A test for Allee effects in the self-incompatible wasp-pollinated milkweed *Gomphocarpus physocarpus*.

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Abstract It has been suggested that plants which are good colonizers will generally have either an ability to self-fertilize or a generalist pollination system. This prediction is based on the idea that these reproductive traits should confer resistance to Allee effects in founder populations and was tested using *Gomphocarpus physocarpus* (Asclepiadoideae; Apocynaceae), a species native to South Africa that is invasive in other parts of the world. We found no significant relationships between the size of *G. physocarpus* populations and various measures of pollination success (pollen deposition, pollen removal, and pollen transfer efficiency) and fruit set. A breeding system experiment showed that plants in a South African population are genetically self-incompatible and thus obligate outcrossers. Out-crossing is further enhanced by mechanical reconfiguration of removed pollinaria before the pollinia can be deposited. Self-pollination is reduced when such reconfiguration exceeds the average duration of pollinator visits to a plant. Observations suggest that a wide variety of wasp species in the genera *Belonogaster* and *Polistes* (Vespidae) are the primary pollinators. We conclude that efficient pollination of plants in small founding populations, resulting from their generalist wasp-pollination system, contributes in part to the colonizing success of *G. physocarpus*. The presence of similar wasps in other parts of the world has evidently facilitated the expansion of the range of this milkweed.

Keywords: Allee effect, *Gomphocarpus physocarpus*, pollen transfer efficiency, pollinarium reconfiguration, wasp pollination, Asclepiadoideae, self-incompatible.

INTRODUCTION

The fitness of individuals is frequently positively related to either the number or density of conspecifics (Allee 1931). This phenomenon has become known as the Allee effect (Groom 1998; Stephens *et al.* 1999; Courchamp *et al.* 1999; Stephens & Sutherland 1999). The basis for Allee effects may be genetic (Charlesworth & Charlesworth 1987; Ellstrand & Elam 1993; Courchamp *et al.* 1999; Herlihy & Eckert 2002) or ecological, as in reduced cooperative interactions between individuals in small or sparse populations (Groom 1998; Courchamp *et al.* 1999). There may also be interactions between ecological and genetic effects. For example, small plant populations may be less attractive to foraging insect pollinators because of reduced floral display and rewards (Schmid-Hempel & Speiser 1988; Klinkhamer & de Jong 1990; Goulson 1999; Thompson 2001). Pollinators that do visit small populations may, in turn, also increase their intrapatch foraging time culminating in increased self-pollination, which in self-compatible plants leads to inbreeding (de Jong *et al.* 1993; Oostermeijer 2003). Self-pollination can also compromise the export of pollen by wasting pollen that potentially could have been exported. This process is known as pollen discounting (de Jong *et al.* 1993).

Most attention has been paid to Allee effects in rare native species (Ward & Johnson 2005). The persistence of small populations is, however, undoubtedly also important for colonizing and invasive species (Liebhold & Bascompte 2003; Van Kleunen & Johnson 2005; Taylor & Hastings 2005). Single founders, in particular, would be more likely to establish populations if they are able to self-fertilize (Baker 1955), since they would be relatively immune from ecological Allee effects due to an absence of pollinators or mates. Available data suggest that there is a tendency for Allee effects to be weakened or absent in self-compatible plant species (Leimu *et al.* 2006).

Several studies have demonstrated that plants in small populations often show markedly increased seed production following supplemental hand-pollinations using pollen from within the same population (Ågren 1996; Ward & Johnson 2005). This provides direct evidence for ecological Allee effects through decreased pollinator visitation in small populations. On the other hand, pollination success is seemingly unaffected by population size in other plant species (Van Treuren *et al.* 1993; Kunin 1993; Kunin 1997; Van Kleunen & Johnson 2005; Grindeland *et al.* 2005).

Asclepiads and orchids are ideal subjects for studying factors that influence pollination success because their pollen is packaged in pollinia which makes it relatively easy to directly quantify rates of pollen removal and deposition in flowers. A further advantage of these plants is that it is relatively easy to calculate pollen transfer efficiency (PTE), a measure of the proportion of removed pollen that reaches stigmas (Johnson *et al.* 2005). PTE has been used to investigate mating patterns in orchids (Johnson *et al.* 2005), rates of selfing (Johnson *et al.* 2004) as well as the evolution of pollen aggregation in the Angiosperms (Harder & Johnson 2008). In general, PTE would be expected to decrease in small populations because of lower levels of pollinator foraging constancy, leading to higher levels of pollen transport losses.

In comparison to North America species, the pollination biology of African milkweeds has been poorly studied (but see Liede & Whitehead, 1991; Pauw, 1998; Ollerton *et al.* 2003; Ollerton & Liede, 2003; Shuttleworth & Johnson 2006). For this study we focused on the asclepiad *Gomphocarpus physocarpus* E. Mey. which is an indigenous weedy species in South Africa where it rapidly colonizes roadsides and other disturbed habitats. It is also invasive in other regions, including Australia, China, Hawaii and other Pacific islands (Orchard 1994; Forster 1994; Wagner.*et al.* 1999). Forster (1994) documented a wide range of wasp pollinators of this species in Australia, but its pollination biology has not been studied in its native range. In South Africa, isolated plants usually set fruit which led us to suspect that the species is genetically self-compatible. We also noticed that pollinaria withdrawn from flowers must undergo gradual reconfiguration before they can be inserted into stigmas. Available evidence suggests that this serves to reduce self-pollination among flowers of orchids and milkweeds (Peter & Johnson 2006).

The aim of this study was to determine the relationship between population size and various measures of pollination success in *G. physocarpus*, including rates of pollinia removal and insertion, pollen transfer efficiency and fruit set. In order to interpret our results we also investigated basic aspects of the reproductive biology of the species, including its breeding system, pollen vectors and post-removal pollinarium reconfiguration.

MATERIALS AND METHODS

Study species

Gomphocarpus physocarpus (Asclepiadoideae, Apocynaceae; Fig. 1A-C) is a common plant occurring in disturbed habitats such as ploughed farming lands and road verges. It is found throughout the southern and eastern parts of South Africa at lower altitudes (Fig. 2). Individual plants produce large numbers of flowers that are arranged in pendant umbels. *G. physocarpus* has floral morphology similar to that found in members of the well studied genus *Asclepias* (Bookman 1981; Wyatt and Broyles, 1994) with actinomorphic flowers each bearing five prominent corona lobes which accumulate copious amount of nectar (Fig. 1C). Flowering occurs throughout the austral summer from September to late April, but peaks in December. We studied populations of *G. physocarpus* at a number of sites in the Eastern Cape and KwaZulu-Natal provinces (Fig. 2).

Breeding systems

To enable the delicate hand-pollinations needed for the breeding system experiment to be conducted under observation with a dissecting microscope, we required potted plants that could be brought into the laboratory (cf. Lipow & Wyatt 2000). This was achieved by propagating plants in 10 litre pots from wild harvested seeds originating from one large population of plants occurring near Port Alfred. Plants were fertilised and well watered. With the aid of a dissecting microscope, three treatments were performed on at least one umbel on each of 7 plants. These flowers where then rebagged to exclude pollinators. Treatments included cross-pollination with pollinia from another plant; self-pollination with pollinia from the same plant; and un-pollinated to test for

autonomous self-pollination. A total of 8 additional umbels were bagged on 4 of these plants and left untreated to test for autonomous self-pollination. Cross and self-pollination treatments were done by inserting two pollinia into two stigmatic cavities. This procedure was based on suggestions by Moore (1946), Moore (1947) and Sparrow and Pearson (1948) who showed that two pollinaria, inserted into separate stigmatic chambers, are needed for effective pollination. The proportion of flowers in each treatment group that set fruit was analyzed using the non-parametric Friedman test which considered each plant as a "block".

In addition to the treatments on the potted plants, additional flowers were bagged on six plants growing in the gardens of the Department of Botany, Rhodes University. These were left untreated as a further test for autonomous self-pollination.

Pollinators

Insects visiting flowers of *G. physocarpus* were collected at a number of sites in KwaZulu-Natal and the Eastern Cape provinces of South Africa between 1997 and 2007 (Fig. 2). Insects were identified and the number of pollinaria and corpusculae attached to each insect was determined. The corpusculum is the mechanical clip that attaches the pollinarium to the pollinating insect (Fig 1D) and in most cases remains attached even after individual pollinia have been deposited (Brown 1833; Frost 1965; Bookman 1981). We observed the total duration that pollinators spent visiting flowers of individual plants in the Mountain Drive population, a natural population near Grahamstown.

Nectar rewards

We measured the standing crop of nectar from randomly selected flowers between 7:00 and 8:30 at the start of pollinator activity. A second measurement was made from 10:30 to 11:30 to determine nectar utilization by pollinators. Nectar volumes were determined using 5μl micropipettes. Nectar concentration was determined using an Atago refractometer. During the second interval of nectar measurements, the nectar quantities of
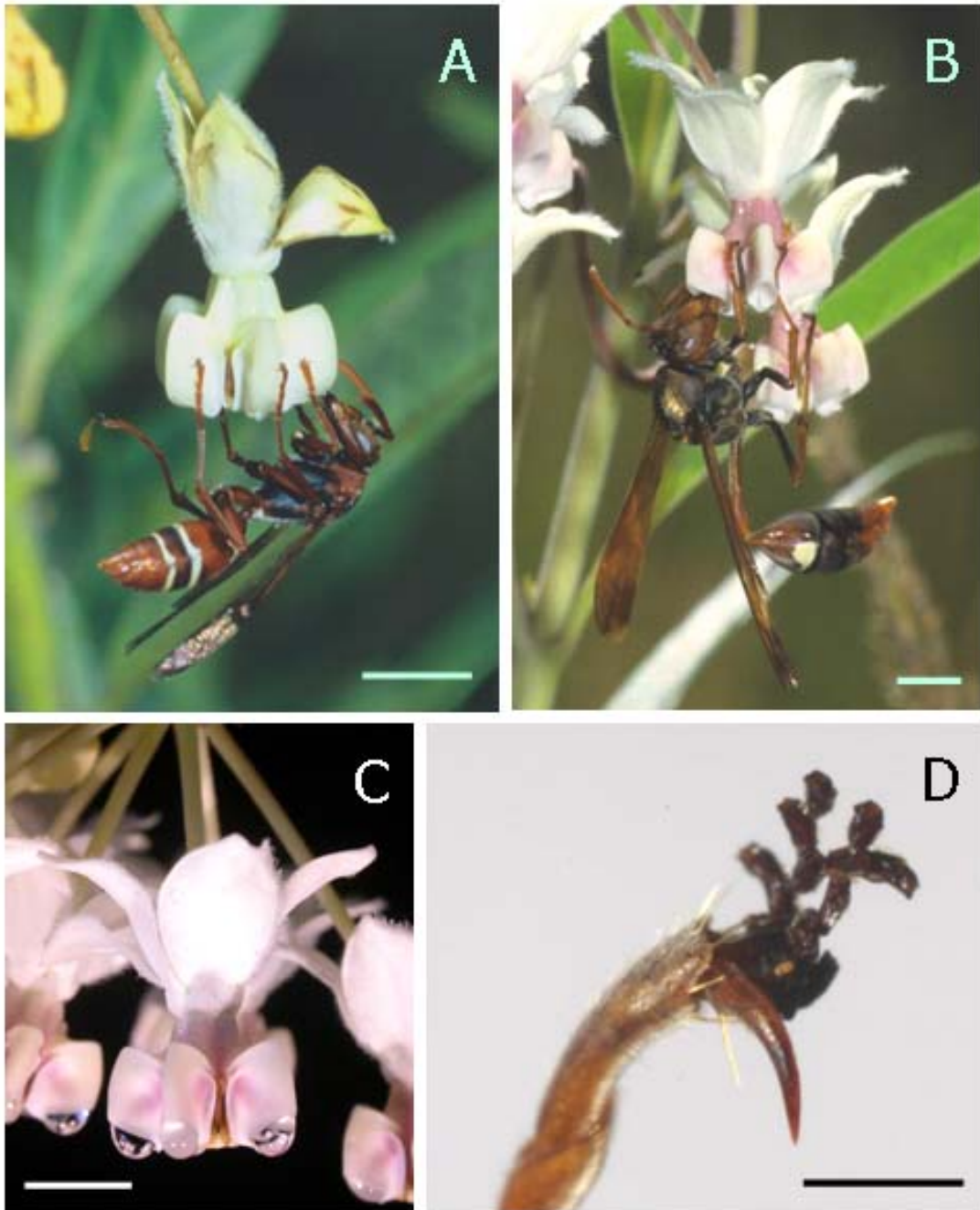
Fig. 1. A) *Polistes* species showing typical position when visiting the flowers of *Gomphocarpus physocarpus*. **B**) *Belonogaster* wasps are equally important pollinators of this species. **C**) Nectaries formed by the corona lobes produce large quantities of accessible nectar. **D**) Multiple corpusculae attached in chains to the arolium (a fleshy pad between the tarsal claws) of a species of *Belanogaster* wasp (Bars: A, B and C = 5 mm, D = 1 mm).

individual corona lobes were very low, so the nectar volume and concentration was determined for the nectar pooled from all five corona lobes of flowers. Changes in the median volumes and concentrations were determined using a Mann-Whitney U-test.

Pollinaria reconfiguration

Pollinaria were removed from freshly harvested flowers using an insect pin. The pollinaria were orientated so that the longitudinal axis could be viewed using a dissecting microscope. To monitor the rate of pollinaria reconfiguration, digital pictures were taken at intervals of approximately 30s. The angle between the pollinia was then measured from these images. Pollinaria reconfiguration is complete once the angle stops changing.
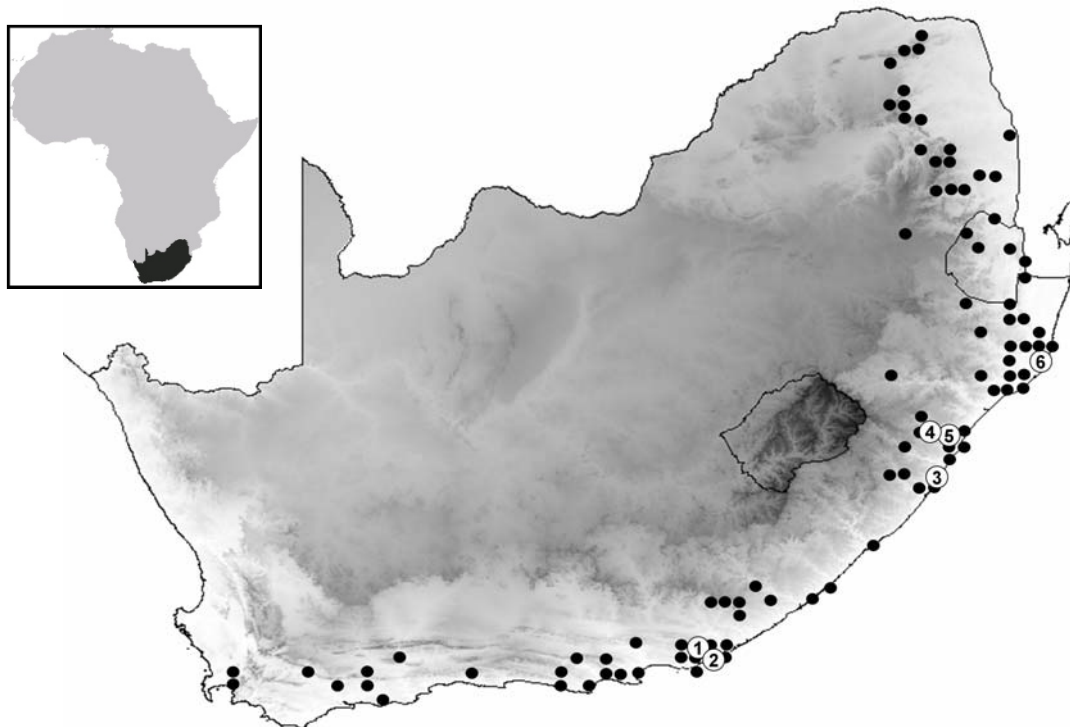
Fig. 2. Distribution of *Gomphocarpus physcocarpus* in South Africa. Study sites in the Eastern Cape include **1**) Mountain Drive and Woest Hill on the Rietberg, Rhodes University, all in the vicinity of Grahamstown; **2**) Kasouga and Kenton-on-Sea. Sites in KwaZulu-Natal include **3**) Vernon Crookes nature reserve; **4**) Hesketh conservation area Pietermaritzberg; **5**) Ashburton and Thornville; and **6**) Cape Vidal and Lake St Lucia.

Cellular mechanisms for pollinaria reconfiguration

Pollinaria were removed from freshly harvested flowers, fixed in acrolein and then dehydrated in an alcohol-butanol series before being embedded in paraplast wax. Whole flowers were also fixed and embedded using this technique to examine pollinaria prior to reconfiguration. Mounted sections of approximately 15 μm thickness were stained with safranin and fast green, and imaged.

Population survey and test for the Allee effect

Nineteen populations between Grahamstown and Kenton-on-Sea were examined (between sites 1 and 2 in Fig. 2). These ranged from single isolated plants to a large population of approximately 1000 plants. We counted all flowering plants in each population.

In small populations we counted the number of fruit on each plant. In large populations a subset of twenty plants was randomly sampled and the number of fruit on each plant counted.

We also randomly sampled one inflorescence from each of twenty plants bearing open but not senescent flowers. In smaller populations, it was sometimes necessary to collect more than one inflorescence per plant to make up a sample of twenty inflorescences. One flower from each of these inflorescences was randomly selected and scored for pollinaria removal and pollinia deposition in stigmatic cavities. These data were also used to calculate pollen transfer efficiencies (PTE) in each population. PTE is the proportion of removed pollinia (removed pollinaria multiplied by two as there are two pollinia per pollinaria) that are deposited in stigmatic cavities.

The relationships between log-transformed population size (predictor variable) and various measures of pollination success (response variables) were determined using univariate regressions. The proportion of flowers pollinated, proportion of flowers with pollinaria removed and PTE were arcsine square-root transformed prior to these analyses.

Table 1. Results of an experiment to determine the breeding system of *Gomphocarpus physocarpus*.

Variable	Unmanipulated	Self-pollinated	Cross-pollinated
Number of plants	13	7	7
Total number of flowers	168	22	22
Number of fruits set	0	0	7
Overall proportion of flowers that set fruit	0	0	0.32
Median proportion of flowers that set fruit per plant	0	0	0.33**
Mean (±SE) number of seeds per fruit.	n/a	n/a	110.3 ± 16.5

** P = 0.007 (Friedman test involving seven plants on which all three treatments were applied)

Table 2. Identity, gender, and means (± standard error) pollinarium loads of insects collected visiting the flowers of *Gomphocarpus physocarpus*. Values in bold are means (± standard error) for individuals belonging to the respective insect families.

Taxon	Study sites[†]	Number captured and sex	Average corpusculae[‡]	Average pollinaria	Average half Pollinaria[§]
Vespidae (Hymenoptera)		**42 F, 6 M**	**10.5 (1.4)**	**1.0 (0.2)**	**1.4 (0.3)**
Belonogaster (*B. dubia, B. lateritia, B. petiolata* and 2 unidentified species)	1, 2, 3, 4, 6	20 F, 3 M	12.5 (3.5)	1.1 (0.4)	1.9 (0.4)
Polistes (*P. fastidiosus* and 7 unidentified species)	1, 3, 4, 5, 6	19 F, 3 M	9.4 (2.9)	1.0 (0.4)	1.3 (0.7)
Ropalidia (three unidentified species)	3, 4, 5	3 F	9.3 (4.1)	1.0 (1.0)	0.7 (0.7)
Pompilidae (Hymenoptera)		**5 F, 11 M**	**1.2 (0.4)**	**0.5 (0.3)**	**0.4 (0.2)**
Hemipepsis (*H. capensis, H. hilaris* and 1 unidentified species)	1	4 F, 11 M	1.0 (0.9)	0.1 (0.1)	0
Batozonellus (unidentified species)	3	1 F	2	2	2
Apidae (Hymenoptera)					
Apis mellifera	1, 5	6 F	4.6 (1.6)	1.9 (0.7)	0.6 (0.4)
Halictidae (Hymenoptera)					
Lassioglossum (unidentified species)	4	16 F	0.2 (0.1)	0.3 (0.1)	0.1 (0.1)
Occasional visitors					
Cerceris sp (Sphecidae: Hymenoptera)	6	F	0	0	0
Scoliidae (Hymenoptera)	1	F	6	3	0
Ichneumonidae (Hymenoptera)	3	F	0	0	0
Formicidae (Hymenoptera)	1	2 F	0	1	0
Muscidae (Diptera)	1, 3	3?	0	0	0
Syrphidae (Diptera)	1	?	0	2	2
Chrysomelidae (Coleoptera)	5	?	0	0	0
Lycidae (Coleoptera)	1	?	1	0	0

1 Numbers refer to study sites given in Figure 2.

2 Corpusculae refers to pollinaria where both pollinia have been removed and only the mechanical clip remains.

3 Half polliniaria are defined as polliniaria where one pollinium has been deposited.

RESULTS

Breeding systems

Only cross-pollinated flowers set fruit indicating that plants in the study population are genetically self-incompatible (Table 1). This difference was statistically significant (Friedmans test, $\chi^2=10.0$, p=0.007).

Pollinators

Flowers were visited by a wide diversity of Hymenoptera, as well as a few Diptera and Coleoptera (Table 2). The majority of the insects bearing pollinaria or corpusculae, however, belonged to the wasp family Vespidae and the bulk of these to two genera, *Belonogaster* and *Polistes*. In most cases the corpusculum of the pollinarium was attached to the arolium (a fleshy pad between the claws of the insects' limbs). In many cases these insects had multiple corpusculae attached in chains (Fig. 1D), indicating that even when the initial attachment sites on the arolium are full, the insect can still remove further pollinaria from the flowers.

Numerous pompilid wasps were collected at the Mountain drive site, however these wasps carried few pollinaria. Honey bees bearing pollinaria were occasionally collected at a number of the sites and small *Lassioglossum* bees (Halictidae) were abundant at the Hesketh site, but only a few of these bees carried pollinaria.

In most of the cases we observed, the wasps approached the plant often from a down wind position before briefly hovering in front of an umbel of flowers and grappling them with their front legs to alight upside down. The insects then hung from the flowers while probing the shallow corona nectar cups for the abundant nectar (Fig. 1A-C). The wasps often clambered from one flower to another in an umbel. In windy conditions wasps sometimes walked between umbels on a plant. With the insects hanging from the drooping flowers the tarsi of the wasps may be drawn through the open proximal end of the stigmatic slit down towards the apex of the gynostegium where the corpusculae are positioned at the termination of the stigmatic slit. When a wider structure, such as an insect limb, is drawn through the stigmatic slit, not only is the stigmatic cavity opened but also the mechanical clip of the corpusculum allowing the arolium to be inserted. As the limb of the insect is drawn further the support of the stigmatic slit no longer holds the clip of the corpusculum open and so the corpusculum close onto the arolium or another corpusculum already attached to the insect, firmly attaching the pollinarium to the insect.

Nectar rewards

The median nectar volume per coronal lobe decreased from 0.63 µl (n = 18) between 7:00-8:30 am, to 0.02 µl (n = 14) between 10:30-11:30am (Fig. 3A). This difference was statistically significant (Mann-Whitney U test, p<0.0001). At the same time, median nectar concentration increased from 16.2% (n = 17) to 38.4% (n = 8; Fig. 3B), but this difference was not significant (Mann Whitney U test, p = 0.071), probably as a result of the smaller sample size and greater variability. The increase in nectar concentration was not sufficiently high to have been caused by evaporation alone as there was a near 18-fold decrease in median nectar volume compared to the two-fold increase from the initial nectar concentration. While evaporation may have had some role in reducing the nectar quantity, our sampling protocol could not separate these effects. Reabsorption of nectar did not occur in this species and bagged flowers accumulated large standing crops of nectar (Fig. 1C).
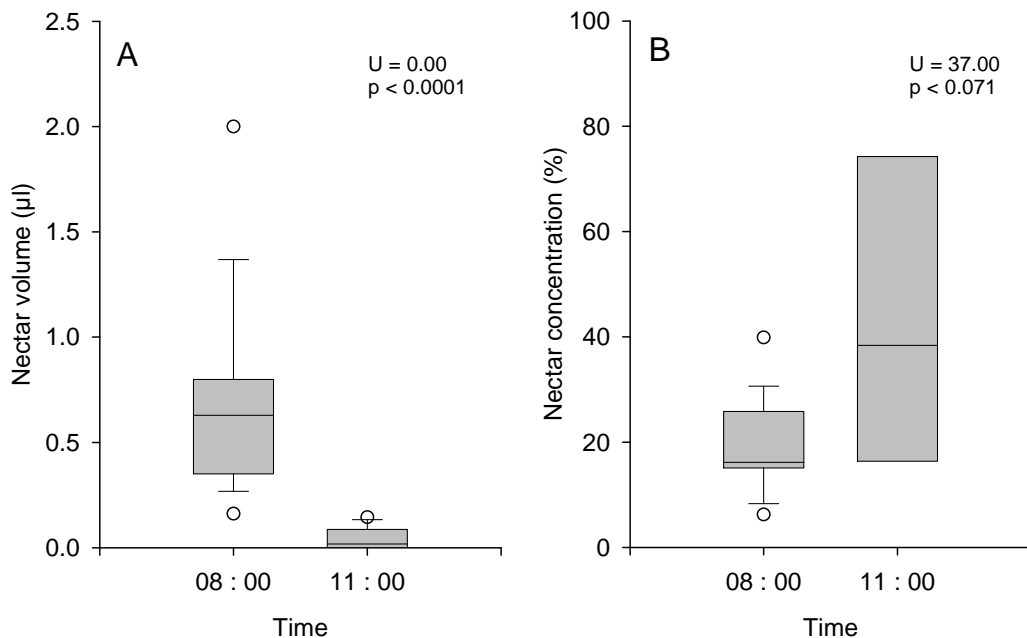

Fig.3. Boxplots indicating changes in **A**) nectar volume and **B**) nectar concentration between the start of insect visitation (7:30 to 8:30) and late morning (10:30 to 11:30) when insect activity decreased. Open circles indicate outliers, details of the statistics are given in the text.

Pollinaria reconfiguration

Pollinaria underwent marked reconfiguration. In a longitudinal plane, the two pollinia were initially at an angle of approximately 90 degrees to one another when freshly removed from the flower (see Wyatt, 1976). Over the course of the reconfiguration this angle was reduced to nearly zero, with pollinaria coming to rest parallel to each other. This reconfiguration took 224 seconds on average (SD = 77, n = 20) and was significantly longer than the average visit duration of 106s (SD = 62, n = 23) by pollinators to individual plants (t_{41} = 5.56, p<0.0001).
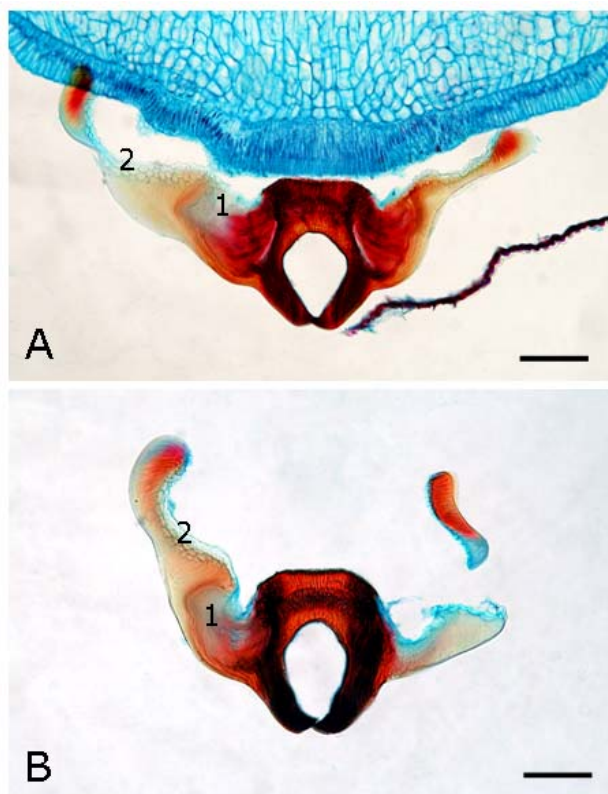

Fig. 4. Anatomy of the corpusculum of *Gomphocarpus*. **A**) Corpusculum still attached to the gynostegium before reconfiguration. **B**) Corpusculum following removal from the flower and reconfiguration. Labels: 1 = thin-walled cells, 2 = large turgid cells.

Cellular mechanisms for pollinaria reconfiguration

Transverse sections through the pollinarium indicated regions of large thin-walled cells located on the inside of the translator arms, next to the corpusculum (Fig. 4). The shape of this region of cell before and after reconfiguration suggests they may play a role in pollinarium reconfiguration (Fig. 4A&B).

In addition there is a layer of apparently turgid cells situated along the inner surface of the translator arm (Fig. 4A). These cells also appear to lose water when pollinia are removed from the flower resulting in the bending of translator arms towards one another (Fig. 4B).

Population survey and test for the Allee effect

We found no significant relationships between population size and various measures of reproductive success in *G. physocarpus*, including the number of fruits per plant (Fig. 5A), the proportion of flowers with pollinaria removed (Fig. 5B), proportion of flowers with at least one pollinium inserted (Fig. 5C) and the proportion of removed pollinia inserted into stigmas (Fig. 5C). Similarly, the efficiency with which removed pollinia are subsequently deposited on stigmas (PTE) showed no correlation to population size (Fig. 5D). Population size and number of flowers per plant were also not significantly correlated (p = 0.20, n = 23).

DISCUSSION

Small populations of *Gomphocarpus physocarpus* can achieve high levels of pollination success (pollen removal and deposition), pollen transfer efficiency and fruit set. Contrary to our initial predictions, the breeding system experiment indicates that at least some populations are genetically self-incompatible and therefore completely reliant on insect flower visitors to transfer pollen among different plants. However, some populations in Australia, where this species is invasive, appear to be self-compatible (M. Ward, University of Queensland, personal communication). Compatibility in milkweeds may vary among individuals and populations (cf. Ivey *et al.* 1999; Lipow & Wyatt 2000). Even in self-compatible milkweeds, cross-pollination usually produces higher fruit set (Ivey *et al.* 1999; Lipow & Wyatt 2000).

Our results show that the pollination system is essentially specialized at the level of functional group (medium-sized vespid wasps), but generalist,
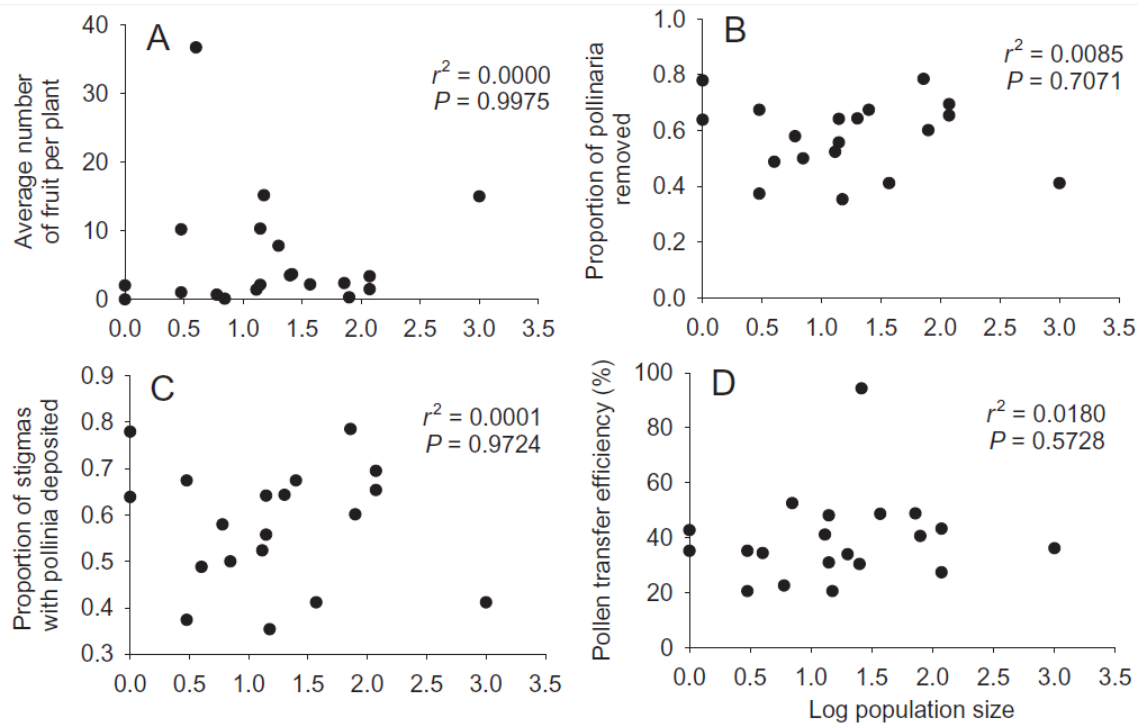
Fig. 5. Measurements of reproductive fitness in relation to population size in *Gomphocarpus physocarpus*. **A**) average fruit set, **B**) pollinaria removal, **C**) pollinia deposition and **D**) pollen transfer efficiency.

and thus flexible, at the species level (cf. Fenster *et al.* 2004). This flexibility is also evident from the similarly wide range of wasp species, mainly vespids, which have been shown to pollinate the species in Australia (Forster 1994). These results contrast with those recently obtained for another South African milkweed, *Pachycarpus asperifolius*, which is pollinated by just 2-3 species of pompillid wasps (Shuttleworth & Johnson 2006). Pollination success and fruit set in *G. physocarpus* is considerably higher than in *P. asperifolius* which may reflect the broader spectrum of insects that can function as its pollinators.

The high levels of pollen transfer efficiency observed in *G. physocarpus* populations are comparable to other weedy milkweeds, such as *G. fruticosa* (15.2%, Harder & Johnson 2008), and *Asclepias curassavica* (2.2-17%, Wyatt 1980). PTE in *G. physocarpus* exceeds that of almost all the species studied by Ollerton *et al.* (2003). High levels of PTE in *G. physocarpus* must be due to a mechanically efficient pollen transfer system combined with high levels of fidelity by vespid wasps.

Floral specialization for pollination by wasps has been considered mainly in the context of brood site mutualisms, as in figs and fig wasps (Weiblen 2002), and sexual deception systems in

orchids (e.g. Steiner *et al.* 1994; Mant *et al.* 2002; Ciotek *et al.* 2006). However, there is increasing evidence that flowers that provide nectar rewards can be specialized for pollination by wasps (Sahagun-Godinez & Lomeli-Sencion 1997; Vieira & Shepherd 1999; Ollerton *et al.* 2003; Fenster *et al.* 2004; Johnson 2005). Other wasp-pollinated milkweeds include *Pachycarpus natalensis* and *Miraglossum verticillare* that are both pollinated by *Hemipepsis hilaris* (Ollerton *et al.* 2003). Our results together with those of Ollerton *et al.* (2003) and Shuttleworth & Johnson (2006) suggest that concentrated nectar may be a characteristic of these wasp-pollinated species.

An important trait associated with many wasps seems to be the accessibility of nectar for these short-tongued insects. In *G. physocarpus* the abundant nectar supply accumulates in the shallow, cup-like corona lobes, accessible to the wasp and heavily utilised by these insects in the first half of the day. An exception to this pattern is the long-tongued masarid wasps which can access concealed nectar (Gess 1996).

Although rates of self-pollination could not be quantified in this study, our data suggest that pollinarium reconfiguration times in *G.*

physocarpus are generally longer than the duration of pollinator visits. As insertions are impeded mechanically until reconfiguration is completed, (C. Peter & G. Coombs, unpublished data), this would strongly promote cross-pollination (Peter & Johnson 2006). Although the possible role of pollinarium reconfiguration in promoting cross-pollination in asclepiads has been discussed previously (Queller 1985), the general association between reconfiguration and pollinator visit times in a range of orchids and asclepiads provides compelling support for the cross-pollination hypothesis (Peter & Johnson 2006). In orchids, reconfiguration of pollinia after removal from the anther sac is thought to occur as a result of differential drying of cell layers of accessory tissue of pollinaria (Peter & Johnson 2006). In *G. physocarpus*, two areas of pollinarium tissue have large, thin walled cells which appear to result in pollinarium reconfiguration when they desiccate.

Allee effects

The absence of Allee effects in populations of *G. physocarpus* is consistent with its weedy life history and relatively generalized wasp pollination system, but nevertheless surprising for a self-incompatible species. Our data suggest that efficient pollination in small populations, combined with a mechanism (pollinarium reconfiguration) that reduces self-pollination enables plants in small populations to achieve levels of fruit set comparable to those in larger populations. Contrary to the expectation of net pollen flow out of small populations, pollen transfer efficiency was unaffected by population size in *G. physocarpus*. This suggests either that pollinators show foraging constancy in small populations or that a net outflow of pollinia from small populations is balanced by an inflow from other populations. A more detailed analysis of pollen fates in this species would require direct labelling of pollinia, as has been done in orchid populations (e.g. Johnson *et al.* 2005) and once in asclepiads (Pleasants 1991).

There are still too few studies for general conclusions to be reached about whether colonizing species are relatively buffered against Allee effects and, importantly, whether Allee effects pose a significant ecological barrier to establishment and persistence (Liebhold & Bascompte 2003; Davis *et*

al. 2004; Taylor & Hastings 2005, but see Groom, 1998). Pollen receipt and fruit set were not affected by population size in the self-compatible but allogamous invasive species *Senna didymobotrya* (Van Kleunen & Johnson 2005). On the other hand, Allee effects have been detected in naturalized populations of the partially self-compatible invasive taxa *Spartina alterniflora* (Davis *et al.* 2004) and in artificial populations of the self-incompatible invasive herb *Raphanus sativa* (Elam *et al.* 2007).

Allee effects on seed production have been documented in the self-compatible colonizing milkweed *Vincetoxicum rossicum*, but the mechanism appeared to be through the inability of small populations to suppress competing vegetation, rather than through an effect of population size on pollination processes (Cappuccino 2004).

CONCLUSION

Despite its reliance on cross-pollination for fruit set, *G. physocarpus* is able to produce seeds efficiently in small populations. Even though the plant seems specialized for pollination by vespid wasps, these pollinators are common and diverse enough not to be a limiting factor for reproduction in small populations. It would be particularly interesting to study the successful naturalization of this species in Australia where it is considered a serious weed (Forster 1994). One possibility is that substitute wasp pollinators are common enough in Australia to allow establishment of small populations. Another is that there has been evolutionary change in the compatibility system. Wyatt and Broyles (1994) document both self-incompatible and self-compatible breeding systems in asclepiads. In addition, several studies have found the breeding systems of milkweeds to be variable between different populations and different individuals within the same population (Lipow *et al.* 1999; Lipow & Wyatt 2000; Leimu 2004).

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