

High self-pollen transfer and low fruit set in buzz-pollinated *Dianella revoluta* (Phormiaceae)

David H. Duncan^A, Adrienne B. Nicotra^A and Saul A. Cunningham^{B,C}

^ABotany & Zoology, Australian National University, Canberra, ACT 0200, Australia.

^BCSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia.

^CCorresponding author; email: saul.cunningham@csiro.au

Abstract. We used pollinator observation, flower manipulation, controlled pollination and pollen-tube analysis to better understand the reproductive ecology of *Dianella revoluta* R.Br., a common species known to have depressed fruit set at fragmented sites. This buzz-pollinated species was found to receive large quantities of self-pollen even during a single pollinator visit, but is only partially self-compatible. This may be the first direct demonstration of pollinator-facilitated, autogamous self-pollen transfer accounting for a significant proportion of stigmatic pollen load. Frequent high self-pollen transfer may account for the observed low rate of fruit development in open-pollinated flowers. Self-pollen tubes reached the base of the style in comparable numbers and at the same rate as outcross pollen tubes, with no sign of pollen-tube competition favouring outcross pollen. Barriers to greater self-fertility occur late, probably through early abortion of selfed ovules. We also investigated what impact overlapping distribution with *D. longifolia* may have on *D. revoluta* pollination and reproduction. Although these species shared pollinators, they differed in terms of frequency of visits. There was also separation of floral phenology within the course of a day.

Introduction

Although many factors influence the susceptibility of plant species to habitat-fragmentation effects, there is agreement that pollination specialisation, and mating system, are likely to be important determinants of a species' level of risk (Bond 1994; Murcia 1996). Native-pollinator faunas have been shown to decline in fragmented landscapes (Aizen and Feinsinger 2002) and, all else being equal, species with fewer potential pollinators have a higher probability of losing theirs during this process of attrition (Bond 1994). Species that are self-incompatible are also likely to suffer reproductive declines because of greater dependence on adequate pollinator service for outcross-pollen receipt. Net reproductive success of plants, however, also depends on many other aspects of a species' biology and life history, and the interplay between those traits. Indeed, a recent meta-analysis by Aizen *et al.* (2002) found that species' self-incompatibility and degree of pollinator specialisation were not good predictors of fragmentation effects, at least not as they are usually assessed.

For many plant species we will need to know more about pollinator behaviour and the pollen-flow dynamics that occur when pollinators visit, in order to gauge the importance of

pollination biology and mating system. For example, ongoing reproductive success may be relatively secure for a self-incompatible species if it is well supplied with outcross pollen. Alternatively, a generalist-pollinated species may be prone to suffer reproductive failure because of being swamped by pollen from other plant species. Ideally, a species' pollinator service should be determined from field studies that consider the outcome of pollinator behaviour at flowers (e.g. Snow 1982) as well as season-to-season variation in pollinator distribution and abundance (e.g. Minckley *et al.* 1999). High-resolution studies on single species are a key part of developing our understanding of fragmentation effects (Aizen and Feinsinger 2002).

In this study, we investigated the reproductive ecology of *D. revoluta*—a common and sometimes dominant understorey species of mainland Australia, where it often forms large clonal patches (Henderson 1987). Despite its considerable distribution and abundance, a study of fragmentation effects in mallee woodland found that fruit-set efficiency of *D. revoluta* was significantly higher in nature reserves than in nearby linear strips (Cunningham 2000). One of the key concerns relating to habitat fragmentation is that effects may take the form of a slow erosion of essential

processes and mutualisms (Kearns *et al.* 1998) and these effects could be evident in common species as well as already threatened taxa.

Despite its abundance, there is no detailed published information about *D. revoluta* reproductive biology. The genus *Dianella* and related taxa were segregated from the Liliaceae and placed into the recircumscribed family Phormiaceae Agardh in 1984 (Henderson and Clifford 1984). *D. revoluta* is a rhizomatous perennial herb that has erect leaves forming tufts or large clumps (Henderson 1987). The blue petaloid flowers are usually pendulous and pedicellate, borne on branching flowering shoots or inflorescences. There are six anthers, which are projected in a ring around the superior ovary and pistil. The anthers remain enclosed with small pores at the apex (poricidal), becoming slits (Henderson 1987).

The poricidal anther morphology of *Dianella* is consistent throughout the genus, and congeneric species are known to be buzz-pollinated by native bees (Buchmann 1983; Bernhardt 1995; Hingston 1999). *D. revoluta*, therefore, is likely to be buzz-pollinated, as are several other species in the study area, including *D. longifolia* var. *longifolia* (R.Br., syn. *D. laevis* R.Br.). To our knowledge, there are only four published records of flower visitors to *D. revoluta*; all were female halictid and anthophorine apid native bees (Houston 2000). *D. caerulea*, studied by Bernhardt (1995) in coastal New South Wales, was also buzz-pollinated by female anthophorine apid and halictid bees. During foraging bouts, bee visitors to *D. caerulea* rarely visited more than one flower on an inflorescence before moving onto another anther. This behaviour, in addition to the fact that inflorescences opened only 5% of their flowers on a given day, ought to strongly promote outcrossing. However, observed rate of fruit set was only 20% and Bernhardt concluded that *D. caerulea* was likely to be self-incompatible.

Certain self-incompatibility systems are correlated with certain morphological traits, allowing speculation about the breeding system of *D. revoluta*. The dry-type stigmatic surface of *Dianella* species is often associated with sporophytic self-incompatibility (SSI; Heslop-Harrison and Shivana 1977; Dahlgren *et al.* 1985). The binucleate pollen of *Dianella* (Heslop-Harrison and Shivana 1977) is, however, more reliably associated with gametophytic self-incompatibility (GSI) systems (Barrett 1988). In GSI plants, inhibition of pollen-tube growth in incompatible combinations acts relatively late, in the style or even at the ovary (Barrett 1988), whereas incompatible combinations are usually rejected at the stigma in SSI plants (Heslop-Harrison and Shivana 1977). Another distinct mechanism of self-incompatibility that occurs after pollen tubes have reached the ovary is 'late-acting self-incompatibility' (LSI). There are no reliable morphological correlates of this type, and the precise

mechanisms are unclear (Gibbs and Bianchi 1999); however, it is frequently identified as the cause of self-incompatibility in studies of species' mating systems (Ramsey 1995; Gribel and Gibbs 2002; Vaughton and Ramsey 2003).

We applied a combination of pollinator observation, flower manipulation, controlled pollination and pollen-tube studies to achieve a broad understanding of the reproductive ecology of *D. revoluta* and to provide insight as to why habitat-fragmentation effects are occurring in this species. We also investigated whether overlapping distribution with *D. longifolia* was likely to affect *D. revoluta* pollination and reproduction.

Materials and methods

Observations of floral and pollination ecology of *Dianella revoluta* were made in a range of mallee-vegetation remnants in nature reserves and along roadsides in central New South Wales, Australia. All experiments took place in Gubbata Nature Reserve (33°38.01'S, 146°33.36'E). The vegetation of the roadsides and reserves consists of shrubby, open mallee woodland dominated by multi-stemmed trees of the genus *Eucalyptus*. The roadsides have similar floristic composition to the reserves but are more disturbed, largely because of soil disturbance associated with road maintenance. All sites are between 150 and 200 m a.s.l. and average rainfall for the area is between 200 and 300 mm year⁻¹.

Floral phenology

In the Spring 2000, 26 open flowers and 24 buds that looked soon to flower were tagged with wire over a period of 4 days. Tagged nodes were observed each day and their status (yet-to-open, open, closed) was recorded. Of the 26 open flowers tagged, none remained open for a second day. Of the 24 buds tagged, all flowered for a single day only. In the same season, the number of buds and flowers on 257 inflorescences was counted once. The number of flowers open on one day was also recorded once at each inflorescence between mid-October and early November 2000. Follow-up counts to assess the rate of fruit set were carried out in mid-December 2000.

Pollinator observation and collection

Insects visiting flowers of *D. revoluta* were opportunistically netted during three consecutive spring seasons, from 2000 to 2002. In addition, regular but *ad hoc* efforts were made to capture flower visitors to *D. revoluta* and to the sympatric congener, *D. longifolia* var. *longifolia*. Insect specimens were deposited in the Australian Museum, Sydney. Other data on pollinator visitation were drawn from observations made at plants, and from viewing video of selected flowers. For the latter, a tripod-mounted, 8-mm video camera was trained on one or more flowers on an inflorescence and then left to record for 90 min. In total, data for *D. revoluta* visitation is drawn from 117 records over 26 sites, 20 days and three seasons. Visitation data for *D. longifolia* is drawn from 59 records over six sites, 5 days and two seasons.

Autogamous self-pollen transfer

Observation of pollinator behaviour at flowers caused us to suspect that the flower-handling of pollinators caused self-pollen transfer to occur within flowers ['facilitated' autogamous selfing (Lloyd 1992)]. In order to determine whether this was so, we devised an anther-blocking technique, which prevents removal of self-pollen from a flower but does not interfere with flower attractiveness. We presented manipulated and intact virgin flowers to foraging bees and compared the pollen load

from a single visit. This method is similar to flower emasculation (removal of anthers, e.g. Eckert 2000).

We used cut inflorescences for this experiment. Trials showed that flowers on cut stems remained open almost as *in situ* flowers, and inflorescences left in vases overnight flowered the following day. Thus, stem hydraulics did not appear unduly affected. Inflorescences with four or more buds in the process of opening were collected early in the morning and placed in vases. Visitors were excluded while the flowers opened. Craft glue (PVA) was applied to cover the apex of anthers on half of the open flowers on each inflorescence. The base of the pedicel of glued flowers was marked so that flowers could be identified from a distance. Inflorescences were then placed in a vase in a moderately dense patch of flowering conspecific plants between 1100–1230 hours and 0200–0330 hours. Flowers were collected after a single pollinator visit and transferred to a vial of 70% ethanol. All flower visits were by female *Lipotriches ferricauda* bees. Bees were seen to visit blocked flowers as readily as unblocked flowers and there was no difference in the length of time spent at blocked compared with unblocked flowers. These observations suggest that bees were not deterred from visiting flowers with blocked anthers. We removed visited flowers quickly to prevent bees from consecutively visiting anther-blocked flowers. In total, data for 35 flowers was obtained (blocked anther $N = 18$, control $N = 17$) over 1 day. In the laboratory, stigmas were stained for 3 min with modified Carlsberg's solution (Ogden *et al.* 1974) and examined under light microscope. The number of conspecific and heterospecific grains was recorded.

Self-compatibility

Late in October 2001, at least two flowers on 22 inflorescences of *D. revoluta* were bagged early in the morning (before they had opened) with fine tulle mesh. A large number of flowers from plants elsewhere in the reserve was collected for use as an outcross-pollen source. These flowers were stored in an airtight bag in cool conditions until pollen application took place.

From midday, mesh bags were removed from flowers while self- or outcross pollen was applied to the stigma with a needle. Outcross pollen was from a single outcross donor flower, although flowers from many donors were used over the treatment as a whole. Pollen was collected from flowers by sliding the needle tip into, and then along, the anther towards the apex. The needle was wiped clean between applications. One additional open flower in each inflorescence was selected for the open-pollinated treatment. In total, 69 flowers were used, including 23 self-, 24 outcross, and 22 open treatments. Self-pollinated flowers were re-covered with mesh until the following day to prevent any subsequent outcross-pollen deposition.

Fruit development can occur rapidly in *D. revoluta*, and once ripe the fruits may drop or be removed. For that reason, 10 days after the treatments were applied we visited all flowers and noted whether or not fruit was developing (ovary swelling). A further 20 days after the treatments were applied, at fruit maturity, we made a final assessment on fruit development and collected all remaining fruits in order to count the number of seeds per fruit.

Pollen competition

We used controlled pollination techniques to test for differences in pollen-tube growth between self- and outcross-pollinated flowers. Thirty-two flowers were covered with mesh bags and hand-pollinated as described above, with either self- ($N = 15$) or outcross pollen ($N = 17$), and then re-bagged. They were collected in three batches, the first batch after 8 h ($N = 7$ for self, 9 for outcross), one batch after 24 h ($N = 4$, 5) and the last after 30 h ($N = 4$, 3).

Stigmas were prepared for pollen-load counts as already described. The styles were cleared in 60% bleach for 2 1/2 h, rinsed and left to stain overnight in decolourised aniline blue (Kearns and Inouye 1993). The

number of pollen grains on the stigmas was counted with a light microscope and the number of pollen tubes at the base of the styles was counted with fluorescent-light microscope.

Results

Floral phenology

Dianella revoluta was predominantly spring flowering over three seasons (2000–2002), although some individuals continued to flower into summer. Flowering was earlier in the hot drought of 2002 than in the previous two seasons and within seasons flowering began earliest in patches along open roadsides and railway reserves (D. H. Duncan, pers. obs.). In 2002, flowering was depressed overall, with many previously healthy plants failing to flower or drying out completely. Inflorescences from 2002 also tended to be smaller and have fewer nodes than either 2000 or 2001.

Individual *D. revoluta* inflorescences produced 18–190 buds in the spring of 2000 (mean \pm s.e., 76 ± 2.3). The average number of flowers open in one day was 3 (± 0.12 , $N = 257$; range 1–11); thus, flowering on an individual inflorescence typically extended over a month. Over the course of three seasons the greatest number of open flowers seen on one inflorescence in a day was 16. The average conversion rate of flowers to fruit from the 2000 survey was 32% (D. H. Duncan, unpubl. data).

Flowers began to open from as early as 0800 hours in the warm, dry spring of 2002, and slightly later in the preceding two seasons. The anther pores opened shortly afterwards, and extended to become short slits after several hours. From about 1500–1600 hours, the flowers began to close and droplets of dark fluid were exuded that eventually bound the perianth together around the androecium and gynoecium. Within a few days of flowering, the fused structure hardened and became brittle. The structure often persisted even as a fertilised ovary expanded behind it; however, it was usually lost before fruit maturation. Open flowers and maturing fruits were commonly found on a single inflorescence. The fruit matured to a deep blue globular berry, up to 1 cm in diameter, depending on the number of seeds. All examined ovaries contained 18 ovules.

In the study area *D. revoluta* often co-occurred with *D. longifolia*. The flowering seasons appeared to overlap little, with *D. longifolia* beginning to flower at the tail of *D. revoluta* flowering season. Likewise, timing of flowering during a given day did not coincide. Whereas *D. revoluta* flowers opened from the early morning and began to close mid-afternoon, *D. longifolia* flowers began to open from 1500–1900 hours and on some inflorescences, they remained open for several hours after dark.

Pollinator visitation

The introduced honeybee *Apis mellifera* was the most abundant bee species observed at most sites, but it was never seen to visit *Dianella* flowers. All pollinators trapped while

Table 1. Insects trapped visiting *Dianella revoluta* flowers

Taxon ^A	Body length (mm)
Apidae (Subfamily Apinae), tribe Anthophorini	
<i>Amegilla</i> (<i>Zonamegilla</i>) sp.	13.5 (n = 3)
Halictidae, subfamily Halictinae	
<i>Homalictus urbanus</i>	5.0 (n = 2)
<i>Lasioglossum</i> (<i>Chilactus</i>) <i>ebeneum</i>	8.6 (n = 5)
<i>Lasioglossum</i> (<i>Parasphecodes</i>) near <i>hiltacum</i>	8.5 (n = 3)
Halictidae, subfamily Nomiinae	
<i>Lipotriches</i> (<i>Austronomia</i>) <i>ferricauda</i>	9.3 (n = 3)
<i>Lipotriches</i> (<i>Austronomia</i>) <i>australasica</i>	11.0 (n = 3)

^ATaxonomy follows (Michener 2000).

visiting *Dianella* flowers (total $N = 41$) were female native bees from the Halictidae and Apidae (Tribe Anthophorini) families (Table 1 and Fig. 1). The larger species (*Amegilla*, *Lipotriches ferricauda* and *Lasioglossum* spp.) were also observed to visit flowers of other buzz-pollinated species in the area (*Senna artemisioides* and *Halgania cyanea*) as well as *Westringia cheelii*, *Prostanthera aspalathoides*, *Philotheca difformis* and *Thysanotus baueri*.

Dianella pollinators ranged in size from 5 mm in length (*Homalictus*) to over 13 mm (*Amegilla*) and different-size flower visitors varied in the way they interacted with a flower when foraging for pollen. Small bees typically visited all anthers by moving around the androecium, sometimes without appearing to contact the stigma. Mid-sized bees (*Lipotriches* and *Lasioglossum*) also visited all anthers of fresh flowers, but in most cases were in constant contact with the style and stigma. However, the large *Amegilla* bees appeared to buzz up to three anthers at a time, their weight and momentum causing the inflorescence branchlet to dip sharply. They would then release and move to another flower, sometimes returning to flowers they had already visited. It was very common for bees of all species to visit several or all flowers on a single inflorescence before moving onto another plant.

Combined collection and observation data across both *Dianella* species indicate that all bee species were observed visiting flowers of one species or other from early morning (0900–1000 hours) to late evening (1800–2000 hours). However, although *D. revoluta* and *D. longifolia* shared the same suite of pollinators, the relative importance differed between species (Fig. 1). For example, *L. ferricauda* represented nearly 60% of observed/trapped visitors to *D. revoluta* compared with ~10% of visits to *D. longifolia*. *Homalictus* bees accounted for only ~5% of visits to *D. revoluta* but were the most common visitors (over 60%) to *D. longifolia*.

Other insect–flower interactions

Flea beetles (*Arsipoda homolaena* Germar, and *Arsipoda* sp. (Chrysomelidae)) commonly chewed open flowers, recently

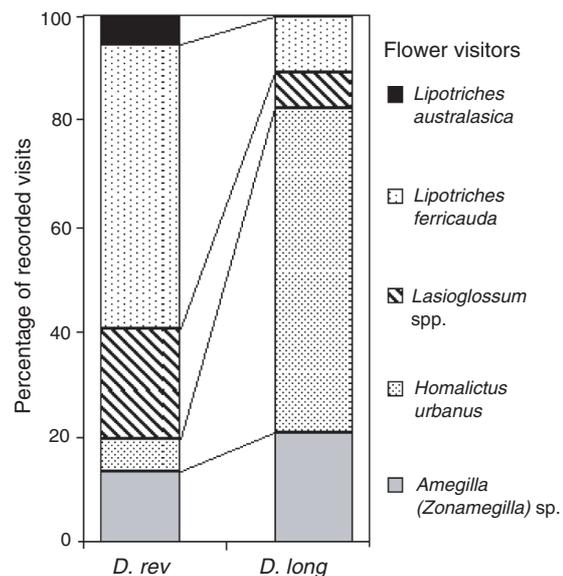


Fig. 1. Accumulated pollinator-visit data for *Dianella revoluta* (90 records) and *D. longifolia* (59 records) from 2000 to 2002. Multiple flower visits from a single bee during a foraging bout count as a single record only.

closed flowers and mature buds. These beetles were apparently responsible for stigma losses of ~40% of study flowers over two seasons.

Thrips were often seen against the dark anthers of open flowers late in the day and subsequently were commonly found in flower samples. Thrips are known to cause *Dianella* flowers to be malformed (Henderson 1987; Wilson 1993), and outbreaks of flower galls were observed to a limited extent in 2000 and 2001 seasons, but very frequently in 2002 (D. H. Duncan, pers. obs.). The majority of thrip specimens were identified as female onion thrips (*Thrips tabaci* Lindeman, Thysanoptera: Thripidae), a cosmopolitan, polyphagous pest of agriculture, also known to be a vector of plant viruses. On other plant species they are known to feed on pollen, flower and leaf tissue as well as preying on other organisms such as mites (Milne and Walter 1998). *Thrips imaginis*, ‘plague thrips’ Bagnall (Thysanoptera, Thripidae), was also identified in flower samples.

Autogamous self-pollen transfer

Significantly more *Dianella* pollen was transferred to the stigma when self-pollen was accessible (median = 45) than when the anthers were blocked (median = 10, Fig. 2, Mann–Whitney U -test: $P < 0.025$). Therefore, much of the pollen transferred during a pollinator visit was self-pollen from within the same flower (facilitated autogamous selfing). This experiment is likely to underestimate the total proportion of stigmatic pollen load that is self-pollen. If pollinators consecutively visit flowers on the same plant then additional geitonogamous self-pollination will occur. The

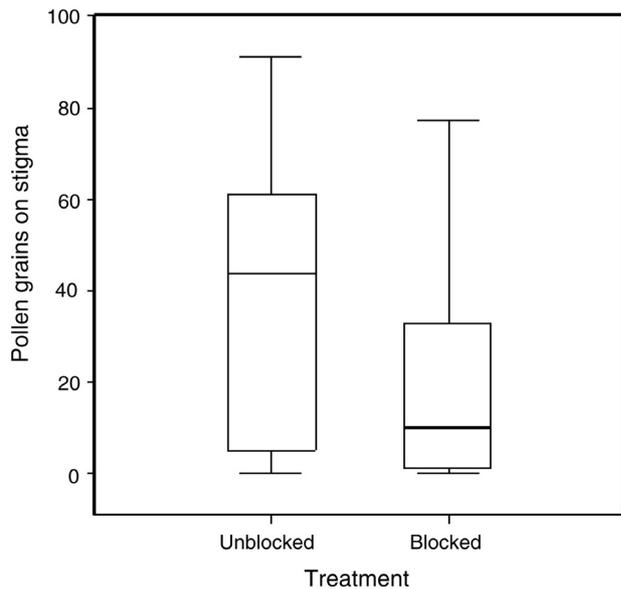


Fig. 2. Boxplot of *Dianella revoluta* pollen deposited during a single pollinator visit. Unblocked anthers received significantly more pollen (Mann–Whitney *U*-test (1-tailed), $P < 0.025$).

amount of non-*Dianella* (heterospecific) pollen deposited on stigmas ranged from 0 to 71 grains (median 2, upper quartile *c.* 9) and did not vary by treatment (Mann–Whitney *U*-test: $P > 0.5$). The absence of any difference among treatments in the amount of heterospecific pollen on stigmas suggests that pollinator behaviour at flowers was similar across treatment types.

Self-compatibility

Application of outcross pollen resulted in a higher rate of fruit formation than did either open- or self-pollinated treatments (Fig. 3a, *G*-test: $P = 0.005$ and $P < 0.001$, respectively). Fruit set in open-pollinated flowers was lower than in outcross-pollinated flowers but higher than for self-pollinated flowers, indicating that some outcross-pollen transfer was happening. There was substantial loss of fruit between the two assessment periods, resulting in very low sample size for the seed per fruit data. Outcross-pollinated flowers yielded more seeds per fruit (mean = 4) than did open-pollinated (mean = 1.5) or self-pollinated (mean = 1) flowers (Fig. 3b). The observed range of seeds per fruit was low overall compared to the number of available ovules (18), and compared to fruit set data reported by Cunningham (2000) for the same species.

Pollen competition

There was no difference detected between self- and outcross-pollen treatments in the number of pollen tubes that grew through to the base of the style (Fig. 4). Tube development per grain was between 10 and 20% after 8 h, and increased to nearly 40% after 1 day. Extra 6 h resulted in

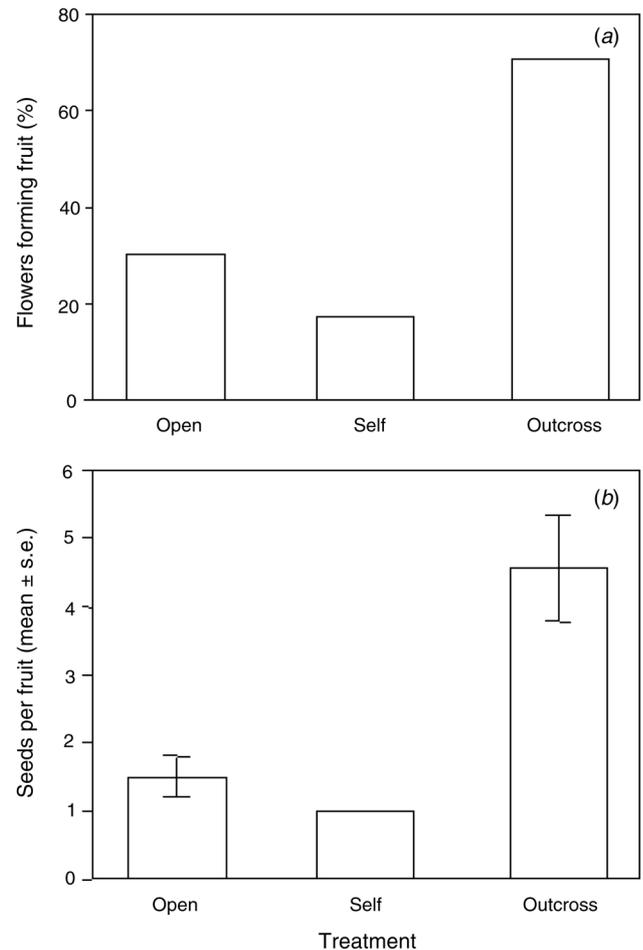


Fig. 3. (a) Percentage of *Dianella revoluta* flowers forming fruit after controlled pollination treatment (outcross: $N = 24$; open and self: $n = 23$), assessed during fruit maturation (+10 days). (b) *D. revoluta* seed number per fruit in mature berries after controlled pollination treatment [$n = 9$ (outcross); $n = 4$ (open and self)], assessed at fruit maturity (+20 days). Standard error could not be calculated for the self-pollen treatment, as all values were 1.

the mean rate increasing only marginally or not at all in the case of self-pollen treatment; however, replication of this aspect was insufficient to make a confident assessment.

Stigmatic pollen loads ranged from about 20 to 160 grains, and pollen-tube numbers ranged from 0 to about 80. The relationship was linear, with no evidence of decelerating increase in pollen-tube numbers at high levels of pollen application. We expected that the relationship should be an asymptotic dose–response curve, assuming a physical limit on the number of pollen tubes that a style can accommodate (e.g. Waser and Price 1991). Although the maximum number of pollen tubes achieved (80) by far exceeds the number of available ovules (18), the shape of these data suggest that we did not succeed in applying a saturating pollen dose.

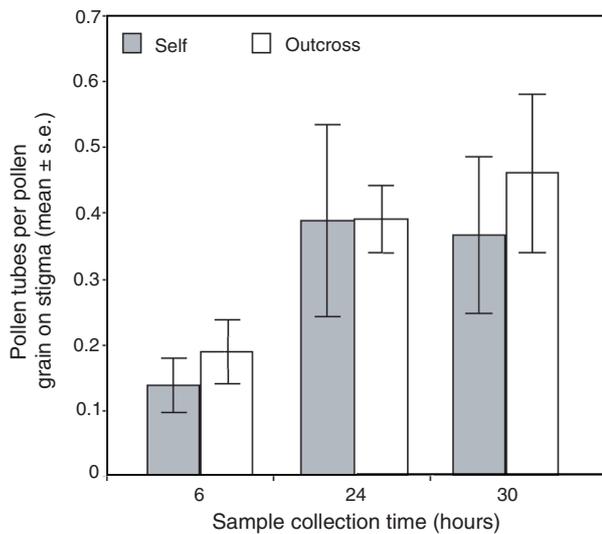


Fig. 4. Number of pollen tubes reaching the base of style per pollen grain on stigma after controlled pollination. Stigmas were collected after 8 h [$n = 7$ (self), 9 (outcross)], 24 h [$n = 4$ (self), 5 (outcross)] or 30 h [$n = 4$ (self), 3 (outcross)].

Discussion

Floral phenology, pollinator behaviour and self-pollination

Over three flowering seasons *Dianella revoluta* was buzz-pollinated by the same small suite of native halictid and anthophorine apid bees (Fig. 1). One species in particular, *Lipotriches ferricauda*, accounted for over 50% of all recorded visits. All taxa are presumed to be solitary or female-group ground nesting (Dollin *et al.* 2000) and pollinate a broad taxonomic range of plant species (Bernhardt 1995; Dollin *et al.* 2000), including pollen-only and nectar-reward flowers. A similar breadth of closely related bee taxa have been recorded visiting *D. revoluta* flowers in Western Australia (Houston 2000), and *D. caerulea* flowers in coastal New South Wales (Bernhardt 1995). Interestingly, Bernhardt observed that the suite of anthophorine apid and halictid bees visiting *D. caerulea* rarely visited multiple flowers on a single inflorescence before moving onto another. In contrast, all bee taxa in this study typically visited numerous flowers on a single inflorescence before moving away, although the nature and duration of flower handling varied among species (D. H. Duncan, pers. obs.).

Flower visits by *L. ferricauda*, the dominant pollinator of *D. revoluta* during this study, resulted in significant quantities of within-flower self-pollen transfer (~50%, Fig. 2). Although this kind of 'facilitated' autogamous selfing is presumed to occur in many species (Lloyd 1992; Larson and Barrett 1999b), this may be the first demonstration that it can account for such a large proportion of stigmatic pollen load. However, Ramsey (1995) elegantly demonstrated that facilitated selfing limits seed set in

Blandfordia grandiflora (Liliaceae). In three previous studies on sources of self-fertilisation, between-flower transfer (geitonogamy) was found to be the dominant mode of selfing (Schoen and Lloyd 1992; Leclerc-Potvin and Ritland 1994; Eckert 2000). We did not quantify geitonogamous transfer for *D. revoluta*, but our data indicate that about half of the conspecific pollen load was facilitated autogamous self-pollen, and the balance a mixture of geitonogamous self- and outcross pollen. In nature, the relative proportion of each pollen type in the total stigmatic load will presumably be a function of the availability of mates and pollinators, and the total number of flowers on a plant.

The potential for geitonogamous selfing in *D. revoluta* is limited to an extent by its floral phenology. Flowering occurred over an extended period and only three flowers (less than 5%) on any one inflorescence are open on a given day. Therefore, although bees often visited most open flowers on an inflorescence before moving on, pollinating bees are likely to move pollen to other plants within a foraging bout, even when there are several flowering inflorescences on a single plant. This is likely to hold true at any time in the flowering season. In contrast, in mass-blooming plants, maximum outcrossing is predicted for individuals that peak early or late relative to the surrounding population (Zimmerman 1988). Studies of other plant species that have granular pollen show that a pollinator may deliver pollen from a single flower visit to more than 50 recipient flowers (Thomson and Thomson 1989). *D. revoluta* can form large clonal patches, however, and in such cases pollinators may affect substantial geitonogamous selfing, which is a widespread cause of selfing in animal-pollinated plants (Eckert 2000).

Self-incompatibility and mating system

Despite an apparent abundance of pollinator activity in an area of relatively high mate density, open-pollinated flowers formed fruit at a rate of only ~30%. In comparison, outcross hand-pollinated flowers set fruit at over 70% (Fig. 3). This result is consistent with those of other pollination studies that were able to separate self- and outcross-pollen loads (Snow 1982; Vaughton and Ramsey 2003). Snow (1982) found that hummingbirds mostly transferred self-pollen to the entirely self-incompatible vine (*Passiflora vitifolia*), and higher rates of fruit set were achieved from hand-pollination with outcross pollen. The failure of open-pollinated *D. revoluta* to set more fruit could be due to outcross-pollen limitation, which has been recorded for many species, at least in some seasons or times within seasons (Burd 1994; Larson and Barrett 1999a). It is possible for example that the majority of pollen on open-pollinated *D. revoluta* plants comes from a combination of within-plant, within-inflorescence and within-flower selfing, as described for *Decodon verticillatus* (Eckert 2000). A high proportion of self-pollen deposited on

the stigma could act to diminish the efficacy of the outcross pollen received by flowers. In addition, some further fraction of the outcross pollen could be from close siblings, whose pollen may also be rejected by the self-incompatibility mechanism (biparental inbreeding). Biparental inbreeding may occur simply because of dispersal of both pollen and seed is a declining function of distance in most species (i.e. population genetic structure: Griffin and Eckert 2003).

There is evidence that 'swamping' by incompatible pollen types may be interfering with adequate levels of outcross-pollen delivery. First, on average, the dominant pollinator species in an area of high floral density transferred self- to outcross pollen at a ratio of at least 2:1 (Fig. 2). Second, despite a poor rate of return from self-pollinated flowers we found no evidence of an early barrier to self-fertilisation, such as temporal separation (dichogamy) or pollen-tube competition.

The evidence from this study suggests that 'late-acting self-incompatibility' (LSI: Seavey and Bawa 1986) is responsible for poor seed set from self-pollination in *D. revoluta*. The precise nature of the mechanism(s) of LSI is an ongoing debate (Gibbs and Bianchi 1999), which we do not intend to join. The important distinction for *D. revoluta* mating system is between LSI and gametophytic self-incompatibility (GSI). LSI differs in that most ovules that are initially self-fertilised subsequently fail at a very early stage of maturation. Those ovules cannot be fertilised by later-arriving compatible pollen tubes. By contrast, GSI typically inhibits the growth of incompatible pollen tubes in the style, allowing later-arriving compatible combinations to successfully fertilise ovules. The similarity between self- and outcross pollen-tube growth in *D. revoluta* (Fig. 4) suggests that LSI is more likely than GSI. A number of studies of other species have found strong evidence for early inbreeding effects rather than physiological self-incompatibility (e.g. Ramsey 1995; Gibbs and Bianchi 1999; Gribel and Gibbs 2002; Pound *et al.* 2003).

That pollen tubes of both self- and outcross pollen have been shown to reach the base of the style in good number does not necessarily mean that this occurs in open-pollinated flowers. Mixed pollen loads have been shown to decrease the amount of pollen germination at the stigmatic surface. Interestingly, not only have self- (e.g. Brewbaker and Majunder 1961) and heterospecific (e.g. Sukhada and Jayachandra 1980) pollen been shown to inhibit conspecific pollen germination but mixed outcross pollen may also perform worse than pollen from a single outcross donor (Németh and Smith-Huerta 2002). Clearly, to delve further into the issue of self-pollen interference *v.* outcross-pollen limitation in *D. revoluta* would require an analysis of controlled application of mixed pollen loads.

Whatever the cause, transfer of incompatible pollen appears likely to be limiting fruit set in the populations used for this study, which were characterised by numerous mates

and apparently abundant pollinators. Where the number of available mates are reduced the amount of outcross pollen received might be expected to be less, as would reproductive output. Indeed, reduced availability of outcross compatible mates may be the best explanation of fragmentation effects found by Cunningham (2000) for *D. revoluta*.

So why should *D. revoluta* allow so much self-pollen transfer and self-fertilisation? Many preferentially outcrossing species exhibit mixed mating, and some degree of reproductive assurance through self-fertilisation may be under strong selection, particularly when reproductive failure is a likely alternative because of mate or pollinator scarcity (Fausto *et al.* 2001). Interestingly, while all sources and types of self-fertilisation have the same genetic consequences, they may have different effects on successful pollen export and reproductive success (Lloyd 1979; Harder and Thomson 1989; Harder 2000). In general, autogamous self-pollination is thought to be relatively advantageous in comparison to geitonogamous selfing. While self-fertilisation can be evolutionarily advantageous, it is mostly when supply of cross-fertilisation is unreliable, and when self-fertilisation is delayed rather than directly competitive with outcross pollination (Lloyd 1979). In *D. revoluta*, we have shown that substantial quantities of competing self-pollen are deposited on stigmas during the same events as outcross pollen. As Lloyd (1992) notes, this mode of selfing occurs least when pollinators are limiting, and most often when they are not. Thus, opportunities seem weighted strongly in favour of self-pollination. Like Ramsey (1995) for *Blandfordia*, we conclude that the magnitude of ovule wastage in this system ought to create a strong selection gradient to reduce self-pollination. However, we need to know more about the circumstances in which outcross pollen is limiting for this species.

It seems possible that the mechanics of buzz-pollination (pollen release by vibration of the anthers while the stigma is receptive) might predispose this and other buzz-pollinated species to particularly high levels of facilitated autogamous self-pollen transfer. Perhaps the advantages that buzz-pollinated anther morphology confer to pollen dispensing and transport (male-flower function) (Harder and Barclay 1994) outweigh the potential costs to higher seed output (female function). In one of the few other detailed accounts of pollen transfer in buzz-pollinated species, Larson and Barrett (1999b) suspected that facilitated selfing might occur in *Rhexia virginica* (Melastomataceae). However, they concluded that infrequent pollinator visitation was likely to best explain limited natural seed set. Having quantified facilitated selfing, we conclude that for *D. revoluta*, excessive selfing is the most likely explanation for low natural seed set. Our work with this species suggests that the system may be ideal for more detailed experimentation (e.g. Schoen and Lloyd 1992) on the sources and consequences of self- *v.* outcross pollination.

Flower visitors

Dianella revoluta seemed amply served by its native bee pollinators over the period of this study. Pollinators were easy to locate at flowers, both within reserves and in roadside vegetation (cf. Gross *et al.* 2003 for *Tetratheca juncea*). At these sites, *D. revoluta* had a limited pool of potential pollinators that exhibit buzz-pollinating behaviour, a subset of female native bees. Arguably, it could be considered to have a relatively specialised system (e.g. Aizen *et al.* 2002). However, those pollinators are not limited to foraging at buzz-pollinated flowers, as demonstrated here by pollinator observation and heterospecific-pollen identification, and as reported in recent community-level studies (Hingston 1999; Harter *et al.* 2002). Therefore, the apparent specialisation of the pollination system is asymmetrical.

Despite sharing pollinators, *D. revoluta* is unlikely to suffer substantial pollen loss or wastage to its sympatric congener *D. longifolia*, because of clear partitioning of floral phenology and the pollinator resource. *D. longifolia* flowering began in the very tail end of *D. revoluta* flowering season, but the within-day separation was more remarkable. Sequential phenology patterns in co-occurring plant species are well documented (Zimmerman 1988, Barth 1991) and have been interpreted as a mechanism for co-occurring plants to partition pollinator and other resources, reduce seed predation or improve prospects for seed dispersal. For such closely related species as these two, avoiding cross-pollination may be an important selection agent for separation of flowering times, as according to Wilson (1993), *Dianella* species are known to hybridise on occasion.

A comparison of visitation data for the sympatric *D. revoluta* and *D. longifolia* indicate that the two species are likely to rely on different taxa for the bulk of flower pollination (Fig. 1). This may simply be a reflection of distinct peaks in pollinator activity over the course of a day. *D. revoluta* was primarily pollinated by mid-sized *Lipotriches* bees, which were shown to deposit substantial amounts of pollen in even a single visit. *D. longifolia*, however, was predominantly visited by the very small *Homalictus* (Table 1) and it is possible that bees of this size—which ‘milk’ or ‘glean’ pollen from anthers rather than buzz—may function as pollen thieves rather than effective pollinators (Michener 2000; Thorp 2000). For example, Bernhardt (1995) suggested that *Homalictus* and *Hylaesus* sp., both very small bees, might not often contact the stigma when harvesting pollen. Although *Homalictus* may not move as much pollen between flowers of *D. longifolia* (in comparison to *Lipotriches* on *D. revoluta*), the low-disturbance pollen-harvesting method could well result in rather less facilitated self-pollen transfer. Whereas the efficacy of native bees as pollinators have been experimentally compared to introduced honeybees (Gross and Mackay 1998; Gross 2001), it would be interesting to

further investigate the relative efficiency of outcross- v. self-pollen transfer among native-bee types for this and other dependent plant species.

Acknowledgments

We thank Andrea Leigh, Meredith Cosgrove and Cassia Read for field and laboratory assistance; NSW National Parks and Wildlife Service for permission to conduct research in Nature Reserves; Dr Michael Batley (Australian Museum), Dr Laurence Mound and Dr Tom Weir (ANIC, CSIRO) who kindly identified bee, thrip and flea beetle specimens, respectively; and two anonymous reviewers for helpful comments on an earlier version of the manuscript. An award from the Australian Flora Foundation to D. Duncan financially supported this research.

References

- Aizen MA, Feinsinger P (2002) Bees not to be? Responses of insect pollinator faunas and flower pollination to habitat fragmentation. In ‘How landscapes change: human disturbance and ecosystem fragmentation in the Americas’. (Eds GA Bradshaw, PA Marquet) pp. 111–129. (Springer-Verlag: New York)
- Aizen MA, Ashworth L, Galetto L (2002) Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter? *Journal of Vegetation Science* **13**, 885–892.
- Barrett SH (1988) The evolution, maintenance, and loss of self-incompatibility systems. In ‘Plant reproductive ecology’. (Eds J Lovett Doust, L Lovett Doust) pp. 98–124. (Oxford University Press: New York)
- Barth FG (1991) ‘Insects and flowers: the biology of a partnership.’ (Princeton University Press: Princeton, NJ)
- Bernhardt P (1995) The floral ecology of *Dianella caerulea* var. *assera* (Phormiaceae). *Cunninghamia* **4**, 9–20.
- Bond WJ (1994) Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society of London, Series B* **344**, 83–90.
- Brewbaker JL, Majumder SK (1961) Cultural studies of the pollen population effect and the self-incompatible inhibition. *American Journal of Botany* **48**, 457–464.
- Buchmann SL (1983) Buzz pollination in angiosperms. In ‘Handbook of experimental pollination biology’. (Eds CE Jones, RJ Little) pp. 73–113. (Van Nostrand: New York)
- Burd M (1994) Bateman’s Principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Botanical Review* **60**, 83–139.
- Cunningham SA (2000) Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. *Conservation Biology* **14**, 758–768. doi:10.1046/J.1523-1739.2000.98609.X
- Dahlgren RMT, Clifford HT, Yeo PF (1985) ‘The families of the monocotyledons. Structure, evolution and taxonomy.’ (Springer-Verlag: Berlin)
- Dollin A, Batley M, Robinson M, Faulkner B (2000) ‘Native bees of the Sydney region: a field guide.’ (Australian Native Bee Research Centre: Sydney)
- Eckert CG (2000) Contributions of autogamy and geitonogamy to self-fertilisation in a mass-flowering, clonal plant. *Ecology* **81**, 532–542.
- Fausto JA, Eckhart VM, Geber MA (2001) Reproductive assurance and the evolutionary ecology of self-pollination in *Clarkia xantiana* (Onagraceae). *American Journal of Botany* **88**, 1794–1800.

- Gibbs PE, Bianchi MB (1999) Does late-acting self-incompatibility (LSI) show family clustering? Two more species of Bignoniaceae with LSI: *Dolichandra cynanchoides* and *Tabebuia nodosa*. *Annals of Botany* **84**, 449–457. doi:10.1006/ANBO.1999.0933
- Gribel R, Gibbs PE (2002) High outbreeding as a consequence of selfed ovule mortality and single vector bat pollination in the Amazonian tree *Pseudobombax munguba* (Bombacaceae). *International Journal of Plant Sciences* **163**, 1035–1043. doi:10.1086/342518
- Griffin CM, Eckert CG (2003) Experimental analysis of biparental inbreeding in a self-fertilizing plant. *Evolution* **57**, 1513–1519.
- Gross CL (2001) The effect of introduced honeybees on native bee visitation and fruit set in *Dillwynia juniperina* (Fabaceae) in a fragmented ecosystem. *Biological Conservation* **102**, 89–95. doi:10.1016/S0006-3207(01)00088-X
- Gross CL, Mackay D (1998) Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomaceae). *Biological Conservation* **86**, 169–178. doi:10.1016/S0006-3207(98)00010-X
- Gross CL, Bartier FV, Mulligan DR (2003) Floral structure, breeding system and fruit-set in the threatened sub-shrub *Tetradlea juncea* Smith (Tremandraceae). *Annals of Botany* **92**, 771–777. doi:10.1093/AOB/MCG201
- Harder LD (2000) Pollen dispersal and the floral diversity of monocotyledons. In 'Monocots: systematics and evolution'. (Eds KL Wilson, DA Morrison) pp. 243–269. (CSIRO: Melbourne)
- Harder LD, Barclay RMR (1994) The functional significance of poricidal anthers and buzz-pollination: controlled pollen removal from *Dodecatheon*. *Functional Ecology* **8**, 509–517.
- Harder LD, Thomson JD (1989) Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *American Naturalist* **133**, 323–344. doi:10.1086/284922
- Harter B, Leistikow C, Wilms W, Truyllo B, Engels W (2002) Bees collecting pollen from flowers with poricidal anthers in a south Brazilian *Araucaria* forest: a community study. *Journal of Apicultural Research* **41**, 9–16.
- Henderson RJF (1987) *Dianella* (Liliaceae). In 'Hydatellaceae to Liliaceae'. (Australian Government Publishing Service: Canberra)
- Henderson RJF, Clifford HT (1984) A recircumscription of Phormiaceae Agardh. *Taxon* **33**, 423–427.
- Heslop-Harrison Y, Shivana KR (1977) The receptive surface of the angiosperm stigma. *Annals of Botany* **41**, 1233–1258.
- Hingston AB (1999) Affinities between southern Tasmanian plants in native bee visitor profiles. *Australian Journal of Zoology* **47**, 361–384.
- Houston TF (2000) 'Native bees on wildflowers in Western Australia.' (Western Australian Insect Study Society: Perth)
- Kearns CA, Inouye DW (1993) 'Techniques for pollination biologists.' (University Press of Colorado: Niwot, CO)
- Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: the conservation of plant–pollinator interactions. *Annual Review of Ecology and Systematics* **29**, 83–112. doi:10.1146/ANNUREV.ECOLSYS.29.1.83
- Larson BMH, Barrett SCH (1999a) The ecology of pollen limitation in buzz-pollinated *Rhexia virginica* (Melastomataceae). *Journal of Ecology* **87**, 371–381. doi:10.1046/J.1365-2745.1999.00362.X
- Larson BMH, Barrett SCH (1999b) The pollination ecology of buzz-pollinated *Rhexia virginica* (Melastomataceae). *American Journal of Botany* **86**, 502–511.
- Leclerc-Potvin C, Ritland K (1994) Modes of self-fertilisation in *Mimulus guttatus* (Scrophulariaceae): a field experiment. *American Journal of Botany* **81**, 199–205.
- Lloyd DG (1979) Some reproductive factors affecting the selection of self-fertilisation in plants. *American Naturalist* **113**, 67–79. doi:10.1086/283365
- Lloyd DG (1992) Self- and cross-fertilization in plants. II. The selection of self-fertilisation. *International Journal of Plant Sciences* **153**, 370–380. doi:10.1086/297041
- Michener CD (2000) 'The bees of the world.' (Johns Hopkins University Press: Baltimore, MD)
- Milne M, Walter GH (1998) Significance of mite prey in the diet of the onion thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). *Australian Journal of Entomology* **37**, 120–124.
- Minckley RL, Cane JH, Kervin L, Roulston TH (1999) Spatial predictability and resource specialisation of bees (Hymenoptera: Apoidea) at a superabundant, widespread resource. *Biological Journal of the Linnean Society* **67**, 119–147. doi:10.1006/BIJL.1998.0295
- Murcia C (1996) Forest fragmentation and the pollination of neotropical plants. In 'Forest patches in tropical landscapes'. (Eds J Schellas, R Greenberg) pp. 19–36. (Island Press: Washington DC)
- Németh MB, Smith-Huerta NL (2002) Effects of pollen load composition and deposition pattern on pollen performance in *Clarkia unguiculata* (Onagraceae). *International Journal of Plant Sciences* **163**, 795–802. doi:10.1086/342034
- Ogden EC, Raynor GS, Hayes JV, Lewis DM, Haines JH (1974) 'Manual for sampling airborne pollen.' (Hafner Press: New York)
- Pound LM, Patterson B, Wallwork MAB, Potts BM, Sedgley M (2003) Pollen competition does not affect the success of self-pollination in *Eucalyptus globulus* (Myrtaceae). *Australian Journal of Botany* **51**, 189–195. doi:10.1071/BT02082
- Ramsey M (1995) Ovule pre-emption and pollen limitation in a self-fertile perennial herb (*Blandfordia grandiflora*, Liliaceae). *Oecologia* **103**, 101–108.
- Schoen DJ, Lloyd DG (1992) Self- and cross-fertilisation in plants. III. Methods for studying modes and functional aspects of self-fertilization. *International Journal of Plant Sciences* **153**, 381–393. doi:10.1086/297042
- Seavey SR, Bawa KS (1986) Late-acting self-incompatibility in angiosperms. *Botanical Review* **52**, 195–218.
- Snow AA (1982) Pollination intensity and potential seed set in *Passiflora vitifolia*. *Oecologia* **55**, 231–237.
- Sukhada K, Jayachandra (1980) Pollen allelopathy: a new phenomenon. *New Phytologist* **80**, 739–746.
- Thomson JD, Thomson BA (1989) Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. *Evolution* **43**, 657–661.
- Thorp RW (2000) The collection of pollen by bees. *Plant Systematics and Evolution* **222**, 211–223.
- Vaughton G, Ramsey M (2003) Self-compatibility and floral biology in subdioecious *Wurmbea dioica* (Colchicaceae). *Australian Journal of Botany* **51**, 39–45. doi:10.1071/BT02054
- Waser NM, Price MV (1991) Outcrossing distance effects in *Delphinium nelsonii*: pollen loads, pollen tubes and seed set. *Ecology* **72**, 171–179.
- Wilson KL (1993) Phormiaceae. In 'Flora of New South Wales'. (Ed. GJ Harden) pp. 73–80. (New South Wales University Press: Sydney)
- Zimmerman M (1988) Nectar production, flowering phenology, and strategies for pollination. In 'Plant reproductive ecology: patterns and strategies'. (Eds J Lovett Doust, L Lovett Doust) pp. 157–178. (Oxford University Press: New York)

Manuscript received 18 September 2003, accepted 28 January 2004