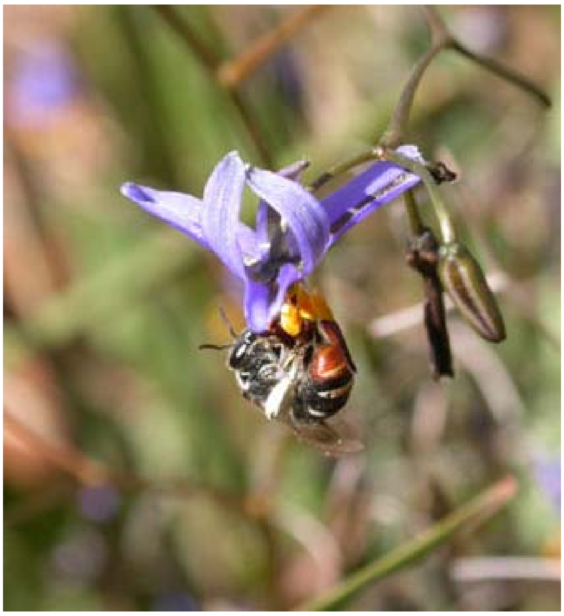
POLLINATION OF BLACK-ANTHER FLAX LILY (*DIANELLA REVOLUTA*) IN FRAGMENTED NEW SOUTH WALES MALLEE

A report to the Australian Flora Foundation



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Preface

This work was undertaken while David Duncan was a PhD Student at the School of Botany & Zoology, Australian National University, under the supervision of Dr Adrienne Nicotra (Botany and Zoology, Australian National University) and Dr Saul Cunningham (CSIRO Entomology). David Duncan took all of the photographs in this report.

The project received financial support from the Australian Flora Foundation, ANU FRGS scheme, and the Sigma Xi Foundation.

The work described in this report will appear in more detail in a number of publications, and in the doctoral thesis of the author. For further information email david.duncan@anu.edu.au. Many people made valuable contributions to the project through data analysis, computer programming, insect identification, fieldwork and laboratory assistance. They are properly acknowledged elsewhere. We thank the NSW National Parks and Wildlife Service for giving us permission to work in parks and reserves of the Riverina.

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SUMMARY

There is international concern that native plant pollination systems may be disrupted as a result of habitat loss and fragmentation processes. One important way that habitat fragmentation may endanger plant populations is by reducing the number of available mates (reducing plant density). Below critical density levels, sexual reproduction in a population can fail. Sustained reproductive failure could lead to population declines and ultimately extinctions of native plant species.

We used the Black-Anther Flax Lily (*Dianella revoluta*), a common understorey species, to look at how local mate density effects the deposition of outcross pollen (pollen from a mate, non-self pollen). We first established that the number of flowers that form fruit is greatest when outcross pollen is deposited on the stigma, and poor with only self-pollen. Yet the average pollinator visit in dense patches of mates results in twice as much self-pollen on the stigma as outcross pollen.

In experimental arrangements of manipulated flowers, we found that the deposition of outcross pollen declines significantly with distance from a pollen source. However, pollen load from other co-pollinated species did not decrease with distance, indicating that pollinator visitation to *D. revoluta* flowers was relatively constant irrespective of isolation from a potential mate. This demonstrates how fragmentation could detrimentally affect reproductive processes in even common species with adequate pollinator service.

In Australia we have little or no formal knowledge of important pollinating species for the vast majority of our flora. Therefore, we have no baseline with which to assess the ongoing health of our overall pollination service. This is of great concern and it is fundamentally important that we gather quality data on plant-pollinator relationships whenever the opportunities arise. Future research on the plant-pollinator interactions, and on effects of habitat fragmentation on the pollination ecology of natural systems, and the spatial scale of concern of these interactions is critically important to conservation and management.

INTRODUCTION

The biggest threats to the conservation of Australia's unique flora are habitat loss and fragmentation [1]. Whereas habitat loss refers to the process of land clearing, habitat fragmentation concerns reduction of the available habitat area, quality and connectivity. Fragmentation is an ongoing concern for the viability of plant and pollinator populations left behind *after* clearing and major land use change.

For most plant species reproductive success is greatest when their flowers are fertilised by pollen from another individual rather than self-pollen (i.e. pollen from the same plant). Some species, however, can also successfully reproduce from self-pollen, which may be important when pollen from other individuals does not arrive. If a species cannot set fruit from self-pollen it is called self-incompatible. Many species are only partially self-incompatible meaning that some proportion of self-pollen grains will fail to fertilise, and some will succeed. There are a range of means by which self-fertilisation may be prevented, in order to maximise opportunities for fertilisation by pollen from another plant [2-4].

Fragmentation may reduce reproductive success in plant species by introducing barriers to pollen movement between populations or individuals. Even in continuous vegetation, successful pollen transport from one plant to a mate faces many difficulties. It has been estimated that less than 1% of pollen that the average plant produces is expected to reach its ideal destination – the receptive stigma of a mate [5]. It comes as little surprise then, that fruit production of plants in small or isolated populations is often lower than that found in larger populations [6]. Within a population, fruit production of isolated plants has been shown to be lower than for plants with nearby mates [7].

Similarly, the diversity and abundance of native insect pollinators and visitation rates have been found to be significantly lower in small patches or at isolated plants compared with larger patches or continuous vegetation [6]. Reduced fruit set, however, could be a consequence of fewer pollinator visits, or it could result from pollinators depositing mostly incompatible pollen [8]. Incompatible pollen may include self-pollen, and pollen from other plant species. Deposition of incompatible pollen can occur because of two common features of plant reproductive ecology. Firstly, when isolated plants get visited, pollinators often visit most available flowers on the plant before moving on – this means that considerable self-pollen can be transferred between flowers on one plant. Secondly, most plants share their pollinators with other plant species and pollinators may visit flowers of more than one species in any foraging excursion. The degree to which a pollinator will stick with a host species, or switch hosts, during foraging is likely to depend on the local density of mates.

Although plant conservation research often focuses on threatened plant species, our work used common species to investigate the effects of fragmentation on fundamental processes of plant reproduction - pollination and fruit set. We did this for two important reasons. Firstly, one of the key concerns relating to habitat fragmentation is that effects may take the form of a slow erosion of essential processes and species interactions [9] and these effects should be evident in common as well as threatened species. Secondly, with sensitive design, large-scale experiments can be undertaken with common species without fear of lasting impacts upon the health of target populations.

THE STUDY

The primary objective of this study was to investigate the effect of flowering plant density on outcross pollen receipt in our target species, the Black-Anther Flax Lily (*Dianella revoluta* R. Br; Phormiaceae). *Dianella revoluta* (Fig. 1) is a common and sometimes dominant understorey species of mainland Australia, where it often forms large clonal patches [10]. Despite its considerable distribution and abundance, a study of fragmentation effects in mallee woodland found that fruit set of *D. revoluta* was significantly higher in nature reserves compared with nearby roadside remnants [11]. Prior to this study, very little published information existed about the reproductive biology of this species. In order to better understand the implications of the study, we also carried out experiments to establish key information on the pollination ecology and reproductive biology of the species in the study area.

Figure 1 - Flower of* Dianella revoluta *(diameter ~ 12mm). Note that the anthers are brown in this part of its range!

The work was undertaken 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 (S 33° 38.01' E 146° 33.36'). Gubbata is a small reserve of approximately 150 hectares in area, set amongst land cleared for wheat and sheep farming. The vegetation of the roadsides and reserves consists of shrubby, open mallee woodland dominated by multi-stemmed trees of the genus *Eucalyptus*. The roadsides often have similar floristic composition to the reserves but are more disturbed, largely due to soil disturbance associated with road and fence maintenance. All sites are between 150 – 200 m above-sea-level and average rainfall for the area is between 200 and 300 mm per year.

Pollination biology and mating system of *Dianella revoluta*

In order to gain an understanding of the species reproductive ecology, we applied a combination of pollinator observation, flower manipulation, controlled pollination, and pollen tube studies. Through these methods we achieved a broad understanding of the reproductive ecology of *D. revoluta* and gained some insight as to why habitat fragmentation effects are occurring in this species. The findings were also critical in understanding the implications of the density experiment described below. We also investigated what impact overlapping distribution with *Dianella longifolia* may have on *D. revoluta* pollination and reproduction.

The effect of flowering plant density on outcross pollen receipt

As far as pollen is concerned it is not necessarily how much pollen you get but where it comes from that determines pollination success of plants. For this experiment, we cleared *Dianella* inflorescences from 800m sections of roadside vegetation adjacent to Gubbata Nature Reserve. Into each roadside section, we placed 1 cut inflorescence of 6 flowers in a vase (Fig. 2) at 0, 20, 50, 100, 200 & 400m. Flowers were left open to visitors for one day (the life of a *D. revoluta* flower).

We were able to develop a technique of blocking anthers with craft glue to prevent pollen removal & self-pollination; therefore pollen could only be delivered to the flowers in this experiment from plants outside the cleared sections.

For each collected flower the number of *D. revoluta* (conspecific) pollen grains on the surface of the stigma was counted. In addition we counted the number of heterospecific (other species) grains, as an indicator of pollinator visitation.

We repeated this experimental design a total of 21 times, in 6 roadside sections, over 2 spring seasons, both adjacent to and away from (> 1km) a reserve.

Figure 2 – The experimental unit, one vase with six blocked-anther flowers

Pollination biology and mating system of *Dianella revoluta*

Buzz-pollination

Dianella revoluta is buzz-pollinated by female native bees [12]. Buzz-pollination or sonication involves bees collecting pollen from anthers by vibrating or shivering their flight muscles [13]. This technique is particularly effective with flowers like those of *D. revoluta* that have enclosed anthers with only a small opening for pollen release. Pollen is actively collected by female bees, which alight, grip onto anthers and vibrate pollen onto their bodies, from where it is usually groomed onto particular surfaces for transport [14]. Enclosed anthers are found in many plant families [13] and at least three other species in the study area are buzz-pollinated (Fig. 3), including *Dianella longifolia*. Buzz-pollinating behaviour is also widespread amongst bee families [13]. Importantly, honeybees (*Apis mellifera*) are incapable of the behaviour and are not known to be effective pollinators of plants with enclosed anthers.

Figure 3 - Buzz-pollinated species co-occurring with **D. revoluta** ***in NSW mallee woodlands, from left to right*** **Halgania cyanea** ***(Boraginaceae),*** **Senna artemisioides** ***(Caesalpiniaceae), and*** **Dianella longifolia**

All flower visitors trapped while visiting *D. revoluta* flowers were relatively common female native bees from two families; the Halictidae and the Apidae (Tribe anthophoriini). The bees were also observed to visit flowers of other buzz-pollinated species in the area (Punty bush (*Senna artemisioides*) and Mallee Blue-Flower (*Halgania cyanea*)) as well as a broad range of other flower types including Mallee Rosemary (*Westringia cheelii)*, Scarlet Mintbush (*Prostanthera aspalathoides*), Small-Leaf Wax Flower (*Philotheca difformis*), and Mallee Fringe-Lily

Figure 4 - Common flower visitors to D. revoluta: from left Amegilla sp. (~15mm tip to tip), Homalictus urbanus (~5mm), and Lipotriches (Austronomia) ferricauda (~9mm), buzz-pollinating a Dianella flower

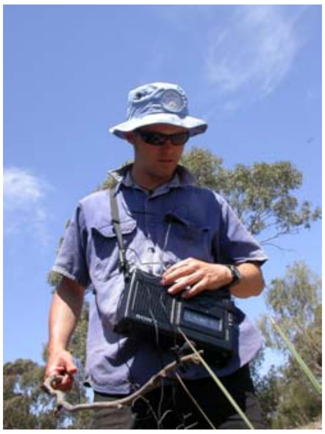

Figure 5 - Recording buzz-pollination with a professional walkman and a lapel microphone clipped to a stick

(*Thysanotus baueri*). *Dianella* flower visitors ranged in size from 5 mm in length (Emerald Homalictus – *Homalictus urbanus*) to over 13 mm (Blue-banded Bee - *Amegilla*) (Fig. 4). It is possible that the small *Homalictus* is not an effective pollinator of *D. revoluta*. Firstly, it gleans pollen by inserting a forelimb into the anther to gather pollen rather than by the more energetic buzzing. Secondly, *Homalictus* bees typically visit all anthers by moving slowly around the anthers, often without appearing to contact the stigma. Several authorities consider small bees of this type "pollen-thieves" as opposed to pollinators [14, 15]. For the mid- and large-size bees, which appear to be in contact with the stigma most of the time during flower visits, buzz-pollination was verified using amplified audio recording (Fig. 5).

Co-occurrence of *Dianella revoluta* and *D. longifolia*

A key issue when closely related species occur in the same sites is avoidance of hybridisation and interbreeding. This is particularly true when the same pollinators visit both species. In the study area *D. revoluta* often co-occurred with *D. longifolia* var *longifolia* and our work uncovered an uncommonly neat example of partitioning of the pollinator resource. Firstly, 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 3 – 7 pm (Eastern Summer Time) and on some inflorescences, they remained open for several hours after dark. The two species shared the same group of flower visitors. However, the relative numeric importance of visitors differed between species. For example, *Lipotriches ferricauda* represented nearly 60% of observed / trapped visitors to *D. revoluta* compared with approximately 10% of visits to *D. longifolia*. *Homalictus* bees accounted for only about 5% of visits to *D. revoluta* but were the most common visitors (over 60%) to *D. longifolia*. Such minimal pollinator overlap may occur because the bees themselves differ in preferred foraging conditions (*Lipotriches* mostly during in the day, *Homalictus* mostly later in the evening) or it may reflect the bee species preference of flower in some way.

Other insect-flower interactions

Whereas pollination is an important positive interaction between *Dianella* and its bee pollinators, detrimental interactions with insects are also important in ultimately determining reproductive success. Flea beetles (*Arsipoda homolaena* Germar, and *Arsipoda sp.* (Chrysomelidae), commonly chewed open flowers, recently closed flowers, and mature buds of *D. revoluta* (Fig. 6). They were apparently responsible for the loss of approximately 40% of flowers in the density experiment (see below).

***Figure 6 - Flea beetles* (Arsipoda *sp.) attacking an open flower of* D. revoluta**

Thrips were a very common sight on open flowers and subsequently were found in many preserved flower samples. Thrips are known to cause *Dianella* flowers to be malformed [16], and outbreaks of flower galls were observed to a limited extent in 2000 and 2001 seasons but very frequently in 2002, a drought year. The majority of thrips specimens were identified as female Onion thrips (*Thrips tabaci* Lindeman, Thysanoptera: Thripidae), a cosmopolitan 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 [17]. *T. imaginis*, "Plague thrips" Bagnall (Thysanoptera, Thripidae), was also identified in flower samples. It is possible that the thrips may affect self-pollen transfer as they are often seen at the anther-openings and moving about on the style and stigma.

Dianella revoluta mating system and self-incompatibility

The timing of flowering (phenology) of *D. revoluta* limits the amount of pollen transfer between flowers of the same inflorescence. Flowering occurs over an extended period and only a small proportion of the flowers on an inflorescence are open on a given day. Therefore, the foraging bouts of pollinating bees include many inflorescences and this is likely to be true at any time in the flowering season.

Despite inflorescence structure and phenology reducing the amount of pollen transfer between flowers on the same inflorescence, we found that self-transfer was very high because of within-flower pollen transfer. 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 about 30%. In comparison, outcross hand-pollinated flowers set fruit at over 70%. The failure of open-pollinated flowers to set fruit more often could be due to outcross pollen limitation, which has been recorded for many species, at least in some seasons or times within seasons [18, 19]. In this case, however, outcross pollen does not appear to be limiting fruit set in *D. revoluta* in any strict quantitative sense. Pollinators regularly deposit at least 10 outcross grains in a single visit, whereas there are at total of only 18 available ovules. In theory, many flowers should get sufficient outcross pollen from only a handful of visits, whereas our observations suggest they are likely to receive many visits. There is evidence that swamping by high self-pollen loads may be interfering with adequate levels of outcross pollen delivery. Firstly, on average, the dominant pollinator species in an area of high mate density still transferred twice as much self-pollen compared with outcross pollen. Secondly, despite a poor rate of return from self-pollinated flowers we found no evidence of an early barrier to self-fertilization. This may indicate that self-pollen tubes reach the ovary but mostly abort before seed development begins.

The effect of flowering plant density on outcross pollen receipt

The results of our manipulated flower experiment suggest three important biological effects. Firstly:

- Average pollen receipt of flowers declined significantly with distance from a pollen source.

This is an important demonstration of a process by which increased distance between individuals or sub-populations may cause decline in reproductive success - even for common species with ample pollinator service. To our knowledge no previous study has been able to combine a technique for isolating self from outcross pollen with a large-scale field experiment. Furthermore, frequent pollinator movements between *Dianella* and other co-flowering species, as indicated by high loads of other species' pollen, suggest that at low mate density much *Dianella* pollen is probably lost to the flowers of other species.

Secondly, three lines of evidence suggest that the native pollinating bee populations have been able to persist in the fragmented landscape, at least around Gubbata Nature Reserve. They are:

- Consistently high average heterospecific pollen load found at isolated flowers within arrays (isolated flowers still get found by pollinators);

- Similar amounts of heterospecific pollen found at arrays distant from the reserve compared with adjacent arrays (ability to be visited doesn't decrease up to several kilometres from the reserve); and

- Some pollen got to flowers hundreds of metres into open fallow wheat field (pollinators appear 'willing' to traverse open ground to visit flowers).

Finally, flower predators damaged more flowers adjacent to the reserve compared with those in far arrays. This suggests that the fragmented landscape of our study site may be less favourable for the flea beetles than pollinating bees.

CONCLUSIONS

Most importantly, this study demonstrated reduced outcross pollen receipt with distance from a conspecific pollen source in a common, partially self-compatible, buzz-pollinated herb. Here is a process by which increased distance between individuals or sub-populations due to fragmentation may cause decline in reproductive success - even for common species with ample pollinator service. Fragmentation is likely to reinforce reproductive isolation in small groups of plants by reducing the frequency of outcross pollination. Presumably, many species can ill-afford further barriers to successful pollen transport in the long-term. On the other hand our results provide encouraging signs that the native bee pollinators of *Dianella revoluta* may be able to persist in roadside remnants and in this experiment were apparently capable of delivering some pollen to the most isolated plants. Also, although the quality of pollination service to plants in fragments may decline, there may be benefits such as reduced flower attack that go some way to countering reduced pollination quality.

Future directions

Despite the broad distribution and abundance of *Dianella revoluta*, when we began this study there was little detailed information available about its reproductive biology. Unfortunately, this remains true of the majority of the Australian flora. Over 80% of Australia's native plants are animal pollinated, mostly by bees, and if we wish to understand how habitat fragmentation effects pollination of native species it is important that we strive to better understand how our pollinator fauna is distributed, and the details of their interactions with plant species. Advances in knowledge of plant-pollinator relationships are required urgently for both threatened and common species, particularly because the pollinators that sustain threatened taxa are likely to rely upon other more common plant species for their own survival.

A body of research is building up investigating pollination success of plants in habitat fragments. To date the results have been mixed, with some species appearing vulnerable, some robust.

So far, studies have been dispersed throughout the country and the plant kingdom, and there has been little opportunity for consolidation or community study within any one area. As researchers and land managers struggle to meaningfully evaluate the relative health of remnant vegetation and habitat fragments it remains critical that research into the integrity of fundamental processes such as pollination continues to be a priority.

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