

# Depressed pollination in habitat fragments causes low fruit set

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In central New South Wales, Australia, flowers of *Acacia brachybotrya* and *Eremophila glabra* plants growing in linear vegetation remnants received less pollen than conspecifics in nearby reserves. Pollen supplementation increased fruit production by both species, indicating pollen limitation of fruit set. Together these observations explain why fruit production by these species was depressed in linear-strip populations relative to nearby reserves. This study confirms that habitat fragmentation can lead to decline in pollination and subsequent fruit set in wild plant populations. Disrupted pollination interactions of the kind documented in this study may offer a substantial challenge to the conservation of biodiversity in fragmented landscapes.

**Keywords:** habitat fragmentation; pollination decline; fruit set decline; plant–animal interactions

## 1. INTRODUCTION

There is growing concern that the rapid human modification of landscapes will disrupt the sometimes loose mutualistic relationships between plants and their pollinators, causing declines in plant reproduction (Kearns & Inouye 1997; Kearns *et al.* 1998; Murcia 1996; Rathcke & Jules 1993). This concern, coupled with fears of the destructive impact of parasitic mites on honeybee populations (Watanabe 1994), has led to warnings of an impending scenario of crop failures and species extinctions, coined the ‘pollinator crisis’ (Allen-Wardell *et al.* 1998; Buchmann & Nabhan 1996). To date, however, concern for the future has accumulated faster than data about the present. In particular, there are no clear examples of habitat fragmentation causing declines in pollination of wild plants that have translated into declines in fruit or seed production. Here I present evidence that fragmentation of woodland habitat in New South Wales, Australia, has led to a decline in pollination of two plant species. In addition, evidence of pollen limitation in these species provides a causal link between declining pollination and previously documented depressed fruit production in fragmented habitats (Cunningham 2000).

The pollinator crisis scenario warns that habitat destruction is reducing the abundance of pollinators, causing a decline in the rate plants are pollinated in fragmented sites that will in turn lead to lower fruit or seed production. Recent research on experimentally fragmented plant populations has demonstrated that this process does indeed pose a plausible threat (Steffan-Dewenter & Tschamtkke 1999). To establish this link between declining pollination and declining reproduction, evidence is required that the reproductive output of plants growing in fragments is pollen limited. Previous studies of the effect of habitat fragmentation on plant reproduction have been suggestive of pollination or fruit set decline, but failed to jointly establish pollination decline and pollen limitation. In their landmark study of 16

species in fragmented chaco forest in Argentina, Aizen & Feinsinger (1994) found four species with both declining pollination and fruit set. Unfortunately, however, they did not have information on pollen limitation in these species, and felt that variation in fruit production by one of the four species (*Prosopis nigra*) was more consistent with resource limitation of fruit set. A number of other studies have documented depressed reproduction in small or low-density populations without an explicit link to fragmentation, or declines in fruit set with fragmentation, without data on pollination (Ghazoul *et al.* 1998; Gigord *et al.* 1999; Groom 1998; Jennersten 1988; Lamont *et al.* 1993; Spears 1987). These declines could alternatively be explained by aspects of the resource environment in fragments that depress reproductive output in the absence of any change in pollination rates. Fragments may be exposed to different amounts of water, light, wind and invasion by competitive weeds (Brothers & Springarn 1992; Murcia 1995). In these circumstances, depressed reproduction in fragments may pose a serious threat to plant populations, but does not necessarily provide evidence of pollination decline.

In central New South Wales, Australia, woodlands have been extensively cleared for agriculture, leaving much of the remnant vegetation in linear strips along roads, railways and fence lines (Sivertsen 1994; Sivertsen & Metcalfe 1995). These linear strips typically have no core area > 20 m from cleared pastureland, but may continue for kilometres with occasional breaks for road crossings. In this region, linear strips are the most common kind of highly fragmented remnant vegetation. In 1997 and 1998 I examined reproduction by two shrub species common in the ‘mallee’ woodlands (Specht 1981) of this region: *Eremophila glabra* and *Acacia brachybotrya*. *Acacia* species (Mimosaceae) are dominant in many plant communities throughout Australia, and most are thought to be pollinated by insects (New 1984; Sedgley *et al.* 1992; Tybirk 1993). *A. brachybotrya* has globose yellow inflorescences. *Eremophila* species are a common component of the flora in large areas of arid Australia. *E. glabra* (Myoporaceae) has zygomorphic tubular red flowers, typical of the bird pollination syndrome, that are visited

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by honeyeaters (Meliphagidae) (S. A. Cunningham, unpublished data).

In 1997 plants growing in linear strips produced significantly less fruit per inflorescence (*A. brachybotrya*) and less fruit per flower (*E. glabra*) than those in reserves (Cunningham 2000). These patterns were apparent in paired contrasts of reserves (> 20 ha of mallee vegetation) and nearby linear strips (< 40 m wide, within 8 km of reserve) replicated in five or six sites (depending on species). In 1998 the differences were less substantial, but in the same direction. *A. brachybotrya* in linear strips produced less fruit per inflorescence in six out of seven site contrasts, and *E. glabra* in linear strips produced less fruit per flower in three out of six contrasts, with little change in the other three (S. A. Cunningham, unpublished data). These observations of depressed fruit production per flower (or per inflorescence) in linear-strip fragments prompted this investigation of pollination rates and pollen limitation.

## 2. MATERIAL AND METHODS

### (a) *Pollen per stigma*

To determine what fraction of flowers were pollinated I collected flowers that had passed through their pollen-receptive stage, examined them under a light microscope, and counted the number of pollen grains on the stigmatic surface. Pollen (or polyads) were compared with pollen (or polyads) collected from the anthers of the same species and were counted only if they were similar in size and shape. These pollination surveys were conducted in the same field sites as the study demonstrating depressed fruit set (Cunningham 2000). The selection of field sites, plants within sites and flowers within plants were determined by availability and therefore were not truly random.

In late spring 1997, I collected 6–12 inflorescences of *A. brachybotrya* (from different individuals), in 11 locations (five pairs, plus one unpaired reserve site) totalling 107 inflorescences. Inflorescences bore  $18 \pm 7$  (1 s.d.) flowers, with no significant site ( $n = 5$  sites) or fragment type (reserve versus linear strip) effects (two-way ANOVA on paired sites). Flowers had zero or one polyad attached to the stigma. In late spring of 1998, I collected one to six (mean =  $2.9 \pm 1.2$ , 1 s.d.) post female-phase flowers from *E. glabra* plants in 12 locations (six site pairs, 13–38 flowers per location,  $n = 335$  flowers). The style was dissected from each flower, cleared in sodium hydroxide, stained with decolourized aniline blue and examined by fluorescence microscopy (Martin 1959). I found 0–72 pollen grains per stigma on flowers (mode = 0). For both species the abundance of zero values precluded parametric analysis, so I compared flowers from reserves (pooled among plants, all sites) to flowers from linear strips (pooled among plants, all sites) using the Mann–Whitney *U*-test.

### (b) *Pollen supplementation*

In spring of 1998 I selected five *A. brachybotrya* plants in linear strips at six sites ( $n = 28$  after the death of two plants) for the pollen supplementation experiment. On each plant I tagged a branch with at least four inflorescences in female phase (selection based on availability) and counted all inflorescences on the branch (mean inflorescences per branch  $59 \pm 42$ , 1 s.d.). Inflorescences were clustered together on the branch and were commonly in contact with one another. Pollen was applied liberally with a paintbrush to ensure maximum pollination of

the four female-phase inflorescences, inevitably distributing pollen to clustered neighbouring inflorescences. Pollen used for supplementation was collected from three plants in a different site, one day prior to application, and mixed well. To assess the rate of natural pollination I tagged a second branch of inflorescences in a similar stage of development, on the same plant, and counted all inflorescences on that branch ( $n = 113 \pm 48$ , 1 s.d.). I returned in late spring 1998 and counted the number of fruits on tagged branches. Mean fruit : inflorescence ratios were similar counting the four inflorescences targeted for pollen supplementation, or all inflorescences on the branch (mean = 0.17). Variance in fruit number was less when considering the whole branch (s.d. = 0.17 versus 0.25). Because the application of pollen dusted so many other inflorescences on the tagged branch I used whole branch data for analysis.

In spring 1998 I selected between two and 12 *E. glabra* female-phase flowers from 26 plants in three linear strips. I added supplemental pollen to half the flowers on each plant (randomly assigned) by rubbing the stigma of each flower with three anthers, each from a different plant in the same location. The remaining flowers were used to assess fruit production from open pollination. I counted fruit in late spring.

Linear strip sites used for pollen supplementation experiments were a subset of the sites used in the study of fruit set declines in 1997 (Cunningham 2000) and 1998 (S. A. Cunningham, unpublished data). I did not assess the effect of pollen supplementation on fruit set in reserves because establishing pollen limitation in these plants would not alter interpretation of reserve versus linear strip differences. Because plants in reserves were, on average, receiving more pollen (figure 1), these plants would still be expected to have higher fruit set even if they proved pollen limited, and would (by definition) be receiving sufficient pollen if they proved not to be pollen limited.

### (c) *Visitation rates*

A video camera was used to record open *E. glabra* flowers for 24.5 h over several days, with one to five flowers in view per session (approximately 43 flower-hours), between 08.00 and 18.30, in reserves and linear strips. Although it was not uncommon to incidentally observe birds visiting *E. glabra* flowers, no such visits were recorded on video. There was similarly little insect activity. *A. brachybotrya* flowers were recorded for nearly 14.5 h, with 4–40 inflorescences in view per session (approximately 320 inflorescence-hours) between 08.15 and 16.30, in reserves and linear strips. On average there was less than one visit per inflorescence per two hours.

## 3. RESULTS

Inflorescences of *A. brachybotrya* collected in linear strips had fewer pollinated flowers than those collected in reserves in all five sites (figure 1a) and the median proportion of pollinated flowers per inflorescence was significantly lower ( $U = 1089.5$ ,  $p = 0.043$ ,  $n = 60$  from reserves, 47 from linear strips). Similarly, flowers of *E. glabra* collected in linear strips had significantly fewer pollen grains per stigma than those collected in reserves in four out of six sites (figure 1b) and the median number of pollen grains per stigma was significantly lower ( $U = 16\ 254$ ,  $p = 0.009$ ,  $n = 162$  from reserves, 173 from linear strips). To remove possible non-independence among flowers on the same plant in the *E. glabra* sample, I

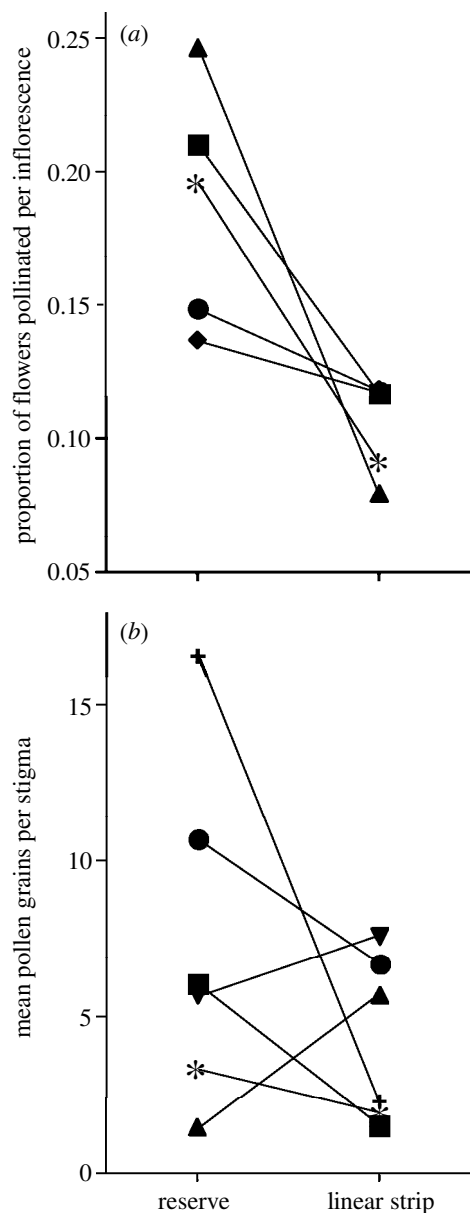


Figure 1. Effect of fragmentation on pollination. Independent contrasts between reserves and linear strips were replicated in multiple sites. Each site is represented by a different symbol, and lines connect each reserve-linear strip pair. (a) Mean proportion of *A. brachybotrya* flowers pollinated per inflorescence (unpaired reserve site not graphed). (b) Mean number of pollen grains per stigma of *E. glabra*.

applied a similar analysis to a reduced data set, using only one (randomly selected) flower per plant, and found a similar result ( $U = 2815$ ,  $p = 0.002$ ,  $n = 58$  from reserves, 57 from linear strips).

The depressed level of pollination in linear strips for these two species could be due to a lower frequency of pollinator visits per flower, or a smaller average pollen load carried by flower visitors in linear strips. Unfortunately it was difficult to detect changes in visit frequency or collect substantial numbers of potential pollinators to measure pollen loads because the rate of pollinator visits was very low for both species, even in reserves (see §2).

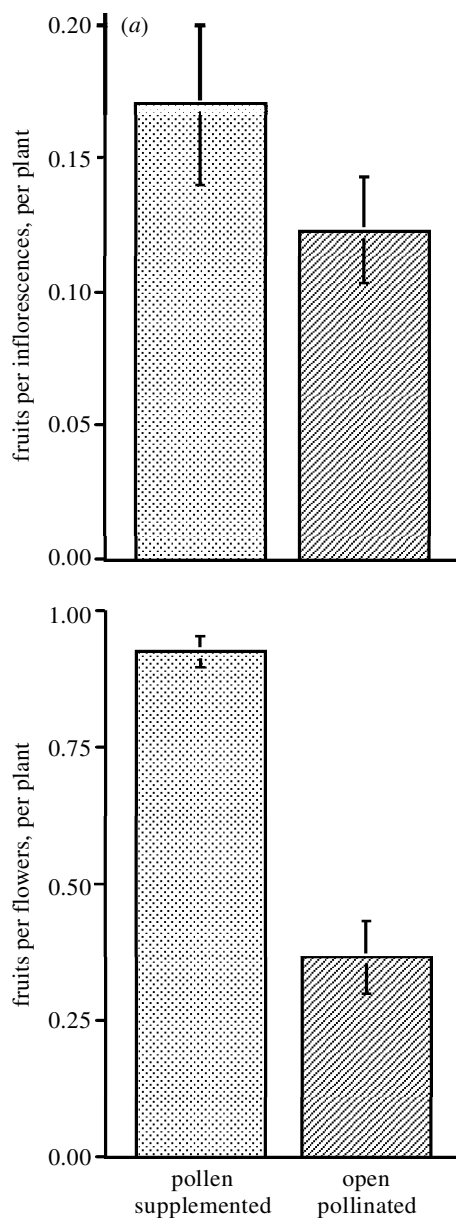


Figure 2. Effect of pollen supplementation on fruit set. (a) Mean number of fruit per inflorescence of *A. brachybotrya* ( $\pm$  s.e.,  $n = 28$  plants). (b) Mean number of fruit per flower of *E. glabra* ( $\pm$  s.e.,  $n = 26$  plants).

Pollen supplementation significantly increased fruit production in *A. brachybotrya* (figure 2a). Two-way nested ANCOVA finds that pollen supplementation significantly increased fruit production ( $F_{1,21} = 4.83$ ,  $p = 0.039$ ), with the number of inflorescences as a covariate ( $F_{1,21} = 26.11$ ,  $p < 0.001$ ), a site effect ( $F_{5,21} = 3.19$ ,  $p = 0.026$ ), plant nested in site effect ( $F_{22,21} = 2.83$ ,  $p = 0.010$ ) and non-significant site by treatment interaction ( $F_{5,21} = 1.23$ ,  $p = 0.333$ ). Inspection of the regression slopes and residuals indicated that the ANCOVA model was appropriate.

Pollen supplementation also increased fruit production per flower in *E. glabra* (figure 2b). The frequency of fruit production was significantly higher among pollen-supplemented flowers (81 out of 89) than open-pollinated flowers (32 out of 89:  $G = 63.6$ , d.f. = 1,  $p < 0.001$ ). This test did not allow assessment of site or plant effects, but variation was relatively low among plants (error bars,

figure 2b) and among sites (site mean proportion of flowers producing fruit  $\pm$  s.e.: pollen-supplemented,  $0.908 \pm 0.053$ ; open-pollinated,  $0.376 \pm 0.147$ ;  $n = 3$  sites).

#### 4. DISCUSSION

Pollen supplementation experiments indicate *A. brachybotrya* and *E. glabra* are pollen limited, and pollination surveys show that flowers receive less pollen when growing in fragmented sites. Together these facts explain depressed fruit production recorded in fragmented sites over two seasons. These data confirm that habitat fragmentation can affect pollination rates, with consequences for reproduction by wild plants in habitat fragments, such as predicted in the pollinator crisis scenario.

It is not clear if the declines in fruit production are sufficient to endanger fragmented populations of *A. brachybotrya* or *E. glabra*, but it is alarming that significant effects of habitat fragmentation can be detected in two relatively common species that are not involved in highly specialized pollinator relationships. It seems likely that plant species that are already at low density, or that are pollinated by a narrow suite of flower visitors, will be more vulnerable to the effects of habitat fragmentation on pollination and fruit production. Given that the majority of the world's ca. 250 000 flowering plants are primarily animal-pollinated (e.g. > 95% of tree species in a tropical rain-forest survey (Bawa *et al.* 1985), ca. 70% of canopy tree species in North America (Regal 1982)) many species could be affected even if only a small fraction of these plant-animal interactions prove vulnerable to disruption. The decline of pollination in commercial crops (Allen-Wardell *et al.* 1998; Buchmann & Nabhan 1996) is likely to receive attention because of the substantial economic risks involved. Unless a comparable research effort is made, however, we may remain oblivious to declines in pollination and reproduction of wild plants that place biodiversity and other natural resources at risk.

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