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Changes in the composition and behaviour of a pollinator guild with plant population size and the consequences for plant fecundity

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Summary

1. Small population size in plants is often associated with decreased fruit set through lower pollinator visitation and reduced offspring fitness as a result of inbreeding. Whether the potentially negative impacts of small population size are realized may be potentially influenced by the behaviour and ecology of pollinators.

2. Here, we investigate changes in both guild composition and pollinator behaviour with plant population size in the bird-pollinated *Anigozanthos flavidus* (Haemodoraceae) and the consequences for fruit set and seed germinability. We used a germination stimulant to reduce the potentially confounding affects of interpopulation variation in dormancy when quantifying seed germinability.

3. All populations were visited in small numbers by western spinebills, while the behaviourally dominant New Holland honeyeaters only visited large populations. Fruit set was extremely high (mean = 96·4 ± 0·6%) regardless of population size.

4. The percentage of foraging bouts interrupted by aggressive interactions tended to be less in small plant populations. The percentage of interrupted foraging bouts was as high as 31% in some populations, suggesting that aggression may frequently promote pollen movement. Western spinebills tended to visit fewer stems per plant than New Holland honeyeaters, a behaviour that potentially favours greater outcrossing.

5. There was no relationship between seed germinability or seeds produced per capsule and plant population size, suggesting that the foraging behaviour of western spinebills provides sufficient pollinator services in small populations to mitigate the potential reduction in seed production and germinability.

6. Our findings highlight that, even within a pollinator guild, differences in behaviour can have important implications for the fitness of plants in small populations, with the potential for some pollinators to provide unexpected levels of resilience.

Key-words: aggression, bird, germination stimulant, pollination, pollinator behaviour, seed dormancy, small population

Introduction

Population size in plants has important consequences for plant reproduction and offspring fitness (Menges 1991; Groom 1998; Hobbs & Yates 2003; Ghazoul 2005). Small populations may provide insufficient resources to attract and sustain pollinators (Thomson 1981; Sih & Baltus 1987; Kunin 1993; Ågren 1996; Johnson *et al.* 2003; Aguilar *et al.* 2006) or pollinators may carry reduced pollen loads (Waites & Ågren 2004) leading to low fruit set. A reduction in seed germinability and offspring vigour may arise

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through increased inbreeding as a result of a smaller pool of potential mates (Ellstrand & Elam 1993; Heschel & Paige 1995). Fitness expressed as seed quantity and germinability will have important consequences for small populations, with low pollinator abundance or increased inbreeding leading to greater extinction risk (Groom 1998; Anderson *et al*. 2011). Whether the potentially negative impacts of small population size are realized for any given plant species may be dependent on the behaviour and ecology of pollinators.

Plant population size can affect the composition of the pollinator community through variation in the minimum number of plants required to attract and sustain different pollinator species (Linhart 1973; Stiles 1975). For example, in hummingbird-pollinated plants, small populations may only attract trap-lining hermits, while larger populations can attract a range of territorial genera (Stiles 1975). While nectar and pollen foraging animals tend to move predominantly between flowers within a plant before switching to neighbouring plants (Pyke 1978, 1981), pollinators exhibit considerable variation in their behaviour and effectiveness, including differences in pollen loads, grooming behaviour, dispersal distances, plant species fidelity and pollen carryover (Primack & Silander 1975; Stiles 1975; Schemske & Horvitz 1984; Wilson & Thomson 1991; Castellanos, Wilson & Thomson 2003; Mitchell *et al*. 2004). As such, differences in plant population size that affect pollinator community composition may have important consequences for plant-mating systems and interpopulation gene flow.

Differences in plant population size are also expected to lead to changes in pollinator behaviour. In small or low-density populations, pollinators may move between plants less frequently, potentially exacerbating inbreeding resulting from small population size (Beattie 1976; Goverde *et al*. 2002). Further, small populations could potentially receive a lower proportion of pollen from other populations through attracting fewer pollinators (Richards, Church & McCauley 1999; though see Cresswell & Osborne 2004). In addition to increasing the risk of population extinction, limitations to seed quantity and quality (i.e. germinability) via pollinator abundance or behaviour will also have evolutionary consequences, with natural selection favouring the use of pollinators that lead to the highest fitness gain for that population size. Specialized pollination systems where only a few pollinator species visit plants provide useful opportunities for comparing the ecological and evolutionary consequences of different pollinator species and testing how this varies with plant population size.

Bird pollination systems provide an interesting test of the consequences of both interspecific differences in pollinator behaviour, and their interaction with plant population size, for seed germinability. While nectarivorous birds are capable of moving long distances as they follow seasonal variation in nectar supply (e.g. Schuchmann 1999; Phillips, Hopper & Dixon 2010), many species form territories and restrict their foraging movements over a small area at least while breeding (Pyke, Christy & Major 1996; Schuchmann 1999). Conversely, some pollinator species target small populations and move widely to locate them (Schuchmann 1999). This variation in strategies could have important consequences for seed production and germinability. The aggressive territorial behaviour exhibited by many nectarivorous birds (e.g. Schuchmann 1999; MacNally & Timewell 2005; Cheke & Mann 2008; Temeles & Kress 2010) may also be important as the pursuit of intruders will interrupt the pattern of nearest neighbour movements. Particularly for floriferous plants, where a single plant may account for a large proportion of a territory, this may represent a significant contribution to outcrossing (Paton 1986). Both territorial behaviour and specialization by nectarivores on small plant populations may provide mechanisms through which bird-pollinated plants avoid inbreeding depression.

Here, we investigate the differences in pollinator guild composition and behaviour with plant population size and their consequences for seed germinability and fruit set. We studied *Anigozanthos flavidus* (Haemodoraceae), a bird-pollinated plant visited by a small number of honeyeater (Meliphagidae) species (Hopper 1993). Following quantification of the specificity of the system, we tested the hypotheses that: (i) pollinator community composition changes with plant population size. (ii) Small populations receive fewer pollinator visits leading to lower fruit set. (iii) Different honeyeater species exhibit different patterns of foraging behaviour. (iv) Foraging behaviour and the frequency of aggressive interactions are related to plant population size (v) Patterns of pollinator behaviour influence plant fecundity and seed germinability.

To quantify pollinator behaviour, we developed a scheme that takes into account critical components that influence pollen movement; stems (inflorescences) visited per plant (self-pollination), the distance moved relative to the nearest neighbouring plant (pollen movement within population) and length of a foraging bout in a population (which may affect pollen movement within populations). We also take advantage of the recent discovery of glyceronitrile as a key agent that stimulates germination in Haemodoraceae (Flematti *et al*. 2011; Nelson *et al*. 2012). This reduces the potential confounding effects of population level variation in the physiological component of dormancy (e.g. Tieu *et al*. 2001) when assessing seed germinability.

Materials and methods

STUDY SPECIES

Anigozanthos flavidus is a bird-pollinated monocot restricted to high rainfall areas in south-western Australia (Hopper 1993). Individual plants form dense clumps with several scapes (referred to hereafter as stems) projecting up to two metres above the foliage (Hopper 1993). Stems contain up to 120 flowers that open sequentially in 3–9 pairs of inflorescences (racemes) with up to *c*. 50 seeds produced per flower. *Anigozanthos flavidus* is self-compatible but

shows a large reduction in seed germination and seeds produced per capsule following self-pollination (Hopper 1980a). Preliminary observations of the pollination biology suggest that the dominant pollinator is the New Holland Honeyeater *Phylidonyris novaehollandiae* (Meliphagidae; Fig. 1) with some populations visited by the Western Spinebill *Acanthorhynchus superciliosus* (Meliphagidae) and brown honeyeater *Lichmera indistincta* (Meliphagidae) (Hopper 1993). All of these honeyeater species are known to feed on nectar from a diverse range of flowers and consume invertebrate prey (Higgins, Peter & Steele 2001).

STUDY SITE

Fourteen populations in the Northcliffe region, 300 km south of Perth, Western Australia, were used to study the relationship between plant population size, pollinator guild composition, pollinator behaviour and seed germinability (Fig. 2). An additional ten populations were used to quantify the specificity of the pollination system of *A. flavidus* across its distribution (Fig. 2). Populations were considered to be discrete groups of plants with few or no individuals present in the nearby bushland. Small- to moderate-sized populations varied considerably in density (0·01–1·14 plants per m^2), while the very largest populations tended to have high density (0·72–1·11 plants per m^2). Generally, populations were separated by distances of >500 m with the exception of occasional individual plants. For the Northcliffe populations, population size was quantified by direct counts of the number of plants. The number of stems was counted for up to 35 random plants (less for populations <35), averaged and then used to calculate the number in the entire population.

POLLINATOR SPECIFICTY OF *ANIGOZANTHOS FLAVIDUS*

Specificity of the pollination system of *A. flavidus* was quantified by combining the observations from the Northcliffe region with a series of 2-h observations at sites across its distribution. Observations were undertaken in December 2010 and January 2011. Due to the similarity in habitat at each site and their close geographic proximity, all populations flower synchronously in the Northcliffe area. During this study period, *A. flavidus* was the only bird attracting plant in flower at the Northcliffe study sites. During observations of pollinator behaviour, all nectarivorous birds present at the site were recorded, including those not foraging on *A. flavidus*. Observations of any insect visitation were also made. No nocturnal observations were made to survey for the presence of honey possums *Tarsipes rostratus* (Tarsipedidae), a common visitor to bird-pollinated plants (Hopper 1980b; Wooller *et al.* 1983), as there are no records of honey possums from the immediate study region and they appear to be absent from the high rainfall forests and swampy sandplains where the study was undertaken (data from the Western Australian Museum and Department of Environment and Conservation, Nature Map 2012).

Fig. 1. New Holland Honeyeater feeding on *Anigozanthos flavidus*. Flowers are held in a series of paired inflorescences (racemes) originating off the main stem. Arrows indicate a pair of inflorescences, which formed the unit of study for the seed germination trials.

POLLINATOR ABUNDANCE AND BEHAVIOUR IN RELATION TO POPULATION SIZE

Observations at each site were made twice, 3 h in the morning and again for 3 h in the afternoon. Morning and afternoon observation periods were made on different days, up to 8 days apart. Birds were not colour-banded so it is not known how many different individuals visited flowers at each population over the course of the study.

An estimate of the quantity of birds visiting each population was made by counting the number of birds present within populations of *A. flavidus* in a three-minute period every 15 min during the six hours of observation. For small populations, bird visitation was a comparatively rare event so any birds visiting the population were recorded. In both cases, abundance was expressed as birds recorded per minute. A regression analysis was undertaken to test for a relationship between plant population size and the number of birds recorded per minute.

We recorded variables that characterize the foraging behaviour of pollinators as is relevant to plant mating systems and pollen movement. The variables recorded were: length of foraging bout; number of plants visited in a foraging bout (pollen movement within population); number of stems visited per plant (self-pollination) and distance travelled between plants. Movements within stems were not quantified as preliminary observations showed that bird activity was too quick to reliably record and pollinators consistently visited most of the open flowers as they worked their way up the stem. The behavioural variables were quantified as follows:

1. Foraging bout length – once a foraging bird was located, timing continued until the bird was out of sight, the bird stopped foraging or three minutes had elapsed. In this event, the bird was recorded as visiting for 180 s.
2. Number of visited plants – during each foraging bout, the number of plants visited was counted until three minutes had elapsed.
3. Number of visited stems – for each plant visited during a foraging bout, the number of stems visited was recorded.
4. Pollinator Movement Index (Phillips, Hopper & Dixon 2010) – we visually estimated the distance the bird travelled to the next plant and the distance to the closest potential forage plant (excluding the plant where the honeyeater had been foraging previously). The ratio was then calculated as distance travelled/distance to nearest plant. This provides an estimate of how far a pollinator moved to a food plant compared with the minimum distance it had to move. Pollinators with a high Pollinator Movement Index move pollen further for a given density of plant.

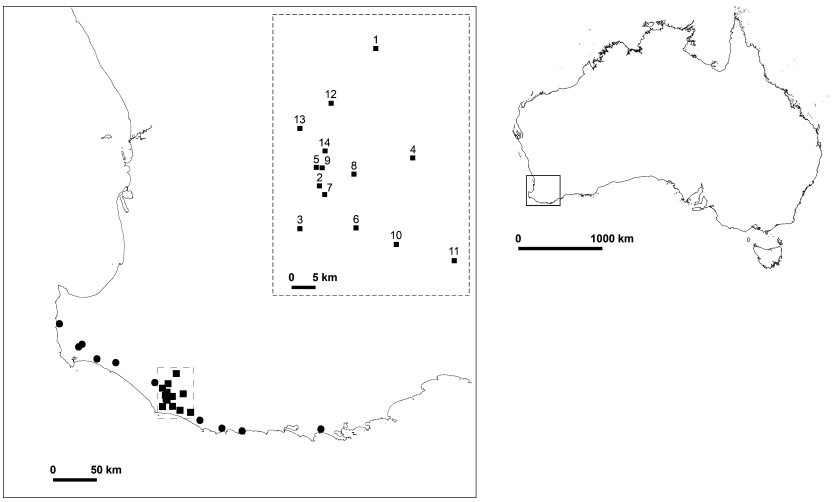

Fig. 2. Locations of study sites of the pollination biology of *Anigozanthos flavidus* in south-western Australia. Squares are sites in the Northcliffe area where detailed pollinator observations have been undertaken. Circles represent sites used to quantify pollinator specificity across the distribution of *A. flavidus*, outside the Northcliffe area.

When a foraging bird left a population of *A. flavidus,* we recorded if the bird departed as a result of interspecific or intraspecific aggression. If an aggressive interaction caused the bird to leave a population, it was noted which species was the aggressor. The percentage of foraging bouts terminated by an aggressive interaction was calculated and arcsin square-root transformed prior to analysis. Regression analysis was used to test for a relationship between (i) the incidence of aggressive interactions and the number of flowering stems and (ii) the incidence of aggressive interactions and a measure of the potential for competition for nectar (birds per minute divided by the number of flowering stems).

For interspecific comparisons of foraging behaviour, observations were pooled across all populations. Mann–Whitney *U*-tests were used to test for differences in foraging behaviour between western spinebills and New Holland honeyeaters. Following the observation that western spinebills occur at more populations than New Holland honeyeaters, we used the same approach to test whether the foraging behaviour of western spinebills change in the presence of New Holland honeyeaters. When testing for relationships between foraging behaviour and population size, populations were only included if greater than ten foraging bouts were observed for that species. Regression analysis was used to test for relationships between each foraging behaviour and the number of plants in a population. All statistical tests were undertaken in JMP 9.0.0 (SAS Institute Inc., Cary, NC, USA).

FRUIT SET IN RELATION TO PLANT POPULATION SIZE

We tested if fruit set differed between small and large populations as a result of differences in pollinator visitation. We quantified the percentage of fruit set (except for sites 4 and 10 which were burnt in management fires before seed set could occur) by counting the percentage of capsules formed for 50 random plants per population (or all plants for populations smaller than this) and averaging across plants. We used the 2nd to 4th pairs of inflorescences from the bottom of the stem (see Fig. 1) as these were sections of the stem in flower during the observation period in all populations.

We used regression analysis to test for a relationship between (i) fruit set and population size and (ii) fruit set and birds recorded per minute. Because the presence of different pollinator species could lead to differing levels of reproductive output, a Student's *t*-test was used to test for differences in fruit set and seeds per capsule between populations where New Holland honeyeaters were present or absent.

We tested for self-pollination to confirm that patterns of seed production and germinability where primarily determined by honeyeater visitation not autogamy. Ten inflorescences were bagged at one population as they were about to open to prevent visitation by pollinators. The average proportion of capsules formed per inflorescence was calculated to estimate the rate of self-pollination.

SEED GERMINABILITY AND SEEDS PER CAPSULE IN RELATION TO POPULATION SIZE

Previous work on the mating system of *A. flavidus* has demonstrated lower seed germinability in selfed compared with outcrossed seed (Hopper 1980a). Here, we investigated the potential for differences in levels of inbreeding between populations by quantifying seed germinability. A reduction in seed germination from inbreeding would result from a combination of both geitonogamy and crosses between closely related plants. Seed was collected from the remaining 13 Northcliffe populations of *A. flavidus*. Seed from one stem from each of 50 plants was collected, sampling plants from the full spatial extent of the population. In populations of fewer than 50 plants, seed was collected from all individuals. Capsules at the point of natural dehiscence were collected from the 2nd to 4th pairs of inflorescences on the stem. These included capsules that were used for the quantification of fruit set. Within 2 days of collection, all plant material was stored at 15 °C/15% RH until processing. Seed was separated from capsules and plant debris by vacuum separation.

Seeds were germinated on a 0·7% (w/v) water agar medium and incubated at a constant 15°C on a 12/12 h light/dark regime. A second treatment involved addition of glyceronitrile, a germination stimulant (Flematti *et al*. 2011), in the agar at a concentration of 50 μM. Prior to plating on agar (± glyceronitrile), seeds were surface sterilized on a 2% (w/v) solution of calcium hypochlorite ($Ca(OCl)_2$) under vacuum for 30 min and rinsed in sterilized water. For both treatments and each population, 25 seeds were plated in each of four Petri dishes. Germination (defined as radicle emergence) was scored over 60 days.

For both treatments, the percentage of seeds germinating was calculated and arcsine square-root transformed prior to analysis. Regression analysis of germination with glyceronitrile against population size was used to test for an effect of inbreeding.

As an additional test for inbreeding in small population sizes, we examined the number of seeds produced per capsule. Immediately after the flowering season concluded, nylon bags were placed over one pair of inflorescence for 15 individuals per population (Northcliffe populations only; 2nd to 4th pairs of inflorescence on the stem) and removed *c*. 2 months after the end of the main flowering season. The total number of seeds for each pair of inflorescences was counted and divided by the number of capsules to give

an estimate of seeds per capsules per plant. A regression analysis was conducted between plant population size and the average number of seeds per capsule per plant.

Results

POLLINATOR SPECIFICITY IN *ANIGOZANTHOS FLAVIDUS*

In the Northcliffe region New Holland Honeyeater (nine of 14 sites), Western Spinebill (14 sites) and brown honeyeater (1 site) were observed foraging on *A. flavidus*. For each species, large amounts of pollen were visible on the forehead (see Fig. 1), the position that comes into contact with the stigma (Hopper 1993). At 12 of 14 sites, the most abundant visitor remained consistent across observation periods. Of the nine sites where New Holland honeyeaters were recorded, they were recorded during both observation periods at eight sites. Similarly, of the 14 sites where western spinebills were recorded, they were recorded during both observation periods at 12 sites.

Several other honeyeater species were regularly observed at the study sites, but were not observed to forage on *A. flavidus*, namely red wattlebird *Anthochaera carunculata* (10 sites), western wattlebird *Anthochaera lunulata* (three sites) and western white-naped honeyeater *Melithreptus chloropsis* (six sites). On average, 1·7 ± 0·2 (SE) species were seen foraging per population, while the total honeyeater diversity averaged 4·3 ± 0·3 (SE) species. Outside of the Northcliffe region, four species were observed foraging on *A. flavidus*; Western Spinebill (nine sites), New Holland Honeyeater (five sites), brown honeyeater (one site) and western wattlebird (one site). Some nectar robbing was observed by silvereyes *Zosterops lateralis* (Zosteropidae) in the manner described in the study described by Hopper (1993), where they slash a hole in the base of the floral tube to reach the nectar. The only observations of insects were attempted flower visitations by exotic honeybees, *Apis mellifera*, at three populations, though they are not considered effective pollinators as they do not make contact with the stigma.

POLLINATOR ABUDANCE AND FRUIT SET IN RELATION TO POPULATION SIZE

The composition of the pollinator guild was strongly correlated with plant population size (Fig. 3). New Holland honeyeaters did not occur at any population smaller than 100 plants. Western spinebills occurred at populations of all sizes, with abundance showing no relationship with population size. At populations of 260 plants or greater, New Holland honeyeaters were always the more common species. The total number of birds observed per minute significantly increased with plant population size (R^2 = 0·608; P = 0·001). The number of plants and the number of flowering stems in a population were strongly correlated (R = 0·935).

Fruit set for open-pollinated inflorescences ranged between 93·4% and 99·1% [mean = 96·4 ± 0·6% (SE)], with observations from subsequent years confirming that fruit set consistently exceeds 90% in all populations (R.D. Phillips, unpublished data). There was no significant relationship between fruit set and population size (R^2 = 0·096, P = 0·320) or fruit set and the number of birds recorded per minute (R^2 = 0·030, P = 0·587). Further, there was no difference in fruit set between populations with or without New Holland honeyeaters [with = 96·7 ± 0·8 (n = 6); without = 96·2 ± 0·8 (n = 6), P = 0·661]. Inflorescences covered with bags to prevent visitation by pollinators yielded 15·3 ± 3·3% fruit set, though some of these self-pollination events may have been caused through incidental brushing of the floral parts as a result of the bagging process.

POLLINATOR BEHAVIOUR AND PLANT POPULATION SIZE

For both species, the Pollinator Movement Index showed a strongly leptokurtic distribution with birds most commonly moving to the nearest neighbouring plant (Fig. 4a). There was no significant difference between species for the Pollinator Movement Index (P = 0·453; Table 1). The number of stems visited per plant also showed a strongly leptokurtic distribution for both species (Fig. 4b). However, New Holland honeyeaters visited significantly more stems per plant than western spinebills ($P \leq$ 0·0001; Table 1), with the average number of stems being 16·6% higher. This difference arose from western spinebills more regularly visiting one stem per plant but less regularly visiting two or three stems per plant compared with New Holland honeyeaters.

New Holland honeyeaters had significantly longer foraging bouts (P = 0·004; Table 1), with the average length of

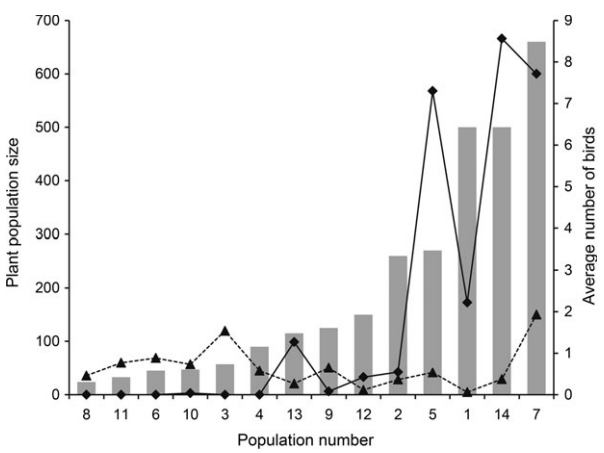

Fig. 3. The relationship of bird abundance and plant population size for *Anigozanthos flavidus*. Average number of birds is the number of birds recorded per minute in censuses made during the foraging observations. Dashed line: Western Spinebill; solid line: New Holland Honeyeater.

a foraging bout being 20·3% longer. Compared with western spinebills, foraging bouts of New Holland honeyeaters more frequently exceeded the three-minute observation time (NHH = 27·2%; WS = 10·9%; Fig. 4c) suggesting that the difference in foraging length is much greater than suggested by comparing mean bout length. There was no significant difference in the number of plants visited per foraging bout ($P = 0{\cdot}333$; Table 1). Neither Western Spinebill nor New Holland Honeyeater showed a significant relationship between number of plants in a population and any aspect of foraging behaviour (Table 2). There was no significant difference in any aspect of the foraging behaviour of western spinebills in the populations with or without New Holland honeyeaters (Pollinator Movement Index: $Z = 0{\cdot}350$, $P = 0{\cdot}554$; stems visited per plant: $Z = 0.\ 346$, $P = 0{\cdot}556$; plants visited per foraging bout: $Z = 2{\cdot}295$, $P = 0{\cdot}130$; foraging bout length: $Z = 3{\cdot}632$ $P = 0{\cdot}057$).

The mean percentage of total foraging bouts per population interrupted by aggressive interactions was $12{\cdot}0 \pm 2{\cdot}5\%$ SE (range = 0–31·2%). In both species, foraging bouts were commonly interrupted by intraspecific aggression, with New Holland honeyeaters being the species more frequently showing aggression (NHH: 9·0% of foraging bouts interrupted; WS: 3·8% of foraging bouts interrupted). Likewise, New Holland honeyeaters commonly ceased foraging to attack western spinebills and silvereyes *Zosterops lateralis* (Zosteropidae), with 3·0% of foraging

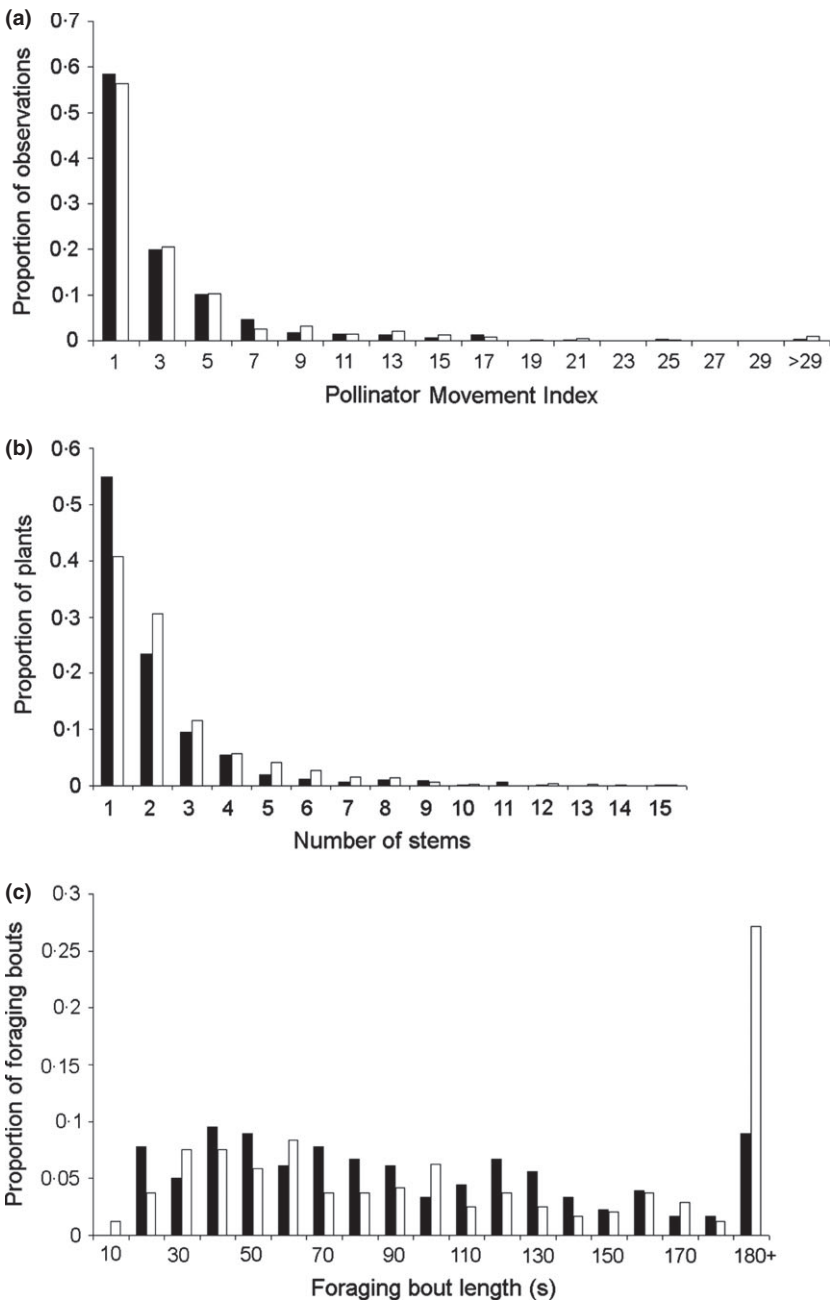

Fig. 4. Comparative foraging behaviour of Western Spinebill (black) and New Holland Honeyeater (white) foraging on *Anigozanthos flavidus*. (a) Pollinator movement index (b) number of stems visited per plant (c) length of foraging bout.

Table 1. Comparative foraging behaviour of New Holland honeyeaters and western spinebills on *Anigozanthos flavidus*

	New Holland Honeyeater	Western Spinebill
Pollinator Movement Index		
Raw mean (±SE)	3·011 ± 0·178	2·626 ± 0·136
Mean rank	700 (N = 732)	686 (N = 654)
Z	−0·750	
P	0·453	
Stems visited per plant		
Raw mean (±SE)	2·360 ± 0·060	2·024 ± 0·065
Mean rank	965 (N = 1020)	831 (N = 792)
Z	−5·779	
P	< 0·0001	
Plants visited per foraging bout		
Raw mean (±SE)	3·98 ± 0·17	4·56 ± 0·27
Mean rank	201 (N = 240)	213 (N = 171)
Z	0·968	
P	0·333	
Foraging bout length (s)*		
Raw mean (±SE)	104·9 ± 3·9	87·2 ± 4·0
Mean rank	222 (N = 239)	188 (N = 175)
Z	−2·864	
P	0·004	

Mean rank, Z-scores and P-values are from Mann–Whitney U-tests.
*All foraging bouts exceeding 180 s were given a value of 180. For New Holland honeyeaters this occurred on 65 occasions, for western spinebills this occurred on 19 occasions.

bouts interrupted to attack another species. On 12·6% of foraging bouts, western spinebills ceased foraging either because they were attacked by a New Holland honeyeater or to attack a Silvereye themselves. All interspecific aggression between New Holland honeyeaters and western spinebills involved New Holland honeyeaters attacking western spinebills, demonstrating their behavioural dominance over this species. During an act of aggression, the attacked birds were at least temporarily driven away from the plant population. There was no significant relationship between the number of flowering stems and the incidence of aggressive interactions (R^2 = 0·274, P = 0·055), though the plant populations with the least aggressive interactions were all small (Fig. 5). There was no significant relationship between the potential competition for nectar (measured as the number of birds present per flowering stem) and incidence of aggressive interactions (R^2 = 0·005, P = 0·812).

SEED GERMINABILITY AND SEEDS PER CAPSULE IN RELATION TO POPULATION SIZE

Seed germination averaged 32·1 ± 4·0% (SE) on water agar (control) while germination after treatment with glyceronitrile averaged 85·8 ± 2·6%, confirming the presence of a physiological component of dormancy. Germination levels showed no relationship with population size (R^2 = 0·15, F = 1·77, P = 0·21). There was no significant difference in germination at sites with and without New Holland honeyeaters (with NHH = 88 ± 2·3% (SE), without NHH = 82·8 ± 5·5%, t = 0·871, P = 0·423). Similarly, there was no relationship between seeds produced per capsule and population size (R^2 = 0·100, P = 0·317) or difference in seeds per capsule between populations with or without New Holland honeyeaters (with NHH = 22·5 ± 0·3 (SE), n = 6; without NHH = 19·6 ± 3·2, n = 6, P = 0·402).

Table 2. The relationship of pollinator behaviour and plant population size for honeyeaters feeding on *Anigozanthos flavidus*. R^2, F, and P values are from regression analyses

	New Holland Honeyeater	Western Spinebill
Pollinator Movement Index		
R^2	0·100	0·193
F	0·445	1·192
P	0·541	0·325
Stems visited per plant		
R^2	0·072	0·003
F	0·311	0·015
P	0·607	0·908
Plants visited per foraging bout		
R^2	0·237	0·001
F	1·244	0·005
P	0·327	0·944
Foraging bout length		
R^2	0·035	0·350
F	0·143	2·692
P	0·724	0·162

Discussion

SPECIFICTY OF THE POLLINATION SYSTEM

Observations at multiple sites across its distribution demonstrated that *Anigozanthos flavidus* has a highly specific pollination system, with almost all visitation by just two bird species, the New Holland Honeyeater and the Western Spinebill. This confirms preliminary observations of Hopper (1993) that these species are the primary pollinators. Further, bird communities in this region show little variation from year to year due to consistent rainfall, suggesting that these results are an accurate reflection of longer term patterns (Higgins, Peter & Steele 2001; Barrett *et al.* 2003). While common in the study area, the western white-naped honeyeater was not observed to forage on *A. flavidus*, most likely due to a short beak that restricts foraging to comparatively open faced flowers (Paton 1986). Similarly, red wattlebirds were also common in the study region but were not observed foraging on *A. flavidus*, potentially due to their large size restricting access to the corolla tube (Paton 1986). The high specificity of this system means that patterns of pollen movement in the majority of populations can be attributed to New Holland honeyeaters and/or western spinebills. While New Holland honeyeaters only occupied large populations of *A. flavidus*, small numbers of western spinebills visited all population sizes, demonstrating that the contribution of the two bird species to pollen movement and seed set will vary with plant population size.

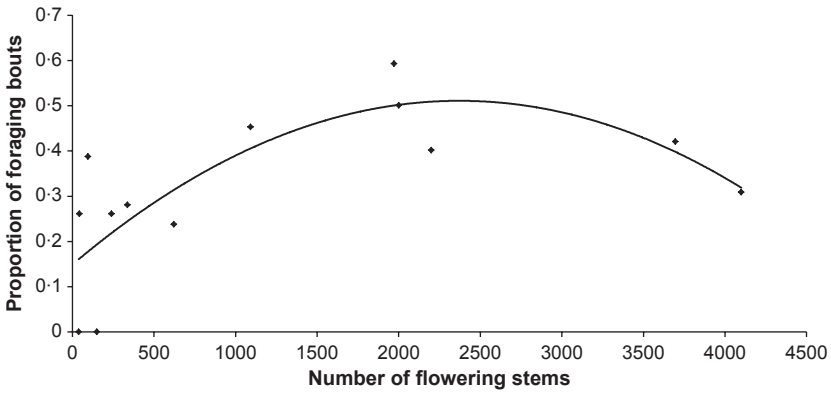

Fig. 5. The proportion of honeyeater foraging bouts interrupted by aggression (interspecific and intraspecific) in relation to the number of flowering stems in populations of *Anigozanthos flavidus*. Proportions have been arcsine square-root transformed.

FRUIT SET AND POPULATION SIZE

In highly specific pollination systems, pollinator availability may limit fruit set through two different mechanisms. In small populations, there are fewer resources to attract and sustain pollinators (e.g. Sih & Baltus 1987; Lamont, Klinkhamer & Witkowski 1993; Yates *et al*. 2007a). Alternatively, if increases in plant population size are not met by concomitant increases in the abundance of the specific pollinator then average fruit set may decrease in very large populations (Johnson, Hollens & Kuhlmann 2012). Despite a highly specific pollination system, fruit set in *A. flavidus* was extremely high regardless of population size. The use of mobile pollinators that consume invertebrate prey may lead to frequent visitation to small and isolated plant populations, as pollinators search more widely for invertebrate food resources rather than being restricted to large patches of flowering plants. Further, at all study sites *A. flavidus* was by far the most abundant flowering species adapted to bird pollination, suggesting that birds may adjust their feeding routes to coincide with these populations. From this system, we predict that specific pollination systems would not lead to low fruit set in small populations if pollinators are common, if they have a flexible diet, or if the reward is sufficiently high that they modify their foraging patterns to maximize visitation.

POLLINATOR BEHAVIOUR AND PLANT POPULATION SIZE

While small populations are predisposed to inbreeding through a limited pool of potential mates (Ghazoul 2005), pollinators have the ability to mitigate this effect. This could potentially occur through pollinators engaging in behaviours that result in high levels of outcrossing (Stiles 1975), comparatively long pollen movements within populations (Krauss *et al*. 2009) or pollen movement between populations (Byrne *et al*. 2007; Llorens *et al*. 2012). In the present study, neither bird species showed a relationship between foraging behaviour and plant population size. However, the predominance of western spinebills in small populations and New Holland honeyeaters in large populations suggests that any difference in the foraging behaviour of these two species will lead to differences in pollen movement with plant population size. While both species most commonly moved to the nearest neighbouring plant, there were differences in the foraging behaviour that may have important implications for pollen movement and seed germinability. Western spinebills more frequently visited a single stem per plant suggesting higher levels of outcrossing. As such, the foraging behaviour of western spinebills has the potential to counteract the negative consequences of small population size on plant and seed fitness through greater outcrossing. While pollinator behaviour can vary between years in response to variables such as the number of flowers, these results are likely to be consistent between years due to the reliable, annual flowering of this species.

Western spinebills spent less time foraging within populations of *A. flavidus* than New Holland honeyeaters. Shorter foraging bouts may potentially lead to more frequent pollen dispersal between plant populations, depending on how many occur within the bird's foraging range. Both New Holland honeyeaters and western spinebills have territories of generally <0·5 ha (Newland & Wooller 1985; Higgins, Peter & Steele 2001), suggesting that most movements will be between a small number of local plant populations. However, both species are known to occasionally move well beyond their territory to utilize significant nectar sources (Newland & Wooller 1985; Higgins, Peter & Steele 2001). Further, New Holland honeyeaters show pronounced differences in territory size through the year (Pyke, Christy & Major 1996; Higgins, Peter & Steele 2001), suggesting that overlap of flowering time and breeding season could have consequences for plants through changes in foraging patterns and therefore pollen dispersal. Evaluation of the role of these honeyeater species in pollen-mediated gene flow needs to be examined through a combination of colour-banding studies to determine home ranges and fluorescent dyes that can be applied to anthers and used to track pollen dispersal (Kearns & Inouye 1993).

AGGRESSION, POLLEN MOVEMENT AND POPULATION SIZE

While rarely studied in terms of the consequences for plants, aggressive interactions between pollinators may

have important consequences for pollen movement through the interruption of optimal foraging behaviour (Pyke 1978; Greenleaf & Kremen 2006). In the present study, the proportion of foraging bouts interrupted by aggression ranged from 0% to 31·2% per population suggesting that in at least some populations, aggression will result in significant interruption to optimal foraging behaviour and increase pollen movement distances. Levels of aggression were highest in populations exceeding 260 plants where New Holland honeyeaters were present. Given that interspecific aggression from New Holland honeyeaters often takes the form of chasing the Western Spinebill away from the plant population, they may play an important role in increasing pollen movement in large populations. From this system, it appears that the role of bird aggression in determining pollen movement will be a complex interplay between plant population size and the composition and dominance hierarchy of bird communities.

SEED GERMINABILITY AND POPULATION SIZE

While small population size is frequently associated with a reduction in seed germinability (e.g. Menges 1991; Heschel & Paige 1995; Le Cadre *et al*. 2008), there was no strong evidence for a relationship between seed germinability and population size in *A. flavidus*. Further, the number of seeds produced per capsule showed no variation across population sizes. Given that *A. flavidus* exhibits reduced germinability and seeds per capsule following self-pollination (Hopper 1980a), it should be evident if small populations are encountering high levels of pollinator mediated inbreeding. However, the absence of such relationships suggests that the foraging behaviour of the Western Spinebill may be important in maintaining high seed germinability in small populations through greater outcrossing. More broadly, mobile pollinators or those with comparatively low nectar requirements may play an important role in maintaining genetic variation in small or isolated plant populations leading to high seed germinability. Given that the consequences of inbreeding can also be expressed postgermination, investigations of seedling vigour resulting form selfed and outcrossed seed would shed further light on the consequences of pollinator behaviour for plant fitness.

CONSEQUENCES OF BIRD POLLINATION

Pollination by birds has been predicted to lead to higher levels of outcrossing, possibly favouring its evolution from insect-pollinated ancestors (Hopper & Moran 1981). This hypothesis has been supported by experiments showing a reduction in outcrossing when birds but not insects are excluded from floral visitation (England *et al*. 2001). Further, Castellanos, Wilson & Thomson (2003) found that bird pollination can lead to greater levels of pollen removal and transfer efficiency. These observations have led to the hypothesis that the evolution of bird pollination will be favoured as a mechanism to avoid inbreeding in lineages characterized by naturally fragmented populations (Hopper 2009). While the present study lacks comparison with insect pollinators, it does lend support for the advantages of bird -pollination in small populations. Our study shows that there is no reduction in fruit set or seed germinability in small populations and that the behaviour of western spinebills may mitigate the potential effects of inbreeding in small populations. While some other studies have suggested that honeyeaters may not rescue very small plant populations from inbreeding or low fruit set (Lamont, Klinkhamer & Witkowski 1993; Yates *et al*. 2007b), these have been undertaken in anthropogenically fragmented landscapes. In natural areas, bird pollination may be an effective strategy of achieving pollination in small populations.

While most nectarivorous Australian birds consume nectar from a wide phylogenetic diversity of plant species (Hopper & Burbidge 1986; Paton 1986; Higgins, Peter & Steele 2001), there is increasing evidence that some plant species are specialized on one or few pollinators. In bird-pollinated plants, many of the more specialized species tend to be those with more tubular flowers, such as *A. flavidus*, where visitation by short-beaked or large-bodied bird species is not possible (e.g., Hopper & Burbidge 1978; Johnson, McQuillan & Kirkpatrick 2010). Alternatively, some plant species rely on a single small honeyeater species because nectar is not provided in sufficient quantity to support larger bodied or territorial birds (Paton 1986). As shown in the present study, this can lead to smaller populations relying on a single species of pollinator. A level of specialization can also be conferred in some habitats through a relatively species poor nectarivorous bird assemblage (Hopper 1993). Following initial assessments that specialized bird pollination systems were largely absent from Australasia (Stiles 1981), this evidence suggests that specificity of bird pollination systems may be much higher than initially proposed.

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