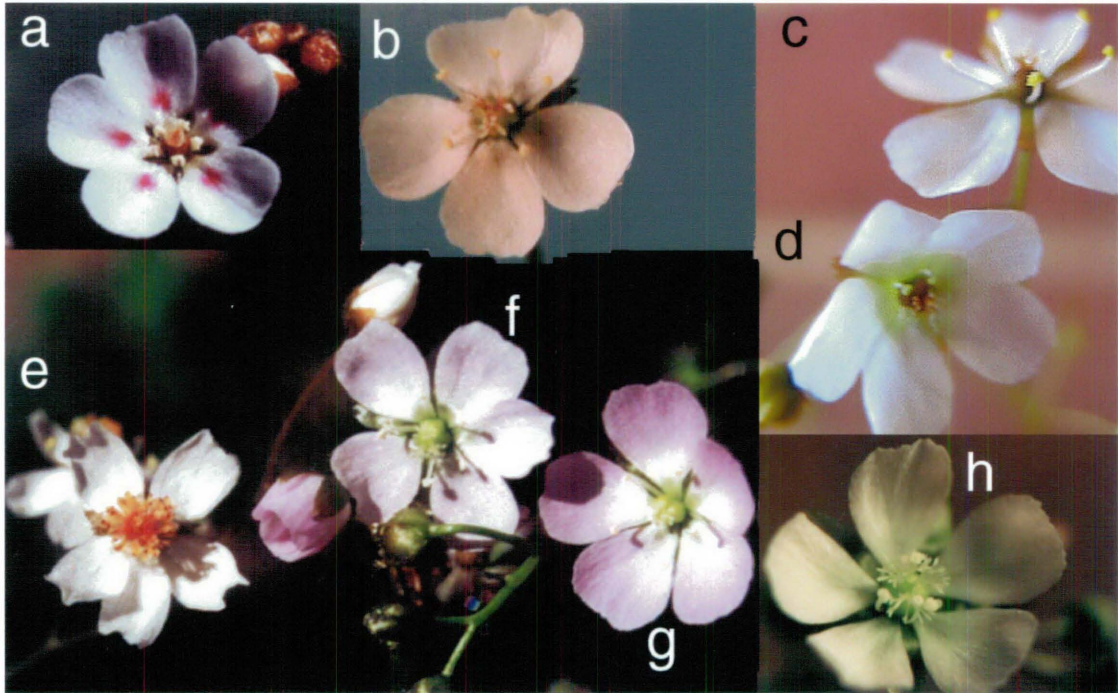
4.3.1 Floral biology of members of the *Drosera peltata* complex

The flowers of the *Drosera peltata* complex are produced on a scorpioid raceme, or rarely a cyme, and open acropetally. At anthesis the flowers are saucer-shaped, up-facing, co-sexual and exhibit pentamerous radial symmetry. Usually plants have only one flower open at a time, although after a period of cool weather, two adjacent flowers on one scape, or flowers on any additionally produced scapes, may open simultaneously. Each flower lasts only for a few hours and is open most often between mid-morning and the early afternoon, when the ambient air temperature rises above about 18 °C. The petals are white or pink, and in the case of *D. bicolor* each petal has a spot of red pigment about its base. The centrally-located ovary is usually green, but may be dark orange in some taxa (Figure 31 a, e). The petals emit a sweet fragrance and the flowers do not appear to produce nectar. The floral architecture, the timing and duration of anthesis, petal colour and fragrance and reward of pollen suggest that the flowers are most likely visited by bees and flies.

4.3.2 Delayed autonomous self-pollination within members of the *Drosera peltata* complex

The flowers of many members of the *Drosera peltata* complex exhibit a delayed autonomous selfing mechanism. Self-pollination is achieved by the inflexion of the filaments. For most of anthesis, flowers exhibit herkogamy (i.e. the anthers and stigmas are spatially separated, Figure 32 a). However, over time the filaments begin to inflex (Figure 32 b, c) until the anthers are brought into contact with the stigmas to facilitate self-pollination. The movement of the filaments precedes the inward movement of the petals (Figure 32 d) towards the end of anthesis. This mechanism was not observed in *Drosera peltata* ‘Western Australian Form’, where the stamens did not inflex. Nor was it seen in *D. bicolor* in which the stamens are shorter than the ovary and the styles are short, so that autonomous self-pollination is highly unlikely to occur even if the filaments did inflex. In both of these taxa, herkogamy is maintained throughout anthesis (Figure 31 a, e).

At ambient temperatures just below 18 °C, two situations were observed. The first was that flowers of the *D. peltata* complex remained closed until the day when



this temperature threshold was reached, and then the flower opened fully. The other situation was that the flower only partially opened, with the petals remaining erect and forming a narrow cylinder containing the gynoecium and the stamens; an arrangement that appeared too narrow for most potential pollinating insects to negotiate (pseudocleistogamic behaviour). The petals wilted at the end of that day. In such partially open flowers, the anthers were observed to either be in contact with the stigmas or were in close proximity, however in the latter case at least some stamens would be pushed into the stigmas by either inflexing filaments or corolla abscission and facilitate autonomous self-pollination. Such flowers did indeed set seed, however in this study these flowers were not differentiated from those that opened fully.

The number of style segments varied between taxa/entities ($F_{11,115} = 7.06$, $P = 0.000$). Three of the four taxa from Western Australia (*D. andersoniana*, *D. gigantea*, and *D. peltata* 'Western Australian Form') have significantly more style segments per flower than taxa/entities from eastern Australia, Asia and the Western Australian *D. bicolor* (Figure 33).

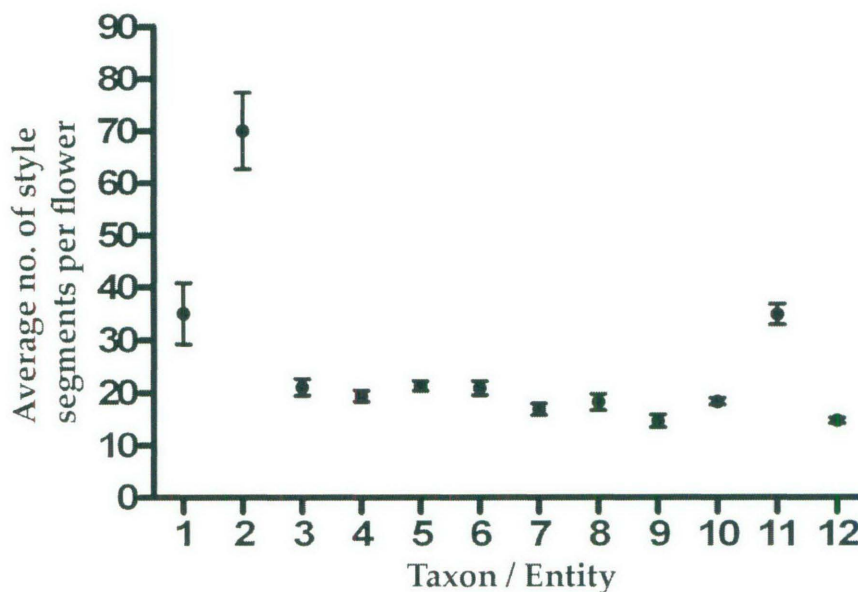


Figure 33. Mean (\pm SE) number of style segments per taxon/entity. Taxon/entity codes: 1 = *D. gigantea*, 2 = *D. andersoniana*, 3 = *D. auriculata*, 4 = *D. bicolor*, 5 = *D. peltata* 'Black Mountain, A.C.T.', 6 = *D. foliosa*, 7 = *D. peltata* 'gracilis', 8 = *D. peltata* 'Isla Gorge, Qld.', 9 = *D. peltata* 'nipponica', 10 = *D. peltata* 'Red Rosette', 11 = *D. peltata* 'Western Australian Form' and 12 = *D. peltata* 'Western Sydney'.

4.3.3 Breeding system of *Drosera peltata* ‘Black Mountain, A.C.T.’

Percent seed set did not differ significantly among pollination treatments ($F_{2,80} = 1.86, P > 0.163$) or populations ($F_{4,80} = 1.71, P > 0.151$). Seed set was about 50% in all pollination treatments (Figure 34). At least 90% of flowers involved in the three treatments set seed.

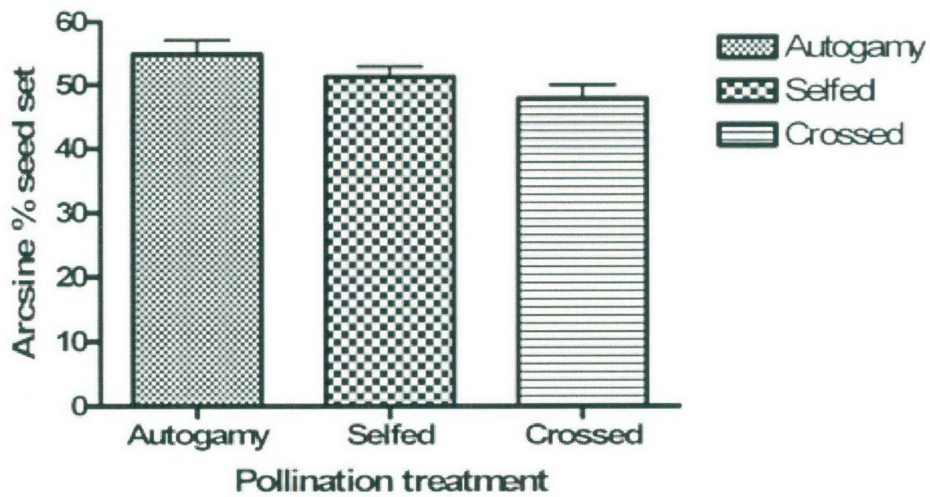


Figure 34. Mean (\pm SE) percent seed set per pollination treatment of five pooled populations of *Drosera peltata* ‘Black Mountain, A.C.T.’. There is no significant difference in fertility between the treatments.

4.3.4 Variation in fertility over geographic distance

Percent seed set of inter-population crosses of *D. peltata* 'Black Mountain, A.C.T.' showed a significant negative relationship with increasing distance between populations ($F_{1,11} = 8.30$, $P = 0.015$, $r^2 = 0.43$, $y = 69.8 - 0.11 x$; Figure 35)

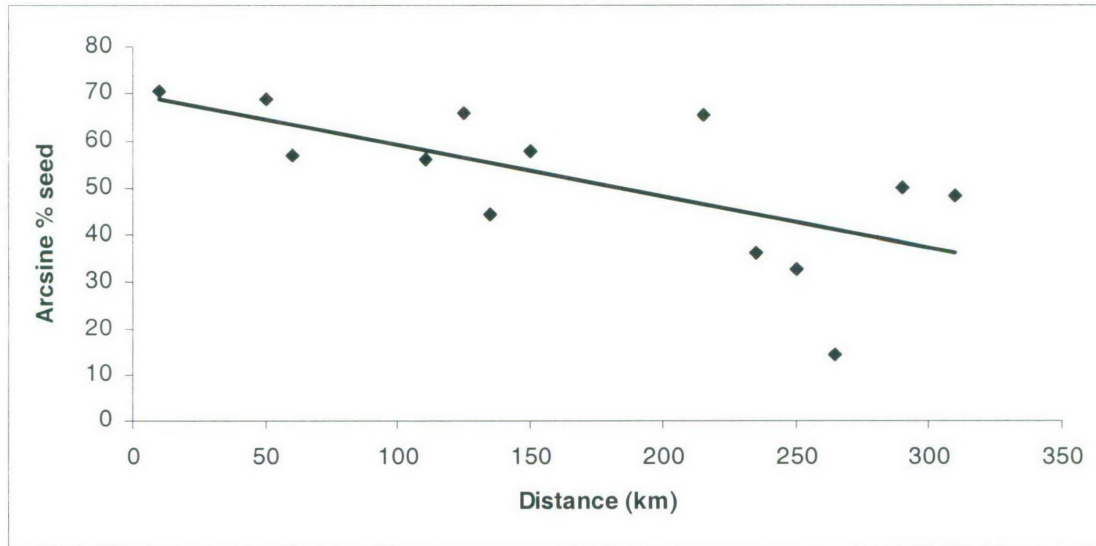


Figure 35. Relationship between arcsine square-root transformed percent seed set and the distance (km) between populations of *D. peltata* 'Black Mountain, A.C.T.' used in the intra-entity pollinations. Least-squares regression showed a significant negative relationship between fertility and distance between populations.

4.3.5 Variation in fertility of between-entity crosses

Seed set did not differ following within and between entity pollinations ($F_{3,77} = 0.40$, $P = 0.750$) and was about 45% in all treatments (Figure 36).

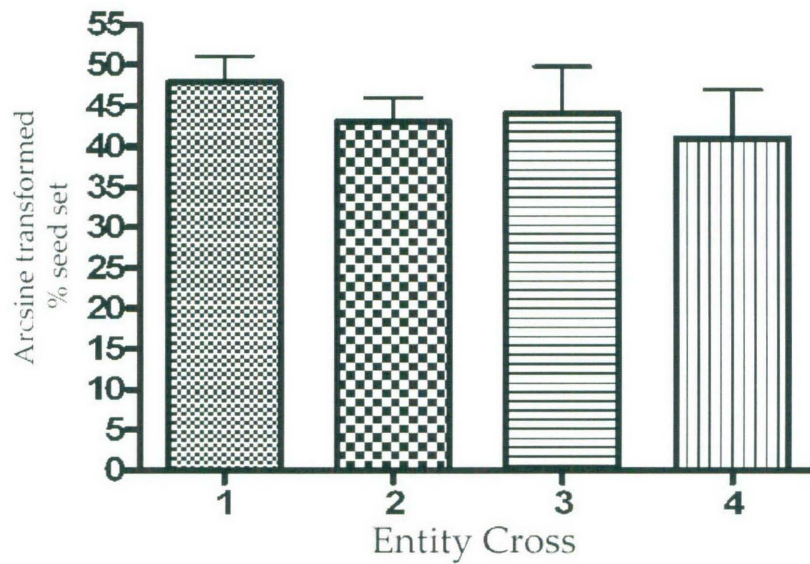


Figure 36. Mean (\pm SE) percent seed of four pollination treatments of *D. peltata* 'Black Mountain, A.C.T.' flowers. 1 = *D. peltata* 'Black Mountain, A.C.T.' crosses as a control; 2 = \times *D. auriculata*, 3 = \times *D. peltata* 'Red Rosette', and 4 = *D. peltata* 'Western Sydney'.

4.3.6 Variation in self-fertility between entities

Seed set in *D. peltata* 'Black Mountain, A.C.T.' and *D. auriculata* were about 50% (Figure 37). No significant difference was found between the entities ($F_{1,34} = 0.80, P = 0.380$).

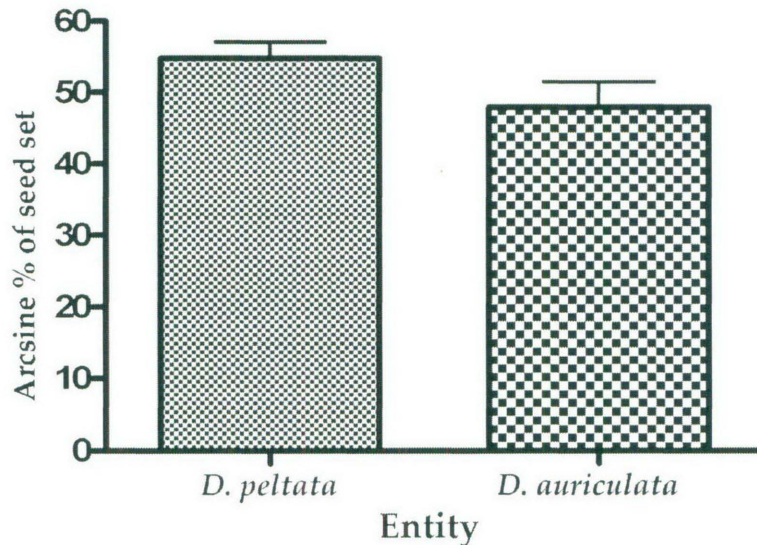


Figure 37. Mean (\pm SE) seed set of *D. peltata* 'Black Mountain, A.C.T.', and *D. auriculata* complex following autonomous self-pollination. Entities did not differ significantly.

4.4 Discussion

The members of the *Drosera peltata* complex studied were found to have a delayed autonomous selfing mechanism. Self-pollination was achieved by inflexing filaments that brought the anthers into contact with the stigmatic surfaces as the flower was beginning to close. The experimental pollinations revealed that the study group had similar fertility following autonomous self-pollination, manual self-pollination and cross-pollination. Percent seed set decreased within *D. peltata* 'Black Mountain, A.C.T.' when pollen from increasingly distant populations was used to pollinate flowers, indicating that outbreeding depression may be operating. Surprisingly, cross-pollinations between different entities were also highly fertile, although hybrids between these entities are not known from the wild.

4.4.1 High fertility of entities in the *D. peltata* complex

Under differing pollination treatments, *Drosera peltata* 'Black Mountain, A.C.T.' was found to be highly fertile, with high percent seed set. This is the first time that such fecundity has been recorded within the genus. Murza and Davis (2005) found *D. anglica* to be self-compatible and facultatively autogamous, although they did not investigate seed set from pollinations between related taxa. Wilson (1994; 1995) reported that *D. tracyi* was self-compatible, but flowers required pollinators to set seeds.

Unlike *D. anglica* (Murza and Davis 2005), plants of *D. tracyi* (Wilson 1994) and the *D. peltata* complex, are susceptible to geitonogamy. This happens under four situations: (1) where more than one flower opens on the same inflorescence on a given day; (2) where a plant develops a terminal panicle instead of a raceme, and flowers on different parts of the panicle open simultaneously; (3) where one or more axillary inflorescence(s) are developed on the same plant and flowers from two or more such scapes open simultaneously; and, (4) where a plant has reproduced clonally, by producing daughter tubers, and the flowering of one or more of these clones coincides with the flowering of the parent plant or other clones. Similar fertility following self- and cross-pollination found in this study indicates that geitonogamy would not reduce seed set in the entities studied. However, other members of *Drosera* subgenus *Ergaleium* commonly produce flowers in panicles and also reproduce asexually to form local clusters of clones (Marchant and George 1982; Lowrie 1987). Some taxa have been found to be self-incompatible (Chen *et al.* 1997: *D. gigantea*, *D. stolonifera* and *D. erythrorhiza*). Therefore geitonogamy is likely to play a variable role in affecting seed set in species of *Drosera*.

This study found *Drosera auriculata* to be self-compatible and autogamous; by contrast, Chen *et al.* (1997), found this taxon to be self-incompatible. They used commercially sourced plants of unknown geographic origin for their study. Therefore it appears that different subpopulations of this species have different breeding systems. This result is not anomalous for interpopulation differences in breeding system in other plant species have been found, such as *Pancratium maritimum* (Amaryllidaceae) (Medrano *et al.* 1999), *Leavenworthia* species (Brassicaceae)

(Solbrig and Rollins 1976), *Hibiscus laevis* (Malvaceae) (Klips and Snow 1997) and *Kalmia latifolia* (Ericaceae) (Rathcke and Real 1993).

Controlled pollinations between different populations of *D. peltata* 'Black Mountain, A.C.T.' showed a decrease in fertility with increasing distance between pollen and seed parents (Section 4.3.3). By contrast, high fertility was found in all other experimental pollinations conducted on the same entity. The result from this study (Figure 35) suggests that outbreeding depression may be operating, and that local populations are adapting to local environmental conditions (Affre and Thompson 1999).

4.4.2 Sympatric entities

Different members of the *Drosera peltata* complex in south eastern Australia grow and flower together in a limited number of sites. This occurs despite the different entities often having a slightly different habitat preference to each other:

- *D. auriculata* grows in moderately well-drained and often shaded habitats.
- *D. peltata* 'Black Mountain, A.C.T.' and *D. peltata* 'Western Sydney' grow in seasonally moist to wet sites in full sun to part shade; the latter taxon was found only in heavy clay soils.
- *D. peltata* 'Red Rosette' typically grows in seasonally to permanently wet to sodden, peaty or clay soils with variable sun exposure.

Two or three members of the complex grow sympatrically at sites at Castlereagh State Forest, Ilford, Mulgoa, Picnic Point, Wallaby Swamp (all in N.S.W.) and Mt. George in South Australia (see Figure 9). At these locations plants grow at the same time and flower together. No hybrids occur as judged by an absence of plants of intermediate morphology between potential parental taxa in the field. Knowledge of sympatric entities that remain distinct has been used to infer, for example, that *D. auriculata* and *D. peltata* are different species (Marchant and George 1982), and even that *D. peltata* consisted of a number of undescribed taxa (Gibson 1993a). In this study experimental pollinations demonstrated that inter-entity pollinations produce substantial numbers of seeds. Under natural conditions, however, hybrid plants may

be less fit than either parent and may be selected against whenever, and wherever they occur.

Future work on the reproductive biology of members of the *D. peltata* complex should involve growing the progeny produced by the different pollination treatments. This was done in the study of *D. anglica* by Murza and Davis (2005) to measure the effectiveness of different modes of pollination and the fitness of resulting progeny. Seeds from inter-entity crosses within the *D. peltata* complex may provide another means of investigating entity limits and taxonomic status.

Whilst members of the complex typically flower in spring, there are some notable exceptions. Plants of *D. peltata* 'gracilis' from Tasmania and montane areas of south eastern Australia flower in late summer (Planchon 1848; Curtis 1993). Similarly, *D. peltata* 'Isla Gorge, Qld.' and *D. peltata* 'nipponica', from coastal north eastern Australia and eastern Asia, respectively (Ohwi 1965; Stanley and Ross 1983), usually flower in late summer to mid autumn. In addition, isolated populations of members of the entities in south eastern Australia and New Zealand are able to flower outside of spring (Salmon 2001). In all cases, this atypical flowering season is determined by local soil temperature and soil moisture regimes. As a consequence, populations of these taxa will be genetically isolated from related entities, due to non-overlapping flowering times.

4.4.3 Delayed autonomous self-pollination

The results of this study documented a delayed autonomous self-pollination mechanism within members of the *Drosera peltata* complex that would provide reproductive assurance (Lloyd and Schoen 1992). In other taxa, plants can achieve autonomous self-pollination via a number of ways (Table 24).

The mechanism behind the inflexing stamens of *D. peltata* flowers is unknown. However, the leaf blade and the stalked retentive glands on the leaves of *Drosera* plants move in order to trap and retain invertebrates (Darwin 1875). The prey are killed, by suffocation, and the soft tissues of their bodies are broken down by acids and enzymes and then absorbed by the plant (Juniper *et al.* 1989). The stalked glands bend by a process of differential cell expansion (Hooker 1916; Hooker 1917; Lloyd

1942; Williams and Pickard 1972; Bopp and Weber 1981; Williams 2002). Perhaps filament curvature to facilitate autonomous self-pollination occurs by a similar mechanism.

Ambient air temperature appears to play a role in the timing and level of floral anthesis in members of the *Drosera peltata* complex. Observations made during this study indicated that temperatures above 18 °C were needed to trigger anthesis. This concurs with the findings of Lyon (1992) who found that bees rarely fly when ambient air temperature is below 18 °C. Therefore flowers of the study taxa may open during conditions that favour activity of pollinating vectors. To date, other studies on the reproductive biology of species of *Drosera* have not investigated the interaction of ambient air temperature and anthesis, and instead have focused on time of day when anthesis occurs (Wilson 1994; Wilson 1995) or have considered flower opening in relation to daily mean temperature (Murza and Davis 2005, Figure 1).

Observations made in this study of pseudo-cleistogamic floral behaviour of the study entities during cool weather concur with those of Murza and Davis (2005) showing differing responses of *D. anglica* flowers to weather conditions on the day of anthesis. Given that pseudo-cleistogamous flowers were produced from precocious plants that flowered in winter or in the early spring, before the main flowering period, it appears that these flowers opt for reproductive assurance through self-pollination in the bud when few pollinating insects are likely to be active (Lyon 1992).

Table 24. Summary of different autonomous self-pollination mechanisms found in different plant groups by other studies.

Autonomous self-pollination mechanism	Example taxa
Movement of the stamens to touch the stigmas	<i>Geranium sessiliflorum</i> (Geraniaceae) (Phillip 1985)
	<i>Aquilegia canadensis</i> (Ranunculaceae) (Eckhert and Schaefer 1998)
	<i>Sanguinaria canadensis</i> (Papaveraceae) (Lyon 1992)
	<i>Kalmia latifolia</i> (Ericaceae) (Rathcke and Real 1993)
Movement of the stigma(s) into the anthers	<i>Hibiscus laevis</i> (Malvaceae) (Klips and Snow 1997)
	<i>Hibiscus trionum</i> (Malvaceae) (Seed <i>et al.</i> 2006)
	<i>Iris versicolor</i> (Iridaceae) (Zink and Wheelright 1997)
Pollen pushed onto stigmas due to floral abscission	<i>Collinsia verna</i> (Scrophulariaceae) (Kalisz <i>et al.</i> 1999)
	<i>Pedicularis dunniana</i> (Orobanchaceae) (Sun <i>et al.</i> 2005)
Growth of the stigma into a zone of accumulated pollen	<i>Cyphia stenopetala</i> (Cyphiaceae) (Leins and Erbar 2005)
Pollen sliding onto the stigma due to gravity	<i>Caulokaempferia coenobialis</i> (Zingiberaceae) (Wang <i>et al.</i> 2005)

In many ways the members of the *Drosera peltata* complex in eastern Australia have the breeding system of a weed. According to Sakai *et al.* (2001), the reproductive attributes of invasive plant species include highly self-compatible flowers, a delayed autonomous mechanism, and a high rate of seed set irrespective of pollen source or pollination mechanism. Such features were found in members of the *D. peltata* complex studied. From the data presented in this chapter, for eastern Australia members of the complex, it appears likely that these taxa have the ability to found a new, viable population from the lodgement of a single seed into a new and

suitable habitat. Therefore members of this complex would comply with “Baker’s Law” (Baker 1955; Baker 1967). This is also seen in such species as *Crotalaria micans* (Etcheverry *et al.* 2003), a colonizing species.

4.4.4 Taxonomic implications of the breeding system of the *D. peltata* complex

The difference in style lengths in taxa and entities of *Drosera* from Western Australia and eastern Australia (4.3.1.1) indicates that different breeding systems occurring in most of the Western Australian taxa compared with those in eastern Australia and Asia. As shown in Figure 31 and 27, *Drosera peltata* ‘Western Australian Form’, along with another Western Australian endemic tuberous sundew, *D. andersoniana*, have more divided styles than members of the *D. peltata* complex in eastern Australia. This pattern of Western Australian members of *Drosera* subgenus *Ergaleium*, section *Ergaleium* having generally longer and more divided style segments is illustrated by Lowrie (1987; 1989; 1998; 1999). There are some exceptions to this rule, however, as shown by multiply-divided styles from *D. macrantha* subsp. *planchonii* in south-eastern Australia (Marchant and George 1982) and simply-divided styles in *D. bicolor* and *D. graniticola* from Western Australia (Marchant and George 1982; Lowrie 1987; Lowrie 1989; Lowrie and Carlquist 1992). Chen *et al.* (1997) found that the Western Australian members of *Drosera* subgenus *Ergaleium*, section *Ergaleium* were self-incompatible. Perhaps the variation in flower structure, petal colour and floral odour in the south western Australian Rainbow Sundews is a response to competition between often-sympatric taxa from scarce pollinators. In the absence of self-compatibility, plants of each taxon may devote resources to uniquely advertise their own flowers to local pollinators with high fidelity. On the other hand, the eastern Australian Rainbow Sundews, except for *D. macrantha* subsp. *planchonii*, have developed self-compatibility and are capable of delayed autonomous-selfing. They are not limited by pollinators because delayed selfing provides for reproductive assurance; which may be examined by measuring the pollen: ovule ratio in a range of species (Cruden 1977). Therefore these plants are able to use less resources in their flowers, which are relatively small and dull within this subgeneric section. Further study on the reproductive biology of Rainbow Sundews appears long overdue.

The high interfertility between entities of this complex supports the idea raised by Rivadavia *et al.* (2002), based on molecular data, that *Drosera* subgenus *Ergaleium* section *Ergaleium* has undergone a rapid radiation. I hypothesise that in the *Drosera peltata* complex recent divergence is not matched by establishment of reproductive barriers. As such, gene flow would still be possible between entities, particularly where they grow sympatrically. If this were the case, it would explain the lack of separation of described and putative entities within the complex in the phenetic analyses. It may be that these entities are still in the process of spreading and obtaining fixed and defined morphologies.

4.5 Conclusions

Members of the *Drosera peltata* complex studied have high fertility from autonomous self-pollination, self-pollination and inter-taxon cross-pollinations. The eastern Australian taxa examined have an efficient delayed autonomous self-pollinating mechanism for reproductive assurance. Each fruit produces many small seeds that appear to be easily dispersed. Due to the delayed nature of autonomous self-pollination each bloom, if open fully, is available for cross-pollination until the flower is nearing the end of anthesis, at which stage the inflexing stamens ensure the anthers contact the stigmatic surfaces. Under cool temperatures, the flowers open partially and are therefore less likely to be cross-pollinated before autonomous self-pollination occurs. Therefore the study taxa potentially have a mixed mating system, with the ability to produce high quality heterozygous progeny from cross-pollinations, or a comparable number of homozygous progeny from autonomous self-pollination, depending on pollinator ability (Holsinger 1991).

The results of pollinations between different populations of the same putative taxon were anomalous compared to the other treatment results. Pollinations showed a significant negative correlation between fertility and distance between populations, with levels of fertility dropping to half of that observed in all other treatments. This is consistent with outbreeding depression and the development of partial reproductive barriers. The occurrence of outbreeding depression indicates that local populations may be rapidly adapting to new environments following dispersal events and that a process leading to speciation has begun. This is particularly the case in south eastern

Australia where the most variation in the complex occurs and where different entities grow sympatrically in several locations. The occurrence of the entities *D. auriculata* and *D. peltata* 'Black Mountain, A.C.T.' in New Zealand indicate that dispersal events are continuing (Salmon 2001).

An efficient mechanism of self-pollination is an effective barrier to gene flow (Lloyd 1979). Gene pools of autonomous self-pollinating populations will rapidly become co-adapted to local environmental conditions (Parker 1992). Inter population cross-pollinations within the wide-ranging *D. peltata* 'Black Mountain, A.C.T.' resulted in reduced percent seed set when the distance between pollen donor and seed parent increased. This is indicative of outbreeding depression, where local adaptation is occurring within individual populations. Therefore members of the *D. peltata* complex appear to be at an interesting stage of evolution, where incipient speciation may be occurring, assisted by infrequent long-distance dispersal events and an effective delayed autonomous self-pollination mechanism.

Further study of *Drosera* is warranted. As Murza and Davis (2005) have stated, the study of reproductive biology of carnivorous plants is in its infancy due to the focus of study on the carnivorous syndrome of these plants. This study has certainly revealed that some species of carnivorous plants are able to employ movement, not only for obtaining additional nutrition, but also for reproductive assurance.