CONSEQUENCES OF SELF-FERTILISATION FOR FECUNDITY AND PROGENY PERFORMANCE IN INVASIVE PLANTS

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FOR MY PARENTS

The Lily

The modest Rose puts forth a thorn,
The humble sheep a threat'ning horn:
While the Lily white shall in love delight,
Nor a thorn nor a threat stain her beauty bright.

William Blake

ABSTRACT

Plants that can self-fertilise should, on average, be more invasive than plants that can not self-fertilise because they can reproduce regardless of the availability of mates and pollinators. Self-fertilisation should have a strong effect on invasiveness because, to become invasive, introduced plants have to pass through bottlenecks of low plant abundance when mates and pollinators are likely to be scarce. Under these conditions, reproduction of plants that can not self-fertilise is often limited by pollen receipt. Selfing may thus contribute to invasiveness by alleviating pollen limitation Allee effects (pollen limitation caused by low abundance) especially as theoretical work indicates that ability to invade and rate of invasion are highly sensitive to fecundity of small and isolated populations and single individuals.

Recently, a correlation between ability to self-fertilise and invasiveness has been observed in several invasive floras, consistent with the hypothesis that species that can self-fertilise should be more invasive. However, it has not yet been demonstrated that this relationship arises from reproductive assurance. To establish the causal basis of a correlation between a plant trait and invasiveness, a mechanism linking that trait to invasiveness must be demonstrated. For this it is necessary to show firstly that the trait actually affects performance in the introduced range and secondly that plant performance affects invasiveness. Self-fertilisation is hypothesised to increase invasiveness by enhancing reproductive performance. The first step in testing this hypothesis is therefore to show that being able to self-fertilise increases fecundity, i.e. that it provides reproductive assurance. However, progeny from self-fertilisation often suffer from inbreeding depression – they perform worse than those from cross-fertilisation – so it is also necessary to show that this cost does not outweigh the reproductive assurance benefit of selfing. So far, reproductive assurance has been assessed in only a few invasive plant species. These studies did not assess inbreeding depression and only one investigated reproductive assurance in relation to abundance, finding no relationship.

In this thesis I have sought to understand the importance of self-fertilisation for reproduction of invasive plants in the introduced range through case studies. In particular, I assessed whether reproductive assurance from self-fertilization alleviates Allee effects via pollen limitation. To do this I tested whether pollen limitation and reproductive assurance were greater at low plant abundance. Further, I conducted progeny trials to assess inbreeding depression, as this cost of selfing potentially negates reproductive assurance benefits. I also conducted observations and experiments to identify the principle pollinators of my study species as reproductive assurance and its relationship to plant abundance depend on pollinator visitation,

The Australian trees *Acacia mearnsii* and *A. dealbata* are highly invasive in the study region of KwaZulu-Natal, South Africa. Through controlled pollination experiments I established that *A. dealbata* was self-compatible and autonomously self-fertilising, while previous studies reported *A. mearnsii* as self-incompatible. I identified the native honeybee *Apis mellifera scutellata* as the principal pollinator of *A. mearnsii*, *A. dealbata* and a co-occuring related invasive species, *Acacia decurrens*, in the study region. I conducted pollen supplementation experiments in two of these species, aiming to indirectly assess reproductive assurance from selfing in the self-compatible *A. dealbata* by comparing pollen limitation between this species and the self-incompatible *A. mearnsii*. In both species, I conducted pollen supplementation in single isolated trees and trees in continuous populations, to test whether pollen limitation was more severe in isolation. These pollen supplementation experiments were inconclusive with respect to pollen limitation but indicated that if there was pollen limitation in *A. mearnsii*, it was not related to isolation. Progeny trials in *A. dealbata* revealed relatively strong inbreeding depression in progeny growth and survival. This suggests that selfed progeny may not reach reproduction, so even if self-fertilisation provides reproductive assurance, it may not contribute to invasion in this species.

As floral morphology of *Acacia* species prohibits the use of emasculation experiments to directly measure reproductive assurance, I conducted further investigations on *Lilium formosanum*, a large-flowered, autonomously self-pollinating invasive geophyte native to Taiwan. I identified the long tongued hawkmoth *Agrius convolvuli* as its primary pollinator in its introduced range in KwaZulu-Natal, South Africa. Trials of progeny from self- and cross-pollination in the field (to 31 months) and in a controlled shade-house environment (to 26 months) showed no evidence of inbreeding depression in germination, growth or survival. Flowering was assessed in the shade-house as most plants did not flower in the field. Only one of five populations showed inbreeding depression in probability of flowering in the second year of growth but none showed inbreeding depression in the third year. Inbreeding depression was thus generally undetectable in *L. formosanum*.

I tested for reproductive assurance and pollen limitation in *L. formosanum* by conducting floral emasculations and pollen supplementations in multiple populations across a range of population size and isolation in three different years. These experiments demonstrated that reproductive assurance was substantial and that pollen limitation was low

or absent. Contrary to expectations, reproductive assurance was not greater in smaller populations and was greater for more isolated populations in only one of three years. However, that study did not include many very small populations. To assess reproductive assurance at very low abundance, I created arrays of emasculated and intact plants within and around naturally occurring populations at two sites. Isolated plants had higher reproductive assurance than did plants placed inside the continuous population at one site, supporting the hypothesis that selfing provides reproductive assurance against pollen-limitation Allee effects. However, in these studies, generally inadequate pollinator visitation was the main reason that *L. formosanum* exhibited reproductive assurance through selfing.

The substantial reproductive assurance and minimal inbreeding depression displayed by *L. formosanum* makes a compelling case for the hypothesis that self-pollination promotes invasion. Nevertheless, demographic modelling will be necessary to assess whether increased fecundity through reproductive assurance results in increased rates of population growth and spread, and hence invasion, in this species. To assess whether reproductive assurance accounts for the relationship between ability to self-fertilise and invasiveness in plants generally, the contribution of self-fertilisation to invasiveness will have to be evaluated for a larger sample of invasive and non-invasive introduced species, using the approaches taken in this thesis, followed up by demographic modelling.

PREFACE

The data described in this thesis were collected in the Republic of South Africa from June 2003 to October 2011. Experimental work was carried out while registered at the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Steven D. Johnson and co-supervision of Professor Mark van Kleunen.

This thesis, submitted for the degree of Doctor of Philosophy in the Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any university. Where use has been made of the work of others, it is duly acknowledged in the text.

Rodger

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James Gordon Rodger

October 2011

I certify that the above statement is correct.

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Professor Steven D. Johnson (supervisor)

October 2011

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Professor Mark van Kleunen (co-supervisor)

October 2011

FACULTY OF SCIENCE AND AGRICULTURE

DECLARATION 1 - PLAGIARISM

I, James Gordon Rodger, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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JGRodger

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James Gordon Rodger

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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS THAT FORM PART OF AND/OR INCLUDE RESEARCH PRESENTED IN THIS THESIS.

PUBLICATION 1

Rodger, J.G. and S.D. Johnson. Native Honeybees are the primary pollinators of invasive, Australian *Acacia* species in eastern South Africa. To be submitted to South African Journal of Botany.

Author contributions: JGR and SDJ conceived the paper. JGR collected and analysed data, and wrote the paper. SDJ contributed comments.

PUBLICATION 2

Rodger, J.G. and S.D. Johnson. Autogamy and inbreeding depression in *Acacia dealbata*: can selfing promote invasion in trees? To be submitted to Annals of Botany

Author contributions: JGR and SDJ conceived the paper. JGR collected and analysed data, and wrote the paper. SDJ contributed comments.

PUBLICATION 3

Rodger, J.G. and S.D. Johnson. Isolation does not increase pollen limitation in honeybee-pollinated invasive trees. To be submitted to Biological Invasions.

Author contributions: JGR and SDJ conceived the paper. JGR collected and analysed data, and wrote the paper. SDJ contributed comments.

PUBLICATION 4

Rodger, J.G., M. van Kleunen and S.D. Johnson. 2010. Does specialized pollination impede plant invasions? International Journal of Plant Sciences **171**:382-391.

Author contributions: JGR, MvK and SDJ conceived the paper. JGR collected and analysed data, and wrote the paper. MvK and SDJ contributed comments.

PUBLICATION 5

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Author contributions: JGR , MvK and SDJ conceived the paper. JGR collected and analysed data, and wrote the paper. MvK and SDJ contributed comments.

PUBLICATION 6

Rodger, J.G., M. van Kleunen and S.D. Johnson. Pollinator failure and reproductive assurance are not correlated with population size and isolation in an invasive lily. To be submitted to Oikos.

Author contributions: JGR, MvK and SDJ conceived the paper. JGR collected and analysed data, and wrote the paper. MvK and SDJ contributed comments.

PUBLICATION 7

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PUBLICATION 9

van Kleunen, M., Rodger, J.G., Glaettli, M. and Johnson, S.D. The implications of uniparental reproduction for colonization and invasion: Baker's Law still rules. To be resubmitted to Quarterly Review of Biology

Author contributions: MvK conceived and wrote the paper, JGR, MG and SDJ contributed comments.

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Contents

Chapter 1

Introduction

Baker's Law and invasive plants

For species to become invasive they must achieve sufficient fecundity in the introduced range to generate population growth. In flowering plants, this will depend on their ability to attract pollinators in the novel range or to reproduce without them. In a seminal and influential paper, Herbert Baker hypothesised that plants would be more likely to be successful colonists if they possessed either uniparental reproduction or generalised pollination systems (Baker 1955). However, until recently, there has been little evidence to link these aspects of reproductive biology to invasiveness.

Observations that colonising organisms, including brine shrimps, agricultural weeds and island plants tended to be self-compatible hermaphrodites led Herbert Baker (1955, 1965) to assert that self-compatibility should enhance colonising ability because: "*With self-compatible individuals a single propagule is sufficient to start a sexually-reproducing colony, making its establishment much more likely than if the chance growth of two self-incompatible yet cross-compatible individuals sufficiently close together spatially and temporally is required".* For angiosperms, he saw an additional advantage to uniparental reproduction in that *"Self-compatible flowering plants are usually able to form some seed in the absence of visits from specialized pollinating insects, which may be absent from the new situation".* He also recognised that clonal forms of uniparental reproduction – apomixis and vegetative reproduction in plants – would provide the same advantage for colonisation as self-fertilisation. This model, proposing an association between colonisation success and uniparental reproduction, later became known as Baker's Law or Rule (Stebbins 1957, Baker 1967), and has been widely applied in biogeography, metapopulation dynamics and invasion biology.

The applicability of Baker's law to long distance natural colonisation has gained partial acceptance due to reports of a high incidence of self-compatibility on oceanic islands relative to comparable mainland areas (e.g. Pandey 1979, Webb and Kelley 1993, Anderson et al. 2001) and the association between uniparental reproduction and range disjunction due to long distance dispersal in many taxa (e.g. Baker 1953, Barrett et al. 1989, Busch 2005), reviewed in Appendix 2). Until quite recently, however, there has been little evidence for an association between uniparental reproduction and invasiveness in plants (Pysek and Richardson 2007). However, some recent studies demonstrate correlations between ability to self-fertilise and invasive status (van Kleunen et al. 2008, Pyšek et al. 2011) or size of area invaded (van Kleunen and Johnson 2007). Similarly Kuster et al. (2008) and Hao et al. (2011) found that among naturalised species, those with uniparental reproduction (self-fertilisation or apomixis) had larger ranges, and the frequencies of uniparental reproduction in certain invasive floras are higher than expected from random sampling of global datasets (Rambuda and Johnson 2004, Hao et al. 2011). Positive associations have also been shown between invasiveness and vegetative reproduction (Pysek 1997, Lloret et al. 2005, Cadotte et al. 2006). Despite some exceptions (Williamson and Fitter 1996, Sutherland 2004, Burns et al. 2011), there is thus a substantial body of evidence for a general positive relationship between uniparental reproduction and invasiveness.

The veracity of the original formulation of Baker's Law can be considered at three levels: firstly, whether there is indeed a correlation between uniparental reproduction and colonisation; secondly, whether such a correlation can be attributed to a demographic advantage conferred by uniparental reproduction; and thirdly, whether such a demographic advantage results from the ability of colonisers to reproduce in the absence of mates and pollinators. Although there is now support for Baker's law, with respect to the correlation between invasiveness and uniparental reproduction, whether this arises from reproductive assurance as envisaged by Baker (1955, 1967), remains to be investigated.

Baker's (1955) original conjecture that uniparental reproduction provides a demographic advantage by allowing reproduction in the absence of mates and pollinators after long distance dispersal is probably not generally applicable for invasive plants. Although propagules may arrive singly when introduction is accidental, many introductions, especially intentional ones, involve a number of propagules arriving together (Wilson et al. 2009). It is also very rare that introduced plants fail to establish relationships with pollinators in their novel range (Richardson et al. 2000). It therefore seems more likely that uniparental reproduction promotes invasion in plants by elevating fecundity when mates and pollinators are in short supply, rather than by preventing complete reproductive failure in their absence.

Pollen limitation and reproductive assurance

Reproductive assurance is the basis of Baker's (1955) ecological prediction that species with uniparental reproduction should be better colonisers, and one of the chief explanations offered for the evolution of selfing from outcrossing, which is probably the most frequent evolutionary transition among flowering plants (Stebbins 1974, Igic 2008). In self-incompatible plants, fecundity is often limited by pollen receipt (pollen limitation), as opposed to resource availability (Burd 1994, Larson and Barrett 2000, Knight et al. 2005). This is due to inadequacies in rates of pollinator visitation, mate availability and efficiency of pollinators brought about by diverse ecological circumstances (Wilcock and Neiland 2002, Knight et al. 2005) as well as intrinsic inefficiencies in cross-pollination in multiflowered plants (Aizen and Harder 2007). Uniparental reproduction can impart reproductive assurance by increasing fecundity when cross-pollen receipt is inadequate (e.g. Herlihy and Eckert 2002, Kalisz and Vogler 2003, Kennedy and Elle 2008b, reviewed in Eckert et al. 2006). The other evolutionary factor favouring self-fertilisation is the advantage it has for gene transmission: individuals that can self-fertilise will transmit two copies of their genes in their own seeds as well as one copy in seeds they sire on other plants. However individuals that can not self will transmit only one copy of their genes through their own seeds and one through seeds they sire on other plants (Fisher, 1941).

The ecological and evolutionary advantages of self-fertilisation are opposed by inbreeding depression – the poorer performance of progeny arising from inbreeding than outbreeding (Jain 1976, Herlihy and Eckert 2002, Kalisz et al. 2004). When inbreeding depression is present and self-pollen pre-emptively fertilises ovules that would otherwise have been outcrossed (seed discounting, Lloyd 1992), self-fertilisation will be detrimental to fitness. In such cases, self-fertilisation may increase fecundity through production of larger numbers of less vigorous selfed seed at the expense of outcrossed seed production. However, inbreeding depression itself is subject to evolution. Inbreeding depression results principally from the accumulation of deleterious recessive alleles in outcrossing populations, although heterozygote advantage probably also plays a role to some extent (Cheptou and Donohue 2011). Selfing exposes deleterious alleles to selection which can result in them being removed (purged) if selfing rates remain high for several generations, potentially leading to increased selection for selfing (Lande and Schemske, 1985, Barrett and Charlesworth 1991, reviewed in Crnokrak and Barrett 2002). This will result in selfing becoming even more advantageous, potentially leading to evolution of almost complete selfing (Lande and Schemske, 1985).

Despite much theoretical work, the adaptive basis of plant mating is still not well understood (Goodwillie et al. 2005). Early models took into account the transmission advantage, purging of inbreeding depression and a decrease in availability of ovules for outcrossing with increased frequency of selfing (Lande and Schemske, 1985). These models suggested that only complete selfing or complete outcrossing should be evolutionarily stable, with selfing evolving whenever the fitness of selfed progeny was more than half that of outcrossed progeny (Lande and Schemske, 1985). However mixed mating species are common in nature and display a wide range of inbreeding depression (Holsinger 1991, Goodwillie et al. 2005). Models incorporating additional factors indicate that mixed mating can be stable under some circumstances. For instance, reproductive assurance can allow mixed mating, despite the presence of sufficiently high inbreeding depression that evolution to complete outcrossing would have occurred in Lande and Schemskes' (1985) model (Morgan and Wilson 2005). On the other hand, Holsinger (1991) presents a model showing that when an increase in selfing also results in a decrease in outcrossing, as might occur through reduced flower size, plants with mixed mating systems do not necessarily evolve to complete selfing, even in the absence of inbreeding depression. Nevertheless, the issue is complex as outcomes also depend on variability in expression of inbreeding depression over time, the timing of self-pollination relative to cross-pollination, the rate of biparental inbreeding and pollen discounting (Goodwillie et al. 2005). Despite sustained effort in this area, most models incorporate only some of these factors and can only account for mixed mating under very restricted conditions (Goodwillie et al. 2005).

While our theoretical understanding of the evolution of plant mating is incomplete, empirical investigation is even sparser (Goodwillie et al. 2005, Eckert et al. 2006). Although a number studies have demonstrated reproductive assurance, few have also assessed the costs of inbreeding depression to provide a test of the overall benefit of selfing for fitness (reviewed in Eckert et al. 2006). Where this has been done, results have been mixed. Reproductive assurance through selfing results in an overall fitness benefit in *Collinsia verna* (Kalisz et al. 2004) and *Collinsia parviflora* (Kennedy and Elle 2008a, b), which have low inbreeding depression. In fact, it is unclear why such species have not evolved to complete selfing. On the other hand, *Aquilegia canadensis* has high inbreeding depression and high seed discounting, making it hard to explain why it has not evolved to complete outcrossing (Herlihy and Eckert 2002). Thus information on the costs and benefits of selfing versus outcrossing is needed for many more species, to gain a general picture of the conditions under which outcrossing, selfing and mixed mating systems occur.

Even if selfing is beneficial for fitness, taking into account reproductive assurance and costs of inbreeding depression (Kalisz et al. 2004), this will only result in a demographic advantage if population growth is limited by fecundity (seed limited, reviewed by Turnbull et al. 2000). Although no studies have so far investigated the demographic impacts of reproductive assurance by self-fertilisation, there is evidence that pollen limitation leads to elevated population extinction rates in the rare *Clarkia concinna* (Groom 1998) and reduces rate of spread in the invasive *Cytisus scoparius* (Parker 1997) and *Spartina alterniflora* (Taylor et al. 2004). This implies that these species could benefit demographically from reproductive assurance. In introduced plants, population growth should often be seed limited

(Ramula et al. 2008), a factors which may account for the success of biological control that targets seed production, through the use of insect seed predators or fungal pathogens, in halting invasive spread (e.g. Dennill and Donnelly 1991, Hoffmann and Moran 1998).

Pollen limitation Allee effects

During the early stages of invasion, abundance will frequently be low, sometimes at introduction when the number of propagules arriving is small, but also at the invasion front during spread, as a result of long distance dispersal within the novel range (Taylor and Hastings 2005, Wilson et al. 2009). Performance of plants and animals is often reduced at low abundance – a phenomenon known as the Allee effect (Stephens et al. 1999). Allee effects on the fecundity of self-incompatible plants ("component Allee effects" *sensu* Stephens et al. 1999) can occur because of reductions in the quantity or quality of pollen received at low abundance (Ågren 1996, Groom 1998, Fischer et al. 2003, Leimu et al. 2006) – in other words increased pollen limitation. Ability to self-fertilise may therefore promote invasion by mitigating pollen-limitation Allee effects (Taylor and Hastings 2005).

Declines in pollinator visitation at low plant abundance (Sih and Baltus 1987, Feinsinger et al. 1991) are in accordance with optimal foraging theory, which predicts that animals will avoid areas of low profitability (Pyke et al. 1977). As the size of food patches decreases and their isolation from one another increases, foragers have to spend more time and energy searching and travelling between them, reducing profitability. Foragers may leave areas of low abundance to search elsewhere, but may also remember profitability of patches they have previously exploited and choose not to return to those that are poor (Pyke et al. 1977, McNamara et al. 2006). Reduced visitation may lead to reduced cross-pollen receipt and for plants that lack autofertility, reduced fecundity (Sih and Baltus 1987, Feinsinger et al. 1991, Ågren 1996, Groom 1998). Apart from reducing visitation rates, low plant abundance may change pollinator behaviour in other ways, to the detriment of cross-pollination. At lower plant densities, pollinators may be less constant, resulting in heterospecific pollen transfer (Kunin 1993, 1997). They may also visit a greater number of flowers per plant, increasing the rate of geitonogamous self-pollination at the expense of cross-pollination (Klinkhamer et al. 1989, Cascante et al. 2002, Johnson et al. 2009).

Although pollination of wind-pollinated plants is not affected by insect behaviour, they can also experience reduced rates of cross-pollination at low density (Davis et al. 2004a, Davis et al. 2004b, Eppley and Pannell 2007, Friedman and Barrett 2009). Wind distributes pollen across the landscape in a non-directed fashion, while pollen-dispersal by animals is usually directed from flower to flower. This may make wind pollinated plants perhaps even more vulnerable to pollen-limitation Allee effects than animal pollinated ones.

In small populations, pollen limitation may occur due to reduced cross-compatibility between individuals and increased expression of inbreeding depression, over and above pollen limitation due to reduced cross-pollen receipt (Leimu et al. 2006). These types of pollen-limitation Allee effects are also referred to as genetic Allee effects (Fischer et al. 2003) and involve limitation of fecundity and progeny performance by pollen quality rather than quantity (Wilcock and Neiland 2002). In self-incompatible plants, fertilisation is prevented when pollen and stigmas share self-incompatibility alleles (Richards 1997). This not only prevents self-fertilisation but also reduces mating between close relatives (Richards 1997). Small populations of self-incompatible plants may have reduced diversity of self-incompatibility alleles, reducing cross-compatibility and hence fecundity (Fischer et al. 2003, Wagenius et al. 2007, Leducq et al. 2010). Mating between relatives (biparental inbreeding) may also be more common in small populations, which, when they are not cross-incompatible, can result in increased expression of inbreeding depression (Fischer et al. 2003, Willi et al. 2005). However, plants with autonomous self-fertilisation do not necessarily have immunity from pollen-limitation Allee effects, as small population size or low density may similarly result in increased expression of inbreeding depression due to increased rates of selfing and biparental inbreeding (Fischer and Matthies 1998, Eppley and Pannell 2007).

Models indicate that invasion rate is highly sensitive to fecundity in small isolated populations and individuals which have the potential to form new invasion foci (Veit and Lewis 1996, Leung et al. 2004). This is because a high proportion of seeds from such isolated plants will disperse into unoccupied habitat, whereas in continuous populations, much seed is dispersed into habitat that is already occupied, and seed production is likely to saturate available establishment microsites (Taylor and Hastings 2005, Price et al. 2008, Waser et al. 2010). Moreover, if suitable habitat is patchy, spread has to occur by long-distance dispersal, likely involving single or small numbers of propagules (e.g.Tobin et al. 2007). Models demonstrate that Allee effects can reduce rate of spread dramatically or even prevent it entirely in the gypsy moth *Lymantria dispar* (Tobin et al. 2007) and the invasive intertidal grass *Spartina alterniflora* (Taylor et al. 2004) *inter alia*, (reviewed in Taylor and Hastings 2005). This suggests that reproductive assurance through self-fertilisation may promote invasion in plants by alleviating pollen-limitation Allee effects.

Pollination relationships in the introduced range

It has been hypothesised that, for plants without uniparental reproduction, those with generalised pollination systems are more likely to become invasive than pollination specialists, as they should be more likely to establish relationships with pollinators in the novel range (Baker 1955, 1965, 1974, Richardson et al. 2000). However, complete reproductive failure due to a lack of pollinators in the introduced range very seldom occurs (Richardson et al. 2000). Detailed studies of the pollination relationships in invasive plants, although previously rare (Richardson et al. 2000, Goulson 2003), are becoming more common (e.g. Schueller 2004, Stout 2007, Gross et al. 2010) and trends are emerging with regard to how plants acquire pollinators following introduction.

Acquisition of pollinators in the novel range has been documented in several species with specialised pollination systems (Dulberger 1981, Forster 1994, van Kleunen and Johnson 2005, Coombs et al. 2009, Geerts and Pauw 2009, Herrera and Nassar 2009), suggesting that pollination specialisation is less frequently a barrier to invasion than previously thought (Baker 1955, 1965). Although the prevalence of pollination specialisation among plants has been debated (Ollerton 1996, Waser et al. 1996, Johnson and Steiner 2000, 2003), where specialisation occurs, it is generally to pollinator functional groups (functional specialisation) rather than to single pollinator species (Fenster et al. 2004, Ollerton et al. 2007). Pollinator functional groups comprise pollinators that interact with flowers in a similar way as a result of common morphological, sensory and behavioural characteristics and frequently belong to the same higher order taxon (Ollerton et al. 2007). Many of the pollinator functional groups onto which plants are specialised are quite widespread so, upon introduction, plants may establish relationships with different members of the same functional group that pollinates them in the native range (Dulberger 1981, Forster 1994, van Kleunen and Johnson 2005, Coombs et al. 2009, Geerts and Pauw 2009, Herrera and Nassar 2009). For instance *Stapelia gigantea* from South Africa, which is functionally specialised for carrion fly pollination, is pollinated by native carrion flies in its introduced range in Venezuela (Herrera and Nassar 2009).

Even if specialisation is seldom a barrier to the establishment of relationships with pollinators in the introduced range, more specialised plants may still suffer more severe pollen limitation (Knight et al. 2005). This is likely because functionally specialised plants are also usually more ecologically specialised – that is they have a smaller number of pollinator species (Armbruster 2006). Their fecundity may therefore be more strongly affected by fluctuations in the abundance of particular pollinator species (Waser et al. 1996).

This has been borne out in the genus *Schizanthus*, where phylogenetic analysis indicates that evolution of autonomous self-pollination and functionally specialised pollination are correlated (Perez et al. 2009).

For plants lacking uniparental reproduction, more generalised pollination systems are likely to reduce pollen limitation in the introduced as well as the native range, potentially leading to greater invasiveness (Richardson et al. 2000). In self-incompatible European plants, those with more generalised pollination systems in their native ranges have spread more widely in their introduced ranges (Chrobock et al. unpublished manuscript). Pollination network studies have shown that honeybees are frequent visitors to many invasive plants with more generalised pollination (Morales and Aizen 2006, Kaiser-Bunbury et al. 2011) and case studies have demonstrated that they play critical role in pollination of certain invasive species (Barthell et al. 2001, Gross et al. 2010). These results suggest that honeybees may facilitate invasion of many species with more generalised pollination requirements.

Research aims and development

Correlations between ability to self-fertilise and invasiveness (van Kleunen and Johnson 2007, Kuster et al. 2008, van Kleunen et al. 2008, Hao et al. 2011, Pyšek et al. 2011) are consistent with the hypothesis that species that can self-fertilise should be more invasive. However, it has not yet been demonstrated that these relationships arise from reproductive assurance, in accordance with Baker’s Law (Baker 1955). To establish the causal basis of a correlation between a plant trait and invasiveness, a mechanism linking that trait to invasiveness must be demonstrated. For this it is necessary to show firstly, that the trait actually affects performance in the introduced range and secondly, that plant performance affects invasiveness.

If species traits are to be used to predict invasiveness it will be necessary to understand to what extent traits that confer increased invasiveness are preadaptions from the native range or whether they are acquired through evolution post-introduction. Unfortunately, there is seldom enough information on trait values from the native and introduced range to evaluate this (although see Davis 2005, Sloop et al. 2009). Of the studies that assess correlation between ability to self-fertilise and invasiveness, some have used traits measured in the introduced range (eg Hao et al. 2011), some have used traits measured in the native range (eg van Kluenen et al.2008) while others do not specify (eg Pysek, 2011). Nevertheless, establishing the effects of traits on invasion is an ecological matter and does not rely on knowledge of their evolutionary history.

Self-fertilisation is hypothesised to increase invasiveness by enhancing reproductive success (Baker 1965, 1974). To demonstrate this, it must first be shown that self-fertilisation increases fecundity in the introduced range – i.e. that it provides reproductive assurance. Thereafter, it must be shown that increased fecundity from reproductive assurance results in increased invasiveness, in terms of population growth and/or rate of spread. Reproductive assurance is likely to be greatest at low plant abundance (eg Ågren, Dabis et al. 2004a, Elam et al. 2007) and fecundity is likely to have its greatest effect on invasiveness in small and isolated populations (Veit and Lewis 1996, Leung et al. 2006). Therefore, to evaluate the effect of self-fertilisation on invasiveness, it is necessary to take into account the relationship between reproductive assurance and plant abundance. Inbreeding depression should also be assessed, as it represents a cost that may outweigh the benefits of reproductive assurance (Herlihy and Eckert 2002). So far, reproductive assurance has been assessed in only a few invasive plant species (Schueller 2004, van Kleunen et al. 2007). These studies did not assess inbreeding depression and only one (van Kleunen et al. 2007) investigated reproductive assurance in relation to abundance. We therefore need further, more detailed case studies of invasive plants in the introduced range to determine whether reproductive assurance causes the higher invasiveness observed for plants that can self-fertilise, relative to those that can not.

In my research I set out to investigate how invasive plants overcome the challenges of low abundance to reproduce in the novel range, a topic which has so far received little attention (van Kleunen and Johnson 2005, Elam et al. 2007, van Kleunen et al. 2007). In particular, I set out to test the hypothesis that reproductive assurance through self-fertilisation mitigates pollen-limitation Allee effects in invasive species. To address this idea, I selected plant species invasive in KwaZulu-Natal, South Africa, for detailed case studies.

In selected invasive plant species, I asked: how are reproductive assurance and pollen limitation related to plant abundance and does self-fertilisation provide reproductive assurance by alleviating pollen-limitation Allee effects? (Chapters 4, 7, 8) As the relationships of reproductive assurance and pollen limitation with abundance depend on plant breeding systems and patterns in pollinator visitation, I also asked: what are the breeding systems (Chapters 3, 5) and pollination systems (Chapters 2, 5) of these species? As the overall benefit of selfing depends not only on reproductive assurance but also on inbreeding depression, I asked: what is the extent of inbreeding depression in these species? (Chapters 3, 6)

I initially chose to test for pollination-limitation Allee effects in Australian leguminous trees, *Acacia mearnsii* (black wattle) and *A. dealbata* (silver wattle), which are highly invasive in the KwaZulu-Natal midlands region of South Africa. I used pollen supplementation experiments to test for pollen limitation Allee effects in these species. However, it was not feasible to directly measure reproductive assurance from selfing in the autogamous *A. dealbata* by emasculation, as the compact nature of inflorescences and small size of flowers prohibits this procedure. I therefore sought another species to develop as a model for understanding the role of self-pollination in promoting invasion.

Subsequent investigations focused on the invasive Taiwanese geophyte *Lilium formosanum* (Liliaceae), which had been shown to be self-compatible and autonomously self-pollinating in KwaZulu-Natal (Rambuda and Johnson 2004) and in Japan, where it had also been shown to have low inbreeding depression (Inagaki 2002). This species was a promising candidate for the investigation of reproductive assurance because it is amenable to floral emasculation, which can be used to measure the fecundity benefit of selfing (Eckert and Schaefer 1998). A particular advantage of using this species is that it has a hawkmoth pollination system (Chapter 5) and because hawkmoths do not forage for pollen it was considered unlikely that emasculation would effect pollinator visitation and deposition of cross-pollen.

During the course of my thesis, I also participated in two literature reviews, one on the relationship between reproductive biology and invasiveness in Australian *Acacia* species (Appendix 1) and the other a review of Baker's Law (Appendix 2).

Experimental and statistical approach

To address how pollen limitation and reproductive assurance relate to population size, I had to carry out pollen supplementation and emasculation experiments in multiple populations. I used relatively few plants per population in these experiments for two reasons. Firstly, for the relationship between population attributes (eg size; isolation from other populations) and plant performance, the effective replicate is the population. Therefore, when there is a trade off between number of populations sampled and replication within populations, statistical power is likely to be increased by increasing the number of populations (Quinn and Keough, 2000). Secondly and more importantly, an underlying problem with experiments in multiple populations that vary in size is that it is not possible to obtain as many replicates in small populations as in large populations. This can lead to sampling effort being biased towards larger populations. Unequal sample size between groups (unbalanced data), makes ANOVA much less robust to violations of the assumptions of normality and homogeneity of variance, rendering test-statistics unreliable (Quinn & Keogh 2002, pp187, 217). The worst case scenario is an association between smaller sample size and higher variance (Quinn and Keogh, 2002), exactly what would be expected for fecundity under a hypothesis of lower pollinator visitation in smaller populations. In the study on the effect of isolation on pollen limitation in invasive Acacia, I compared isolated to non-isolated trees (in continuous populations, Chapter 4). As isolated trees occur singly by definition, I also sampled a single (non-isolated) tree in each population to ensure equal sampling effort. Similarly, for *Lilium formosanum* (Chapter 7), I chose to replicate treatments on three individuals per population as I did not expect to encounter many populations with fewer than three individuals. I then attempted to maximise number of populations to obtain as much statistical power as possible.

In the experiment on the effect of isolation from populations for *L. formosanum* (Chapter 8), I only used two sites, in contrast to Chapters 4 and 7. As I was manipulating the spatial distribution of plants, there was no restriction on the number of plants I could sample either in continuous populations or isolated from them. I also used few populations in this experiment because there were not many ‘safe’ populations for treated plants, which were frequently lost due to roadside maintenance and weed clearing operations in the experiments for Chapter 7. I wanted to minimise loss of plants as the experiment was extremely labour intensive. I regarded two populations as sufficient for the experiment on breeding system of *L. formosanum* as its breeding system had already been described in South Africa in one population (Rambuda and Johnson 2004). I would have preferred to assess breeding system in more than one population of *A. dealbata* to test for variation among populations but the experiment was very time consuming for this species so I was not able to repeat it. In consequence the inbreeding depression experiment for *A. dealbata* (Chapter 3) similarly could only be conducted using seed from a single population. I tested for inbreeding depression in *L. formosanum* in four populations for field experiments and five for shade house experiments. I did not test for a relationship between inbreeding depression and population size because some results were already available for the experiment on population size and reproductive assurance (Chapter 7) and as there was no indication of higher reproductive assurance in smaller populations, I did not expect them to have experience greater purging of genetic load.

While plant fecundity is expressed in terms of seeds produced per flower, I conducted separate analyses of its components – fruit-set and seeds per fruit. There are two reasons for doing so. In the first place, fruit-set and seeds per fruit may be governed by separate processes For instance resource limitation may play a greater role in one than the other, so separating them may reduce error variance. Secondly, seeds per flower data tends to be highly zero-inflated as all flowers that do not produce fruit obviously produce zero seeds. It is considerably easier to analyse fruit-set, as a binomial variable, separately from seeds per fruit for flowers that do set fruit. I conducted analyses of fruit set using generalised linear models for binomial data with a logit link function (Payen 2011). Seeds per fruit data frequently were unbalanced between experimental treatments as treatments often differed in fruit set. I therefore usually analysed seeds per fruit in REML analysis of variance, which better accounts for unbalanced sample sizes (Quinn and Keough 2002, Payne et al. 2011).

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Chapter 2

Native Honeybees are the primary pollinators of invasive, Australian *ACACIA* species in eastern South Africa

James G. Rodger and Steven D. Johnson

Abstract

Although pollinators are vital for reproduction of many invasive plant species in their introduced ranges, the identities of these pollinators are often unknown. We investigated the pollination of three invasive Australian *Acacia* species, *A. dealbata*, *A. decurrens* and *A. mearnsii* in their introduced range in KwaZulu-Natal, South Africa. Based on abundance, rate of visitation and pollen loads, the native African honeybee, *Apis mellifera scutellata*, is identified as the primary pollinator of all three of these *Acacia* species in the study region. Although Australian *Acacia* species (previously subgenus *Phyllodineae*) did not evolve with honeybees, they all have similar generalist flowers and upon introduction, are likely to attract, and be pollinated by honeybees, which are native or introduced over most of the world.

Introduction

Most animal-pollinated plants receive visits from suitable pollinators when introduced to new environments, facilitating invasion (Richardson et al. 2000). Plants may be pollinated in novel environments by virtue of generalised pollination requirements (Baker 1965, 1974, Goulson and Derwent 2004, Stout et al. 2006); if they are specialised for pollination by widespread groups of pollinators (Herrera and Nassar 2009, Rodger et al. 2010) or if suitable pollinators have also been introduced (Liu and Pemberton 2009, Gross et al. 2010). Self-incompatible, non-clonal species will be unable to invade without pollinators but even autonomously self-fertilising species may benefit from pollinators through production of fitter, outcrossed progeny. Pollinators may also influence biological invasion through the effects of gene flow on local adaptation (Blossey and Notzold 1995, Colautti et al. 2010, Verhoeven et al. 2011). Pollination thus plays a crucial role in the invasion of numerous plant species, yet for many of these it has not been investigated in the introduced range.

Acacia sensu strictu previously subgenus *Phyllodineae*, prior to controversial retypification (McNeill et al. 2006), comprises approximately 1020 species of trees and shrubs, almost all native to Australia. 386 Australian *Acacia* are known to have been introduced to other regions and of these 23 have become invasive (Richardson et al. 2011). A concerted effort is underway to assess what characteristics, if any, distinguish invasive from non-invasive species, although this is hampered by a general paucity of data (Castro-Díez et al. 2011, Gallagher et al. 2011, Gibson et al. 2011, Morris et al. 2011).

We set out to describe the pollination system of invasive Australian *Acacia dealbata*, *A. decurrens* and *A. mearnsii,* all of which produce large crops of seeds in their invasive range in KwaZulu-Natal, South Africa. Specifically, we measured relative abundance, rate of visitation to inflorescences and pollen loads to assess the relative importance of different visitor groups for pollination (Ne'eman et al. 2010).

Methods and materials

Study species and area

The mimosoid tree species *Acacia dealbata*, *A. decurrens* and *A. mearnsii* are native to Eastern Australia and have become invasive in South Africa and elsewhere following introduction for forestry (Henderson 2007). These three species are closely related and belong to the mearnsii clade of former subgenus *Phyllodineae* (Murphy et al. 2010, Miller et al. 2011). They have sweetly scented, compact globular yellow (*A. dealbata*, *A. decurrens*) or cream (*A. mearnsii*) inflorescences arranged in loose racemes. Inflorescences are composed of numerous minute flowers with abundant stamens which are the main visual attractant. Pollen is the only reward as no floral or extrafloral nectar is produced in these species. Anthers produce eight disc shaped polyads, each composed of 16 individual pollen grains (Kenrick, 2003). As most stigmas receive only a single polyad due to their small size (Moncur et al. 1991, Kenrick 2003), geitonogamous self-pollination is likely to result in pollen limitation of fruit-set in self-incompatible species and seed discounting in those that are self-compatible. Bees are regarded as the principal pollinators of most Australian *Acacia* species investigated so far in their native range, including *A. mearnsii* (Bernhardt 1987, 1989). The African honeybee, *Apis mellifera scutellata*, has also previously been observed as a pollinator *A. mearnsii* in its introduced range in South Africa (WRI 1950, Rambuda 2001).

Observations were conducted in KwaZulu-Natal, South Africa, between Camperdown, 29° 44.010´ S, 30 31.374´ E 760m ASL), Nottingham Road (29° 22.9´ S, 29° 54.500´ E, 1500m ASL) and Greytown (29° 03.795´ S, 30° 35.565´ E, 1050m ASL) (Table S1-S3). The natural vegetation in this area consists of species rich grassland with isolated forest patches but many parts are highly transformed by agriculture, forestry and human settlement. Populations used included plantations and invading stands.

Pollinator observations

Floral visitors were counted in transects of 50-100m along the edge of *Acacia* populations (Table S1) to estimate relative abundance of different visitors. One transect was taken per population with an observer (JGR) walking the length of each transect, noting frequency of different visitor groups on inflorescences. Transects length was not standardised as we did not aim to compare visitor abundance between populations or *Acacia* species. Observations were made during the morning when insects were most active on the flowers. Transects were conducted in six populations of *A. mearnsii* in October 2004 and 14 populations of *A. decurrens* in August to September 2006. For *A. dealbata* two populations were surveyed in August 2005 and one in August 2004. In the latter population, the transect was surveyed on four separate days and average counts, rounded to the nearest whole visitor, were used for analysis. We tested for differences in abundance between visitor groups in generalized linear models with a Poisson error distribution and a logarithmic link function in Genstat 12 (Genstat 2009), with population as a blocking factor. Population was entered before visitor group to control for variation abundance between populations. Tests were performed for each *Acacia* species separately. For each *Acacia* species, all visitors groups observed on that species (shown in Fig. 1) were included in the analysis. The unit of observation was the count for each visitor group in each population. The significance of visitor group was assessed from quasi-F statistics in sequential analysis of deviance, analogous to F-statistics in ANOVA with type I sums of squares (Payne 2011). Contrasts were performed comparing abundance of honeybees to other visitor groups. Significance levels of contrasts between abundance of honeybees and other groups were evaluated against the t-distribution.

We recorded the rate of visitation (inflorescences per minute) of individual syrphid flies (Syrphidae) and honeybees (*Apis mellifera*) to inflorescences in *A. mearnsii*, *A. dealbata* and *A. decurrens*. These were the only relatively abundant visitor groups observed to regularly move between inflorescences. Although beetles were not uncommon in some populations, they were sedentary and very seldom moved between inflorescences (J.G. Rodger, pers. obs.). Visitors that move more frequently between flowers within trees are also likely to move more frequently between trees. Although it would have been informative to also measure the frequency with which visitors moved between trees and number of inflorescences visited per tree, insects were often lost from sight before they moved to the next tree. Individual insects were observed for periods ranging from 20 to 120 seconds and the number of inflorescences visited during this time recorded. Observations were conducted in two populations for each *Acacia* species (Table S2) and visitation rate compared between syrphid flies and honeybees with unequal variance t-tests, as groups differed in sample size and/or variance (Ruxton, 2006) except for the *Acacia dealbata* and *Acacia decurrens* populations at Boston, where sample size for syrphid flies was too small for statistical comparison.

Floral visitors to all three species were captured for identification purposes and to measure pollen loads at several sites in the KwaZulu-Natal midlands in 2004 and 2005 flowering seasons (Table S3). Visitors were identified at least to family, and to subfamily where possible. Pollen loads of body parts (excluding the corbiculae) were assessed as a proxy for pollinator effectiveness in terms of grains deposited per visit (Ne'eman et al. 2010). For some plant species pollen loads may be a poor indicator of pollinator effectiveness, because it represents a balance between pollen removal from anthers and deposition: visitors that remove pollen well but deposit it poorly may have the highest pollen loads. However, at least in *Acacia*, pollen load should give a reasonable indication of potential to effect pollination as stigmas and anthers are exposed on the inflorescence surface, so all visitors should contact them. Visitors were killed individually in glass phials containing a piece of ethyl-acetate soaked filter paper. Pollen was removed from the ventral surface (including legs and mouthparts) of visitors by swabbing with a cube of fuchsin-stained gelatine of dimensions ± 1.5mm (Kearnes and Inouye 1993), and counted under a light microscope at 100X magnification.

Pollen loads of different visitor groups collected were compared using ANOVA with Tukey posthoc tests on log-transformed pollen load (number of *Acacia* polyads). Corbicular pollen loads of bees were excluded from analysis as this pollen is generally unavailable for pollination (Michener, 1990). All visitor groups for which at least three observations were available were included in analyses (Table S4). This included some visitor groups which were not detected in the abundance surveys (Table S1). For individuals that carried pollen, pollen purity was calculated as number of *Acacia* polyads/(number of *Acacia* polyads + number of heterospecific pollen grains) in order to assess the potential for heterospecific pollen transfer, which can inhibit fertilisation. Although we were not able to distinguish between polyads of the different *Acacia* species, no other *Acacia* species flowered in the vicinity of *A. mearnsii* and *A. dealbata* populations from which insects were collected for pollen load analysis. Some of the *Acacia* polyads collected from *A. decurrens* might be from *A. dealbata* as these species overlap in flowering time and there were flowering *A. dealbata* trees in the vicinity of the *A. decurrens* used for pollinator capture. However, as pollinators moved most frequently over short distances (J.G. Rodger, pers. obs.), most polyads should be conspecific. Pollen purity (arcsine-square root transformed) was compared statistically between all groups for which there were three or more observations (Table S4). t-tests assuming unequal variances (Ruxton, 2006), were used to compare honeybees and syrphid flies for *A. dealbata* and *A. decurrens* as there were insufficient numbers of observations for other groups. ANOVA was used to compare pollen purity of visitor groups for *A. mearnsii*.

Results

Apis mellifera individuals foraged for pollen on inflorescences by moving around and scraping the inflorescence surface with their front legs. Diptera were less active, most of the time standing still and probing anthers with the proboscis. Beetles were all highly sedentary, seldom being observed moving between flowers. Most, including Cetoniinae and Rutelinae (Scarabaeidae) and Eumolpinae (Chrysomelidae) were destructive feeders on floral parts. No nectar feeding behaviour was observed, consistent with absence of floral nectar in *Acacia sensu strictu*.

Honeybees were the most abundant visitor group overall followed by syrphid flies, identified as *Betasyrphus sp* and *Allograpta sp*. (Fig. 1). For *A. dealbata*, honeybees were significantly more abundant than syrphid flies, the only other visitor group recorded in transect surveys (Quasi-$F_{1, 2} = 106.66$, $p = 0.009$). For *A. decurrens*, abundance differed significantly between pollinator groups (Quasi-$F_{1, 13} = 27.11$, $p < 0.001$). Contrasts showed that honeybees were significantly more abundant than syrphid flies ($t = 7.40$, $df = 39$, $p < 0.001$) , small beetles ($t = 5.01$, $df = 39$, $p < 0.001$), and butterflies ($t = 5.01$, $df = 39$, $p < 0.001$). For *A. mearnsii*, visitor abundance did not differed significantly between pollinator groups (Quasi-$F_{1, 5} = 0.19$, $p = 0.682$). Honeybees visited significantly more inflorescences per minute than syrphid flies (22-38 versus 2-5.8 respectively; Fig. 2) on *A. dealbata* at Curry's Post ($t = 7.57$, $df = 11.41$, $p < 0.001$); on *A. decurrens* at Mooi River ($t = 12.83$, $df = 13.99$, $p < 0.001$) and on *A.mearnsii* at Lions River ($t = 10.08$, $df = 15.45$, $p < 0.001$) and on *A. mearnsii* at Midmar ($t = 10.20$, $df = 4.18$, $p < 0.001$).

Pollen loads differed significantly between pollinator groups in *A. dealbata* ($F_{2, 24} = 12.4$, $p < 0.001$), *A. decurrens* ($F_{2, 18} = 36.4$, $p < 0.001$) and *A. mearnsii* ($F_{6, 38} = 9.1$, $p < 0.001$). Honeybees carried an order of magnitude more *Acacia* pollen than all other visitor groups for each *Acacia* species (Table S4). Values for honeybees were (mean ± se): 65.8 ± 20.4 polyads for *A.dealbata*, 131.6 ± 26.8 for *A. decurrens*, 140.6 ± 67.8 for *A. mearnsii*). Two non-*Apis* bees collected from *A. mearnsii* carried on average 121 *Acacia* polyads. The fruit chafer *Cytrothyrea marginalis* carried the next most pollen with 15.6 ± 12.0 grains (only collected from *A. mearnsii*. Pollen loads of syrphids were 1.8 ± 1.1 for *A. decurrens*, 1.0 ± 0.6 for *A. dealbata* and 2.7 ± 2.2 for *A. mearnsii*. Tukey posthoc tests revealed that honeybees had significantly more pollen than all other visitors to each *Acacia* species ($p < 0.05$) but comparisons among other groups were not significant. Pollen purity (*Acacia* polyads/(number of *Acacia* polyads + number of heterospecific pollen grains) on *Apis mellifera* was always high: 0.78 ± 0.09 on *A. dealbata*, 0.97 ± 0.02 on *A. decurrens* and 0.97 ± 0.02 on *A. mearnsii*. Pollen purity was significantly greater for honeybees than syrphid flies on *A. dealbata* ($t = 2.64$, $df = 11.4$, $p = 0.022$) and there was a non-significant trend in the same direction for *A. decurrens* ($t = 2.89$, $df = 3.06$, $p = 0.227$) (Table S4). For *A. mearnsii*, pollen purity did not differ significantly among visitor groups ($F_{4, 21} = 2.29$, $p = 0.094$) although Ruteline beetles (1.00 ± 0.0) and *Apis mellifera* (0.97 ± 0.2) tended to have purer loads than the remaining visitor groups – Syrphid flies (0.64 ± 0.23) and the beetles *Anaspis sp* (0.50 ± 0.29) and *Cyrtothyrea marginalis* 0.67 ± 0.20).

Discussion

All evidence suggests that the native honeybee, *Apis mellifera*, is the primary pollinator of *A. dealbata*, *A. decurrens* and *A. mearnsii* in KwaZulu-Natal, South Africa. *Apis mellifera* is more abundant (on *A. dealbata* and *A. decurrens*), has a higher rate of visitation to inflorescences and higher pollen loads compared to other visitor groups (on all three *Acacia* species). The high purity of pollen loads carried by *A. mellifera* (78-97%), suggests that *Acacia* pollination is unlikely to be substantially affected by heterospecific pollen transfer. Nevertheless, individuals of *Apis mellifera* usually visited several inflorescences on the same tree in succession before moving to another tree (J.G. Rodger, pers. obs.), suggesting that reproductive success is likely to be pollen-limited due to high rates of geitonogamous self-pollination. Supplemental cross-pollination on *A. mearnsii*, which is self-incompatible, increased fruit-set from 2.83% to 9.20% of inflorescences (Chapter 4), consistent with this hypothesis.

Syrphid flies, the second most abundant visitor group for all three species, are likely to play only a minor role in pollination as, in addition to being less abundant (Fig. 1), they carry much less pollen and visit flowers at a much slower rate than honeybees (Fig. 2). Abundance of beetles may have been underestimated in our transect surveys, due to their sedentary behaviour. However, this behaviour, in addition to their destructive flower feeding, also makes it very unlikely that they play an important role in pollination.

Apis mellifera was the only bee species observed in the transect surveys (Fig S1), although two specimens were captured from *A. mearnsii* for pollen load analysis. This is interesting, given that non-*Apis* bees are important pollinators of many native plants in the study region (Johnson et al. 2009). This suggests that pollen of these species may be low in quality or unprofitable to collect for some other reason for most bee species. There was also a trend for lower abundance of visitors on *A. mearnsii*, compared to the other two species (J.G. Rodger, unpublished results), perhaps due to *A. mearnsii* flowering later in the season when more alternative sources of forage are available (J.G. Rodger, pers. obs.) and demand for pollen for rearing brood may be lower.

In general, it seems that Australian *Acacia* species have similar pollination relationships in their native and introduced ranges. In the native range, Australian *Acacia* species that lack nectar, such as these three species we studied, are visited by a range of pollen feeding insects, including bees, beetles and flies (Bernhardt 1989). These are the three orders recorded from *A. dealbata*, *A. decurrens* and *A. mearnsii* in this study. However, Bernhardt (1989) regarded Diptera and Coleoptera as ineffective visitors as most *Acacia* species are self-incompatible and he thought it unlikely that they would effect much cross-pollination. He suggested these species are moderately specialised, with pollination mainly by pollen collecting bees (Bernhardt 1989). Bees visitors in Australia comprise a fairly narrow subset of native bee faunas in addition to *A. mellifera,* which is introduced there (Bernhardt et al. 1984, Bernhardt 1987, Moncur et al. 1991, Sedgley et al. 1992).

Apis mellifera has been recorded from all Australian *Acacia* species examined in their introduced ranges so far. An *A. mangium* × *A. auriculiformis* hybrid introduced in Thailand is pollinated mainly by introduced honeybees and one other bee species (*Ceratina sp*) (Sornsathapornkul and Owens 1998). Previous surveys in the same region as this investigation have only recorded *Apis mellifera* visting *A. mearnsii* (WRI 1950, Rambuda 2001) and *A. mellifera* was also recorded as a floral visitor in Brazil (Alves and Marins-Corder 2009). Rambuda (2001) found pollen loads of 93.1 ± 26.4 polyads (mean ± se, comparable to the 140.6 ± 67.8 found in this study. *Apis mellifera* was also identified as the most important visitor to *Acacia saligna* in its invasive range in the Western Cape, South Africa (M. R. Gibson, University of Stellenbosch, unpublished results).

Absence of suitable pollinators is unlikely to be a barrier to invasion for Australian *Acacia* species as bees, and in particular *A. mellifera*, are present on all continents to which *Acacia* could be introduced. *A. mellifera* pollinates many invasive species in their introduced ranges (Rambuda 2001, Goulson 2003, Morales and Aizen 2006, Kaiser-Bunbury et al. 2011), likely promoting their invasion. One of the implications of this study is that invasion of alien *Acacia* species may be facilitated by managed native honeybees. It would be interesting to determine if bee-keepers have unwittingly promoted the spread of invasive plants by artificially bolstering honeybee populations in farming areas where Australian *Acacia* species have become seriously invasive (Goulson 2003).

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Figures

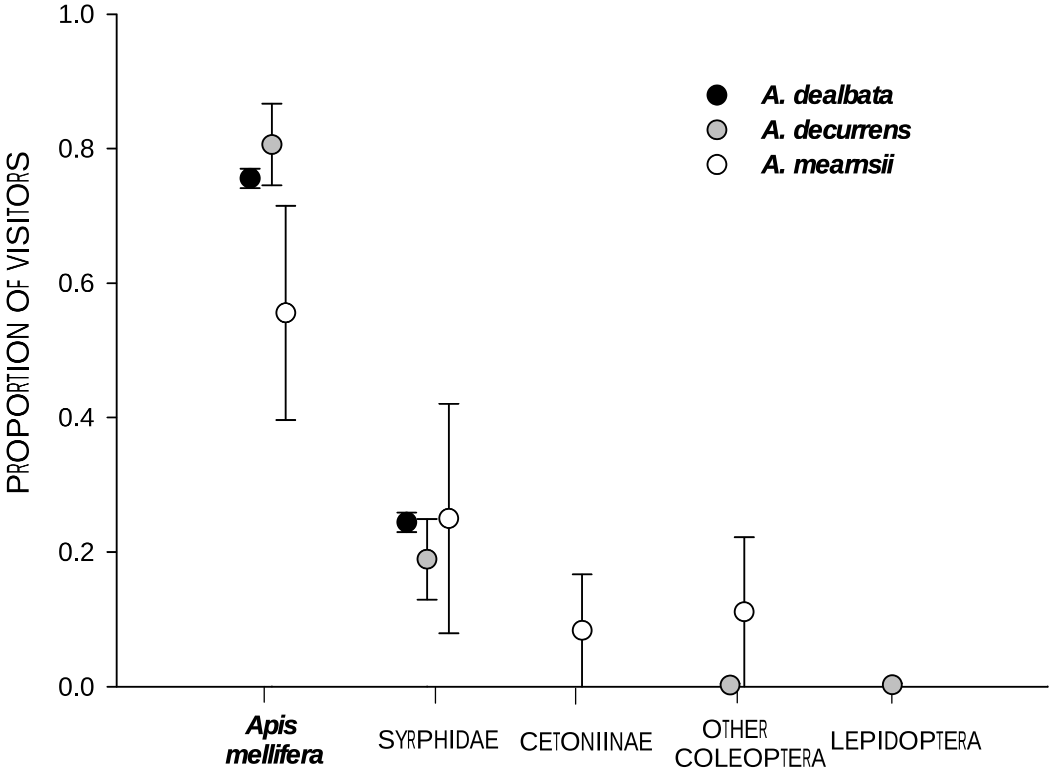

Figure 1 Mean ± SE proportional abundance of floral visitor groups in populations of *Acacia dealbata*, *A. decurrens* and *A. mearnsii*. n = 3 populations for *A. dealbata*, n = 11 populations for *A. decurrens*, n = 6 populations for *A. mearnsii*. For *A. decurrens*, only a single individual was observed over all transects in the categories 'Other Coleoptera' and 'Lepidoptera'.

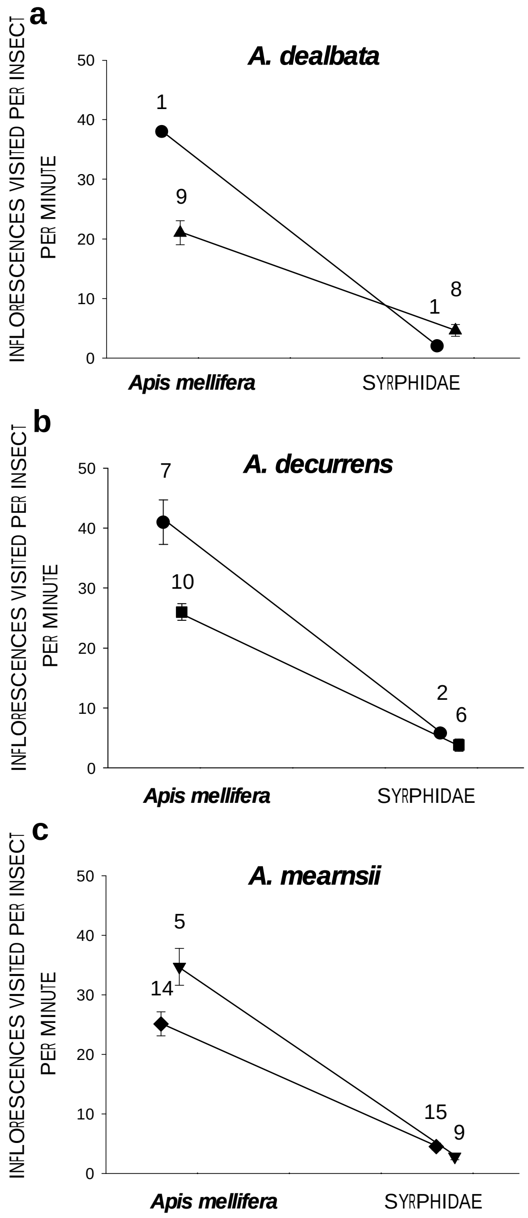

Figure 2 Inflorescences visited per insect per minute by different visitors for *Acacia mearnsii* (a), *Acacia dealbata* (b) and *Acacia decurrens* (c). Symbols and bars indicate means and standard errors, with sample sizes above bars. Sites used were at Boston (circles), Curry's post (upward pointing triangles), Mooi River (squares), Lions River (diamonds) and Midmar (downward pointing triangles).

Appendix

Table S1 Populations of exotic *Acacia* species used for transect surveys of visitor abundance.

Species	Population description	Date
A.dealbata	Dalcrue farm, on road from Nottingham	11, 14, 18 &
	Road to Loteni	19/08/2004
	Howick – Curry's post road	22/08/2005
	Close to Curry's post	22/08/2005
A. decurrens	Corner of Howick – Curry's post and Tweedie roads	04/09/2004
	Lodge at Curry's post	04/09/2004
	Boston – Dargle Road	19/08/2005
	Mooi River – Greytown road	25/08/2005
	Pietermaritzburg – Greytown Road, Albert falls resort	11/08/2006
	Pietermaritzburg – Greytown Road, after Albert Falls resort 500m	11/08/2006
	Albert Falls – Cramond road, among pines after turn off to Cramond police station	11/08/2006
	Corner of Karkloof and Morton's Drift roads	11/08/2006
	On Karkloof road, 2.7 km on Karkloof side of intersection with Morton’s Drift road.	11/08/2006
	Plantation 12km after start of dirt on Karkloof road	11/08/2006
	Lion's River – Curry's post road in sight of freeway	19/08/2006
	Howick – Curry's post road after Old Haliwell	19/08/2006
	Howick – Curry's post road after the lodge	19/08/2006

	Curry's Post – Balgowan road	19/08/2006
A. mearnsii	R103 South of Lynfield Park, Idube corner	10/2004
	R103 Lynnfield Park area near Lion Park	10/2004
	R103 between Bloemendal farm and Wartburg road on left opposite lifestock yard	11/10/2004
	On Dargle road, after the woodturner, opposite a sharp curve sign at the top of a hill	14/10/2004
	Dargle road shortly before turn off to Hebron Haven adjacent to settlement on North side of road	15/10/2004
	Midmar	10/2004

Table S2 Populations used for visitation rate observations.

Species	Population description	Date
A. dealbata	Boston – Dargle Road	19/08/2005
A. dealbata	Howick – Curry's post road	22/08/2005
A. decurrens	Boston – Dargle Road	19/08/2005
A. decurrens	R622, Mooi River – Greytown road	25/08/2005
A. mearnsii	R103 Lion's River	10/09/2005
A. mearnsii	Midmar Dam	10/09/2005

Table S3 Localities of insects collected for identification and pollen load analysis on exotic *Acacia* species in KwaZulu-Natal. AM = *Apis mellifera*, OH = Other Hymenoptera, CM = *Cyrtothyrea marginalis*, EU = Eumolpinae, RU = Rutelinae, OC = Other Coleoptera, AN= *Anaspis sp*, SY = Syrphidae, OD = Other Dipetera.

Acacia species	Date	Locality	AM	CM	OH	EU	RU	OC	AN	SY	OD
Acacia dealbata	19/08/2005	Boston – Dargle Road, Close to Boston	1							2	
	22/08/2005	Curry's Post	3							3	3
	12/08/2004	Dalcrue Farm, on Loteni Road from Nottingham Road	4							2	
	22/09/2004	Hilton	2								
	23/08/2004	Impendle turn off, Boston – Dargle Road									1
	06/08/2005	Loteni Road from Nottingham Road	2							3	
	08/2004	KwaZulu-Natal Midlands								1	
Acacia	06/08/2005	Boston – Dargle	4								

decurrens		Road, about 3km before Dargle								
	20/08/2005	Boston – Dargle Road, Plantation close to Boston	4							
	25/08/2005	Mooi River – Greytown Road, about 30km from Greytown	6			3				4
Acacia mearnsii	12/10/2004	Road from Bloemendal farm to Wartburg Road				1				
	14/10/2004	Dargle road West of Dargle Supply Store	1	2				2		
	14/10/2004	Dargle road, West of population Dargle Supply Store		4						
	14/10/2004	Dargle road, after the woodturner, opposite a sharp curve sign at the top	1							

	of a hill						
14/10/2004	Dargle road, just before school coming from Dargle		1				
05/10/2004	R103, Camperdown						1
14/10/2004	Dargle Valley			1			1
17/10/2004	Hilton Avenue just before Hilton College, Hilton						2
21/10/2004	Howick, opposite Umgeni Valley Nature Reserve entrance					1	
28/09/2004	R103 South of Lynfield Park, Idube racetrack corner	1					
21/10/2004	Karkloof Road, just after beginning of dirt				3		
15/10/2004	Lion's River – Caversham Road						

19/10/2005	Lions' River, on Midmar side of R103 and N3 turn off		1			7
16/10/2004	Opposite Hilton Hotel, Hilton Avenue, Hilton				1	
07/10/2004	R 103 Merrievale	1		1		
16/10/2004	R103 Balgowan, North of junction with road from N3 Balgowan offramp				4	
04/10/2004	R103, 1km South of Lynfield Park	2				
10/2004	KwaZulu-Natal Midlands	3	1			

Table S4 Number of *Acacia* pollen grains carried and pollen purity on visitors to *Acacia dealbata*, *A. decurrens* and *A. mearnsii*. Entries show mean ± SE (n).

Visitor group	*ACACIA DEALBATA*		*ACACIA DECURRENS*		*ACACIA MEARNSII*	
	Load	Purity	Load	Purity	Load	Purity
Apis mellifera Hymenoptera, Apidae	65.8 ± 20.4 (12)	0.78 ± 0.09 (11)	131.6 ± 26.8 (14)	0.97 ± 0.02 (14)	140.6 ± 67.8 (9)	0.97 ± 0.02 (9)
Other Bees Hymenoptera					121.0 (2)	0.62 (2)
Betasyrphus sp and *Allograpta sp,* Diptera, Syrphidae	1.8 ± 1.1 (11)	0.33 ± 0.15 (7)	1.0 ± 0.6 (4)	0.50 ± 0.29 (4)	2.7 ± 2.2 (7)	0.64 ± 0.23 (4)
Other Diptera	0.3 ± 0.3 (4)	1 (1)			5.0 (1)	0.63 (1)
Cyrtothyrea marginalis Coleoptera, Scarabaeidae, Cetoniinae					15.6 ± 12.0 (7)	0.67 ± 0.20 (5)
Coleoptera,					5.8 ± 2.1	1.0 ± 0 (5)

Scarabaeidae, Rutelinae			(8)	
Coleoptera, Chrysomelidae, Eumolpinae	0.3 ± 0.3 (3)	1 (1)	3.3 ± 1.9 (3)	0.91 (1)
Anaspis sp, Coleoptera, Mordellidae			1.4 ± 1.3 (7)	0.5 ± 0.29 (3)
Other Coleoptera			0.5 ± 0.0.5 (4)	0.5 (2)

Chapter 3

Autogamy and inbreeding depression in *ACACIA DEALBATA*: can selfing promote invasion in trees?

James G. Rodger and Steven D. Johnson

Abstract

The ability to self-fertilise may promote invasiveness in plants by assuring reproduction when mate and pollinator availability are inadequate, provided that the benefit of increased fecundity via selfing is not outweighed by inbreeding depression. However, knowledge of breeding systems and inbreeding depression, in particular, is lacking for most introduced plants. We set out to obtain this information for the invasive Australian tree *Acacia dealbata* in its introduced range in South Africa, to assess the potential contribution of selfing to this invasion. Controlled pollinations indicated that the population we examined was completely self-compatible and autonomously self-pollinating, although this species is reportedly self-incompatible in its native range in Australia. This suggests that self-fertilisation may have played a role in its invasion in South Africa. However, substantial inbreeding depression was detected in this study, with seeds per pod, progeny survival and progeny growth being lower following self- than cross-pollination. Progeny arising from self-pollination also had a higher frequency of certain traits – yellow colouration of leaves and pink or white colouration of stems – which were associated with lower rates of survival. Progeny from open pollination were intermediate between those from selfing and outcrossing in their performance and possession of traits associated with lower survivorship, suggesting that *A. dealbata* has a mixed mating system in South Africa. High levels of inbreeding depression in *A. dealbata* and other self-compatible trees suggests that selfing is less likely to contribute to invasiveness in this life form than in herbaceous plants. We recommend that genetic and demographic studies be conducted to assess whether selfed progeny survive to reproduce and contribute to success of invasion.

Introduction

It has been proposed that the ability to self-fertilise promotes invasiveness in plants by providing reproductive assurance in the introduced range where mates and pollinators may be in short supply (Baker 1965a, 1974, Barrett 2011). Due to a paucity of information on breeding systems of introduced plants, few studies have tested for correlations between ability to self-fertilise and invasiveness, until very recently (Williamson and Fitter 1996, Sutherland 2004). As a positive relationship was not detected in earlier studies, ability to self-fertilise has not been included in lists of traits that predict invasiveness (Mack 1996, Kolar and Lodge 2001, Pysek and Richardson 2007). However, several recent studies have found a positive association between invasiveness and self-fertilisation (van Kleunen and Johnson 2007, van Kleunen et al. 2008, Burns et al. 2011, Hao et al. 2011, Pyšek et al. 2011) suggesting that, under some circumstances at least, ability to self-fertilise promotes invasiveness.

Studies which have assessed the reproductive assurance benefit of selfing for invasive plant species have found that they may rely on autonomous self-fertilisation for up to 90% of fecundity in the introduced range (van Kleunen et al. 2007, Chapter 7, although see Schueller 2004). Invasion by self-incompatible species may occur less readily because these plants lack selfing capabilities and are thus more likely to have their fecundity limited by pollen receipt. For example, the invasive intertidal grass, *Spartina alterniflora*, which is largely self-incompatible and wind pollinated, suffers reduced fecundity at the edges of populations where stigmas receive insufficient outcross pollen (Davis et al. 2004a, b). Similarly, fecundity of the invasive shrub *Cytisus scoparius* is severely pollen limited due to inadequate pollinator visitation in prairie habitat in Washington State, USA (Parker 1997). Models indicate that autonomous self-pollination could provide an appreciable demographic advantage through reproductive assurance: in the absence of pollen limitation, invasion rate would be substantially higher in both species (Parker 1997, Taylor et al. 2004).

Although selfing can promote fecundity it also frequently results in inbreeding depression: reduced performance of inbred relative to outbred progeny (Husband and Schemske 1996). In the presence of inbreeding depression, selfing can actually be detrimental to plants that have the opportunity to outcross, if ovules which would otherwise be cross-fertilised are pre-emptively selfed, reducing the amount of seed produced by outcrossing (seed discounting, Lloyd 1992). Whether a demographic advantage accrues from self-pollination will thus depend on opportunity for outcrossing, as well as the magnitude of inbreeding depression. Inbreeding depression is quantified as δ = 1- (performance of selfed progeny)/(performance of crossed progeny), where selfed and crossed progeny mean those arising from selfing and outcrossing (Husband and Schemske 1996, Herlihy and Eckert 2002). The presence of inbreeding depression allows estimation of selfing rate (s) or its complement, outcrossing rate (t) using the formula $s = (p_x - p_o)/(p_x - p_s)$ (Charlesworth 1988). This can be applied wherever data are available for performance in terms of fruit set, seed-set or progeny quality for cross (p_x)-, self- (p_s) and open-pollination (p_o) (Charlesworth 1988). The higher the rate of selfing and the lower the level of inbreeding depression expressed, the more likely it is that selfing is important for invasion.

Although trees are predominantly self-incompatible, many can produce some seed by selfing (Duminil et al. 2009). However, they also experience higher inbreeding depression than herbaceous plants on average (Barrett et al. 1996, Husband and Schemske 1996, Duminil et al. 2009), suggesting that they may not realise benefits from reproductive assurance (Lande et al. 1994, Morgan 2001) and casting doubt on whether selfing would, in fact, promote invasion in trees. Trees have generally higher outcrossing rates than herbaceous plants (measured from seeds or seedlings), probably because high levels of inbreeding depression select against traits that promote selfing (Barrett et al. 1996, Scofield and Schultz 2006, Duminil et al. 2009). In fact, very few woody plants are known to be fully self-compatible or to have predominantly selfing mating systems (Barrett et al. 1996 but see Hardner and Potts 1995, 1997, Ishida 2006, 2008, Robertson et al. 2011). High inbreeding depression in trees may be a result of their size, as a greater number of mitotic cell divisions should result in more mutations (Scofield and Schultz 2006) or their longevity, as the cumulative effects of inbreeding depression increase throughout life (Morgan 2001). In trees, large floral displays often encourage pollinators to visit multiple flowers on the same plants, resulting in high rates of geitonogamous self-pollination (De Jong et al. 1993), which will result in seed discounting in self-compatible plants. Therefore, for woody plants, it is particularly important to consider inbreeding depression when assessing the potential of selfing to provide a demographic advantage through reproductive assurance.

Intriguingly, the Kapok tree *Ceiba pentandra* (Malvaceae), which colonised Africa by long distance dispersal from South America, is completely self-compatible in Africa (Baker 1965b cited in Dick et al. 2007) but populations in South and Central America range from self-incompatible to at least partially self-compatible (Lobo et al. 2005 and references therein). It has been surmised that reproductive assurance through selfing played a role in this successful long distance colonisation (Dick et al. 2007). If this is the case, it would indicate that reproductive assurance through selfing is not necessarily negated by inbreeding depression and could play a role in invasion, as well as natural long distance colonisation in trees.

We aimed to assess whether self-fertilisation potentially contributes to its invasiveness in the Australian tree *Acacia dealbata* in its introduced range in South Africa. To this end we conducted a controlled pollination experiment to assess breeding system and inbreeding depression during seed development; estimated primary selfing rate from seeds per fruit and conducted a progeny trial to assess inbreeding depression in seedling germination, growth and survival.

Materials and Methods

Study Species and study population

Acacia dealbata (silver wattle) is a major invader of grassland and forest habitat in the moist eastern summer rainfall regions of South Africa (Henderson 2007). It originates from temperate south-eastern Australia, where it occurs as a forest pioneer (Sherry 1971) and in grassy woodland communities (Broadhurst and Young 2006). Extensive plantations of *A. dealbata* were established in KwaZulu-Natal, South Africa, in the nineteenth century and it remains an important source of fuelwood, although it is no longer grown commercially (Poynton 2009).

The genus *Acacia* as previously defined (*Acacia s.l.*) has been split into five genera based on phylogenetic analysis of molecular characters (Miller and Bayer 2001) with *A. dealbata* and the rest of subgenus *Phyllodineae*, a mostly Australian clade, remaining as *Acacia s.s.* following controversial retypification (McNeill et al. 2006, Smith et al. 2006). Invasiveness of *Acacia* species in general may be largely due to their high seed production, long lived seed bank and efficient seed dispersal (Milton and Hall 1981, Gibson et al. 2011). However the only factor shown to distinguish between invasive and non-invasive *Acacia* taxa is age at first reproduction, with species reproducing before two years of age being more likely to be invasive than those reproducing only later (Gibson et al. 2011). Fruit production appears especially high in *A. dealbata*, suggesting that it might be self-pollinating. Although it has not been shown to reproduce younger than two years of age elsewhere (Gibson et al. 2011), in the KwaZulu-Natal, South Africa, small plants of 1-2m in height do bear fruit (J.G. Rodger, pers. obs.) indicating fairly precocious reproduction.

In KwaZulu-Natal, South Africa, *A. dealbata* flowers in early spring from July to September. It bears racemes of compact yellow globose inflorescences containing 22-42 minute protogynous flowers (Broadhurst and Young 2006). Inflorescences are highly integrated units that act as blooms and produce only one or a few fruits each (Kenrick and Knox 1989, Gibson et al. 2011). As in other species in *Acacia s.l.*, each anther produces eight disc-shaped, compound pollen grains (polyads), and the cup-shaped stigma neatly holds one polyad, so all the seeds in a fruit are frequently full sibs (Knox and Kenrick 1983, Muona et al. 1991). Many species of *Acacia* show variation among individuals in fruit set following self-pollination, likely due to a gametophytic self-incompatibility system with some non-functional self-incompatibility alleles, in addition to severe inbreeding depression (Kenrick and Knox 1989b). Variation in self-compatibility might also result from variation in ploidy, as polyploidy often results in the breakdown of self-incompatibility systems (Richards 1997), In Australia, a survey of seedlings grown from seed collected in seven natural populations found *A. dealbata* to be mainly diploid, with one population containing 6% tetraploids and two containing about 1% triploids (Blakesley et al. 2002). Allozyme analysis of *A. dealbata* seedlings from six populations in its native range in Australia indicates a highly outcrossing mating system (t_m = 0.89-1.00). In Australia it is probably pollinated by honeybees (introduced in Australia) and native bees, which are likely to bring about high rates of geitonogamous self-pollination in plants with large display size, like *A. dealbata* (Bernhardt et al. 1989). In light of this, the high outcrossing rates measured in Australia suggest that it is generally self-incompatible there (Broadhurst et al. 2008).

The population studied was a stand of mature trees, about 20 by 200m along a watercourse on the farm Dalcrue, near Nottingham Road in the KwaZulu-Natal Midlands (29° 22.9’ S, 29° 54.5 E, 1500m ASL). Although information on age, number of founders and other aspects of history of the stand was not available, expression of strong inbreeding depression indicates that the population is not genetically homogeneous (see Discussion).

Controlled pollinations

Controlled pollination experiments were used to assess the ability of *A. dealbata* to self-fertilise in its introduced range. Pollinations were carried out between the 6th and 18th of August 2004. Four treatments were applied to branches on 16 trees: hand cross-pollination, hand self-pollination, an un-pollinated control from which visitors were excluded (bagged control) and open (natural) pollination. Treatments were carried out on the terminal portions of branches. Nylon mesh bags were used to exclude floral visitors from all except open pollinated branches. Although in some plants, bagging flowers may reduce fecundity of open pollinated flowers by reducing opportunity for geitonogamous self-pollination, this would not be an appreciable factor in this case as each tree had hundreds to thousands of branches in flower simultaneously. All inflorescences which had already opened were removed from treated branches at the start of the experiment. Hand-cross and hand-self pollinations were applied to one branch per tree; bagged control treatments to four branches per tree and three branches per tree were marked for open pollination. More branches were used for the bagged control and open pollinated treatments to ensure that sufficient fruit were obtained for comparison of seeds per fruit between treatments.

Wire spirals were attached to bagged branches to minimise contact between flowers and the bag, except that for bagged controls, two bags per tree had wire spirals and two bags did not. This was to assess whether contact between flowers and the bag would promote self-pollination. A paired samples t-test showed that fruit set (arcsine-square root transformed) did not differ between control-bagged branches with and without wire (t = , df = 10, p = 0.232), so data were pooled. Data were also pooled for all open pollinated branches on each tree. A median of 81 inflorescences was used per branch over all treatments (minimum = 11, maximum = 337).

Pollen for cross-pollination was obtained from a tree at least 20m away, which ensured that pollen donors and recipients were separated by a few intervening plants. Due to intensive honeybee foraging, branches were bagged to ensure availability of pollen. Pollen was applied by rotating donor inflorescences against recipients and treated inflorescences were marked on the stalk with a Sharpie Pen (Sanford tm). The proximity of stigmas to anthers makes it likely that some self-pollination occurred in all treatments. However, emasculation to prevent self-pollination is not practical in these plants due to the compact nature of inflorescences and small size of flowers. Inflorescences with dehisced anthers were chosen as donors and all inflorescences which opened on self and cross-pollinated branches during the experiment were pollinated on their first day and again a day later as there is some asynchrony in anthesis between flowers on an inflorescence.

Fruit set was scored just prior to maturity in November 2004, as proportion of inflorescences that set at least one fruit. Fruit-set is frequently scored in this way in *Acacia* as inflorescences are highly integrated and function as blooms (Kenrick & Knox 1989, Gibson et al. 2011). Early inbreeding depression was assessed from seeds per pod. Treated branches were bagged prior to maturity to prevent loss of seeds and fruits due to abscission and dehiscence. Number of full seeds per pods was scored when pods were collected at maturity in December that year. Seeds were regarded as full if they were swollen and if they did not break when held between the fingernails and squeezed gently. As some pods had dehisced in the bags prior to scoring, we obtained mean number of seeds per pod as number of seeds/number of pods pooling all branches for each treatment on each tree. We attributed any reduction in seeds per fruit under self- compared to cross-pollination to inbreeding depression, although detailed microscopic work would be needed to reliably exclude the possibility that residual self-incompatibility was also responsible.

Progeny trials

Seeds from the breeding system experiment on *A. dealbata* were sown on the 14th of April, 2007 at the University of KwaZulu-Natal, Pietermaritzburg. Seeds were nicked with a razor blade to remove a small piece of the seed coat and placed in separate cells in trays filled with Top Crop wattle mixture growing medium, a mixture of composted pine bark and coco fibre. Seedlings were maintained in a shade-house to an age of three months. To assess inbreeding depression and its impact on performance under open pollination, germination, survival and growth were assessed for progeny from self-, cross- and open-pollination. Germination was scored at one month (only three dead seedlings were recorded and no germination was observed subsequently) and presence of live seedlings (cumulative survival) three months after sowing. Seedling height was measured to within 1mm after one month and again after 3 months from sowing. Growth was calculated as (height at three months–height at one month). Certain unusual characters were apparent in the seedlings and we scored their presence at the same time as germination, in order to assess whether they were associated with reduced performance. Predominantly yellow and slightly yellow variants were observed for leaf colour, as opposed to the normal green. Red colouration was observed, either visible on the leaf margin and the dorsal surface of the leaf edging the margin, or only on the margin itself, although most leaves lacked any red colouration. Stems colour variants were either white slightly tinged with pink (hereafter white) or pink, as opposed to the normal green.

Statistical analyses

Breeding system was assessed by comparing differences between treatments in proportion fruit set in ANOVA in SPSS 15 (SPSS 2008). Proportion fruit set for each treatment on each tree was arcsine-square root transformed and tree was included as a factor in the analysis. Tukey posthoc tests were used to compare pairs of treatment means in all analyses.

Early inbreeding depression was assessed from seeds per pod. Data from bagged control and hand self-pollination were pooled as sample sizes were low and because these treatments are expected to be equivalent for seeds per fruit. Although these treatments might differ in seeds per fruit if there was a higher level of fruit-set in one treatment and a trade off between proportion fruit-set and seeds per fruit, no significant difference was found in fruit-set (Fig 1). While there was a slight trend for more seeds per pod in the bagged-control treatment than the hand self-pollination treatment, this was not significant in an independent samples t-test ($t = 0.47$, $df = 15$, $p = 0.643$). Crossed, open pollinated and selfed treatments were compared in REML analysis of variance as sample sizes were unbalanced. Rank-transformed data was used as no other transformation provided acceptable homogeneity of variance. Although rank-transformation can result in type I error rates above the nominal level for interaction terms (Seaman et al, 1994), we did not include an interaction term in our model. We thus did not assess whether inbreeding depression varied among maternal trees. Significance of the fixed effect (treatment) was evaluated from the Wald F-statistics and significance of the random term (tree) was evaluated by comparing change in deviance in the model when the term was dropped to a chi-squared distribution with one degree of freedom (Payne, 2009). Tree was included as a random factor and differences between pairs of treatments were assessed with Fisher's unprotected least significant difference test.

An index of self-incompatibility (ISI = fruit-set for self-pollination/cross pollination, Lloyd 1965 cited in Zapata and Arroyo 1978, Kenrick and Knox 1989a) was calculated using untransformed fruit set data (proportion of inflorescences setting fruit). Means and confidence intervals were generated by bootstrapping with replacement, 10 000 times using Poptools Add in for Excel (Hood 2009). Fruit-set values were available from 11 trees for cross-pollination and nine for self-pollination. In each round, resampling with replacement, 11 values were drawn for cross pollination and nine values for self-pollination. The means for cross- and self-pollination were used to calculate ISI in each round, and the bootstrapped mean and confidence intervals were generated from the 10 000 rounds. Previously, indices less than or equal to 0.2 have been taken to indicate self-incompatibility, 0.2-1 partial self-incompatiblity and greater than 1 self-compatibility (Zapata and Arroyo 1978, Kenrick and Knox 1989a). However, we take ISI values of greater than or equal to 0.8 to indicate self-compatibility as values greater than one should only occur due to experimental or sampling error. An autofertility index (AFI) was calculated from fruit set as bagged control/self pollination (Lloyd and Schoen 1992; Eckert et al. 2006) with means and 95% confidence limits calculated by bootstrapping as for ISI. Where both hand-cross and -self or hand-cross and control-bagged treatments were available for the same tree, ISI and AFI values were calculated for each individual to examine potential variation in ability to self-fertilise.

Charlesworth (1988) suggested a rough method for calculating outcrossing rate with the formula selfing rate $s = (p_x - p_o)/(p_x - p_s)$ where data are available for any measure of fruit set, seed set or progeny performance for cross- (p_x), self- (p_s) and open-pollination (p_o). We performed this calculation on seeds per pod for *A. dealbata*. Although Charlesworth (1988) gives a calculation for the variance, this requires data from each tree for each treatment, which we did not have for several trees. Instead we bootstrapped mean values of cross, self and open pollination 10 000 times as for ISI, calculating 's' each time, to generate a bootstrapped estimate of s with 95% confidence limits. Early inbreeding depression was calculated from medians of tree values for seeds per pod for selfing (pooling selfed and control-bagged treatments) and outcrossing using the formula $\delta = 1-$ performance of selfed progeny/performance of outcrossed progeny (Husband and Schemske 1996).

Germination, survival from one to three months and cumulative survival from sowing to three months (i.e. inclusive of germination) were evaluated in generalised linear mixed models in R 2.12.0 (R Development Core Team, R 2010)) with the lmer function in the lme4 package, using Laplace approximation (Bates and Maechler 2010). Tray and tree were included as random factors and pollination treatment as a fixed factor. Significance of random factors was tested by dropping them one at a time from the full model, and testing change in log-likelihood against the chi-squared distribution with one degree of freedom. z-values from the full model were used to evaluate whether performance of progeny from self and open pollination were lower than that for cross pollination.

Inbreeding depression was assessed for height at one month, growth between one and three months and height at 3 months using REML analysis of variance in Genstat 12 (Genstat 2009). Significance was evaluated using Wald F-statistics for the fixed term, pollination treatment. Treatments were compared using Fischer's Unprotected Least Significant Difference (ULSD) tests to compare pairs of treatments. For random terms – tree and tray – significance was evaluated by comparing the change in deviance when the term was dropped to a chi-squared distribution with one degree of freedom (Payne 2009). Cumulative inbreeding depression was calculated as $1 - (1 - \delta_{\text{seeds per fruit}}) \times (1 - \delta_{\text{survival 3 months}}) \times (1 - \delta_{\text{height 3 months}})$, where medians were used for seeds per fruit, predicted values from the generalised linear mixed model for survival and predicted values from the REML models for height. Inbreeding depression was estimated for germination, survival and growth-measurements as for seeds per fruit. For germination and cumulative survival, backtransformed values from generalised linear mixed models (employing logit transformation) were used to calculate δ.

We tested whether the frequencies of seedling traits (leaf and stem colouration) differed among pollination treatments, again using generalised linear mixed models with Laplace approximation in R, as described above for germination and survival, as data were binomial. The response variable in each case was the presence or absence of the relevant character: eg all seedlings with predominantly yellow leaves coded 1 and all others coded zero. Pollination treatment was included as a fixed factor and tray and tree as random factors. Further, we tested whether these unusual characters were associated with reduced survival from germination to three months in a generalised linear mixed model with Laplace approximation. Leaf yellowness, leaf redness, and stem colour were included as fixed factors, each with three levels, pollination treatment was also included as a fixed factor and tree and tray were included as random factors.

Assumptions of analyses were assessed from qq plots and plots of residuals against fitted values for ANOVA and REML analysis of variance. For generalised linear mixed models with Bernoulli (yes-no) data such as those presented here, there are no clear guidelines for interpretation of residuals. While overdispersion, can be problematic with other distributions, it can not occur in this case (Zuur et al. 2009, p 253). Although previous studies of *A. dealbata* estimated minimum outcrossing rates using seedling characters which segregated in 3:1 and 15:1 ratios (Philp and Sherry 1946, Moffet 1956), we were unable to do so as the majority of families, including from cross-pollination, showed variation for each trait examined. The necessary assumption that all individuals showing rare characters came from selfing therefore seems rather unrealistic.

Results

Controlled pollinations

In *Acacia dealbata* fruit set (proportion of inflorescences setting fruit) did not differ significantly among pollination treatments according to ANOVA ($F_{3, 32} = 1.04$, $p = 0.387$; Fig. 1a), although there was significant variation among trees ($F_{14, 32} = 6.90$, $p < 0.001$). Index of self-incompatibility (ISI) calculated from proportion fruit set was 0.78 (lower confidence limit (LCL) = 0.37, upper confidence limit (UCL) = 1.43) and autofertility index (AFI) 0.65 (LCL = 0.30, UCL = 0.83) indicating that *A. dealbata* is self-compatible and autonomously self-pollinating. ISI values could be calculated for eight individual trees with median = 0.93 and range 0-1.5 and AFI values also for eight trees with median = 1.15 and range 0.54-2.53.

Pollination treatment did, however, have a significant effect on seeds per pod ($F_{2, 28.1}$ = 3.39, p = 0.048; Fig. 1b). Median values were 1.89 seeds for cross-pollination, 0.98 seeds for self-pollination and 1.04 seeds for open pollination (Fig. 1b). Fisher's unprotected least significant difference test indicated that cross-pollinated pods had significantly more seeds than self-pollinated (p = 0.015) but open versus self (p = 0.208) and open versus cross (p = 0.137) comparisons were non significant (Fig 1b). Inbreeding depression for seeds per pod was estimated as δ = 0.481 from median values. The selfing rate (s) estimated from the number of seeds per pod was 0.716 but with broad 95% confidence limits (LCL = 0.029. UCL = 1.044).

Progeny trials

Inbreeding depression was apparent in progeny trials, but only became significant at three months from sowing (Tables S1-S6; Fig. 2). Germination levels were between 80 and 90% for all treatments with a trend for higher germination for seeds from cross-pollination than self-pollination (p = 0.123) and open pollination (p = 0.100) (Table S1; Fig. 2a). Height of seedlings at one month did not differ significantly between treatments (Wald-$F_{2, 1033.7}$ = 1.75, p = 0.174), although the rank order of the treatment means – cross > open > self – was consistent with inbreeding depression and mixed mating under open pollination (Table S2; Fig. 2b). Selfed seedlings had significantly lower survival from one to three months than crossed seedlings (p = 0.020) although the difference between open and cross was not significant (p = 0.278) (Table S3, Fig 2c). Growth from one to three months did not differ significantly between treatments (Wald-$F_{2, 702}$ = 2.44, p = 0.088; δ for growth = 0.08), with rank order of treatments being open > cross > self (Table S4, Fig 2 d). Cumulative survival to three months was significantly lower for progeny arising from self-pollination compared to those arising from cross-pollination (p = 0.004), with a trend for higher survival for progeny from cross-pollination than open pollination (p = 0.089; Table S5; Fig. 2e). Inbreeding depression (δ) for survival was 0.25. Treatments differed significantly in seedling height at three months (Wald-$F_{2, 830}$ = 4.12, p = 0.017; δ for height = 0.06) with the rank order of treatments being cross > open > self (Table S6; Fig. 2f). Cumulative inbreeding depression was estimated as 0.65 from pollination to three months and 0.32 from sowing to three months.

Seedlings arising from self- and open pollination had a higher frequency of predominantly yellow leaves than those arising from cross-pollination, although only self-pollination was significant (Table S7: $p = 0.046$ and $p = 0.070$ respectively). The frequency of slightly yellow leaves did not differ between pollination treatments (Table S8: self versus cross, $p = 0.295$: open versus cross, $p = 0.93$). Pollination treatment also did not affect the frequency of seedlings with red colouration on both the dorsal surface and margins of leaflets (Table S9: self versus cross, $p = 0.179$; open versus cross, $p = 0.293$) or with red colouration only on margins (Table S10: self versus cross, $p = 0.253$; open versus cross, $p = 0.916$). There was a higher frequency of white stems among seedlings from self- and open compared to cross-pollination but this was only significant for self-pollination (Table S11: $p = 0.003$ and $p = 0.161$ respectively). There was a significantly higher frequency of pink stems among seedlings from self- and open compared to cross-pollination (Table S12: $p = 0.018$ and $p = 0.049$ respectively). All these traits varied significantly among maternal trees ($p < 0.05$; Tables S7-12)

Leaf yellowness and stem colour were significantly associated with lower rates of survival between germination and three months, but leaf redness was not (Table S13). Seedlings with predominantly yellow leaves had dramatically and significantly lower survival between germination and three months than green-leaved seedlings ($p < 0.001$), but slightly yellow-leaved seedlings did not differ from green-leaved seedlings in survival ($p = 0.489$). Seedlings with white and pink stems both had significantly lower survival than green-stemmed seedlings ($p < 0.001$ and $p = 0.002$ respectively). There was a non-significant trend for higher survival in seedlings with red pigmentation on their leaves than those without it ($p > 0.1$).

Discussion

The *Acacia dealbata* population examined was highly self-compatible (ISI = 0.78) and autonomously selfing (AFI = 1.08) with no significant differences in fruit set between pollination treatments (Fig. 1a). This is unusual, but not unprecedented in *Acacia s.s.* Only three out of ten other *Acacia s.s.* species for which controlled pollination experiments have been conducted can be considered at least partially self-compatible: *A. ulicifolia* (ISI = 0.96) *A. paradoxa* (ISI = 0.86) and *A. saligna* (ISI = 0.74) (Kenrick and Knox 1989b, Gibson et al. 2011). The rest were strongly self-incompatible (ISI < 0.2) (Moffett and Nixon 1974, Bernhardt et al. 1984, Kenrick and Knox 1989b, Morgan et al. 2002, summarised in (Gibson et al. 2011).

Substantial inbreeding depression was detected in *A. dealbata*, over seed development and progeny growth and survival to three months, with a cumulative value of $\delta = 0.65$. Seeds per pod (Fig 1b) and germination of seeds (Fig 2a) from open pollination were similar to those from self-pollination suggesting a mixed mating system with relatively high rate of selfing. Over the course of the trial, performance of progeny arising from open pollination became more similar to those from cross-pollination (Fig 2a-d), presumably due to rate of self fertilisation being high but proportion of self-fertilised progeny decreasing over time due to higher mortality of selfed than outcrossed progeny. Unfortunately, the primary selfing rate estimate of $s = 0.716$ obtained from seeds per fruit for cross-, self- and open pollination following (Charlesworth 1988) was not helpful as 95% confidence limits are very broad (0.029-1.044), due to a combination of small sample size and high variation in seeds per fruit from self- and cross-pollination among trees. Presence of inbreeding depression does, at least, indicate that there was reasonable genetic diversity in the study population. If genetic diversity was low, then shared deleterious alleles would make performance of progeny from cross-pollination similar to that of selfed progeny (Angeloni et al. 2011).

Although controlled pollinations and progeny trials suggest a high rate of selfing in the population investigated in this study, Australian populations of *A. dealbata*, have been found to be highly outcrossing, with selfing rates measured from 0-0.11 in isozyme analysis of seedling progeny arrays from six populations (Broadhurst et al. 2008). This strongly suggests self-incompatibility in the native range. Unfortunately, direct comparisons are not possible as controlled pollinations have not been conducted in Australia and isozyme analysis has not been conducted in South Africa. The discrepancy between results from Australia (Broadhurst et al. 2008) and this study (Fig 1a) suggests that there may have been preferential introduction of self-compatible genotypes to South Africa. This is plausible as other *Acacia* species display variation in self-compatibility (Kenrick and Knox 1989b) and the range of values of ISI (0-1.5) and AFI (0.17-2.58) for individual trees in this study suggests that variation in the ability to self-fertilise also occurs in *A. dealbata*. Self-compatible trees may even have been inadvertently selected for introduction if they were more fecund than self-incompatible ones, due to a lack of pollen limitation of fruit set (Larson and Barrett 2000).

High inbreeding depression, as found in *A. dealbata* in this study is typical for trees (Barrett et al. 1996, Husband and Schemske 1996, Duminil et al. 2009), including *Acacia s.s.* Self-pollination results in fewer seeds per pod compared to open- or cross-pollination in *A.*

dealbata in this study (Fig. 1b) and for *A. baileyana*, *A. mearnsii*, *A. decurrens* and *A. melanoxylon* (Moffett and Nixon 1974, Morgan et al. 2002, J.G. Rodger, unpublished results). Selfed progeny of *A. decurrens* and *A. mearnsii*, like *A. dealbata* (Fig. 2), also displayed inbreeding depression in growth and survival, segregating for lethal and less severe deleterious traits at the seedling stage as well as during later growth (Philp and Sherry 1946, Moffet 1956, Moffett and Nixon 1974). The expression of characters associated with lower rates of survival was higher in selfed- than crossed-progeny in *A. dealbata* in this study. It is possible that these characters (Table S11), most of which seemed discrete rather than continuous, reflect the expression of particular deleterious recessive alleles in selfed progeny, although this remains speculative as we were not able to determine the ratios of segregation. Progeny were only maintained to three months in this experiment, by which time substantial inbreeding depression in terms of survival from sowing ($\delta = 0.25$), and to a lesser extent growth ($\delta = 0.06$), was evident, and this would almost certainly have increased over time. This level of inbreeding depression to three months is comparable to that in *Eucalyptus regnans* (Hardner and Potts 1997), *Fuchsia excorticata* and *Sophora microphylla* (Robertson et al. 2011), which all showed substantial inbreeding depression at later stages.

Habitually selfing plants generally experience lower levels of inbreeding depression (Husband and Schemske 1996) as selfing exposes deleterious recessive alleles to selection (Crnokrak and Barrett 2002). Self-compatible trees, however, often display high inbreeding depression, even if they have mixed mating systems or are predominantly selfing (Hardner and Potts 1997, Ishida 2006, Robertson et al. 2011). Theory indicates that when rate of mutation is high, as is characteristic of trees (Scofield and Schultz 2006), there is a threshold in selfing rate below which genetic load can not be removed. This is because virtually all selfed progeny will be homozygous for at least one lethal recessive so will fail to reach reproduction (Lande et al. 1994, Morgan 2001). The high levels of both early and late inbreeding depression displayed in *A. dealbata* in this study, and those found in other self-compatible trees (Hardner and Potts 1997, Ishida 2006, Robertson et al. 2011) are consistent with this scenario.

Inbreeding depression will detract substantially from the reproductive assurance benefit of selfing in *A. dealbata* and other self-compatible trees. It has been argued that in such species selfed progeny hardly ever reach maturity, particularly when they have to compete with outbred progeny (Hardner and Potts 1997, Ishida 2006, Robertson et al. 2011). In *Magnolia obovata*, for instance, the primary selfing rate was estimated as over 0.8 but selfed progeny probably have little demographic input to populations as the lifetime quotient of inbreeding depression was 0.97 (Ishida 2006). This casts doubt on whether self-pollination can promote invasion in trees. However, sufficiently benign environmental conditions and high selfed seed production would potentially allow some selfed progeny to survive to reproduction, at least in the absence of competition from outcrossed progeny. This would occur in the case of trees completely isolated from conspecifics by long distance dispersal, where only selfed progeny would be produced. In addition, in populations with mixed mating, dispersal over long and intermediate distances would ensure that some selfed progeny would escape competition with outcrossed progeny. Invasiveness in conifers (Richardson et al. 1994, Richardson and Rejmanek 2004) and in *Acacia* (Gibson et al. 2011) is associated with precocious reproduction. This would also favour survival of selfed progeny to reproduction compared to later reproducing species, as cumulative effects of inbreeding depression increase over time (Duminil et al. 2009). However if establishment of selfed progeny played an appreciable role in invasion, then this would be expected to result in purging (although see Cheptou and Donohue 2011) leading to a reduction in inbreeding depression in the introduced compared to the native range or a gradient of decreasing inbreeding depression with distance from point of introduction in the introduced range (Pujol et al., 2009).

Thus despite high inbreeding depression, the possibility that selfing promotes invasion (and natural long distance colonisation, Dick et al. 2007) in trees can not yet be rejected. To address this question, we suggest that future studies use genetic markers such as microsatellites to test whether, for self-compatible trees such as *A. dealbata*, selfed progeny do survive to reproduce during invasion.

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Figures

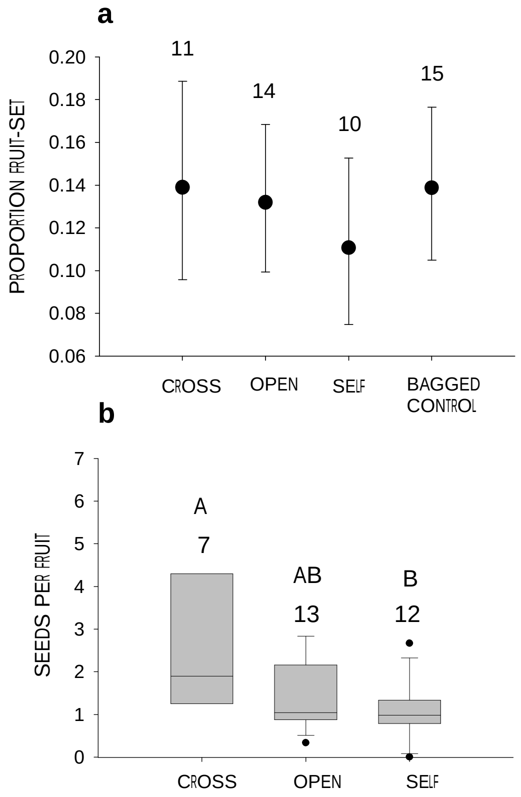

Figure 1 Proportion fruit set (infructescences per inflorescence) (a) and full seeds per pod (b) in controlled pollination experiment on *Acacia dealbata*. Numbers above bars represent sample sizes (number of plants). Back transformed means ± standard errors from arcsine-square root transformed data shown in (a), Treatments are significantly different where they do not share a letter in (b). In (b) whiskers indicate 10th and 90th percentiles where sample size is large enough to calculate them.

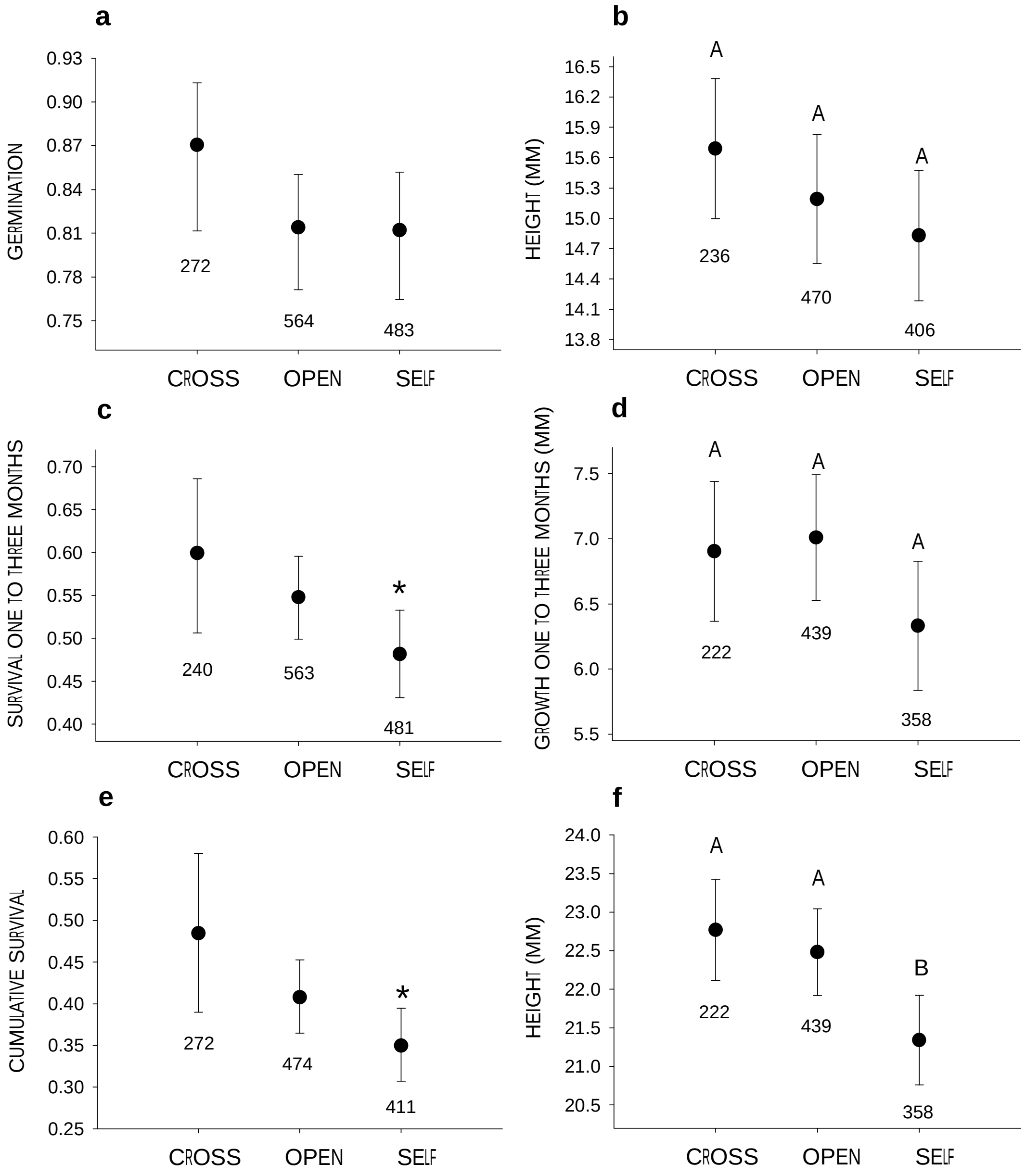

Figure 2 Performance of progeny from cross-, self- and open pollination. Proportion germination at one month (a); height of seedlings at one month (b); survival from one to three months (c), growth from one to three months (d), cumulative germination and survival (ie proportion of seeds with live seedlings) at three months (e); height at three months (f). Bars represent predicted means and standard errors from statistical models; numbers below bars represent number of progeny assessed. Progeny represent seven trees for cross-pollination, ten for open pollination and nine for self-pollination in germination and survival analyses (a, c, e) and seven, nine and nine

trees respectively for growth and height. For germination and survival(a, c, e) * indicates a treatment that is significantly different from cross-pollination ($p < 0.05$); for growth and height (b, d, f) treatments not sharing a letter above the bar are significantly different, as assessed by Fischer's unprotected least significant difference tests.

Appendix

Table S1 GLMM of germination for *Acacia dealbata* seedlings from cross-, self- and open pollination.

Fixed effects	Estimate	se	z	p
Intercept	1.906	0.446	4.27	< **0.001**
Self versus Cross	-0.442	0.287	-1.541	0.123
Open versus Cross	-0.429	0.261	-1.646	0.100
Random effects	Log-likelihood change	X^2	df	p
Tree	39.59	79.18	1	< **0.001**
Tray	6.35	12.7	1	< **0.001**

Table S2 REML analysis of plant height at one month from sowing for *A. dealbata* seedlings from cross-, self- and open pollination.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Pollination treatment	3.51	2	1033.7	1.75	0.174
Random effects	Deviance change	df			p
Tray	53.90	1			< **0.001**
Tree	37.81	1			< **0.001**

Table S3 GLMM of survival from one to three months for *Acacia dealbata* seedlings from cross-, self- and open pollination.

Fixed effects	Estimate	se	z	p
Self versus Cross	-0.4768	0.20	-2.33	0.020
Open versus Cross	-0.2121	0.20	-1.09	0.278
Random effects	Log-likelihood change	X^2	df	p
Tree	-8.39	16.78	1	**<0.001**
Tray	-49.19	175.16	1	**<0.001**

Table S4 REML analysis of growth to three months in progeny trial of *A. dealbata* seedlings from cross-, self- and open pollination.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Pollination treatment	4.89	2	701.9	2.44	**0.088**
Random effects	Deviance change	df			p
Tray	11.14	1			< **0.001**
Tree	35.936	1			< **0.001**

Table S5 GLMM of cumulative survival (sowing to three months) for *Acacia dealbata* seedlings from cross-, self- and open pollination.

Fixed effects	Estimate	se	z	p
Self versus Cross	-0.559	0.193	-2.90	**0.004**
Open versus Cross	-0.311	0.183	-1.70	0.089
Random effects	Log-likelihood change	X^2	df	p
Tree	16.71	33.43	1	**<0.001**
Tray	77.65	155.31	1	**<0.001**

Table S6 REML analysis of plant height at three months from sowing, in progeny trial of *A. dealbata* seedlings from cross-, self- and open pollination.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Pollination treatment	8.24	2	830	4.12	**0.017**
Random effects	Deviance change	df			p
Tray	23.75	1			< **0.001**
Tree	2.61	1			0.106

Table S7 GLMM of frequency of predominantly yellow leaves in *Acacia dealbata* seedlings from cross-, self- and open pollination.

Fixed effects contrasts	Estimate	se	z	p
Self versus cross	0.86	0.43	1.99	**0.046**
Open versus cross	0.77	0.42	1.81	**0.070**
Random effects	Log-likelihood change	X^2	df	p
Tree	14.57	29.14	1	< **0.001**
Tray	0.00	0.00	1	1.000

Table S8 GLMM of frequency of slightly yellow leaves in *Acacia dealbata* seedlings from cross-, self- and open pollination.

Fixed effects contrasts	Estimate	se	z	p
Self versus cross	0.68	0.65	1.05	0.295
Open versus cross	1.01	0.60	1.68	0.093
Random effects	Log-likelihood change	X^2	df	p
Tree	2.75	5.49	1	**0.019**
Tray	3.19	6.39	1	**0.011**

Table S9 GLMM of frequency red colouration on both dorsal surface and margins of leaves in *Acacia dealbata* seedlings from cross-, self- and open pollination.

Fixed effects contrasts	Estimate	se	z	p
Self versus cross	-0.30	0.22	-1.34	0.179
Open versus cross	-0.22	0.21	-1.05	0.293
Random effects	Log-likelihood change	X^2	df	p
Tree	27.64	55.28	1	< **0.001**
Tray	26.51	53.02	1	< **0.001**

Table S10 GLMM of frequency of red colouration on margins only of leaves in *Acacia dealbata* seedlings from cross-, self- and open pollination.

Fixed effects contrasts	Estimate	se	z	p
Self versus cross	0.53	0.47	1.14	0.253
Open versus cross	-0.05	0.48	-0.11	0.916
Random effects	Log-likelihood change	X^2	df	p
Tree	1.98	3.96	1	**0.047**
Tray	1.00	2.00	1	0.158

Table S11 GLMM of frequency of white stems in *Acacia dealbata* seedlings from cross-, self- and open pollination.

Fixed effects contrasts	Estimate	se	z	p
Self versus cross	1.11	0.38	2.93	**0.003**
Open versus cross	0.55	0.39	1.40	0.161
Random effects	Log-likelihood change	X^2	df	p
Tree	2.58	5.16	1	**0.023**
Tray	0.15	0.29	1	0.590

Table S12 GLMM of frequency of pink stems in *Acacia dealbata* seedlings from cross-, self- and open pollination.

Fixed effects contrasts	Estimate	se	z	p
Self versus cross	0.71	0.30	2.37	**0.018**
Open versus cross	0.58	0.29	1.97	**0.049**
Random effects	Log-likelihood change	X^2	df	p
Tree	8.06	16.12	1	< **0.001**
Tray	22.37	4.37	1	**0.037**

Table S13 GLMM of the effects of qualitative seedling characters on survival from germination to three months.

Fixed effects	Contrast	Estimate	se	z	p
Pollination treatment	Self versus cross	-0.28	0.22	-1.28	0.200
	Open versus cross	-0.09	0.21	-0.41	0.680
Leaf yellowness	Predominantly yellow versus green	-2.68	0.67	-4.00	< **0.001**
	Slightly yellow versus green	0.29	0.41	0.69	0.489
Leaf redness	Red from top and sides versus green	0.11	0.17	0.62	0.535
	Red from sides only versus green	0.57	0.37	1.54	0.123
Stem colour	White versus green	-1.63	0.36	-4.48	< **0.001**
	Pink versus green	-0.62	0.20	-3.11	**0.002**
Random effects		Log-likelihood change	X^2	df	p
Tree		8.64	17.29	1	< **0.001**
		93.95	187.90	1	< **0.001**

Chapter 4

Isolation does not increase pollen limitation in honeybee-pollinated invasive trees

James G. Rodger and Steven D. Johnson

Abstract

Shortages of mates and pollinators, as well as inefficient pollen transfer, often result in pollen limitation in plants, particularly at low abundance. Pollen limitation has received much attention in terms of the threat posed to the survival of rare plant species. However, relatively few studies have investigated how it may limit biological invasion. We assessed pollen limitation of fecundity in two invasive, mass flowering, insect-pollinated tree species in South Africa. *Acacia mearnsii* is largely self-incompatible, with low levels of fruit set after self-pollination while *A. dealbata* is completely self-compatible and autonomously self-fertilising in the study area. In these species, inbreeding depression during seed development results in fewer seeds per fruit in selfed relative to outcrossed pods. We carried out pollen supplementation experiments and scored visitation to isolated and non-isolated individuals of both species. We predicted that the self-incompatible *A. mearnsii* would suffer pollen limitation of fruit set, and that this would be more severe in isolated than non-isolated individuals, but that fruit set would not be pollen limited in the self-compatible and autonomously self-pollinating *A. dealbata*. We expected lower pollinator abundance on isolated than non-isolated trees. We assessed pollen limitation of seeds per fruit in *A. dealbata* and predicted it would be pollen limited due to geitonogamous self-pollination, and more severely so in isolated than non-isolated trees. Pollen supplementation increased fruit set of *A. mearnsii* by a factor of 3.25 compared to natural pollination, indicating pollen limitation, but this effect was not related to isolation (50m-450m from continuous populations). Pollen supplementation had no effect on fruit set or seeds per fruit in *A. dealbata*. Pollinator abundance was also not related to isolation in either species. These and other results suggest that in trees the effects of isolation are substantially mitigated by large displays and highly mobile pollinators. We propose that pollen limitation in trees is only likely to limit invasion if long distance dispersal events exceed the distances over which their pollinators will routinely forage.

Introduction

Fecundity in plants is often limited by pollen receipt due to shortages of mates and pollinators, as well as ineffective transfer of pollen by pollinators (Burd 1994, Larson and Barrett 2000, Knight et al. 2005). Low abundance often results in more severe pollen limitation (Sih and Baltus 1987, Ågren 1996, Kunin 1997), constituting a type of Allee effect – a reduction in population growth or individual fitness due to low abundance (Stephens et al. 1999). Pollen limitation has therefore received much attention as a threat to survival of rare plant species, particularly in the context of habitat fragmentation (Lamont et al. 1993, Fischer and Matthies 1998, Groom 1998, Fischer et al. 2000, Ward and Johnson 2005). However, relatively few studies have investigated pollen limitation in invasive plants (e.g. Parker 1997, Cappuccino 2004, Davis et al. 2004b, van Kleunen and Johnson 2005, meta-analysis by Burns et al. 2011).

Low abundance may reduce both the amount of pollen deposited and its suitability, resulting in quantitative and qualitative pollen limitation respectively (Aizen and Harder 2007). When plants are sparsely distributed, they are less profitable for pollinators to exploit, and often experience reduced visitation, reduced pollen receipt and increased quantitative pollen limitation (Sih and Baltus 1987, Groom 1998, Taylor et al. 1999, Duncan et al. 2004). In small populations of self-incompatible plants, pollen limitation may also occur due to reduced availability of compatible mates (Fischer et al. 2003, Wagenius et al. 2007, Leducq et al. 2010). At low abundance pollinators also visit more flowers per plant, increasing geitonogamous self-pollination (Klinkhamer et al. 1989) and, potentially, qualitative pollen limitation. Most self-compatible plants are affected by inbreeding depression to some extent, so they will also be subjected to greater pollen limitation if low abundance results in higher rates of selfing or biparental inbreeding (Husband and Schemske 1996, Angeloni et al. 2011).

Trees generally have very large display size, which carries both benefits and costs in terms of pollen limitation (De Jong et al. 1993). Large display size is beneficial in that it increases overall visitation. However, it also results in pollinators visiting more flowers per plant, increasing geitonogamous self-pollination (Klinkhamer et al. 1989, De Jong et al. 1993, Karron et al. 2004). This is probably a very common source of qualitative pollen limitation in trees as most of them are self-incompatible (Barrett et al. 1996). The few trees that are self-compatible display extremely high levels of inbreeding depression (Hardner and Potts 1995, 1997, Ishida 2006, 2008, Robertson et al. 2011), so they are likely to experience qualitative pollen-limitation as a result of seed-discounting following geitonogamous self-pollination. Seed discounting occurs when self-fertilisation pre-emptively sequesters ovules and resources that would otherwise have been devoted to outcross seed-production (Lloyd and Schoen 1992, Aizen and Harder 2007).

Low abundance is a feature of the early stages of biological invasion, so pollen-limitation Allee effects have the potential to inhibit invasion, especially as rate of spread can be dramatically increased by establishment of outlying invasion foci following long distance dispersal (Kot et al. 1996, Clark et al. 2001). Baker (1965, 1974) suggested that because ability to self-fertilise alleviates pollen limitation, it would predispose plants to invasiveness. This hypothesis has been supported by several studies showing positive correlations between invasiveness and ability self-fertilise (van Kleunen and Johnson 2007, van Kleunen et al. 2008, Burns et al. 2011, Hao et al. 2011, Pyšek et al. 2011). Although pollen limitation is common in invasive plants (Burns et al. 2011), among them its relationship with abundance (Davis et al. 2004b, van Kleunen and Johnson 2005) or effect on invasion dynamics (Parker 1997, Taylor et al. 2004) has seldom been assessed. However, in the invasive intertidal grass *Spartina alterniflora*, a pollen-limitation Allee effect (Davis et al. 2004a, b) slows down rate of spread by 31% (Taylor et al. 2004).

To assess the potential role of pollen limitation in inhibiting invasion in tree species, we conducted pollen supplementation experiments (Bierzychudek 1981) to test for pollen-limitation Allee effects in two invasive tree species, the self-incompatible *Acacia mearnsii* and the self-compatible *A. dealbata*. To assess whether Allee effects in pollen limitation occurred, we compared isolated and non-isolated individuals of both species. We also recorded pollinator abundance to assess whether this explained any potential effects of isolation on pollen limitation. We predicted that fruit-set would be pollen limited in the largely self-incompatible *A. mearnsii* but not in the self-compatible *A. dealbata*. We expected seeds per fruit to be pollen-limited in *A. dealbata* because this species displays inbreeding depression during seed development (Chapter 3) and high rates of geitonogamous pollination are likely due to large display size. We also expected that pollen limitation, if it did occur, would be more severe when plants occurred at low abundance.

Materials and Methods

Study Species and Region

The closely related *Acacia mearnsii* (black wattle) and *Acacia dealbata* (silver wattle) are prominent invaders in the moist eastern summer rainfall regions of South Africa. Both species were introduced to South Africa around the middle of the 19th Century and were widely planted, although only *A. mearnsii* is still used commercially (Poynton 2009). In *Acacia*, globose inflorescences are highly integrated structures that function as blooms and usually set only one or a few fruit. Fruit-set is therefore often assessed as proportion of inflorescences setting fruit (Kenrick and Knox 1989, Gibson et al. 2011). 22-42 flowers per inflorescence have been recorded in *A. dealbata* (Broadhurst and Young 2006) and 27.9 ± 5.6 in *A. mearnsii* (Moncur et al. 1988). In *A. mearnsii*, inflorescence contain variable ratios of male and hermaphrodite flowers, with percent of hermaphrodites recorded as 7% in one year and 52% in another (Moncur et al. 1991) *Acacia mearnsii* is largely self-incompatible (, Moncur et al. 1991), with fruit set (proportion of inflorescences setting fruit) of 0.51 ± 0.10, n = 8 for cross pollination and 0.10 ± 0.05, n = 11 for self-pollination in a plantation investigated in South Africa (Bloemendal farm; J. G. Rodger, unpublished results). *Acacia dealbata* is highly outcrossing in its native range, implying self-incompatibility (Broadhurst et al. 2008) but in South Africa appears completely self-compatible and autonomously self-pollinating (Chapter 2). Both species suffer inbreeding depression, with fewer seeds per fruit and lower rates of progeny growth and survival following self-pollination than cross-pollination (Philp and Sherry 1946, Moffet 1956, Moffett and Nixon 1974, Chapter 3). In KwaZulu-Natal, *A. dealbata* flowers in early spring from late July to early September and *A. mearnsii* from late August to October. Flowers of both species are arranged in racemes of compact globose inflorescences (heads) which are bright yellow in *A. dealbata* and creamy coloured in *A. mearnsii.* Inflorescences are protogynous but asynchrony between inflorescences allows geitonogamous self-pollination by insects (Moncur et al. 1988). In *Acacia s.l.* each anther produces eight disc-shaped, compound pollen grains (polyads), and the cup-shaped stigma neatly holds one polyad, so usually only a single polyad is deposited per stigma and all the seeds in a fruit are full sibs (Knox and Kenrick 1983, Muona et al. 1991).

All trees used in the study were in the KwaZulu-Natal Midlands between Camperdown (29° 50 S, 30° 40 E), Craigie Burn Dam (29° 15 S, 30° 15 E) and Underberg (29° 50 S, 29° 20 E), at altitudes between 700m and 2000m. Natural vegetation is grassland with isolated forest patches but large areas have been converted to agriculture and forestry.

Pollen supplementation experiments

We tested for pollen limitation of fecundity by carrying out pollen supplementation on inflorescences of isolated and non-isolated individuals of *Acacia mearnsii* and *A. dealbata*.

We predicted that the largely self-incompatible *A. mearnsii* would suffer pollen limitation of fruit set and that this would be more severe in isolated than non-isolated individuals, but that fruit set would not be pollen limited in the self-compatible and autonomously self-pollinating *A. dealbata*. Further, we predicted that seeds per fruit would be pollen limited due to geitonogamous self-pollination and more severely so in isolated than non-isolated trees. This was assessed for *A. dealbata*, but not *A. mearnsii*, due to low sample sizes for seeds per fruit in the latter.

Trees were located within sight of roads in the KwaZulu-Natal midlands and Drakensberg. Trees in isolation, separated from all others by at least 50m, were compared to non-isolated trees, occurring in populations of 2 or more individuals, with one focal tree selected for each population. Basal circumference was measured for each focal tree, as well as population size for non-isolated trees and isolation distance (to nearest flowering neighbour) for isolated trees except that in *A. dealbata* there were four trees isolated by more than 500m for which isolation distance was recorded as >500m. These trees were given an isolation distance of 500m for analysis. Non-isolated trees were given an isolation distance of zero. All individuals separated from each other by less than 50m were considered part of the same population. As small populations might also suffer some effects of isolation, we measured population size as a covariate for statistical analyses. Population size was obtained by counting flowering stems in populations up to 200 and estimated by multiplying mean stem density from three 10m by 10m quadrats per population by population area for larger populations.

Pollen supplementations were conducted between the 30th of September and the 17th of October 2004 for *Acacia mearnsii* and the 3rd and 27th of August 2005 for *A. dealbata*. Inflorescences in which anthers had dehisced (male stage) were used as pollen donors and were always taken from a different population to the recipient. Branches were cut and could be kept in water for up to two hours. Inflorescences with anthers not yet dehisced (female stage) were used as recipients for pollen supplementations and for controls for natural fruit set. Supplementation was carried out by rotating donor inflorescences against recipients. Five branches were used for each treatment (pollen supplementation and naturally-pollinated control) on each focal tree with five inflorescences per branch for *A. mearnsii* and ten for *A. dealbata*, giving 25 and 50 inflorescences per treatment per tree respectively. Branches were marked with coloured nylon wool and inflorescences were marked on the stalk using permanent marker (Sharpie Pen, Sanford tm). Fruit set data was obtained from 34 non-isolated and 20 isolated trees for *A. mearnsii* and 21 non-isolated and 20 isolated trees for *A.*

dealbata. Median isolation distance was 150m with a range off 50-450m for *A. mearnsii* and 135m with a range of 50 to 700m in *A. dealbata*. Median population size was 15 with a range of 2-31000 trees for *A. mearnsii* and 17 with a range of 2-45000 for *A. dealbata*.

Fruit set (proportion of inflorescences setting fruit) was measured two months from pollination (8th - 12th December 2004) for *A. mearnsii* and 3 months from pollination (2nd - 13th November 2005) for *A. dealbata*. Both species have fewer seeds per fruit from self-pollination than cross-pollination (Moffet 1956, Chapter 2, Kay Nixon, unpublished results). Therefore, if there is a high rate of geitonogamous self-pollination, number of seeds per fruit is likely to be pollen limited. For both species mature fruit were collected in December 2005. This was 14 months from pollination for *A. mearnsii* and four months from pollination for *A. dealbata*. However, very few mature fruit were obtained for *A. mearnsii*. Although some fruit were lost through natural abortion before they matured, it is likely that some mature fruit abscised prior to collection as there were abscised, mature fruit on the ground below a number of trees at the time that fruit were collected. Therefore, for *A. mearnsii*, pollen limitation of fruit-set was assessed from data three months after pollination rather than at maturity and pollen limitation of seeds per fruit could not be assessed. For *A. dealbata*, mean seeds per fruit was obtained for each treatment on each tree and seeds were counted if they were swollen, glossy black in colour and did not break when squeezed gently between the fingernails.

To test for pollen limitation of fruit set in each species and whether pollen limitation was greater in isolated plants, proportion fruit set (arcsine transformed) was analysed in a ANOVA in SPSS 15 (SPSS 2008) with pollen supplementation and isolation as fixed factors, tree as a random factor nested within isolation class (isolated or non-isolated), basal circumference, isolation distance ($\log_{10}$ transformed) and population size ($\log_{10}$ transformed) as covariates and including supplementation-by-isolation, supplementation-by-circumference, supplementation-by-isolation distance, supplementation-by-population size interactions. Terms were entered in the order they appear in Tables 1-3. Circumference, isolation, isolation distance and population size were tested against tree and other effects were tested against the residual. Type I sum of squares were used to accommodate the nesting of tree within isolation and to allow effects of isolation distance and population size to be tested after accounting for isolation per se. Results from the two species were not analysed together as data were not strictly comparable, given that fruit-set was measured at an immature stage in *A. mearnsii* and close to maturity in *A.dealbata.* Moreover, the two species flower at different times and the experiments were conducted in different years. Fruit set analyses could also have been conducted in generalised linear modelling or mixed modelling frameworks, but arcsine square root transformed data met the assumptions of ANOVA, so this simpler approach was used instead. Pollen limitation for number of full seeds per fruit was assessed for *A. dealbata* in ANOVA using the same model as for fruit set. To quantify the level of pollen limitation, an index of pollen limitation was calculated as PL = 100 × (1 - control/supplemented) (Larson and Barrett 2000). PL was calculated for fruit set in *A. mearnsii* (insufficient mature fruit were obtained from *A. mearnsii* to allow analysis of seeds per fruit) and for fruit set and seeds per fruit for *A. dealbata*.

Visitation

When supplementations were carried out for *A. mearnsii* in 2004 and *A. dealbata* in 2005, abundance of visitors on focal trees was measured by walking once around the canopy and recording identity and number of visitors observed, to determine whether isolated trees were less visited. 48 trees were inspected for *A. dealbata* and 60 trees for *A. mearnsii*. Observations were conducted throughout the day for *A. mearnsii* but only in the mornings for *A. dealbata* as visitation appeared to drop off sharply in the afternoon in the latter species. In addition, visitors were recorded in the same way for isolated and non-isolated trees between the 22nd of August and 7th of September 2004 (66 trees in total). The effect of isolation on number of visitors observed at focal trees was analysed in generalised linear models using the Poisson distribution and the logarithmic link function in Genstat 12 (Genstat 2009). Significance was tested by comparing Quasi F-statistics calculated from change in deviance to the F-distribution (Payne, 2009). As the models included only a single term (isolation), change in deviance for the model with the term compared to an intercept-only model was calculated. These models assume that residual deviance is equal to residual degrees of freedom and Quasi F-values are normally obtained by dividing mean change in deviance by one. Where residual mean deviance exceeded residual degrees of freedom the model was considered over-dispersed and Quasi-F values were corrected for over-dispersion by dividing mean change in deviance for each effect by the mean change in deviance of the appropriate error term, analogous to calculation of F-values in a mixed-model ANOVA (Payne, 2011a). Where models were under-dispersed (residual change in deviance < residual degrees of freedom) no action was taken (Zuur et al. 2009). Analyses were run for number of *Apis mellifera*, Cetoniinae and all visitors for *A. mearnsii*; *A. mellifera*, Syrphidae and all visitors were assessed for *A. dealbata*. Visitor groups other than *Apis mellifera,* Cetoniinae and Syrphidae were found only on one focal tree (Table 4) so they were not subjected to separate analyses.

Results

Pollen supplementation significantly increased fruit set in *A. mearnsii* by a factor of 3.25 from 2.83% to 9.20% indicating that fruit set was pollen-limited (PL = 0.69; Table 1; Fig. 1a). Pollen limitation was also not related to isolation distance (Table 1, Fig S1a) or population size (Table 1, Fig S1c) and the interactions of supplementation with isolation distance and population size were also not significant (Table 1). In *A. dealbata* the effect of pollen supplementation was not significant for fruit set (PL = 0.11; Table 2; Fig. 1b) or seeds per fruit (PL = 0.12; Table 3; Fig. 1c) and the effect of isolation and its interaction with supplementation were also not significant. In *A. dealbata* there were also no significant interactions of pollen supplementation with isolation distance Table 2, Fig. S1b) or with population size (Table 2; Fig. S1d). There was no interaction between isolation distance and pollen supplementation for seeds per fruit in *A. dealbata* (Table 3, Fig. S2a) and there was in fact a non-significant trend for greater pollen limitation of seeds per fruit in larger populations (Table 3; Fig. S2b).

Abundances of visitors was generally low (Table 4) but not lower in isolated than non-isolated trees (Table 5). Isolation had no effect on abundance of visitors to *A. mearnsii* for *Apis mellifera*, Cetoniine beetles and all visitors (Table 5). For *A. dealbata* in 2004 isolation had no effect on abundance of *A. mellifera* but abundance of syrphids and all visitors was greater for isolated trees. For *A. dealbata* in 2005, isolation had no effect on visitation of Syrphidae. For *A. mellifera* and all visitors in that year, isolation had no significant effect for *A. mellifera* and all visitors (Table 5) when an outlier – an isolated tree which had 40 *Apis mellifera* on it, was excluded from analysis. When the outlier was included, isolated trees had significantly more visitors for *A. mellifera* ($p = 0.017$) and all visitors ($p = 0.027$).

Discussion

Fruit set was pollen-limited in the self-incompatible *A. mearnsii* (Table 1; Fig. 1a) but neither fruit set nor seeds per fruit were pollen-limited in the self-compatible *A. dealbata* (Tables 2, 3; Fig. 1b, c). As a result of large display size, even the relatively low percentage natural fruit set in *A. mearnsii* (2.83%) can result in substantial fruit production in absolute terms. In light of this, and that pollen limitation in *A. mearnsii* was not exacerbated by isolation at the distances of 50-450m obtained in this study (Tables 1; Fig. 1a), it is unlikely that pollen limitation affects ability of this invasive species to spread in the study region. However, reduced fruit-set due to pollen limitation still potentially reduces rate of spread by reducing the number of long distance dispersal events (Clark et al. 2001).

The occurrence of pollen limitation at fruit initiation in *A. mearnsii* is in agreement with previous controlled pollination experiments, in which hand cross-pollination consistently results in higher fruit set than natural pollination (Moffett and Nixon 1974, Moncur et al. 1988, J.G Rodger, unpublished results). This may be due to low pollinator visitation, as *A. mearnsii* is relatively poorly visited compared to *A. dealbata* and *A. decurrens* in the study region (Chapter 1) or geitonogamous self-pollination, as honeybees, the principal pollinators (Chapter 1), visit many flowers on an individual before moving to the next (Moncur et al. 1991, J. G. Rodger, pers. obs.). Most stigmas in *Acacia* receive only one polyad due to their small size (Moncur et al. 1991, Kenrick 2003), so geitonogamous self-pollination is likely to cause seed discounting. In an Australian study on *A. mearnsii*, although 37% of stigmas received polyads, only 1.3% of heads initiated fruit and this discrepancy was attributed to geitonogamous self-pollination by honeybees (Moncur et al. 1991). However, pollen limitation of fruit set of *A. mearnsii,* as assessed at three months after pollination in this study, may also not translate into pollen limitation of fecundity at fruit maturation (14 months after pollination). There can be considerable abortion of fruit over the course of development (Moncur et al. 1991, J.G. Rodger, pers. obs.) and we were unable to assess pollen limitation at maturity due to abscission of fruits before collection.

The lack of pollen limitation of fruit set in the autonomously self-fertilising *A. dealbata* (Fig. 1b) suggests that selfing provided reproductive assurance in this species, as its floral biology and pollination relationships are very similar to those of the self-incompatible *A. mearnsii* in which fruit set was pollen limited. However, the difference in pollen limitation between the two species might also be due to some confounding factor rather than the difference in ability to self-fertilise. Unfortunately, reproductive assurance could not be assessed directly by emasculation due to the small size of flowers and compact construction of inflorescences in *Acacia*.

Acacia dealbata has inbreeding depression during seed development and probably has a high selfing rate in the study region due to its high capacity for autonomous and pollinator facilitated self-pollination (Chapter 2). Another self-compatible tree, *Magnolia obovata*, displays selfing rates from 0.6-0.8 at seed production (Ishida et al. 2003, Ishida 2006). It is therefore surprising that pollen supplementation did not increase seeds per fruit in *A. dealbata*. However, qualitative pollen limitation may be underestimated if flowers have already been pollinated with self-pollen before supplementation occurs (Aizen and Harder 2007). This may well have occurred in *A. dealbata* as inflorescences are heavily visited by honeybees, which move predominantly between flowers within plants (Chapter 2). In controlled pollination and progeny trials in *A. dealbata* (Chapter 3), there was cumulative inbreeding depression of 0.75 over seed development through to survival three months from sowing. Because of this and because rates of seed discounting are probably also high, it seems unlikely that selfing benefits fitness and contributes to invasion in *A. dealbata*, even though it may provide reproductive assurance at the level of fruit set.

Isolation did not increase pollen limitation in *A. mearnsii* (Table 1; Fig. 1a). Despite uncertainty over whether pollen limitation of fruit set was carried through to fruit maturation, this study at least provides no evidence for a pollen-limitation Allee effect. Similarly, in the highly rewarding invasive shrub *Senna didymobotrya*, which is pollinated by large carpenter bees, pollen-receipt and fruit set were not related to population size, over a range of 1-200 individuals with isolation distance of at least 300m between populations (van Kleunen and Johnson 2005). Plant abundance probably has little effect on pollen limitation in these species because they have large displays, so single individuals are still profitable resource patches for pollinators, even when isolated by several hundred metres. Consistent with this explanation, pollinator abundance was not found to be lower in isolated than non-isolated trees in this study (Table 5). Large insects such as honeybees, the primary pollinators of introduced *Acacia* species in the study area (Chapter 1), are easily able to pollinate over the distances of isolation (50-700m) in these experiments (Visscher and Seeley 1982, Lowe et al. 2005, Kramer et al. 2008).

The lack of evidence for pollen-limitation Allee effects in this study and that of van Kleunen and Johnson (2005) contrasts to herbaceous plants where small patches or single individuals become unprofitable for pollinators to exploit when separated from larger populations by distances of tens to hundreds of metres, resulting in high levels of pollen limitation (Groom 1998, Duncan et al. 2004, Elam et al. 2007) and potentially leading to extinction of small patches (Groom 1998). Many trees, however, occur naturally at densities of less than one per hectare (Lowe et al. 2005, Kramer et al. 2008). Moreover, although distances of hundreds to thousands of metres between habitat fragments and single individuals usually result in decreased fecundity, outcrossing rates and progeny performance in trees, these effects are usually small (e.g. Dick et al. 2003; Dick 2001, Rocha and Aguilar 2001, Cascante et al. 2002, Fuchs et al. 2003).

Most studies that have examined the effects of forest fragmentation and degradation on reproductive success in trees have concluded that, despite some negative effects, trees occurring singly or in fragments still make meaningful genetic and demographic contributions to population persistence (reviews by Lowe et al. 2005, Kramer et al. 2008). Honeybees have been observed to routinely forage 6km from their nest of their nest with a maximum 10km (Visscher and Seeley 1982), suggesting that isolation distances upwards 6-10km would probably produce severe pollen-limitation Allee effects for honeybee pollinated trees, such as the *Acacia* species in this investigation. Genetic studies have documented maximum long distance seed dispersal distances in trees of between 3 and 22km (Godoy and Jordano 2001, Gaiotto et al. 2003, Bacles and Ennos 2008). Spread of invasive trees may thus be limited either by long distance seed dispersal or the pollination of individuals established thereafter. Further data will be needed to distinguish between these two possibilities. This study and the one on *Senna didymobotrya* (van Kleunen and Johnson 2005), however, suggest that in trees and shrubs, including those that are self-incompatible, isolated individuals can make important contributions to invasion.

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Tables

Table 1 ANOVA for fruit-set in pollen supplementation experiment on *Acacia mearnsii*. ndf = numerator degrees of freedom, ddf = denominator degrees of freedom

Effect	**SS**	**ndf**	**ddf**	**MS**	**F**	**p**
Circumference	0.03	1	45	0.03	0.55	0.462
Isolation	0.04	1	45	0.04	0.71	0.405
Distance	0.01	1	45	0.01	0.14	0.713
Population size	0.00	1	45	0.00	0.03	0.854
Tree (Isolation)	2.70	45	41	0.06	1.93	0.018
Supplementation	0.41	1	41	0.41	14.11	0.001
S * C	0.00	1	41	0.00	0.05	0.821
S * I	0.04	1	41	0.04	1.23	0.275
S * D	0.01	1	41	0.01	0.35	0.559
S * PS	0.03	1	41	0.03	1.08	0.306
Error	1.23	41		0.03		

Table 2 ANOVA for fruit-set in pollen supplementation experiment on *Acacia dealbata*. ndf = numerator degrees of freedom, ddf = denominator degrees of freedom

Effect	**SS**	**ndf**	**ddf**	**MS**	**F**	**p**
Circumference	0.01	1	36	0.01	0.13	0.719
Isolation	0.05	1	36	0.05	0.75	0.392
Distance	0.01	1	36	0.01	0.22	0.640
Population size	0.09	1	36	0.09	1.41	0.243
Tree (Isolation)	2.16	36	31	0.06	8.49	< 0.001
Supplementation	0.00	1	31	0.00	0.00	0.960
S * C	0.00	1	31	0.00	0.55	0.464
S * I	0.01	1	31	0.01	1.42	0.243
S * D	0.00	1	31	0.00	0.11	0.737
S * PS	0.00	1	31	0.00	0.55	0.465
Error	0.31	31		0.01		

Table 3 ANOVA for seeds per fruit in pollen supplementation experiment on *Acacia dealbata*. ndf = numerator degrees of freedom, ddf = denominator degrees of freedom

Effect	**ndf**	**ddf**	**MS**	**F**	**p**
Circumference	1	18	0.09	0.06	0.807
Isolation	1	18	0.77	0.55	0.467
Distance	1	18	0.67	0.48	0.496
Population size	1	18	4.62	3.32	0.085
Tree (Isolation)	18	6	1.39	1.21	0.437
Supplementation	1	6	0.29	0.25	0.632
S * C	1	6	0.10	0.09	0.778
S * I	1	6	0.12	0.11	0.756
S * D	1	6	2.25	1.95	0.212
S * PS	1	6	5.50	4.78	0.071
Error	6		1.15		

Table 4 Mean visitors per tree and (in brackets) percentage of trees on which they were observed for different visitor groups on *Acacia mearnsii* in 2004 (61 trees) and *Acacia dealbata* in 2004 (66 trees) and 2005 (49 trees) in single tree inspections.

	2004	2004	2005
Visitor Group	*Acacia mearnsii*	*Acacia dealbata*	*Acacia dealbata*
Apis mellifera	0.30 (0.16)	0.18 (0.09)	0.23* (0.18)
Syrphidae		0.11 (0.08)	0.16 (0.12)
Wasps	0.02 (0.02)		
Coleoptera, Scarabeidae, Cetoniinae	0.21 (0.07)		
Other Coleoptera	0.32 (0.02)	0.015 (0.015)	

*excluding an outlier on which 40 *A. mellifera* was observed. Including that observation results in an average of one *A.mellifera* per tree for *A. dealbata* in 2005

Table 5 Effect of isolation on abundance of visitors to exotic *Acacia* species in generalised linear models with the Poisson distribution and logarithmic link function.

Acacia species	Visitor group	Deviance	df	Mean deviance	Quasi-F	p
A. mearnsii (2004)	*Apis mellifera*	0.37	1, 59	0.37	0.30	0.586
	Cetoniinae	2.05	1, 59	2.05	1.62	0.209
	All visitors	1.07	1, 59	1.07	0.47	0.494
A. dealbata (2004)	*Apis mellifera*	1.41	1, 64	1.41	1.55	0.217
	Syrphidae	8.25	1, 64	8.25	17.75	**<0.001**
	All visitors	5.93	1, 64	5.93	4.44	**0.039**
A. dealbata (2005)	*Apis mellifera**	0.11	1, 46	0.11	0.16	0.695
	Syrphidae	0.43	1, 47	0.43	0.59	0.447
	All visitors*	0.07	1, 46	0.07	0.06	0.803

* analysis excludes an outlier with forty *A. mellifera* and two Syrphids

Figures

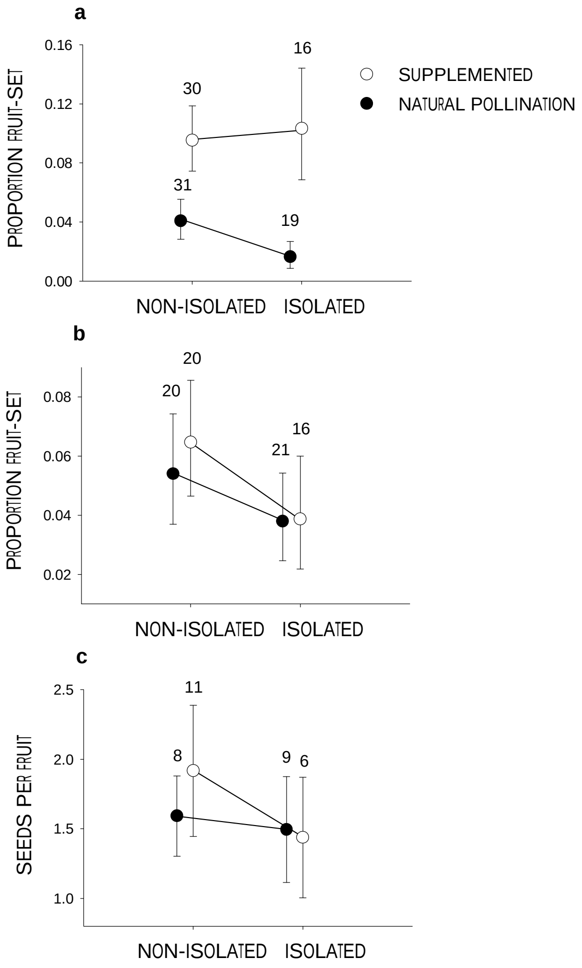

Figure 1 Effect of pollen supplementation on fruit set in *Acacia mearnsii* (a) and *A. dealbata* (b) and seeds per fruit in *A. dealbata* (c) in isolated and non-isolated trees. Analyses of fruit set conducted on arcsine square root transformed data; back-transformed means ± SE shown. Mean ± SE shown for seeds per fruit.

Appendix

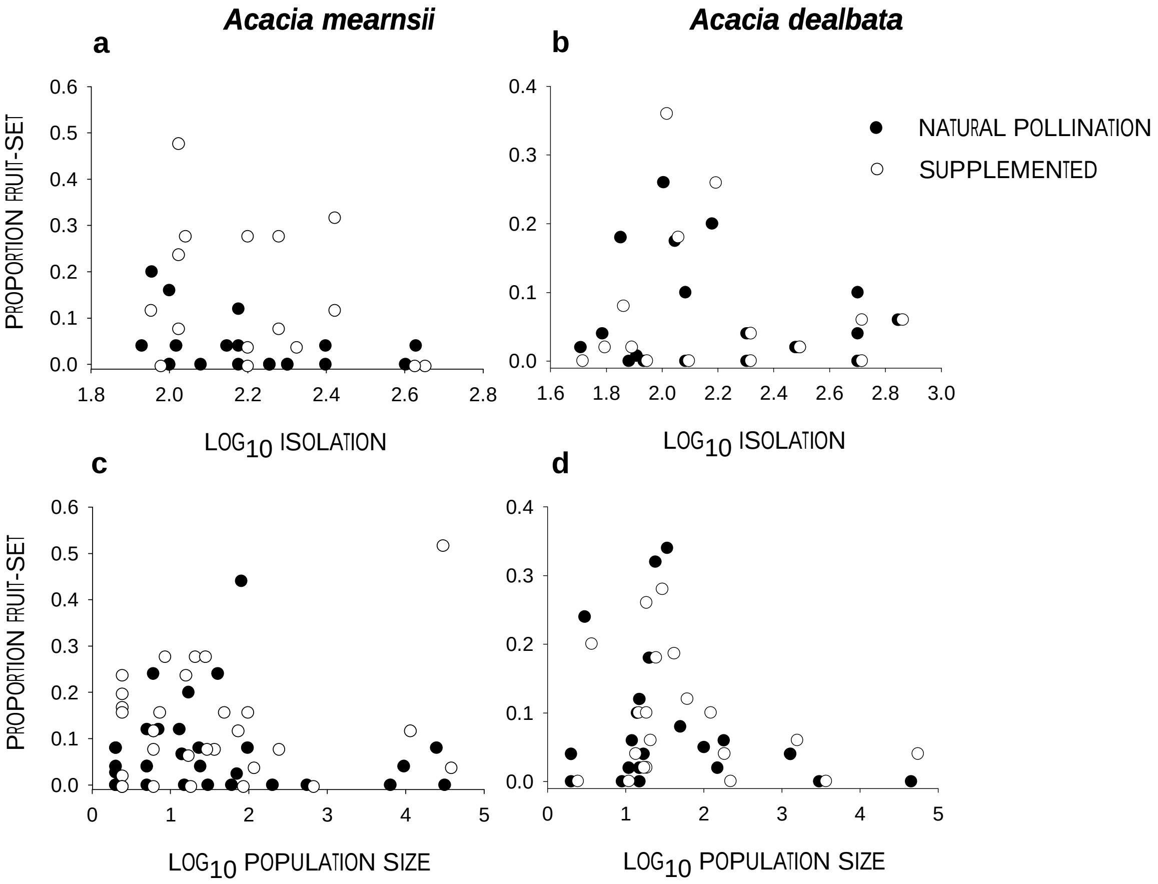

Figure S1 Fruit set for naturally pollinated and pollen supplemented inflorescences in relation to isolation distance of isolated trees (a, b) and population size of non-isolated trees (c, d) in *Acacia mearnsii* and *A. dealbata*.

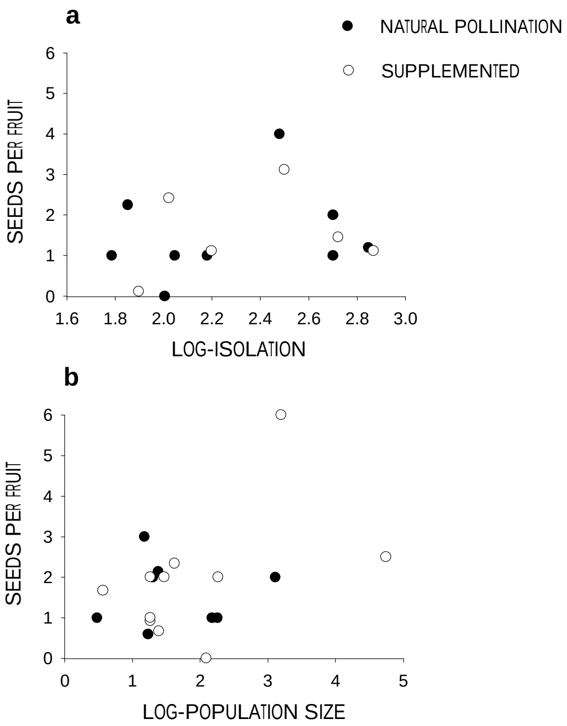

Figure S2 Seeds per fruit for naturally pollinated and pollen supplemented inflorescences in relation to isolation distance of isolated trees (a) and population size of non-isolated trees (b) in *Acacia dealbata*.

Chapter 5

Does specialized pollination impede plant invasions?

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DOES SPECIALIZED POLLINATION IMPEDE PLANT INVASIONS?

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Generalized pollination systems and autonomous self-fertilization are traits that have been linked with plant invasiveness. However, whether specialized pollination requirements pose a significant barrier to plant invasions is not yet clear. Likewise, the contribution of pollinators to the fecundity of facultatively self-pollinating invasive plant species is poorly understood. We addressed these issues using the self-compatible and autonomously self-pollinating *Lilium formosanum*, which also has large, showy flowers that are adapted for pollination by hawk moths. We investigated the pollination of this lily—which is indigenous to Taiwan—in KwaZulu-Natal, South Africa, where it is invasive. The long-tongued hawk moth *Agrius convolvuli* was identified as the primary pollinator on the basis of field observations, pollen load analysis, presence of lepidopteran scales on stigmas, and higher seed production in emasculated flowers exposed at night than in those exposed during the day. Remarkably, this moth is native to much of the Old World, including Taiwan and South Africa. Autonomous self-pollination resulted in seed production, but at a reduced level relative to the seed production of open- and hand-pollinated flowers, which was significant in one out of two populations examined. Thus, pollinators potentially contribute to invasion by increasing seed production and genetic variability through cross-pollination, although contributions of pollinators to seed set versus that of autonomous self-pollination may vary between populations. We conclude that specialized pollination requirements do not present a barrier to invasions when plants are specialized to pollinators or pollinator functional groups with very wide distributions.

Keywords: Baker's law, breeding system, biological invasion, prediction, reproductive assurance, sphingophily.

Online enhancement: appendix.

Introduction

Plants with generalized pollination systems are hypothesized to be more invasive than pollination specialists (Baker 1965, 1974; Richardson et al. 2000), because highly specialized species are unlikely to encounter their particular pollinators in novel environments whereas plants with generalized requirements should be able to utilize alternative pollinators in most environments (Goulson and Derwent 2004; Stout et al. 2006; but see Valentine 1977). Specialization could inhibit invasion if adaptations for pollination by particular pollinator functional groups or species (evolutionary specialization; Armbruster 2006) actually prevent other potential flower visitors from visiting or effectively pollinating. Such evolutionary specialization then results in functionally specialized pollination systems—where plants are served by few functional groups—and/or ecologically specialized systems, involving few pollinator species (Ollerton et al. 2007). These three types of specialization are not always associated with one another (Ollerton et al. 2007). Plants that have specialized pollination systems may invade novel regions if the pollinator taxon or functional class pollinating them in their native range occurs naturally or has also been introduced in the novel range (Liu and Pemberton 2009), if they switch pollinators (Valentine 1977), or if they can reproduce autonomously. We need more information on the pollination of invasive species in their introduced ranges to assess the degree to which pollination specialization is a barrier to invasion in plants (Richardson et al. 2000).

The existence of certain widespread pollination syndromes and corresponding pollinator functional groups (sensu Fenster et al. 2004) suggests that such specialization to a functional class may less frequently impede plant invasions than might be supposed from Baker's (1965, 1974) simplistic interpretation of pollinator species and functional groups with quite restricted distributions. For example, there is a group of mostly self-incompatible red-flowered plants in the Western Cape province of South Africa that comprise a pollination guild and that show convergent adaptation for pollination by the butterfly *Aeropetes tulbagheae*, which is restricted to rocky mountaintops and gorges in South Africa and Zimbabwe and which is the exclusive pollinator of these plants (Johnson and Bond 1994). No species in this guild is known to be invasive. On the other hand, the carrion fly–pollinated and self-incompatible *Stapelia gigantea* from South Africa sets abundant seed in Venezuela, where it is invasive, as a result of pollination by native carrion flies (Muscidae, Calliphoridae, Sarcophagidae;

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Herrera and Nassar 2009). Several invasive plant species with other specialized pollination syndromes are also visited by members of the same pollinator functional groups in both their native and their introduced ranges (Forster 1994; van Kleunen and Johnson 2005; Geerts and Pauw 2009; Herrera and Nassar 2009). Moreover, if plants with specialized pollination systems have evolved selfing to cope with vulnerability to fluctuation in pollinator populations (Fenster and Marten-Rodriguez 2007; Perez et al. 2009), then this may also constitute a preadaptation for invasion.

Baker (1955, 1967) proposed that autonomous self-pollination would promote colonization after long-distance dispersal. Recent studies have supported his prediction of a high frequency of self-compatible taxa among invasive plants (Rambuda and Johnson 2004; van Kleunen and Johnson 2007; van Kleunen et al. 2008; but see Sutherland 2004). Autonomous self-pollination therefore seems likely to be an important mechanism of reproductive assurance for invasive plants. When pollinators and mates are available, however, self-pollination can decrease parental fitness because selfed progeny generally have lower viability and vigor than do outcrossed progeny (Husband and Schemske 1996). Pollinators potentially contribute to invasiveness, even in plants that self-pollinate autonomously, by increasing the quality and genetic variability of progeny through outcrossing and by increasing quantity of seed produced if autonomous self-pollination does not result in fertilization of all ovules (Aizen and Harder 2007). Of the autonomously self-pollinating invasive species studied, pollinators increased seed set in *Nicotiana glauca* (Geerts and Pauw 2009), *Opuntia stricta*, *Carpobrotus acinaciformis* (Bartomeus and Vila 2009), and *Crotalaria retusa* (Jacobi et al. 2005), but not in *Muntingia calabura* (de Figueiredo et al. 2008). Pollinators may thus promote invasion, even in autonomously self-pollinating plant species.

The invasive geophyte *Lilium formosanum* is self-compatible and autogamous (Inagaki 2002; Rambuda and Johnson 2004). Flowers conform to the hawk moth pollination syndrome (Vogel 1954; Waser 2006), and the plant is reputed to be pollinated by hawk moths in its introduced range in Japan (H. Inagaki, personal communication), although to the best of our knowledge its pollination has not been studied in the native range in Taiwan. *Lilium formosanum* is therefore a very promising candidate for investigating the relevance of pollination specialization for invasion and the importance of animal pollinators versus autonomous self-pollination. *Agrius convolvuli*, the most common large hawk moth in the South African invasive range of *L. formosanum*, is also indigenous in Japan and Australia (Pittaway and Kitching 2007). A very similar congener, *Agrius cingulatus*, occurs in the southern United States (Opler et al. 2006), where *L. formosanum* is also invasive. That transcontinental plant invasion might be facilitated not merely by pollinators of the same functional type as those in the plant's native range but by the very same species is remarkable.

In this study we investigated the degree of specialization of the pollination system of *L. formosanum* in its invaded range in KwaZulu-Natal, South Africa, and the potential for animal pollinators versus autonomous self-pollination to contribute to fecundity. Specifically, we asked the following questions: (1) Does this plant experience effective biotic pollination, and if so, by which pollinating agents? And (2) can pollinators potentially increase fecundity over that which is achieved by autonomous self-pollination alone (i.e., is seed set pollen limited under purely autonomous self-pollination)?

Materials and Methods

Study Species

Lilium formosanum Wallace (Liliaceae) is native to Taiwan but has become invasive in the moist eastern parts of South Africa (Henderson 2001) and Australia (AVH 2007), in the southern United States (USDA 2007), and in the southern islands of Japan (Inagaki 2002). It is a geophyte that produces annual aboveground parts during summer and, in South Africa, that flowers between January and April (May). In South Africa it invades anthropogenically disturbed habitats such as road verges and plantation edges; it also invades natural grassland and may therefore pose a threat to native plant species. Stems are unbranched, 0.3–2.5-m tall, with a terminal candelabra-like inflorescence of one to eight flowers (fig. 1). The flowers are white, trumpet shaped, and strongly scented at night, with nectar borne near the base of the flower, far from the sexual parts. Nectar volume (mean ± SE, 47.0 ± 7.3 μL; $n = 10$), sugar concentration ($36.1\% \pm 2.0\%$; $n = 10$), and anther and stigma height (122.5 ± 2.9 mm and 140.5 ± 2.9 mm, respectively; $n = 22$) as measured in a population of *Lilium formosanum* near Hilton (lat. 26°30.528′S, long. 30°17.216′E) are consistent with flowers pollinated by *Agrius* hawk moths in South Africa and elsewhere (Alexandersson and Johnson 2002; Martins and Johnson 2007). Individual flowers abscise 4–7 d after anthesis (median = 6 d; J. G. Rodger, unpublished results). Capsules take ~10 wk to mature and contain a few hundred to over 1000 flat, dry, winged seeds.

Controlled pollination experiments have shown that the species is self-compatible and autonomously self-pollinating in its introduced ranges but that it does not display nonpseudogamous apomixis (Inagaki 2002; Rambuda and Johnson 2004). A trend for lower seed set with autonomous self-pollination than with hand pollination (Rambuda and Johnson 2004) suggests that pollinators may contribute to fecundity. A molecular marker study indicated that the species has a mixed mating system in Taiwan, its native range (Hiramatsu et al. 2001). The stigma usually projects beyond the anthers, and the anthers normally dehisce on the day of anthesis. Self-pollination is effected by pollen falling or being shaken by the wind off the anthers and onto the stigma, at times by contact between stigmas and anthers in flowers, and during perianth abscission when anthers are often dragged across the stigma (Inagaki 2002; J. G. Rodger, personal observation), although stigma receptivity is low at perianth abscission (J. G. Rodger, unpublished results).

Study Sites

All populations examined were located in the invasive range in the KwaZulu-Natal Midlands and coastal regions (between lat. 29°25′ and 30°55′S and between long. 30°05′ and 30°50′E, and from 0 to 1500 m a.s.l.). They were located either in disturbed grassland adjacent to exotic tree

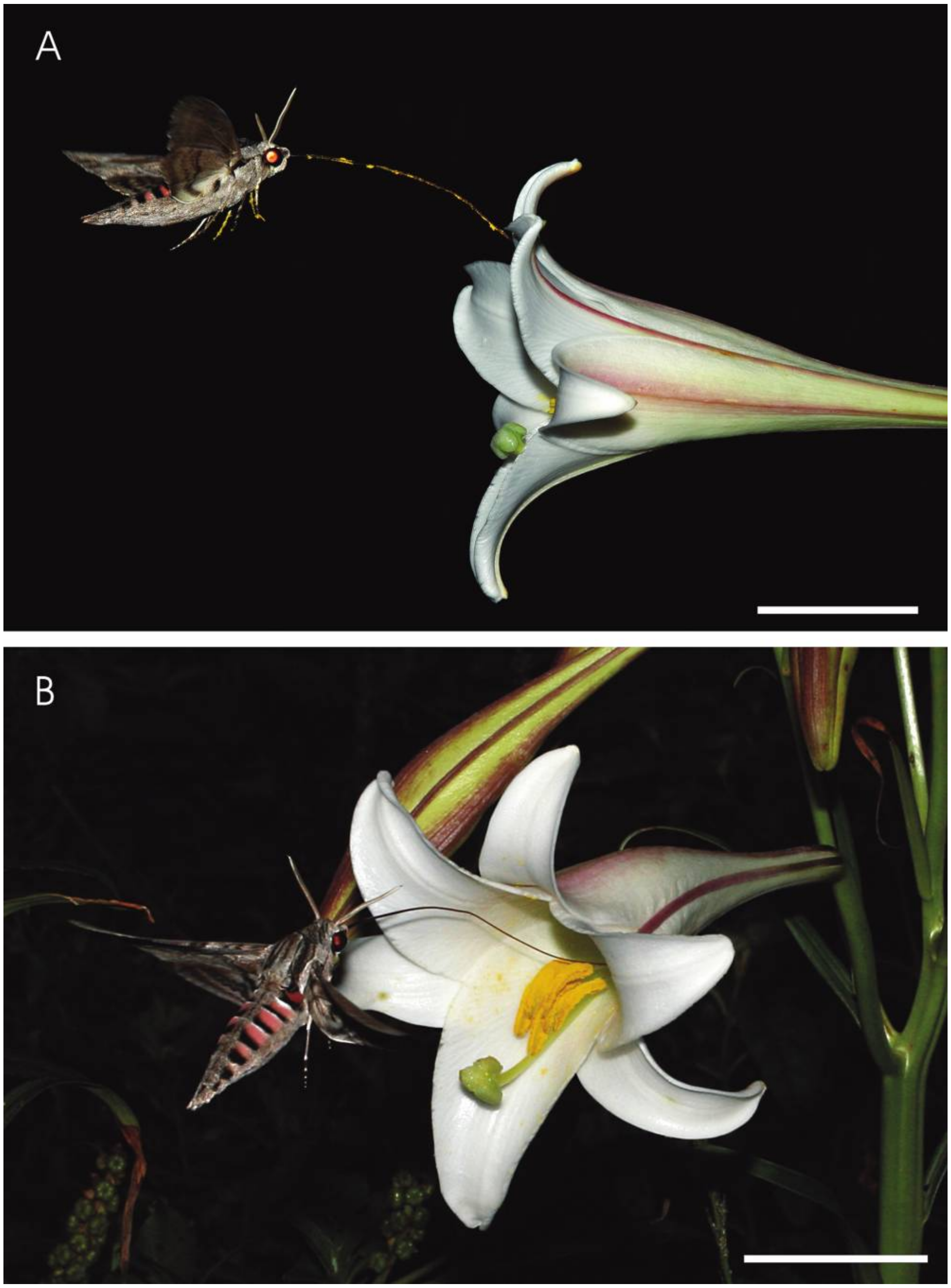

Fig. 1 *Agrius convolvuli* visiting the flowers of *Lilium formosanum*. *A*, Side view of a flower approached by a hawk moth carrying pollen on its proboscis and legs. (Photograph by S. D. Johnson.) *B*, Front view of a flower showing the position of the anthers and stigma, which will contact the underside of the approaching moth's body. Scale bar = 30 mm. (Photograph by C. Botes.)

plantations and invaded by *Rubus cuneifolius* or in grassy road verges. For logistical reasons, different experiments were not all performed at the same sites; details of sites used are provided in each case.

Visitor Observations and Pollen Loads

Relative abundances of nocturnal visitor species were assessed in 20 nocturnal observation sessions performed in the KwaZulu-Natal Midlands between January and March of 2006 and 2007. In 2006, nine observation sessions were performed in disturbed grassland at Wahroonga Farm (lat. 29°36.598′S, long. 30°07.990′E; 1350 m a.s.l.) and four were performed in disturbed grassland at Cedara (lat. 29°31.904′S, long. 30°16.117′E; 1042 m a.s.l.). In 2007, three sessions were performed at Wahroonga Farm and four were performed in grassland alongside a road in the Karkloof (lat. 29°20.190′S, long. 30°16.669′E; 1096 m a.s.l.). One to three observers watched as many flowers as possible for ~1 h, beginning just after sunset. Visitation normally decreased in frequency toward the end of the observation period. The number of flowers visited and the identity and behavior of all visitors were recorded.

In addition to direct observations of moths, we indirectly assessed moth visitation in 2007 by examining flowers in 27 populations for the presence of lepidopteran scales. These populations were located in the KwaZulu-Natal Midlands and the coastal regions (lat. 29°25′–30°55′S, long. 30°05′–30°50′E). Scale inspections were conducted for a median of 19 randomly chosen flowers per population: 18–24 flowers per population were inspected, except where there were fewer than 18 flowers per population, in which case all of the flowers were inspected, and for two populations in which 60 and 48 flowers were inspected, respectively. Stigmas and perianths were examined with the aid of a ×20 magnifying hand lens. Although scales are not always deposited during visitation (J. G. Rodger, personal observation), scale deposition is likely to be correlated with visitation. A χ^2 test was used to test for variation between populations in scale deposition for the 19 populations in which 18 or more flowers were inspected.

Abundance of diurnal visitors was assessed by inspecting up to 20 flowers in each of 24 populations (a total of 415 flowers) during 2007 (a subset of those scored for scale deposition). Inspections were performed between 0900 and 1600 hours. The number of individuals of each species of visitor was scored in each flower at the moment it was inspected. This snapshot method was used because virtually all diurnal visitors we observed spent long periods of time in individual flowers. In a period of over 70 d in the field that was spread across 5 yr of work on *L. formosanum* flowers, we have observed only four honeybees (*Apis mellifera*) and one bee of another species visiting the flowers. Three of the *A. mellifera* individuals foraged for pollen, one robbed nectar by inserting its proboscis through the tepals, and none were observed contacting the stigma. The one non-*Apis* bee observed on the flower was not observed to forage. No other fast-moving diurnal visitors were observed.

Visitors were captured for identification and to quantify their pollen loads. Fuchsin-stained gelatin was used to remove pollen from their bodies (Kearnes and Inouye 1993). The abundances of both *Lilium* and other pollen grains were counted under a compound microscope at ×100 magnification (there was no other species of *Lilium* flowering in the vicinity of study populations). *Agrius convolvuli* individuals were daubed on the head, the mouthparts, and the ventral part of the body, as these were the parts observed to contact anthers and that sometimes carried visible pollen loads. Other species were daubed all over the body. Pollen purity was calculated for each individual as the number of *L. formosanum* pollen grains divided by the total number of pollen grains. For the most abundant nocturnal and diurnal visitor species (*A. convolvuli* and the cetonid beetle *Cyrtothyrea marginalis*, respectively), log pollen loads were compared using *t*-tests and pollen purity was compared using Mann-Whitney *U*-tests.

Day-Night Pollinator Exclusion Experiment

The relative importance of diurnal and nocturnal visitors for fertilization was investigated in two populations, Wahroonga and Cedara (see above), in February 2006. Flowers were emasculated and exposed to pollinators either during the day ($n = 11$ at Wahroonga and $n = 8$ at Cedara) or at night ($n = 11$ at Wahroonga and $n = 7$ at Cedara). Flowers were emasculated before anthesis to prevent self-pollination and were covered with nylon mesh bags to exclude visitors. Only a single flower was treated on any particular individual plant. From anthesis onward, flowers were exposed to visitors by removing and replacing bags just after sunset and just after sunrise, as appropriate. Treatments were performed over five consecutive days in each population. Emasculation potentially affects visitation by removing the pollen reward and by changing the appearance of the flowers. Moth visitation is unlikely to be affected by emasculation, however, as they do not forage for pollen; indeed, moths appeared to visit emasculated flowers as readily as nonemasculated flowers.

Fruits were harvested at maturity, fruit set was scored, and seed set per flower was estimated. Seeds were counted as such if they contained an embryo that was at least half the length of the seed, excluding the wing. Where flowers set fruit, number of seeds was estimated by first calculating mean number of seeds per milligram in two samples of 50 seeds and unfilled ovules from each fruit and then by multiplying this value by the total seed mass for each fruit.

Fruit set (a binomially distributed variable) for nocturnally and diurnally accessible flowers were compared using bootstrapping for the two populations separately. In each population, fruit set for replicates of each treatment was bootstrapped, with replacement from fruit set values of the two treatments combined 1000 times. The difference in the proportion of flowers that set fruit between nocturnal pollination and diurnal pollination was calculated for the original data and for each bootstrap randomization. We present *P* values as the proportion of randomizations in which the difference in fruit set proportion for the two treatments in the bootstrapped data sets was greater than or equal to that observed for the actual data (two tailed). This estimates the probability of obtaining a difference between the treatments by chance that is as great or greater than that actually observed.

Number of seeds per fruit (for flowers that formed fruit) was compared between treatments using a *t*-test assuming equal variances (the significance level did not change when a *t*-test for unequal variances was used). This was performed for the Cedara population only, as there was no fruit set for the diurnally exposed treatment at Wahroonga.

Breeding System

Lilium formosanum is known to be self-compatible, and it self-pollinates autonomously (Inagaki 2002; Rambuda and Johnson 2004), but a trend for higher fecundity with hand pollination than with autonomous self-pollination (Rambuda and Johnson 2004) suggests that seed set is pollen limited under autonomous self-pollination. Therefore, to test whether animal pollinators increase seed set, we conducted further controlled-pollination experiments in two populations.

The two populations used were a freeway-verge population adjacent to the Mariannhill Toll Plaza on the N3 between Pietermaritzburg and Durban (lat. 29°49.415′S, long. 30°48.014′E; 395 m a.s.l.) and a disturbed grassland in Richmond (lat. 29°52.705′S, long. 30°16.672′E; 871 m a.s.l.); these were observed in January and February 2006. Flowers were assigned randomly to one of five treatments: four of these were bagged with nylon mesh bags before anthesis to exclude visitors, and the fifth was left open to pollinators (open pollinated) as a control for the effectiveness of hand pollination compared with natural pollination and to test for pollen limitation under open pollination. Only one flower was treated on any single plant. In two of the treatments, 1–2 d after anthesis, flowers were either self-pollinated or cross-pollinated with pollen from a plant located at least 2 m away in order to test for self-compatibility. Cross-pollinated flowers were emasculated at the time of bagging to prevent self-pollination. In the bagged control treatment, flowers were left unmanipulated in bags to test for autonomous self-pollination. In another treatment, bagged flowers were emasculated before anthesis and left unpollinated to test for the possibility of nonpseudogamous apomixis. Ten flowers were used per treatment per population, except where 20 flowers were used for open pollination at Mariannhill and 15 were used for the bagged control treatment at Richmond. Number of seeds per flower was measured (as described for the day-night pollinator exclusion experiment) to evaluate differences among treatments.

The effects of treatment and population on fruit set were examined by bootstrapping as in the day-night pollinator exclusion experiment, but several comparisons were made in each treatment to test separate hypotheses (Resampling Stats 2009). Tests were performed separately for each population. Hand self- and cross-pollination treatments were compared to test for self-compatibility. Because very similar fruit set levels occurred in these two treatments, their data were pooled for remaining contrasts. To test for autonomous self-pollination, the bagged control was compared with hand pollination. Open pollination was compared with hand pollination to test for the effectiveness of hand pollination and for pollen limitation. To test whether pollinators increased fruit and seed set above levels achieved by autonomous selfing, we compared open pollination with the bagged control treatment. Because there was no fruit set whatsoever in the emasculated flowers, no statistical comparison with any other treatment was performed. Numbers of seeds per fruit (for flowers that set fruit) were compared using *t*-tests for the same set of comparisons detailed above, except for those involving the emasculation treatment, where no fruits were set, and for the bagged control treatment at Mariannhill, which resulted in only three fruits. We used *t*-tests for equal variances (significance levels did not change when a *t*-test for unequal variances was used). For flowers that set fruit, seed-to-ovule ratios were also calculated and cross- and self-pollination were compared with *t*-tests to test for early-acting inbreeding depression.

An index of self-compatibility was calculated as the ratio of mean number of seeds per flower for self-pollination to mean number of seeds per flower for cross-pollination (Kenrick and Knox 1989) for each population. Similarly, an index of autonomous self-pollination was calculated as the ratio of mean number of seeds per flower in the bagged control treatment to mean number of seeds per flower for self-pollination (see Eckert et al. 2006).

Statistical Software

All statistical analyses were conducted in SPSS 15 (SPSS 2008), except for bootstrapping, for which the Poptools add-in for Microsoft Excel was used (Hood 2009).

Results

Visitor Observations

Agrius convolvuli (Sphingidae), a long-tongued hawk moth, was by far the most abundant nocturnal visitor. Individuals were observed in 2006 during three out of four observation sessions at Cedara and five out of nine at Wharoonga, and in 2007 during zero out of three sessions at Wharoonga and one out of four at Karkloof. During all nocturnal observations, a total of 28 individual insects were observed, of which 27 were *A. convolvuli*. The one other individual was an unidentified large, nonsphingid moth species. *Agrius convolvuli* was responsible for 155 of the 156 observed visits to flowers. *Agrius convolvuli* individuals hovered while inserting their very long (mean ± SE, 101.2 ± 6.3 mm; $n = 7$) tongues into the perianth tube, and they settled very briefly onto the anthers, making contact with the stigma, before leaving the flower (fig. 1). Visits typically lasted a few seconds; where more than one flower was open on the same plant, the flowers were often visited sequentially. Substantial pollen deposits were observed on previously virgin emasculated flowers after visitation by *A. convolvuli*, demonstrating effective cross-pollination (J. G. Rodger, personal observation). Although many nonsphingid moths were active at the sites, except for the single exception mentioned above, they did not visit *Lilium formosanum*.

Lepidopteran scales conforming to those of *A. convolvuli* were found on flowers in 22 out of 27 populations. Scales were generally deposited on the stigma or on perianth lobes near the entrance to the flower. In only one case was a dense deposit of scales observed deep down in the perianth tube, below the level where the body of *A. convolvuli* could have fit. This indicates that smaller, shorter-tongued moths do occasionally visit *L. formosanum*. In the 19 populations for which 18 or more flowers were inspected, a range of 0%–68.4% of flowers had scales (mean ± SE, 25.3% ± 5.0%). Frequency of scale deposition varied significantly between these ($\chi^2_{18} = 52.24$, $P < 0.001$), suggesting that pollinator visitation varies between populations.

Cyrtothyrea marginalis (Scarabaeidae, Cetoniinae) was the only diurnal visitor found in more than one population that also had a mean pollen load of more than one *L. formosanum* pollen grain (see tables A1 and A2 in the online edition of *International Journal of Plant Sciences*). They were present in 15 out of 24 populations inspected, with 0.241 ± 0.049 (mean ± SE) beetles per flower (averaged across populations). These beetles fed on the anthers or forced themselves deep down into the perianth tubes to get to the nectar. They were observed to contact the stigma, which they sometimes used as a launch pad for taking off. Other common visitors were minute beetles, flies, aphids, and thrips. These insects carried extremely low pollen loads and are very sedentary (J. G. Rodger, personal observation), and so they could contribute only negligibly to pollination. Information regarding number of populations in which each species of visitor was observed, abundance, and pollen loads is contained in tables A1 and A2.

Lilium formosanum pollen loads on *A. convolvuli* were an order of magnitude greater ($t_{35} = 3.65$, $P < 0.001$) than were those on *Cyrtothyrea marginalis* (45.8 ± 17.9, $t_{35} = 3.65$, $P < 0.001$). Pollen purity of *A. convolvuli* was 0.89 ± 0.05 ($n = 7$), and that of *C. marginalis* was 0.66 ± 0.07 ($n = 30$), but there was no significant difference between the two species ($z = -1.090$ m, $P = 0.138$; see tables A1 and A2). Results of statistical comparisons were qualitatively identical when other Cetoniinae were pooled with *C. marginalis*.

Day-Night Pollinator Exclusion Experiment

Emasculated flowers that were accessible to pollinators during the night had significantly higher fruit sets than did those accessible during the day at Wahroonga ($P < 0.001$) but not at Cedara ($P = 0.152$). However, fruit from nocturnal pollination at Cedara contained significantly more seeds than did fruit from diurnal pollination ($t_8 = 7.189$; $P < 0.001$; fig. 2*b*).

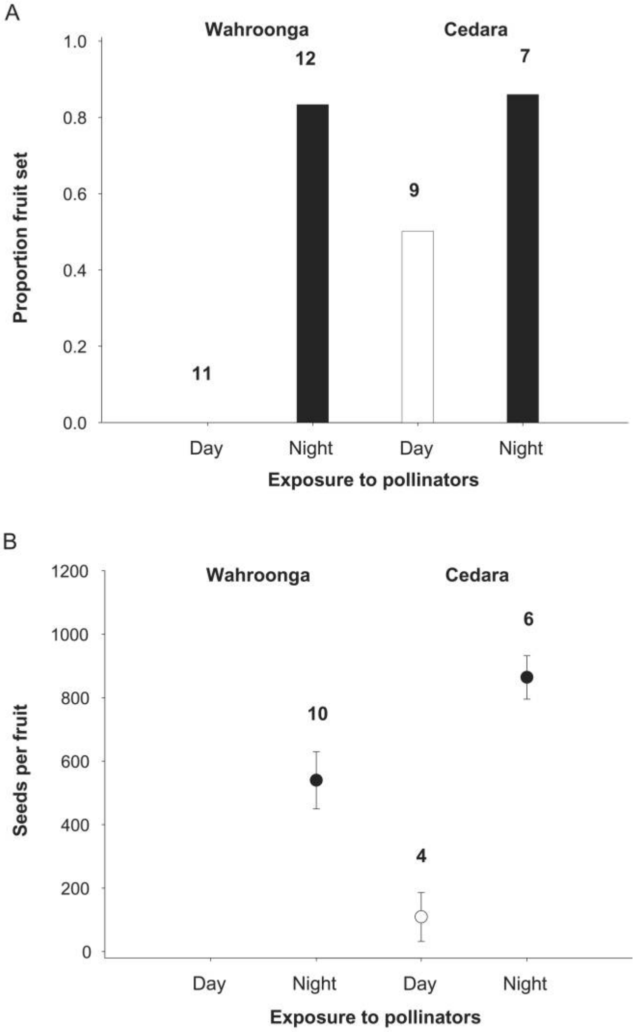

Fig. 2 Proportion fruit set (*A*) and number of seeds per fruit (mean ± SE; *B*) in a day-night pollinator exclusion experiment in two populations of *Lilium formosanum*. Numbers above bars are sample sizes.

Breeding System

Fruit set proportion and mean number of seeds per fruit are shown in figure 3*A* and 3*B*, and *P* values for comparisons between treatments are presented in table 1. Fruit set and seed set were very similar between cross- and self-pollination treatments at both Richmond and Mariannhill, indicating complete self-compatibility. Values for these two hand-pollination treatments were therefore pooled for comparison with other treatments in further comparisons. No fruit were produced in bagged emasculated flowers in either population, indicating an absence of nonpseudogamous apomixis. Fruit set was similar between the hand pollination and the open pollination groups, and seed set was higher under hand pollination, although this was significant only at Mariannhill. This shows that the hand pollination technique was adequate in both populations and that seed set was pollen limited at Mariannhill. Fruit set was lower under autonomous self-pollination (bagged control treatment) than under hand pollination, but it was significantly so only at Mariannhill, demonstrating that seed set is pollen limited under autonomous self-pollination for that population. Similarly, autonomous self-pollination resulted in lower fruit set than did open pollination, but this was significant only at Mariannhill, indicating that pollinators contributed to seed set there.

Seed-to-ovule ratios (means ± SE) at Richmond were 0.56 ± 0.06 for cross-pollination, 0.64 ± 0.07 for self-pollination, 0.53 ± 0.05 for bagged control, and 0.52 ± 0.07 for open pollination, and at Mariannhill they were 0.72 ± 0.04 for cross-pollination, 0.84 ± 0.03 for self-pollination, 0.83 ± 0.07 for bagged control, and 0.63 ± 0.07 for open pollination. There was a slightly higher seed-to-ovule ratio in cross-pollination versus self-pollination at Mariannhill ($t_{15} = 2.143$, $P = 0.050$) but not at Richmond ($t_{14} = 0.782$, $P = 0.446$), suggesting inbreeding depression during early seed development in the former population.

Indices of autonomous self-pollination were 0.54 for Richmond and 0.30 for Mariannhill. Indices of self-compatibility of 1.17 at Richmond and 1.04 at Mariannhill show complete self-compatibility.

Discussion

Our field observations and experiments show that the hawk moth *Agrius convolvuli*, which is also indigenous in the native range of *Lilium formosanum* in Taiwan, is the primary pollinator of *L. formosanum* in its invasive range in KwaZulu-Natal, South Africa. *Lilium formosanum* thus displays high levels of both functional and ecological specialization (Armbruster 2006; Ollerton et al. 2007) in South Africa. *Agrius convolvuli* potentially contributes to invasion in *L. formosanum* by increasing seed set, as autonomous self-pollination results in the fertilization of significantly fewer ovules than do open and hand pollinations in one of the two populations studied here

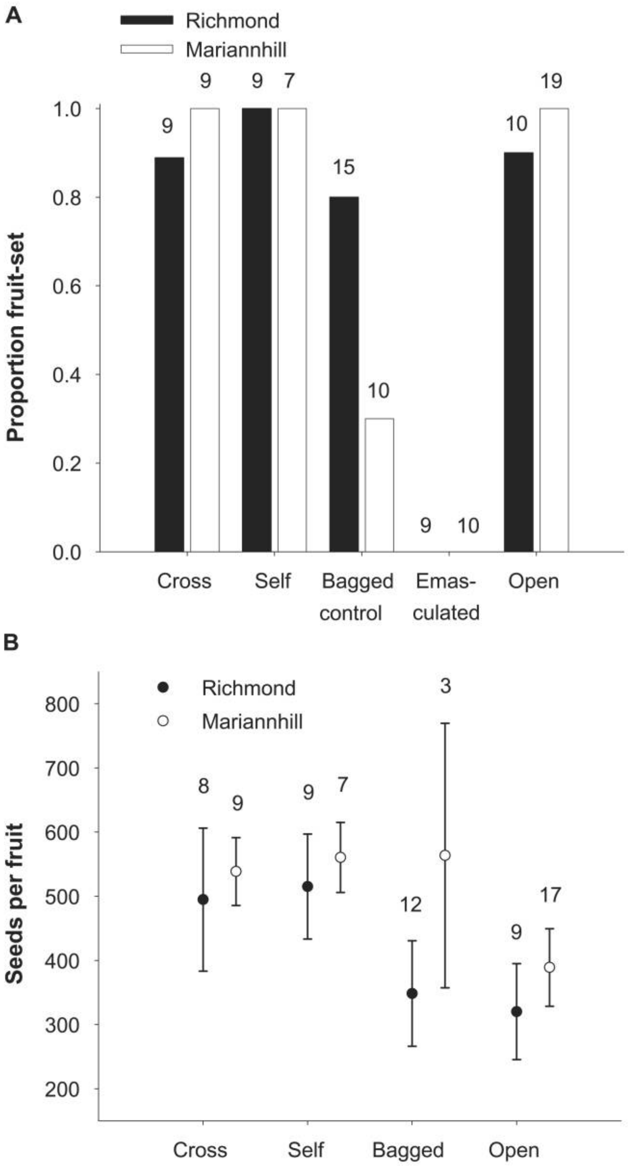

Fig. 3 Proportion fruit set (*A*) and number of seeds per fruit (mean ± SE; *B*) in a breeding system experiment in two populations of *Lilium formosanum*. Numbers above bars are sample sizes.

(table 1; fig. 3). Relative importance of pollinators versus autonomous self-pollination may vary between populations, however. Nevertheless, specialized pollination requirements are not a barrier to invasion when plants are adapted to widespread pollinators.

All evidence indicates that *A. convolvuli* is the primary pollinator of *L. formosanum* in the study region. Nocturnal visitors were shown to be highly effective pollinators, while diurnal visitation resulted in very low or no seed production (fig. 2). *Agrius convolvuli* was far and away the most abundant nocturnal pollinator observed in these populations (table A1). The finding of lepidopteran scales on flowers in the majority of the investigated populations across the distribution range in KwaZulu-Natal is also consistent with *A. convolvuli* being the main pollinator. These scales were also found on *L. formosanum* flowers on Long Tom Pass in Mpumalanga (a neighboring province of KwaZulu-Natal), South Africa (lat. 25°8.480′S, long. 30°37.266′E; 2000 m a.s.l.; S. Morita and M. Profitt, personal observations). Variation in scale deposition among populations suggests that different populations differ markedly in terms of visitation. While there are many other sphingid species in the study area, only one much rarer species, *Coelonia mauritii*, has a tongue that is as long as that of *A. convolvuli*. Other species are probably not able to reach the nectar because of the length of the flower tube.

Although one diurnal species, the fruit chafer beetle *Cyrtothyrea marginalis*, was found in many KwaZulu-Natal populations, it carried an order of magnitude less *L. formosanum* pollen than did *A. convolvuli* (tables A1 and A2), and as such it probably makes a minor contribution to cross-pollination when the hawk moths are absent or rare. All other visitors were either too rare or carried too little pollen to contribute meaningfully to pollination.

The breeding system experiment showed that, at least in some populations, pollinators contribute to fecundity of *L. formosanum* in South Africa, as there was significantly lower reproductive success under autonomous self-pollination than under open pollination and hand pollination in one of the two populations studied (fig. 3; table 1). This is due to insufficient self-pollen grains being transferred autonomously, as *L. formosanum* is completely self-compatible (fig. 3; table 1; Inagaki 2002; Rambuda and Johnson 2004). An earlier study in South Africa (Rambuda and Johnson 2004) and the other population in this study showed nonsignificant trends for lower reproductive success under autonomous self-pollination versus open pollination, and there was also no difference between seed set for these treatments in Japan (Inagaki 2002). It appears, at least in South Africa, that populations vary in their capacity for autogamy.

The seed-to-ovule ratio at Mariannhill was higher under cross pollination than under self-pollination, probably as a result of inbreeding depression during early seed development; however, this did not affect number of seeds per fruit, which suggests compensation for inbreeding depression by high ovule number (Porcher and Lande 2005). Moreover, no inbreeding depression was detected in terms of seed mass, seed abortion, proportion germination, and survival up to 6 mo, which suggests that progeny produced by selfing, whether autonomous or pollinator mediated, contribute to parental fitness and potentially to invasion (J. G. Rodger, M. van Kleunen, and S. D. Johnson, unpublished results).

Hawk moth pollination probably comprises a combination of facilitated within-flower self-pollination, geitonogamy, and cross pollination. It is likely that autonomous self-pollination contributes to invasion in *L. formosanum* by providing reproductive assurance when pollinators are scarce or absent, and when *A. convolvuli* is abundant, it contributes to invasion by increasing seed set above the levels achieved by autonomous self-pollination alone. Outcrossing mediated by these pollinators may also result in increased genetic variation and favorable new gene combinations, enhancing local adaptation. As capacity for autogamy and visitation (using scale deposition as an index) appears to vary between populations (fig. 3; Rambuda and Johnson 2004), so too may the relative importance of pollinators and autonomous self-pollination.

Table 1

Statistical Comparison of Fruit Set and Number of Seeds per Fruit for Different Treatments in Breeding System Experiments Conducted at Richmond (R) and Mariannhill (M)

		Seeds per fruit		
Hypothesis tested, treatments compared, population	Fruit set *P*	*t*	df	*P*
Self-compatibility hypothesis:				
Cross-pollination versus self-pollination:				
R	.116	.15	15	.883
M	[a]	.29	14	.779
Pollen limitation, hand pollination technique:				
Hand pollination[b] versus open pollination:				
R	.29	1.75	24	.93
M	[a]	2.15	33	.039
Autonomous self-pollination:				
Hand pollination versus bagged control:				
R	.209	1.50	27	.144
M	<.001	[c]		
Contribution of pollinators to seed set:				
Open pollination versus bagged control:				
R	.101	.245	19	.809
M	<.001	[c]		

[a] Test omitted because all flowers in both treatments set fruit.

[b] "Hand pollination" refers to the pooled hand self- and cross-pollination treatments.

[c] Test omitted because sample size was three for the bagged control group.

We were unable to establish when selfing occurs relative to outcrossing, although stigma receptivity is reduced at perianth abscission. Because stigmas are receptive and pollen is released on the first day of anthesis, it is likely that self-pollination occurs, possibly incurring a cost of reduced outcrossing and a potential for local adaptation (J. G. Rodger, M. van Kleunen, and S. D. Johnson, unpublished results).

Recent studies indicate that a number of invasive species displaying functionally specialized pollination systems in their native ranges are adapted to pollinators that belong to widespread functional groups (sensu Fenster et al. 2004), which potentially facilitates their invasion. In another autonomously self-pollinating invader, *Nicotiana glauca*, which is adapted to hummingbird pollination in Argentina, pollination by native sunbirds in South Africa also increases seed production above levels achieved by autonomous selfing (Geerts and Pauw 2009; see also Schueller 2004). The carrion fly–pollinated *Stapelia gigantea* (Herrera and Nassar 2009) and the vespid wasp–pollinated *Gomphocarpus physocarpus* (Coombs et al. 2009; Forster 1994), which are both pollinator dependent, have also reestablished their pollination systems after transcontinental invasion. *Senna didymobotrya* is self-compatible, but it requires buzz pollination by large bees. It is pollinated by *Xylocopa flavorufa* in its invaded range in South Africa (van Kleunen and Johnson 2005), by *Xylocopa pubescens* in horticulture in Israel (Dulberger 1981), and presumably by *X. flavorufa* and other *Xylocopa* species in its native range in tropical Africa. Thus, the potential role of pollinators in promoting invasion should not be ignored, even in autonomously self-pollinating, self-compatible plants.

The pollination of *L. formosanum* has not, to the best of our knowledge, been studied in its native range. While we cannot exclude the possibility that other pollinator functional groups contribute to pollination there, it seems unlikely that they are important. Many pollinator functional groups occurring in Taiwan also occur in South Africa, and they were not observed to visit *L. formosanum*. Most nectar feeders are probably excluded by the inaccessibility of the nectar. Although pollen is readily accessible, bees were almost never observed on the flowers (see "Materials and Methods"), suggesting that *L. formosanum* pollen is unattractive to them.

There is a guild of plants in southern Africa (e.g., *Gladiolus longicollis*, *Crinum delagoense*) with similar floral morphologies and scents that represents an apparent convergent evolution for pollination by *Agrius convolvuli* and the much rarer hawk moth *Coelonia mauritii* (Alexandersson and Johnson 2002; Martins and Johnson 2007; S. D. Johnson and R. A. Raguso, unpublished research). *Lilium formosanum* may threaten some of these plants by competing for pollinators. However, *L. formosanum* flowers mainly from January to March, and most of the native plants in the guild finish flowering around the end of January. There may even be an indirect facilitative effect if the presence of *L. formosanum* results in higher abundance of *A. convolvuli* (S. D. Johnson and R. A. Raguso, unpublished research).

Aside from *A. convolvuli* (Pittaway and Kitching 2007), we are not aware of any species of pollinator for which plants have become evolutionarily specialized (Armbruster 2006) and that is naturally so widespread. Generally, plants adapted to a particular functional group will be pollinated by different members of that functional group in their native and introduced ranges. Although *A. convolvuli* belongs to a fairly narrow pollinator functional group of hawk moths with tongue length on the order of 10 cm (Haber and Frankie 1989; Agosta and Janzen 2005), it was the only member of this group that was observed in this study. *Agrius convolvuli* occurs over the entire native and invaded ranges of *L. formosanum* except in North America, where a morphologically similar congener, *Agrius cingulatus*, occurs (Opler et al. 2006). In our study area, *A. convolvuli* also visits the invasive

Ipomoea alba, which is native to the tropical and subtropical parts of the New World, and *Hedychium gardenarianum*, which comes from northern India (S. D. Johnson and R. A. Raguso, unpublished research). The broad geographical range of *Agrius* hawk moths, particularly *A. convolvuli*, means that there is much potential for them to facilitate the invasion of plant species moved from one part of their range to another.

The hypothesis that plants with specialized pollination requirements will be unlikely to find pollinators when introduced to novel environments (Baker 1965, 1974) does not take into account that pollinator functional groups and even particular pollinator species may be very widespread. Studying examples involving specialization to pollinators as diverse as carrion flies, vespid wasps, hawk moths, and nectar-feeding birds allows us to refine the original prediction: specialized pollination requirements should be an impediment to plant invasion to the extent that the pollinator species or functional group (Fenster et al. 2004) involved is restricted in its geographic distribution.

Acknowledgments

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Table A1. Nocturnal visitors to *Lilium formosanum**

Order	Visitor	Number of populations	Number of individuals	Number captured	Mean *Lilium* pollen load	SE of pollen load	Number with pollen	Mean pollen purity	SE of pollen purity
Lepidoptera	*Agrius convolvuli* (Sphingidae)	3	27	7	867.4	533.1	7	0.89	0.05
Lepidoptera	Lepidoptera sp.	1	1	0					

* Number of insects seen visiting during 20 nocturnal observation sessions in 2006 and 2007 with number of populations in which each was found; the total number of each observed and *Lilium formosanum* pollen loads of captured insects.

Table A2. Diurnal visitors to *Lilium formosanum**

Order	Visitor	Number of populations	Number of individuals	Number captured	Mean Lilium pollen load	SE of pollen load	Number with pollen	Mean pollen purity	SE of pollen purity
Coleoptera	*Cyrtothyrea marginalis* (Scarabaeidae, Cetoniinae)	16	70	30	45.8	17.9	27	0.66	0.07
Coleoptera	*Atrichelaphinis tigrina* (Scarabaeidae, Cetoniinae)	0	0	1	908.0		1	1	
Coleoptera	*Pachnoda sinuata* (Scarabaeidae, Cetoniinae)	0	0	1	42.0		1	0.98	
Coleoptera	Hopliini sp. (Scarabaeidae)	1	2	2	4.5		2	0.35	
Coleoptera	Coleoptera sp.	15	28	4	0.5	0.5	2	0.50	
Coleoptera	Mordellidae sp.	1	1	0					

Coleoptera	Eumolpini sp. (Chrysomelidae)	1	1	0					
Coleoptera	Other Coleoptera spp.	3	5	0					
Diptera	Scatopsidae sp.	8	70	35	0.3	0.2	5	0.70	0.20
Diptera	Other Diptera spp.	4	3	0					
Hymenoptera	Formicidae spp.	12	59	8	0.3	0.2	4	0.50	0.29
Hymenoptera	Apocrita sp.	2	4	0					
Hymenoptera	Hymenoptera sp.	1	1	0					
Hemiptera	Aphididae sp.	9	>370	0					
Hemiptera	Heteroptera sp.	1	1	0					
Thysanoptera	Thysanoptera sp	10	31	0					
Blattodea	Blattodea sp.	0	0	1	0.0		0.0		
Orthoptera	Orthoptera sp.	1	1	0					
Dermaptera	Dermaptera sp.	1	1	0					

* Number of insects found in diurnal inspection of 415 flowers in 24 populations in 2007, with number of populations in which each was found; the total number of each observed and *Lilium formosanum* pollen loads of captured insects from 2006 and 2007.

Chapter 6

Minimal inbreeding depression in the invasive geophyte *LILIUM FORMOSANUM*

James G. Rodger, Mark van Kleunen and Steven D. Johnson

Abstract

The ability to self-fertilise should enhance invasiveness in plants because it compensates for shortages of mates and pollinators in novel environments. However, inbreeding depression – a reduction in performance of inbred relative to outbred progeny – may erode this benefit of self-fertilization. In the presence of inbreeding depression, mixed mating will result in qualitative pollen limitation of fitness. However, selection against the deleterious recessive alleles responsible for inbreeding depression may result in their removal, if selfing rate remains high for many generations. *Lilium formosanum* is a self-compatible and autonomously self-pollinating geophyte native to Taiwan and invasive in many parts of the world. In South Africa, where this study was carried out, cross-pollination by hawkmoths accounts for a maximum of one third of seed production, with at least the remaining two thirds coming from self-pollination. To assess whether the reproductive assurance benefit of self-fertilization was eroded by inbreeding depression, we compared number of seeds, mass per seed and subsequent survival and growth of progeny of *L. formosanum* from cross- and self- pollination. We also included open (natural) pollination in our comparisons of seed and progeny performance, to assess the potential impact of inbreeding depression on progeny performance under open pollination. Given a mixed mating system, open pollination should be intermediate between self- and cross-pollination if inbreeding depression is present. We found no difference in the number or mass of seeds arising from self-, cross- and open pollination. Furthermore, there was no evidence of inbreeding depression in germination, survival and growth, both in a common shade-house environment to 26 months from sowing and in the field to 31 months. For plants in the shade-house, there was evidence for inbreeding depression for some populations for proportion of plants flowering in the second year but no evidence for inbreeding depression for flowering in the third year. Overall, these results suggest that deleterious alleles have been substantially purged in South African populations of *L. formosanum* and that self-fertilisation is likely to make a demographically meaningful contribution to invasiveness.

Introduction

It has been hypothesised that ability to self-fertilise contributes to invasiveness in plants by providing reproductive assurance, as plants are likely to experience shortages of mates and pollinators when introduced to novel environments (Baker 1965, 1974, Barrett 2011). Reproductive assurance occurs when cross-pollen receipt is inadequate and self-pollination increases fecundity (Eckert et al. 2006). Although several recent studies have documented positive correlations between ability to self-fertilise and invasiveness (van Kleunen and Johnson 2007, van Kleunen et al. 2008, Hao et al. 2011, Pyšek et al. 2011 but see Sutherland 2004, Burns et al. 2011) it has not yet been shown that this is due to reproductive assurance.

Even with reproductive assurance, being able to self-fertilise is not necessarily beneficial for plants as this benefit of selfing may be negated by inbreeding depression and seed discounting (Herlihy and Eckert 2002). Inbreeding depression – poorer performance of inbred relative to outbred progeny – is near universal in flowering plants, although its intensity varies greatly (Darwin 1876, Husband and Barrett 1996, Duminil et al. 2009). Adaptations that allow self-pollination often reduce outcrossed seed production to levels below those which could be achieved from the amount of cross-pollen received when self-fertilisation pre-empts ovules and resources that would otherwise have been devoted to cross-fertilisation, reducing production of outcrossed seed (seed discounting). Seed discounting may reduce fecundity if inbreeding depression is expressed during seed development and reduce mean progeny performance if inbreeding depression is expressed later. Seed discounting can occur if self-pollination occurs before or during the period when flowers are able to receive cross-pollen but not if self-pollination occurs only after the opportunity for cross-pollination has passed (Lloyd 1992). If seed discounting and inbreeding depression occur in combination, they constitute a cost of self-fertilisation. Although a number of studies have documented reproductive assurance benefits of selfing (Eckert et al. 2006), few have assessed inbreeding depression and seed discounting in order to determine whether self-pollination confers a net benefit (Herlihy and Eckert 2002, Kalisz et al. 2004, Vaughton and Ramsey 2010). For invasive plants, only a handful of studies have tested whether selfing increases fecundity (Schueller 2004, van Kleunen et al. 2007, Chapter 7) or results in inbreeding depression (Sloop et al. 2009).

The transition from self-incompatibility to self-compatibility is one of the most frequent evolutionary events in flowering plants (Stebbins 1974, Igic et al. 2008).

Reproductive assurance is regarded as one of the main selective forces driving this transition but inbreeding depression is a barrier that must be overcome for self-compatibility to evolve (Husband and Schemske 1996; Pannel and Barrett, 1998). Inbreeding depression results principally from the accumulation of deleterious recessive alleles in outcrossing populations, although heterozygote advantage probably also plays a role to some extent (Cheptou and Donohue 2011). Selfing exposes deleterious alleles to selection, which can result in them being removed (purged) if selfing rates remain high for several generations, potentially leading to increased selection for selfing (Lande and Schemske, 1985, Barrett and Charlesworth 1991, reviewed in Crnokrak and Barrett 2002). This will result in selfing becoming even more advantageous, potentially leading to evolution of almost complete selfing (Lande and Schemske, 1985). This transition is generally accompanied by reductions in flower size, anther-stigma separation and pollen to ovule ratios (Sicard and Lenhard 2011).

Selection favours selfing not only because of reproductive assurance but because plants that can self-fertilise will act as fathers and mothers to their own seeds, as well as siring seeds on other plants (the genetic transmission advantage, Fisher 1941). Only taking into account the transmission advantage, inbreeding depression, and the reduction in availability of ovules for outcrossing as a result of self-fertilisation, models predict that selection favours increased selfing when fitness of selfed progeny is greater than half that of outcrossed progeny, particularly when high population selfing rates limit availability of ovules for pollen to cross-fertilise (Lande and Schemske, 1985). This leads to the further prediction that only complete outcrossing and complete selfing will be evolutionarily stable (Lande and Schemske, 1985). However, there is much empirical evidence for the stability of mixed mating systems in nature (Holsinger 1991; Goodwillie et al. 1995), so more recent theoretical work has invoked additional factors. Taking into account reproductive assurance (Morgan and Wilson, 2005), for instance, can help explain mixed mating when inbreeding depression is relatively high. On the other hand, Holsinger (1991) presents a model showing that when an increase in selfing is concomitant with a decrease in outcrossing, as might result from reduced flower size, mixed mating systems can be maintained even in the absence of inbreeding depression.

In populations with low selfing rates, plants generally display inbreeding depression in all stages of life from seed development and germination through growth, survival and reproduction. However, in habitually selfing populations inbreeding depression is greater in later stages (Husband and Schemske 1996, Angeloni et al. 2011). This is likely because alleles which are highly deleterious and expressed earlier in life are more easily purged than less recessive, more mildly deleterious ones expressed later (Angeloni et al. 2011). Alternatively, inbreeding depression may only be apparent in later life because it is masked by maternal effects in early stages (Wolfe 1993). Therefore, it is important to include later life-history stages in progeny trials when testing for inbreeding depression.

For answering ecological and evolutionary questions, inbreeding depression should ideally be assessed in natural environments, which are usually more stressful than controlled common environments (Armbruster and Reed 2005). However, microsite variability in the field may obscure genetic effects (Armbruster and Reed 2005). Inbreeding depression may also vary between populations as a result of both genetic and environmental factors (Byers and Waller 1999, Cheptou and Donohue 2011). Progeny trials to assess inbreeding depression should therefore be conducted both in the field, in multiple populations, and in a controlled common environment.

Lilium formosanum is a self-compatible, autonomously self-pollinating geophyte invasive in South Africa as well as Japan, the southern United States and north-eastern Australia. In South Africa autonomous self-fertilisation increases its fecundity threefold relative to the level resulting from pollinator mediated cross-pollination (Chapter 7). Self-pollination occurs both during and after the period when flowers can receive cross-pollen (J. G. Rodger, unpublished data), so seed discounting probably occurs. To evaluate whether self-fertilisation is likely to contribute to its invasiveness, we tested for inbreeding depression by comparing seed mass and subsequent progeny performance following cross- and self-pollination, in a common shade-house environment and in the field in multiple populations. To assess potential effects of inbreeding depression on progeny performance under open (natural) pollination, we also included seed and progeny from open pollination in our trials.

Methods and materials

Study species

Lilium formosanum produces erect, unbranched stems of 0.3-2.5m with strap-shaped, cauline leaves and terminal candelabra-like inflorescences of one to eight flowers. Apart from autonomous self-pollination (Inagaki 2002, Rambuda and Johnson 2004), *L. formosanum* also displays other “weedy-traits” (Baker, 1974) such as fast growth and early reproduction, and tends to occur in disturbed habitats in its introduced range in South Africa and Japan (Hiramatsu et al. 2001). In its introduced range in South Africa, *L. formosanum* is effectively pollinated by the native hawkmoth *Agrius convolvuli* (Rodger et al. 2010) but it still relies on self-fertilisation for two thirds of seed production (Chapter 7). Immature plants produce rosettes of a few narrow, strap-shaped leaves or occasionally erect stems without flowers. Flowering occurs from January to April, although individual populations only flower for about two weeks. Capsules take about ten weeks to mature and contain a few hundred to over a thousand dry, flat, winged seeds. Although germination can take place immediately if sufficient moisture is present, stored seeds may retain viability for at least a year (T. M. Miles, pers. comm.). However, persistence in the seed-bank is very low under natural conditions (J.G. Rodger, unpublished results).

Pollination experiments

Inbreeding depression in self-compatible plants is frequently assessed by carrying out controlled self- and cross-pollination using bagged flowers and assessing performance of resulting progeny over seed development, germination, growth, survival and reproduction (Husband and Schemske 1996, Angeloni et al. 2011). When inbreeding depression is present and autonomous selfing occurs, maternal fitness may be limited by cross-pollen receipt. That is, even though the total amount of pollen deposited is sufficient, because some ovules are fertilised by self-pollen, mean progeny performance is lower than if only cross-pollen was received. This qualitative pollen-limitation can be detected if mass per seed and performance of progeny from natural fertilisation (open pollination) are lower than those from cross-fertilisation.

Controlled pollinations were used to generate seed in six well separated populations (Table S1) in the midlands of KwaZulu-Natal, South Africa. Among these populations, the least separated were 1.8km from each other (Hilton and Cedara) and the most separated were 18.9km from each other (Lions River from Hilton). Controlled cross- and self-pollinations were conducted in these populations and additional flowers were marked for open pollination at the same time. For cross-pollination, flowers were emasculated in bud, covered in nylon mesh bags to exclude pollinators, and pollinated with pollen from another plant at least 2m away, 1-2 days after anthesis. For self-pollination, flowers were bagged while still in bud, pollinated with their own pollen 1-2 days after anthesis, and then emasculated. The numbers of flowers assigned to treatment groups (typically one flower per plant) in each population ranged from 5-12 for cross-pollination, 6-21 for self-pollination and 13-21 for open pollination. In two populations (Richmond and Mariannhill), seed from autonomous self-pollination was pooled with seed from hand-self pollination for analyses of mass per seed and progeny performance (there was no difference in mass per seed between autonomous and hand self-pollination, J. G. Rodger, unpublished results). Fruits were harvested at maturity and mass per seed was obtained by weighing a sample of 20 seeds. Seeds were defined as containing a linear embryo at least half their length, excluding the wing. A study in two of the populations used here (Richmond and Mariannhill) found no difference in the numbers of seeds in fruits arising from cross-and self-pollination (Chapter 5) and this result was unchanged when assessed for all six populations included here (J.G. Rodger, unpublished results).

Common environment trials

Seed from six different populations was sown in a shade house in November 2006 (Table S1). Batches of about 50 seeds (range 4-69, median 49, 98% of samples > 20 seeds, 91% of samples > 40 seeds) were sown in 15 × 23cm trays in the shade house in the University of KwaZulu-Natal botanical gardens on the 23rd of November in 2006. The exact number of seeds in each batch was known and only a single sample was used from any particular treatment and maternal plant (typically only one treatment was applied to each maternal plant). We used 5-17 (median 8) replicates per treatment per population, depending on number of fruits available. Trays were randomised across two benches and kept moist by an automatic irrigation system. Number of plants was scored on the 24th of February 2007, incorporating germination and survival up to three months. An index of size was obtained as length of the longest leaf × number of leaves for four randomly chosen plants per tray. A mean was obtained for each tray and this was $\log_2$ transformed to improve normality and homoscedascity prior to analysis. Only 4 plants were measured per tray as measurement was rather time-consuming. We applied 5ml of 2:3:2 (N: P: K ratio) fertiliser to each tray in February 2007. In December 2007, 12 months from sowing, plants were separated from their potting medium and number of plants was counted again. For each tray, up to six plants were potted up into 15cm plant pots at this point. Estimates of mean bulb, root and aerial mass per plant at 12 months were obtained for each tray, providing a measure of size based on all surviving progeny, rather than a subsample. For each tray, bulb, root and aerial mass of each of the plants potted up was estimated by regression from aerial and bulb dimensions (see appendix). The remaining plants in each tray were separated into roots, bulbs and aerial parts. For each tray estimated masses of plants potted up was added to measured mass of the remaining plants and this sum was divided by the number of plants to obtain mean mass per plant. Each plant potted up was given 5ml of 2:3:2 fertiliser at potting up (12 months) and one year later (24 months). Plants were scored for survival at the beginning of January 2009, 25 months from sowing. Flowering occurred twice, in the second year between 16 and 22 months and in the third year between 25-26 months from sowing. In each case presence or absence of flowers was scored and in the second flowering event, number of flowers was recorded.

Field trials

In 2006, seed from five of the six populations used for common environment trials was sown in the field (Table S1). In each case, seed was sown in the population of origin, except for seed from Cedara, which was sown at another site, about 20km away (Lions River). This was done because of time constraints: growth trials had to be conducted at Lions River for demographic analyses; seed from cross- and self- pollination were not available from that site and it was impractical to conduct trials at both sites. Sowing was conducted between the 16th and 29th of September 2006. Samples of about 50 full seeds (range 17-73, median 50, 99.6% of samples > 20 seeds, 94% > 40 seeds) were sown, each in a 5cm diameter subplot, by scattering seeds onto the surface and covering lightly with soil and leaf litter. A metal peg with a numbered metal tag was placed in the centre of the area where each sample was sown. The exact number of seeds sown was known in each case. As we had limited numbers of fruits available, for about half the fruits we sowed two samples of seed, each in a different plot. Samples of seed were grouped in 11 to 13 plots in each population and samples were sown about 45cm apart in a grid. Results were obtained for 5-17 samples (median 8) per treatment per population in the first census. Sample sizes for each treatment in each population in different censuses shown in Table S1.

Establishment of plants from natural seed dispersal is a potential source of error in sowing trials in natural populations. To assess whether this might influence results of our experiments we included two control subplots, in which we did not sow seed, in each plot. The number of seedlings was counted at 3 months (5th-20th December 2006). Only seedlings within a circle of 10cm diameter, centred on the tag, were scored. Number of leaves and length of longest leaf were measured for four random seedlings, if available. An index of plant size was calculated as $\log_2$ (number of leaves × length of longest leaf). Further surveys of survival and size were conducted in four populations at 7 months (May 2007), 18 months (April 2008) and 31 months (May 2009) (Table S1). At 31 months, a few plants were no longer in rosette stage but had produced erect stems, some of them with flowers. The comparison of size for May 2009 was restricted to plants still in the rosette stage. Very few plants established in control subplots relative to subplots in which seeds were sown, and there were more plants in sowing than control subplots in all years and populations except Richmond in 2008 and Richmond and Baynesfield in 2009 (J. G. Rodger, unpublished results). Omitting these populations from analyses in these years did not affect significance of pollination treatments (J. G. Rodger, unpublished results). We are therefore confident that plants establishing from natural seed dispersal were not an appreciable source of error in our experiments.

Statistical analyses

Field and common environment experiments were not compared formally as they differed in sowing and census dates. However, the following overall measurements of performance were calculated, pooling all populations and pollination treatments: survival to 18 months in the field (proportion of plants surviving); survival to 24 months in the shade-house (proportion of plant surviving to potting up at 12 months × proportion plants surviving from 12-24 months after potting up); proportion of surviving plants flowering at 31 months in the field and at 16-22 months and 25-26 months in the shade house.

We assessed inbreeding depression in germination, survival and flowering with in generalised linear models. Significance was evaluated from quasi-F values in an accumulated analysis of deviance, analogous to a type I ANOVA. In this approach, terms are added to the model sequentially and quasi-F values calculated from change in deviance are tested against the F-distribution. These models assume that residual deviance is equal to residual degrees of freedom and quasi F-values are therefore usually obtained by dividing mean change in deviance by one. Where residual mean deviance exceeded residual degrees of freedom the model was considered overdispersed and quasi-F values were corrected for overdispersion by dividing mean change in deviance for each effect by the mean change in deviance of the appropriate error term, analogous to calculation of F-values in a mixed-model ANOVA (van Kleunen et al. 2000, Payne 2011a). Where models were underdispersed (residual change in deviance < residual degrees of freedom) no action was taken (Zuur et al. 2009). Mass per seed and size of progeny were analysed using REML (restricted maximum likelihood) analysis of variance, to accommodate unbalanced sample sizes.

Separate analyses were performed for the different censuses in the shade-house trials because a fraction of plants were potted up after the second census and subsequent measurements were conducted on these plants only. Measurements before and after potting up are conducted on different units (see ‘Common environment trials’ above). For field progeny trials, separate analyses were conducted for each census as data were not available for all samples at all censuses; as not all plots that were not found in every census; and in the case of size, low cumulative survival reduced sample size for plant size in later censuses. All analyses were performed in Genstat 12 (Genstat 2009).

For generalised linear models of survival at 3 and 12 months in the shade-house, a logit link function was used with number of plants present as the response and number of seeds sown as the binomial total. The following terms were entered (in this order): block, mean mass per seed, population, pollination treatment and pollination treatment-by-population. This order allowed us to control for maternal effects via seed mass, before testing for the effect of population. We included mass per seed to control for maternal effects which can obscure inbreeding depression (Wolfe 1993). Although, mass per seed could also be affected by inbreeding depression, it did not differ significantly between pollination treatments (see Results). Results for inbreeding depression were also qualitatively identical when it was excluded (J.G. Rodger, unpublished results). Maternal plant was not entered as a factor as seed from each maternal plant was sown in a single tray and survival was measured for the whole tray as a proportion. Pollination treatment was tested against its interaction with population. Survival after potting up (from 12 months to 24 months) and flowering as a binary variable at 15-21 months and 25-26 months were also analysed in generalised linear models for binomial data with a logit link function. Number of flowers at 25-26 months was analysed in a generalised linear model for Poisson data with a logarithm link function. The following terms, entered in this order, were included: bulb diameter at potting up, population, pollination treatment, population-by-pollination treatment and maternal plant. Maternal plant was included here as four individuals were potted up per maternal plant. Bulb diameter at potting up did not differ systematically between pollination treatments (J.G. Rodger, unpublished results) and was included to reduce noise for performance subsequent to potting up. Results were qualitatively identical when it was excluded from analysis (J.G. Rodger, unpublished results). The significance of pollination treatment was tested against its interaction with population.

Cumulative survival in the field was analysed similarly to survival in the shade-house, with terms entered in the following order: population, plot within population, mass per seed, pollination treatment and pollination treatment-by-population. This allowed maternal effects via mass per seed to be tested after controlling for environmental effects and the effect of treatment to be tested after controlling for both environmental and maternal effects. Pollination treatment was tested against its interaction with population. Population was entered first in analyses of field survival because we wanted to know whether survival differed between populations in general. Maternal plant was not included as a factor as, in about half of all cases, only one sample was sown per parent.

Mass per seed and size of progeny in shade-house and field trials were assessed for inbreeding depression using REML analysis of variance, because this better accommodates unbalanced designs than standard ANOVA (Payne et al. 2011). Data were unbalanced with respect to pollination treatment because different numbers of fruit were available for cross, self and open pollination and with respect to plot because of variation in survival between plots. In these analyses fixed terms were sequentially added to a model containing the random terms and the significance of these fixed terms was evaluated from Wald F-statistics. The random terms were sequentially added to the model containing all the fixed terms, and the significance of these random terms was evaluated by comparing change in residual deviance to a chi-squared distribution with one degree of freedom (Payne et al. 2011). In the analysis for mass per seed, pollination treatment was included as a fixed factor and population and the population-by-pollination treatment interaction as random terms. For analyses of progeny size, number of plants in each tray (shade-house experiment) or in each subplot (field experiment) was included as a predictor to take into account any facilitative or competitive effects of seedlings on each other. For analyses of size at three months and biomass at twelve months in the shade-house, terms were added in the order block, mass per seed, number of plants and pollination treatment for fixed terms and population and population-by-pollination treatment for the random effects. Biomass measurements at 12 months were $\log_2$ transformed prior to analysis to improve normality of residuals. For analyses of size in the field, fixed terms were added in the order mass per seed, number of plants and pollination treatment; random terms were added in the order population, then population-by-pollination treatment. Due to mortality, differences in size between cross and self-pollination could only be compared for the Richmond and Cedara populations in 2008 and only for the Richmond population in 2009.

Results

General performance

Pooling all treatments and populations 2.24% of seeds sown in the field gave rise to surviving plants at 18 months whereas in the shade-house at 24 months this figure was 17.3%. In the field 6.7% of surviving plants flowered at the 31 month census and none in earlier censuses whereas in the shade-house 25% of surviving plants flowered from 16-22 months and 92% of surviving plants flowered at 25-26 months (See also Figs 1-3).

Mass per seed

Pollination treatment did not significantly affect mass per seed (Table S2; Fig. 1).

Performance in common shade-house environment

There was almost no evidence of inbreeding depression from the progeny trials in the shade house up to flowering. The effects of pollination treatment and the pollination-by-population interaction were not significant for germination and size at three months; survival and biomass at 12 months; survival after potting up (12-24 months) or flowering at 25-26 months (Tables 1, S3-S12; Figs 2, 3). The only test consistent with inbreeding depression was for proportion of plants flowering at 16-22 months, where the interaction between population and pollination was significant (although the main effect of pollination was not; Tables 1, S9; Fig 3a). Results for some populations, particularly Richmond, were consistent with inbreeding depression (ie cross > open > self; Fig 3a). However, the Thornville population in fact showed the reverse pattern and this would have contributed to the significance of the interaction. We therefore analysed the data again, excluding Thornville. As the interaction remained significant, we interpret this as evidence for inbreeding depression in some populations for flowering between 16 and 22 months.

Performance under field conditions

There was no conclusive evidence of inbreeding depression in progeny performance in the field up to three years from sowing. The effects of pollination treatment and the pollination by population interaction were not significant for germination and size at 3 months, cumulative survival and size at 7 months, cumulative survival and size at 18 months and size at 31 months (Tables 2, S13-S20; Fig. 4). For cumulative survival to 31 months, there was a significant pollination treatment by population interaction, although the main effect of pollination treatment was not significant (Table S19, Fig 4g). For the three populations with some surviving plants, survival was lower for plants from self- than cross-pollination for Hilton – as expected under inbreeding depression but treatments were similar for Richmond However survival was higher for progeny from self- than cross pollination for Cedara (the opposite to what would be expected under inbreeding depression; Fig 4 g). As the interaction became non-significant when Cedara was excluded we do not regard this as conclusive evidence for inbreeding depression.

Discussion

We obtained very little evidence for inbreeding depression in *Lilium formosanum* in its invasive range in South Africa. Cross-pollination did not result in greater seed mass (Fig 1a) or better germination, growth or survival of progeny than self-pollination, either in the common, shade-house environment (Figs 2, 3) or the field (Fig 4). Progeny from cross-pollination did not have a higher likelihood of flowering or have a higher number of flowers than those from self-pollination in the shade-house between 25 and 26 months (Fig. 3). The only reasonably convincing evidence was for flowering in some populations in the shade house between 16-22 months (particularly Richmond in Fig 3a) where a higher proportion of crossed than selfed progeny flowered, with open pollinated being intermediate.

Although results from field and common environment experiments were not compared formally, as they differed in sowing and census dates, performance was generally much lower in the field than in the shade house, strongly suggesting that the field is the more stressful environment (Figs 1-3). In previous experiment number of seeds per fruit did not differ between cross and self-pollination (Chapter 5; J.G. Rodger, unpublished data). Emasculation experiments showed that self-fertilisation increased fecundity of *L. formosanum* threefold in its introduced range in South Africa (Chapter 7). In light of the apparent complete lack of inbreeding depression throughout its life-history, reproductive assurance may well contribute to invasiveness.

In contrast to our findings, Inagaki (2000), working on *L. formosanum* in its introduced range in Japan, found that seeds arising from self-pollination experienced lower germination (16%) than those arising from cross-pollination (47%). However, as also found in this study, growth did not differ between selfed and crossed progeny (Inagaki 2002). The apparent difference in inbreeding depression at the seed germination stage between South Africa and Japan could reflect differences in purging among source populations, as mating system varies among native populations in Taiwan (Hiramatsu et al. 2001), or differences in purging and bottlenecks following introduction to the two regions. Similarity in performance between progeny from cross- and self- fertilisation may be due not only to selective purging, but also to fixation of deleterious alleles by inbreeding. However, this would result in generally poor performance and would probably preclude invasion, so this is an unlikely scenario for successful invaders such as *L. formosanum*.

Although little information is available on inbreeding depression and contribution of self-fertilisation to fecundity in invasive plants, most of it is consistent with the hypothesis that offspring arising from selfing contribute demographically to invasiveness. The lack of inbreeding depression found in this study in conjunction with high levels of reproductive assurance (Chapter 7), suggests that selfing is likely to contribute to population growth and spread, and hence invasiveness in *L. formosanum*. *Datura stramonium* has high levels of reproductive assurance in its introduced range in South Africa (van Kleunen et al. 2007) and generally low or absent inbreeding depression in its introduced range in California inbreeding suggesting that reproductive assurance through selfing contributes to its invasiveness too (Stone and Motten 2002). Inbreeding depression has also been found to be low in *Carpobrotus acinaciformis*, and absent in *C. edulis* (Suehs et al. 2004) and *Rhododendron ponticum* (Stout 2007) in their introduced ranges. Evidence for concomitant evolution of self-fertilisation and purging of inbreeding depression during invasion by intertidal grass species in the genus *Spartina* provides compelling evidence that reproductive assurance through selfing is advantageous during invasion. (Daehler 1999, Sloop et al. 2009). On the other hand, high inbreeding depression in the autonomously self-fertilising tree *A. dealbata* suggests that selfing probably does not contribute to invasion in that species (Chapter 3). Therefore, further investigation is needed to establish whether invasive flowering plants are generally characterized by lower than average levels of inbreeding depression than other angiosperms, and whether reproductive assurance through self-fertilisation is generally important for invasiveness in self-compatible plants.

Lilium formosanum is closely related to the self-incompatible *L. longiflorum* (Nishikawa et al. 1999, Lee et al. 2011), which occurs on the Ryukyu archipelago of Japan to the North of Taiwan (Hiramatsu et al. 2001). As self-incompatibility is basal in most groups of flowering plants, it is likely that *L. formosanum*, or the lineage leading to it, evolved self-compatibility relatively recently (Hiramatsu et al. 2001). *Lilium formosanum* also occurs in more disturbed habitats, and is faster growing than *L. longiflorum* (Hiramatsu et al. 2002), suggesting that self-compatibility may have evolved as part of a weedy life history strategy (Pannell and Barrett 1998). On the other hand these species show adaptations for specialised pollination by hawkmoths, which has been demonstrated in *L. formosanum* (Chapter 5), and reproductive assurance may also evolve in response to greater unreliability of pollen receipt under specialised pollination (Waser et al. 1996, Perez et al. 2009, Marten-Rodriguez and Fenster 2010). Phylogenetic analyses in *Lilium* and other groups are desirable to shed light on factors associated with evolution of self-compatibility (e.g. Perez et al. 2009).

Although *L. formosanum* is almost certainly self-compatible in its native range, the almost complete absence of inbreeding depression observed in this study and substantial reproductive assurance revealed by emasculation experiments (Chapter 7) raise the question of why complete selfing has not evolved in these populations in the introduced range in South Africa. *Collinsia verna* similarly experiences low levels of inbreeding depression ($\delta \approx 0.1$) and significant reproductive assurance (ability to self increases seed production by about 8%) (Kalisz et al, 2004). It seems most likely that outcrossing is maintained in cases such as these through selection on male function, as suggested Holsinger (1991). However, in *L. formosanum* variation in stigma anther separation has been observed both within and between populations in the study region (J. G. Rodger, unpublished results) with some populations have relatively small flowers and a little or no stigma anther separation. This suggests that some populations in the introduced range may, in fact, have evolved to higher levels of selfing. If this is indeed the case, investigation of selection on selfing and outcrossing in different population of *L. formosanum* would likely provide important insights into the maintenance of mixed mating systems (Goodwillie et al 2005).

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Tables

Table 1. Tests for inbreeding depression in common environment trials with *L. formosanum*. ns = not significant, ** = $p < 0.01$.

Date	Performance measure	Pollination	Pollination-by-population
3 months	Germination	ns	ns
	Size	ns	ns
12 months	Cumulative survival	ns	ns
	Bulb biomass	ns	ns
	Root biomass	ns	ns
	Aerial biomass	ns	ns
16-22 months	Flowering (yes-no)	ns	**
24 months	Survival from potting up (12-24 months)	ns	ns
25-26 months	Flowering (yes-no)	ns	ns
25-26 months	Flowering (number of flowers, including zeros)	ns	ns

Table 2. Tests for inbreeding depression in field trials with *L. formosanum*. ns = not significant, ** = $p < 0.01$.

Date	Performance measure	Pollination	Pollination-by-population
3 months	Germination	ns	ns
	Size	ns	ns
7months	Cumulative survival	ns	ns
	Size	ns	ns
18 months	Cumulative survival	ns	ns
	Size	ns	ns
31 months	Cumulative survival	ns	**
	Size	ns	ns

Figures

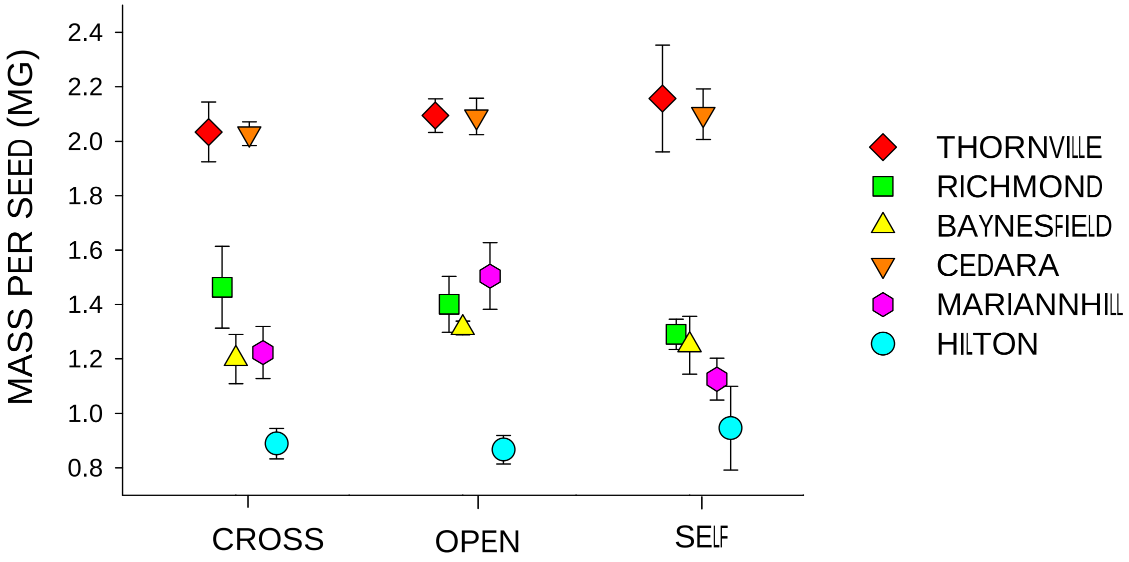

Figure 1 Mass per seed (mean ± standard errors) for seed from cross-, self- and open pollination in *Lilium formosanum* in its invasive range in South Africa.

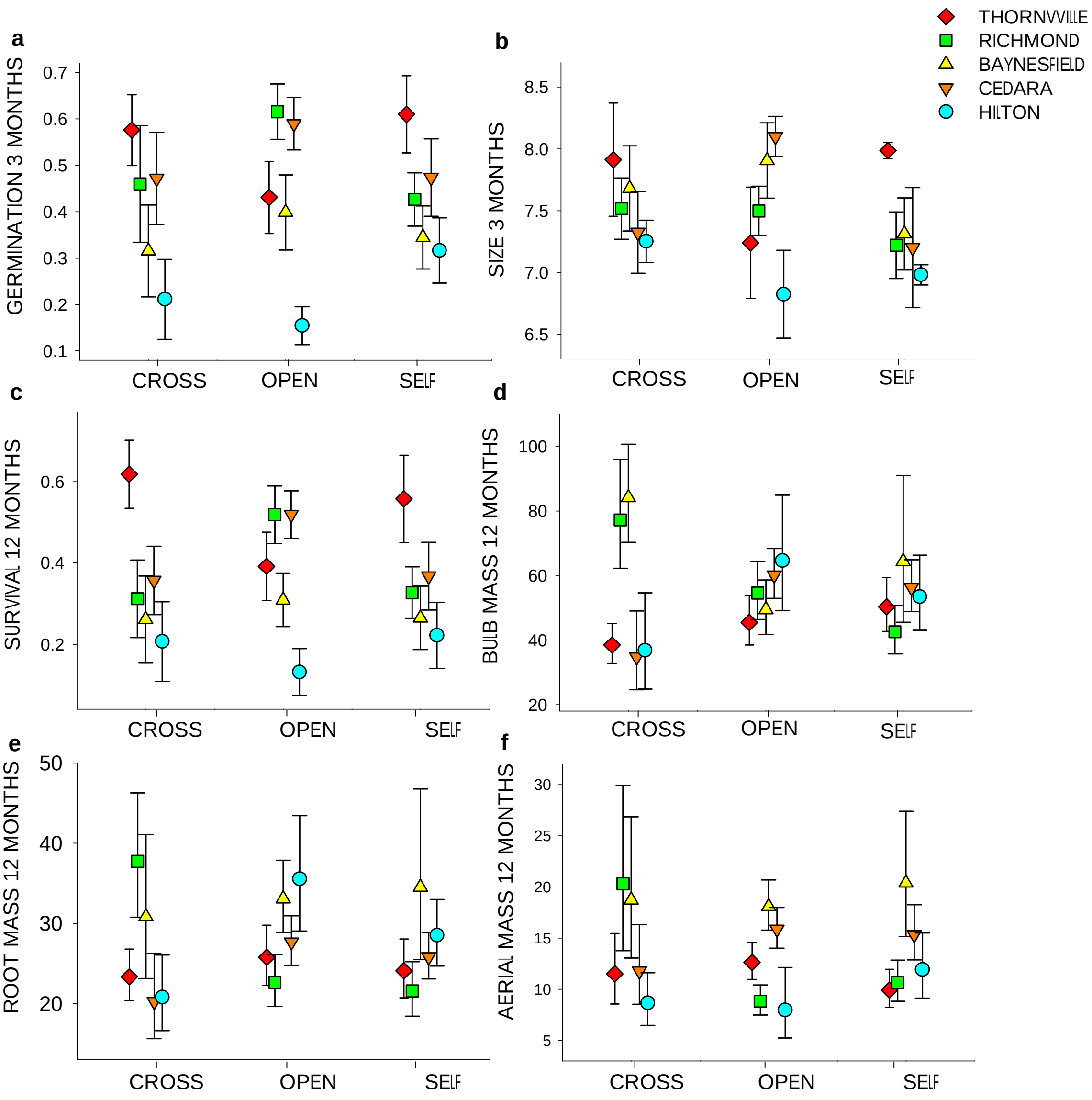

Figure 2 Germination (a) and size (b) at three months and cumulative survival (c) and biomass (d-f) at 12 months for *Lilium formosanum* progeny from cross-, self- and open pollination in the shade-house. For survival estimated marginal means ± standard errors shown, back-transformed from the logit scale. For biomass measurements at 12 months, means and standard errors backtransformed from the $\log_2$-scale shown. Symbols as for Fig. 1.

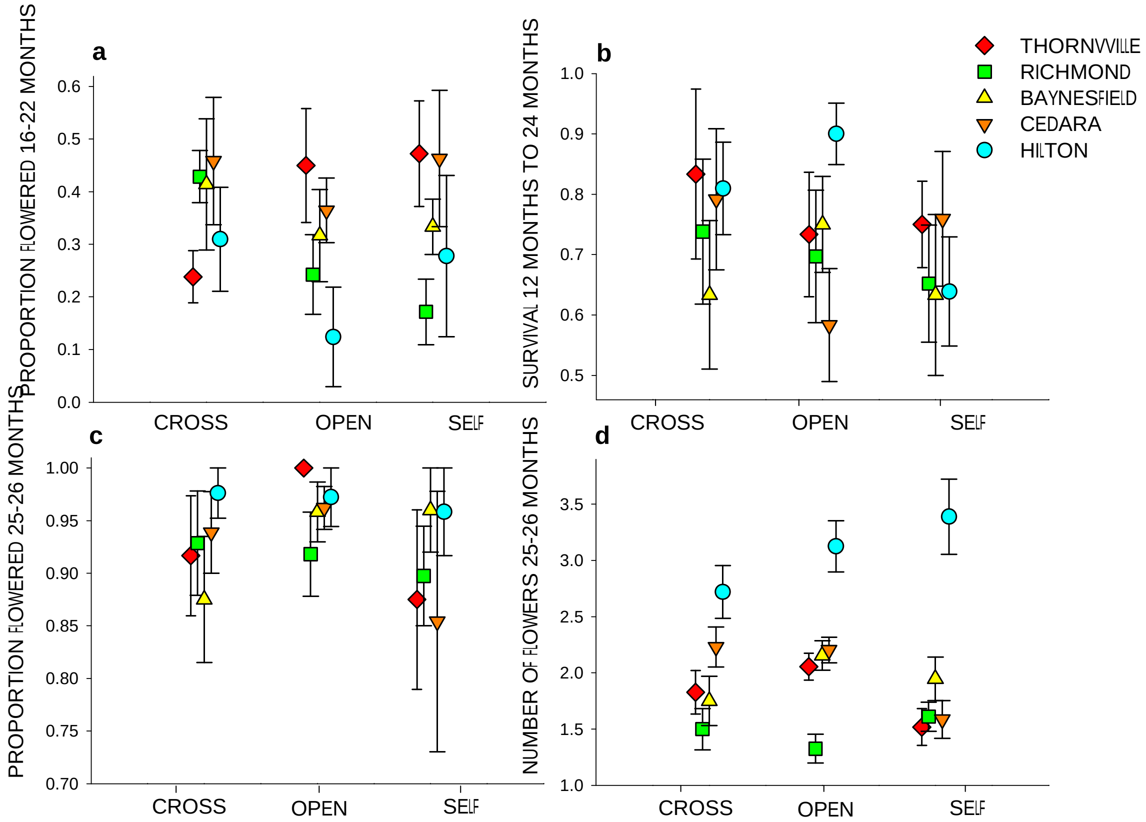

Figure 3 Proportion of *Lilium formosanum* progeny from cross-, self- and open pollination that flowered at 16-22 months (a) and 25-26 months (c), number of flowers at 25-26 months (d) and proportion survival from potting up to 24 months (b). Symbols as for Fig. 1. Means ± standard errors shown

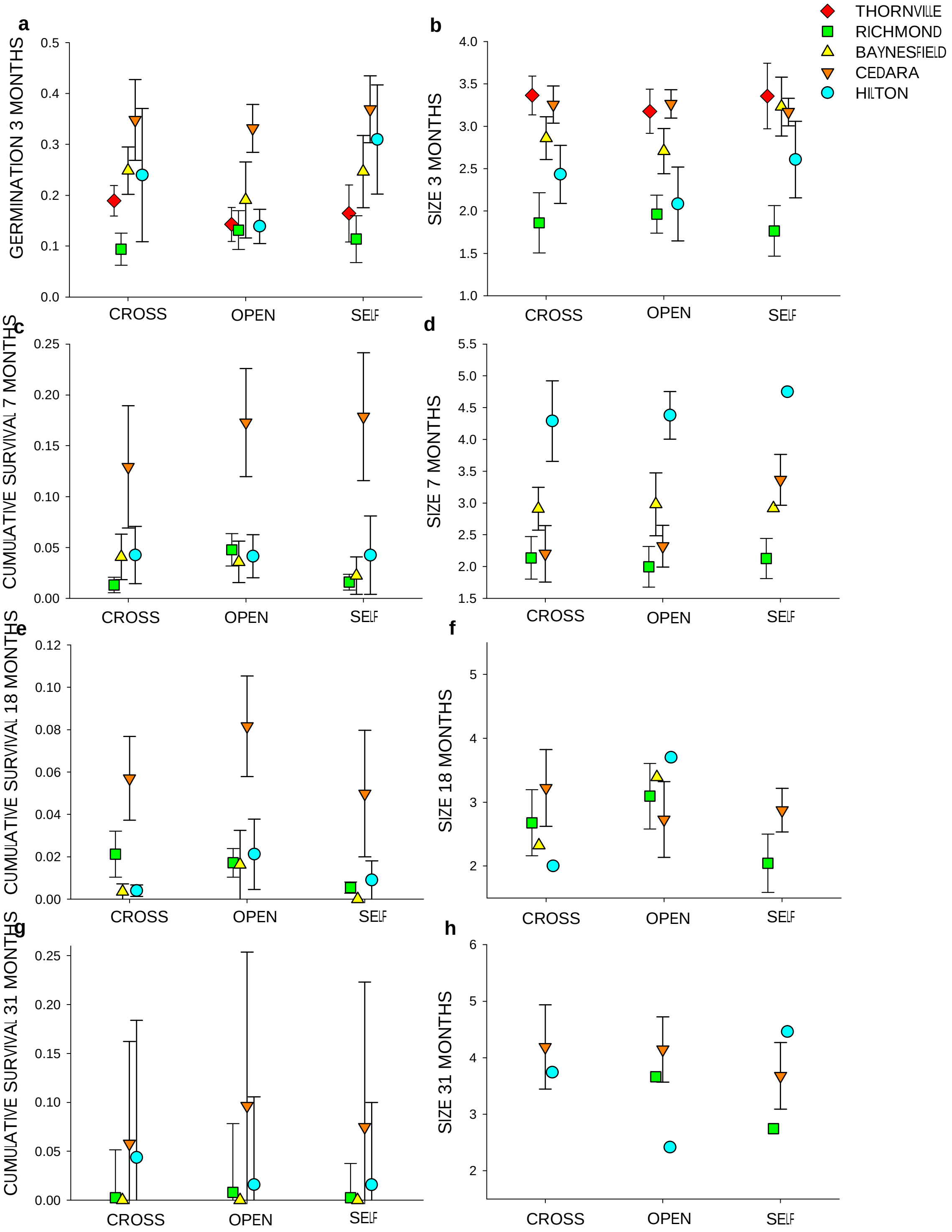

Figure 4 Cumulative survival (a, c, e, g) and size (b, d, f, h) of *Lilium formosanum* progeny from cross-, self- and open pollination in the field, sown in September 2006 and scored at 3 months, 7 months, 18 months and 31 months. Symbols as for Fig. 1. Means ± standard errors shown.

Appendix A. Supplementary tables

Table S1 Sample sizes for analyses of progeny trials to assess inbreeding depression. Populations used were Thornville (Th), 25 plants, 29° 44.312´ S, 30° 22.780´ E; Richmond (Ri), 6000 plants, 29° 52.624´ S, 30° 16.777´ E; Baynesfield (Ba), 1100 plants, 29° 45.151´ S, 30° 21.448´ E; Cedara (Ce), 80 plants, 29° 31.904´ S, 30° 16.117´ E; Mariannhill (Ma), > 1000 plants, 29° 49.415´ S, 30° 18.657´ E and Hilton, (Hi), 3000 plants, 29° 32.383´ S, 30° 17.087´ E. Field trials for Cedara population conducted at Lions River 29° 27.014´ S, 30° 07.113´ E. Populations were located in the KwaZulu-Natal Midlands (South Africa), in disturbed grassland except for the Lions River site, which was on a steep earth bank.

Trial	Performance measure	Age	Replicates	Population	Pollination treatment		
					Cross	Open	Self
Pollination	Mass per seed	-	Maternal plants	Th	7	13	6
				Ri	9	18	21
				Ba	5	21	6
				Ce	12	18	14
				Ma	9	19	10
				Hi	9	35	10
Common Shade-house	Germination	3 months	Maternal plants	Th	7	12	6

environment			Ri	7	12	17
			Ba	5	12	6
			Ce	9	16	11
			Hi	8	6	7
Size	3 months	Maternal plants	Th	7	11	6
			Ri	7	12	16
			Ba	4	9	6
			Ce	8	14	10
			Hi	5	6	7
Cumulative survival	12 months	Maternal plants	Th	7	12	6
			Ri	6	11	17
			Ba	4	12	6
			Ce	9	15	10
			Hi	7	10	6
Biomass	12 months	Maternal plants	Th	7	10	6

			Ri	6	10	16
			Ba	4	9	6
			Ce	8	14	9
			Hi	7	7	5
Flowering	16-22 months	Maternal plants (total progeny)	Th	7 (36)	10 (51)	6 (33)
			Ri	7 (28)	11 (57)	17 (84)
			Ba	7 (35)	10 (57)	5 (27)
			Ce	8 (37)	16 (84)	9 (37)
			Hi	7 (30)	7 (26)	6 (26)
Survival after potting up at 12 months	24 months	Maternal plants (total progeny)	Th	7 (36)	10 (51)	6 (33)
			Ri	7 (28)	11 (57)	17 (84)
			Ba	7 (35)	10 (57)	5 (27)
			Ce	8 (37)	16 (84)	9 (37)
			Hi	7 (30)	7 (26)	6 (26)
Flowering	25-26 months	Maternal plants (total	Th	6 (29)	10 (36)	6 (27)
			Ri	7 (22)	10 (43)	15 (59)

			progeny)	Ba	6 (24)	10 (45)	5 (19)
				Ce	6 (26)	15 (54)	8 (29)
				Hi	7 (25)	7 (24)	6 (18)
Field	Germination	3 months	Subplots	Th	11	21	11
				Ri	13	16	28
				Ba	11	9	7
				Ce	11	11	11
				Hi	7	8	6
	Size	3 months	Subplots	Th	10	8	9
				Ri	7	11	14
				Ba	10	9	5
				Ce	10	11	10
				Hi	7	8	5
	Cumulative survival	7 months	Subplots	Ri	12	17	28
				Ba	13	13	8
				Ce	10	10	10

			Hi	10	13	7
Size	7 months	Subplots	Ri	3	9	9
			Ba	4	3	2
			Ce	5	8	7
			Hi	4	5	2
Cumulative survival	18 months	Subplots	Ri	8	15	19
			Ba	12	9	8
			Ce	10	9	10
			Hi	11	12	6
Size	18 months	Subplots	Ri	3	6	4
			Ba*	1	1	0
			Ce	6	6	5
			Hi*	1	1	0
Cumulative	31	Subplots	Ri	8	13	16

survival	months					
			Ba	5	9	3
			Ce	8	10	11
			Hi	9	8	7
Size	31 months	Subplots	Ri*	0	2	2
			Ba*	0	0	0
			Ce	5	5	5
			Hi*	2	1	1

* not included in statistical analyses due to low sample size.

Table S2 REML analysis of mass per seed for cross-, self- and open pollination for *Lilium formosanum* in its invasive range in South Africa.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Pollination Treatment	2.72	2	10.4	1.35	0.300
Random effects	**Deviance change**		**df**		**p**
Population	218.44		1		< **0.001**
Population × Pollination Treatment	0.27		1		0.603

Table S3 Generalised linear model of germination of progeny from cross-, self- and open pollination in the shade-house up to 3 months. The relationship between mass per seed and survival was positive.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Block	195.01	1	124	195.01	16.97	< **0.001**
Mass per seed	301.99	1	124	301.99	26.28	< **0.001**
Population	116.44	4	124	29.11	2.53	**0.044**
Pollination treatment	4.42	2	8	2.21	0.21	0.816
Population × pollination	84.91	8	124	10.61	0.92	0.500
Residual	1424.67	124		11.49		

Table S4 REML analysis of size of progeny from cross-, self- and open pollination in the shade-house at 3 months. Number of plants and mass per seed were positively related to size.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Block	3.07	1	122	3.07	0.082
Mass per seed	8.9	1	46.3	8.9	**0.005**
Number of plants	75.92	1	120.5	75.92	<**0.001**
Pollination treatment	1.11	2	11.6	0.56	0.588
Random effects	**Deviance change**		**df**		**p**
Population	0.00		1		1.000
Population × pollination	0.75		1		0.386

Table S5 Generalised linear model of cumulative survival of progeny from cross-, self- and open pollination in the shade-house to 12 months. The relationship between survival and mass per seed was positive.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Block	174.49	1	121	174.49	14.21	< **0.001**
Mass per seed	342.34	1	121	342.34	27.88	< **0.001**
Population	96.24	4	121	24.06	1.96	0.105
Pollination treatment	4.59	2	8	2.30	0.13	0.878
Population × pollination	139.51	8	121	17.44	1.42	0.195
Residual	1485.83	121		12.28		

Table S6 REML analysis of mean bulb dry mass of progeny from cross-, self- and open pollination in the shade-house at 12 months.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Block	1.97	1	117.9	1.97	0.164
Mass per seed	0.55	1	43.2	0.55	0.460
Number of plants	0.07	1	117.8	0.07	0.799
Pollination treatment	1.15	2	10.2	0.58	0.579
Random effects	Deviance change		df		p
Population	0.00		1		1.000
Population × pollination	0.23		1.00		0.630

Table S7 REML analysis of mean root dry mass of progeny from cross-, self- and open pollination in the shade-house at 12 months. Root-biomass was negatively related to number of plants surviving.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Block	5.78	1	117.9	5.78	**0.018**
Mass per seed	1.57	1	43.6	1.57	0.217
Number of plants	7.37	1	117.8	7.37	**0.008**
Pollination treatment	3.21	2	10.6	1.6	0.247
Random effects	Deviance change		df		p
Population	0.00		1		1.000
Population × pollination	0.18		1		0.671

Table S8 REML analysis of mean aerial dry mass for progeny from cross-, self- and open pollination in the shade-house in at 12 months.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Block	8.03	1	116.8	8.03	**0.005**
Mass per seed	1.33	1	40.1	1.33	0.255
Number of plants	0.29	1	117	0.29	0.592
Pollination treatment	0.19	2	7.1	0.1	0.909
Random effects	Deviance change		df		p
Population	3.53		1		0.060
Population × pollination	0.03		1		0.862

Table S9 Generalised linear model of flowering at 16-22 months as a binary variable, for progeny from cross-, self- and open pollination in the shadehouse. Bulb diameter was positively related to probability of flowering.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Bulb diameter	70.36	1	514	70.36	70.36	< **0.001**
Population	12.80	4	118	3.20	1.80	0.133
Pollination Treatment	4.91	2	8	2.46	0.89	0.447
Population × pollination	21.99	8	514	2.75	2.75	**0.006**
Maternal plant	209.33	118	514	1.77	1.77	< **0.001**
Residual	501.31	514		0.98		

Table S10 Generalised linear model of survival from potting up at 12 months to 24 months for progeny from cross-, self- and open pollination in the shade house.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Bulb diameter	2.25	1	514	2.25	2.25	0.134
Population	6.71	4	118	1.68	0.60	0.663
Pollination Treatment	2.35	2	8	1.18	0.78	0.490
Population × pollination	12.06	8	514	1.51	1.51	0.152
Maternal plant	329.92	118	514	2.80	2.80	< **0.001**
Residual	423.63	514		0.82		

Table S11 Generalised linear model of flowering from 25-26 months as a binary variable for progeny from cross-, self- and open pollination in the shade-house.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Bulb diameter	3.17	1	355	3.17	3.17	0.076
Population	3.32	4	109	0.83	0.93	0.450
Pollination Treatment	3.05	2	8	1.52	1.75	0.235
Population × pollination	6.99	8	355	0.87	0.87	0.539
Maternal plant	97.51	109	355	0.89	0.89	0.753
Residual	151.63	355		0.43		

Table S12 Generalised linear model of number of flowers at 25-26 months for progeny from cross-, self- and open pollination in the shadehouse.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Bulb diameter	4.00	1	355	4.00	4.00	0.046
Population	49.33	4	109	12.33	24.77	< **0.001**
Pollination Treatment	1.34	2	8	0.67	0.55	0.598
Population × pollination	9.79	8	355	1.22	1.22	0.284
Maternal plant	54.26	109	355	0.50	0.50	1.000
Residual	215.61	355		0.61		

Table S13 Generalised linear model of germination of seed from cross-, self- and open pollination in the field to 3 months.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Population	480.23	4	47	120.06	7.27	< **0.001**
Plot (Population)	776.68	47	114	16.53	1.71	**0.011**
Mass per seed	0.26	1	114	0.26	0.03	0.871
Pollination treatment	0.41	2	8	0.21	0.03	0.970
Population × pollination	53.96	8	114	6.75	0.70	0.692
Residual	1099.64	114		9.65		

Table S14 REML analysis of size of progeny from cross-, self- and open pollination in the field at 3 months. Number of plants and mass per seed were positively related to size.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Mass per seed	11.36	1	58.1	11.36	**0.001**
Number of plants	29.15	1	128.7	29.15	< **0.001**
Pollination treatment	0.43	2	108.7	0.21	0.809
Random effects	Deviance change		df		p
Population	18.33		1		< **0.001**
Population × Pollination	0.00		1		1.000
Plot (Population)	1.07		1		0.301

Table S15 Generalised linear model of cumulative survival of progeny from cross-, self- and open pollination in the field to 7 months.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Population	296.89	3	40	98.97	7.57	< **0.001**
Plot (Population)	522.63	40	91	13.07	3.35	< **0.001**
Mass per seed	1.07	1	91	1.07	0.27	0.602
Pollination treatment	5.59	2	6	2.80	0.49	0.634
Population × pollination	34.03	6	91	5.67	1.45	0.203
Residual	354.79	91		3.90		

Table S16 REML analysis of size of progeny from cross- and self- and open pollination in the field to 7 months.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Mass per seed	0.02	1	47.8	0.02	0.886
Number of plants	2.16	1	49.1	2.16	0.148
Pollination treatment	1.85	2	6.2	0.92	0.447
Random effects	**Deviance change**		**df**		**p**
Population	17.21		1		< **0.001**
Population × Pollination	0.37		1		0.543
Plot (Population)	4.60		1		0.032

Table S17 Generalised linear model of cumulative survival of progeny from cross-, self- and open pollination in the field to 18 months.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Population	115.51	3	36	38.50	7.85	< **0.001**
Plot (Population)	176.49	36	75	4.90	3.11	< **0.001**
Mass per seed	1.57	1	75	1.57	1.00	0.322
Pollination treatment	15.09	2	6	7.55	2.48	0.164
Population × pollination	18.26	6	75	3.04	1.93	0.087
Residual	118.34	75		1.58		

Table S18 REML analysis of index of size for progeny from cross-, self- and open pollination in the field at 18 months.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Mass per seed	2.25	1	21.5	2.25	0.148
Number of plants	4.69	1	24.3	4.69	**0.040**
Pollination treatment	0.38	2	2.4	0.19	0.841
Random effects	Deviance change		df		p
Population	0.07		1		0.791
Population × Pollination	0.44		1		0.507
Plot (Population)	2.95		1		0.086

Table S19 Generalised linear model of cumulative survival of progeny from cross-, self- and open pollination in the field to 31 months (omitting Baynesfield population, which had no surviving plants in any pollination treatment).

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Population	150.86	2	26	75.43	7.21	**0.003**
Plot (Population)	272.08	26	53	10.47	6.38	< **0.001**
Mass per seed	1.98	1	53	1.98	1.21	0.277
Pollination treatment	2.96	2	4	1.48	0.21	0.816
Population × pollination	27.59	4	53	6.90	4.21	**0.005**
Residual	86.88	53		1.64		

Table S20 REML analysis of size of progeny from cross-, self-and open pollination in the field at 31 months (for Cedara population only).

Fixed effects	Wald	ndf	ddf	Wald-F	p
Mass per seed	0.51	1	15.3	0.51	0.486
Number of plants	1.37	1	12.2	1.37	0.264
Pollination treatment	1.17	2	9	0.58	0.578
Random effects	Deviance change		df		p
Plot	3.05		1		0.081

Appendix B. Estimation of dry mass from dimensions of plant parts

Bulb-dimensions and aerial dimensions were measured for six randomly chosen plants per tray, which were then potted up. The remainder of seedlings from each tray were separated into bulbs, roots and aerial parts and dried at 90°C for two weeks before being weighed to within 0.1mg. To obtain estimates of the total dry mass of bulbs, roots and aerial parts for each tray, we added estimates of the dry mass from the plants that were potted up to the measured dry mass of the remaining plants in each tray.

To estimate biomass for plants that were potted up, relationships between plant dimensions (mm) and dry mass (mg) were obtained from a subsample of the plants that were not potted up. For these, bulb and aerial dimensions were measured and roots, bulb and aerial parts were dried separately and then weighed to within 0.1mg. A multiple regression including bulb height and mean diameter ((maximum diameter +minimum diameter)/2) was compared to regressions based on each predictor alone with Schwarze's Bayesian Information Criterion (BIC) and the model including only mean diameter was chosen. The curve fitting procedure in SPSS 15 was used to compare linear, quadratic and cubic relationships and on the basis of BIC a quadratic relationship was chosen. Bulb mass was estimated as -6.879 (mean bulb diameter) + 1.595 (mean bulb diameter)^2 + 10.279 ($F_{2,\,196} = 744.519$, $p < 0.001$, $r^2 = 0.884$). Using the same procedure as for bulb mass, root mass was also estimated from mean bulb diameter. Predictive power was improved by square root transforming root mass and then fitting a straight line relationship. Root mass was estimated as $(0.092 + 0.724\sqrt{mean\ bulb\ diameter})^2$ ($F_{1,\,197} = 329.808$, $p < 0.001$, $r^2 = 0.626$, r^2 calculated in original units = 0.530). BIC indicated that mass of aerial parts for rosettes was best estimated when all the available measurements – number of leaves, length of longest leaf, breadth of longest leaf) – were included in the model. Predictive power was also improved by transforming mass prior to running regression. This transformation was obtained by the Box-Cox method. Shoot mass of rosettes was estimated as ((-0.903 + 0.526 × number of leaves + 0.190 × length of longest leaf + 0.257 × breadth of longest leaf) × 0.225 + 1)$^{4.44}$; ($F_{3,\,170}$=234.769, $p < 0.001$, $r^2 = 0.802$, r^2 in original units = 0.71). Shoot mass of plants with erect stems was estimated as -35.785 + 44.435 × stem basal diameter +1.375 × stem height ($F_{2,\,20} = 22.709$, $p < 0.001$, $r^2 = 0.694$).

Chapter 7

Pollinator failure and reproductive assurance are not correlated with population size and isolation in an invasive lily

James G. Rodger, Mark van Kleunen and Steven D. Johnson

Abstract

An ability to self-fertilise should promote plant invasiveness because it provides reproductive assurance when availability of mates and/or pollinators is inadequate. This benefit of self-fertilisation is predicted to be greatest during bottlenecks of low abundance, as fecundity is more likely to be limited by cross-pollen receipt under such conditions. We investigated this idea in *Lilium formosanum*, a self-compatible hawkmoth-pollinated invasive geophyte, in its introduced range in KwaZulu-Natal, South Africa. We used floral emasculation and controlled hand-pollinations to assess the contributions of self-pollination and pollinator-mediated cross-pollination to fecundity in populations varying in size and isolation, over three consecutive years. Fecundity of emasculated flowers, which could not self-pollinate, was only 33% of that of unmanipulated flowers indicating that, despite having large attractive flowers, *L. formosanum* depends largely on its ability to self-fertilise for fecundity. Fecundity of emasculated flowers was just 29% of that of hand-pollinated flowers, showing that fecundity would be severely pollen-limited if plants were not self-fertilising. Deposition of lepidopeteran scales, an indication of hawkmoth visitation, did not differ between emasculated and intact flowers, suggesting that reductions in fecundity caused by emasculation can not be attributed to hawkmoths avoiding emasculated flowers. Contrary to expectation, this substantial degree of reproductive assurance derived from self-fertilisation was not related to population size and increased with population isolation in only one of three years. Reproductive assurance therefore enhanced fecundity of *L. formosanum* in the introduced range mainly because visitation was generally inadequate, rather than because it was reduced at low plant abundance.

Introduction

It has been proposed that autonomous self-pollination promotes the reproduction and spread of introduced plant species because it assures their fecundity in the event of inadequate availability of mates or pollinators in the introduced range (Baker 1955, 1965; Barrett 2011). While positive associations between ability to self-fertilise and invasiveness of introduced plants have now been reported (Rambuda et al. 2010,van Kleunen and Johnson 2007, van Kleunen et al. 2008, Hao et al. 2010,Pyšek et al. 2011 but see Sutherland 2004, Burns et al. 2011), it is not known if this is because self-fertilisation provides reproductive assurance. Although this hypothesis ultimately rests on whether reproductive assurance provides a demographic advantage for introduced plants, we do not yet know the extent to which introduced plants attain reproductive assurance from self-fertilisation.

Reproductive assurance occurs when cross-pollen receipt is inadequate and self-pollination increases fecundity, although selfing will only be of overall benefit if this advantage is not outweighed by inbreeding depressions and gamete discounting (Eckert et al. 2006). Reproductive assurance is thus the extent to which plants actually rely on self-pollination for fecundity, as a function of pollinator and mate availability in their environment (Lloyd and Schoen 1992; Eckert et al. 2006). Autonomous self-pollination in self-compatible plants – also termed autofertility – confers the highest level of reproductive assurance as it allows plants to reproduce irrespective of both mate and pollinator availability (Lloyd, 1992; Eckert et al, 2006). Although a number of studies using floral emasculations of open-pollinated flowers have demonstrated that ability to self-pollinate does result in reproductive assurance (Kennedy and Elle 2008, Kalisz et al. 2003, Herlihy and Eckert 2002; reviewed in Eckert et al, 2006), to our knowledge this has only been assessed in two invasive species in their introduced ranges. The hawkmoth-pollinated *Datura stramonium* depended on reproductive assurance through self-pollination for 83% of its fecundity (van Kleunen et al, 2007), while the hummingbird-pollinated *Nicotiana glauca* was well visited and did not depend on self-fertilisation for fecundity (Schueller 2004). Further studies are thus needed before general conclusions can be drawn about the importance of self-fertilisation for fecundity of introduced plants.

Self-fertilisation is especially likely to promote plant invasions because, to become invasive, introduced plants have to pass through bottlenecks of low abundance in which cross-pollen receipt is likely to be limited (van Kleunen et al, 2007). Although some introductions involve large number of propagules, in many cases only a few or even single propagules arrive at a particular location (Wilson et al, 2009). Spread frequently involves long-distance dispersal within the introduced range, initially resulting in scattered small populations or single individuals. Smaller and more isolated populations of self-incompatible plants often experience increased pollen limitation both for wind-pollinated (e.g. Davis et al. 2004b) and biotically-pollinated plants. The latter are prone to pollen limitation when plants in small populations are less profitable for pollinators to exploit (Sih and Baltus 1987, Ågren 1996, Groom 1998). Such negative effects of low abundance on individual performance and population growth (eg Groom, 1998), known as Allee effects (Stephens, et al. 1999), can dramatically decrease rate of spread, even preventing invasion altogether (Veit and Lewis 1996, Leung et al. 2004, Taylor et al. 2004). It is thus vital that investigations aiming to assess the importance of reproductive assurance and pollen limitation in the invasion process consider these effects in relationship to plant abundance (Taylor and Hastings, 2005). As pollinator visitation and pollen limitation may vary between plant populations and years, (Herrera, 1988; Campbell et al, 1997; Burd, 1994), studies of reproductive assurance and pollen limitation should also be conducted in multiple populations and years if results are to be generalised (Knight et al, 2005).

Although it was previously thought that plants with specialised pollination systems might often fail to attract suitable pollinators in the novel range (Baker, 1955), such extreme specialisation is rare. It seems that even specialised plants, when introduced, can establish relationships with pollinators that are functionally equivalent to those in their native range (Richardson et al. 2000, Rodger et al. 2011). Nevertheless for plants lacking the ability to self-fertilise, pollen limitation is generally more severe for those that are pollination specialists (Knight et al. 2005), likely because they are more vulnerable to fluctuations in the availability of particular pollinator species (Memmott et al. 2004; Waser et al. 2006; Perez et al. 2009). Reproductive assurance may therefore make a greater contribution to invasiveness of plants with specialised than with generalised pollination systems.

Reproductive assurance implies that mating will consist of variable proportions of outcrossing and selfing, yet theoretical work has not yet resolved how mixed mating can persist in populations without evolving to complete outcrossing or selfing (Goodwillie et al. 2005). Selfing may be selected not only because it provides reproductive assurance but also because plants with the ability to self-fertilise can act as mothers and fathers to their own seeds, as well as siring seed on other plant (the genetic transmission advantage, Fisher 1941). Unfortunately, all models so far developed cover only restricted sets of conditions, limiting their predictive power (reviewed in Goodwillie et al. 2005). Models that including only purging of inbreeding depression, the genetic transmission advantage and the reduction in the availability of ovules for cross-fertilisation due to increased population levels of self-fertilisation predict that only complete outcrossing or selfing should be evolutionarily stable (eg Lande and Schemske, 1985). Models incorporating additional complexity, for instance density and frequency dependence in selection, find conditions under which mixed mating can persist (eg Holsinger 1991, Morgan and Wilson 2005). However, empirical studies assessing the benefits of self-fertilisation have lagged behind theoretical work with relatively few studies assessing the selective benefit of self-fertilisation (Eckert et al. 2006). Investigations of reproductive assurance in invasive species may therefore yield insight into mating system evolution, as well as plant invasiveness.

Lilium formosanum is an autonomously self-pollinating and specialised hawkmoth-pollinated geophyte that is invasive in South Africa. We explored the contributions of self-fertilisation and pollinators to fecundity of this species in its introduced range in South Africa, by asking the following specific questions. 1) What is the magnitude of reproductive assurance derived from self-fertilisation? 2) What would be the magnitude of pollen limitation if *L. formosanum* was self-incompatible? 3) Are the magnitudes of reproductive assurance and potential pollen limitation greater in smaller and more isolated populations of *L. formosanum*?

Methods

Study species and sites

Lilium formosanum is a bulbous perennial with erect, annual stems. Each stem terminates in an inflorescence of 1-8 white, nocturnally scented, trumpet-shaped flowers (Rodger et al. 2010). In South Africa, its principal pollinator is the native hawkmoth *Agrius convolvuli* (Rodger et al. 2010). Studies in its introduced range in Japan (Inagaki 2002) and South Africa (Rambuda and Johnson 2004) show that it is completely self-compatible and can self-pollinate autonomously. In South Africa, ability to self-pollinate autonomously (autofertility) varies – in some populations, fecundity under autonomous self-pollination is equivalent to that under hand-pollination but in others is somewhat lower (Rodger et al. 2010), so pollen limitation is possible. In its native range in Taiwan (where it is endemic), it is regarded as self-compatible. A molecular-marker study in the native range showed that fixation indices (F_{is}) of populations range from 0.032 to 0.901, suggesting a mixed mating system (Hiramatsu et al, 2001). As no inbreeding depression is evident in progeny up to flowering between two and three years of age (Rodger et al. 2010, Chapter 6), the reproductive assurance attained through selfing is likely to be important for population growth and spread.

Experiments were conducted from January to March in 2005, 2006 and 2007. Naturalised populations used for experiments were all in KwaZulu-Natal, South Africa. In the study area, flowering occurs from January to April with individual populations flowering for about two weeks. These populations were mainly in disturbed grassland adjacent to exotic tree plantations or on grassy road verges with a few populations in exotic forests or in otherwise pristine natural grasslands and indigenous forests, and ranged from 10 to 1700m above sea level. Population size was taken as the number of stems with open flowers for populations of up to 250 flowering stems and was extrapolated from a count in a quarter or a half of the area occupied by the population in larger populations. We used 50m as the minimum separation distance between populations, allowing us to span a large range of isolation from almost no separation to over 15km from the nearest population. An index of population isolation was calculated as the $\log_{10}$ of the mean distance (m) to the nearest three populations and population size was taken as the number of flowering stems. Data were obtained from 37 populations in 2005, 20 populations in 2006 and 22 populations in 2007 (Appendix A). Although many populations were accessible or available in only one of the three study years, eight populations were studied in two or three years (Appendix A).

Emasculation and pollen supplementation experiments

Emasculation experiments can be used to distinguish between the contributions of self-pollination (reproductive assurance) versus pollinator-mediated cross pollination to fecundity (Lloyd 1992, Eckert et al. 2006). Fecundity of emasculated flowers, which can not self-pollinate, estimates the contribution of pollinators to fecundity. The reduction in fecundity experienced by emasculated relative to intact flowers is a measure of reproductive assurance (Schoen and Lloyd 1992, Kalisz and Vogler 2003). Flowers were emasculated by opening buds and removing anthers with alcohol-sterilised forceps and for naturally pollinated controls, buds were opened and forceps inserted. We considered it unlikely that emasculation would affect pollinator visitation to *L. formosanum*, as hawkmoths do not forage for pollen, and *A. convolvuli* readily visits emasculated flowers (J. Rodger, pers. obs). To assess this, I inspected stigmas of emasculated and intact flowers for the presence of lepidopteran scales in three populations in 2006 and in four populations in 2007, for 7-17 flowers per treatment per population.

We attempted to obtain three (2005 and 2007) or ten (2006) flowers per treatment per population (Appendix A). In 2005, a single bud was emasculated on each of three plants per population and three flowers were similarly allocated as controls. In 2006, we attempted to obtain ten flowers per treatment per population for a more accurate measure of reproductive assurance at the population level. In 2007, three flowers were used per treatment per population except in four populations for which we needed measures of within-population variation for a separate study. We chose low levels of replication within populations for two reasons. Firstly, we wished to avoid a bias in sampling effort against small populations, which of course have fewer individuals available to receive experimental treatments. This is important because analysis of variance is less robust to unequal variance and non-normality when data is unbalanced (Quinn and Keough, 2002). Secondly, when assessing the relationship between population attributes (eg size; isolation from other populations) and plant performance, the effective replicate is the population, so statistical power is likely to be increased by maximising the number of populations at the expense of sample size per population (Quinn and Keough, 2002).

Control flowers were on separate plants to emasculated flowers in 2005, and on the same plants in other years. It is theoretically possible that in some circumstances emasculating one flower on a plant would reduce pollen receipt and seed set of the remaining intact flowers on the same plant by reducing pollen available for geitonogamous self-pollination. However, this is unlikely to be important in these experiments, firstly because there is effective autonomous self-pollination (see Discussion), and secondly because number of flowers present (in populations) would generally have been adequate to ensure cross-pollen availability, despite emasculations, as population size was seldom very small (Table A1). We also found no evidence for an effect of geitonogamous self-pollination on fecundity (J.G. Rodger, unpublished results).

Even when there is reproductive assurance from selfing, fecundity may be pollen limited, as autofertility may not be sufficient to fertilise all ovules that can be developed into seeds in terms of resource availability. In this case an increase in fecundity following pollen supplementation indicates the extent of pollen limitation (Bierzychudek 1981). Pollen supplementation experiments were used to test for pollen limitation in the same populations used for emasculations, but on different plants, and with the same sample-size regimes (Appendix A). Supplementation consisted of saturating the stigma with outcross pollen from a plant at least 5m away in the same population. Conducting emasculation and supplementation in the same populations also allows one to assess how pollen limited self-fertilising plants would be, if they lacked the ability to self-fertilise. This is indicated by the difference in fecundity between emasculated and pollen supplemented flowers and is termed pollinator failure (Kalisz and Vogler, 2003).

Fruits were harvested for seed counting at maturity, 10-12 weeks after pollination. Seeds were counted if they contained an embryo that was at least half the length of the seed, excluding the wing. For each fruit, I measured the mass of the entire contents and the mass and number of seeds in a random sub-sample containing approximately 50 seeds and used this information to calculate seeds per fruit. All seeds were counted in fruits containing fewer than 50 seeds.

Data Analysis

We calculated the overall reproductive assurance benefit of selfing for each year as the proportional reduction in fecundity caused by emasculation: RA = 100 × (1 – emasculated/control) (Eckert et al. 2006) with fecundity defined as seeds per flower (percentage fruit set × mean seeds per fruit). Means of population mean fecundity for control and emasculated treatments were used in these calculations. We similarly calculated pollen limitation as the proportional increase in seeds per flower caused by supplementation: pollinator limitation = 100 × (1 - control/supplemented) Larson and Barrett 2000). An index of pollinator failure, the estimate of what pollinator limitation would have been in the absence of autonomous self-fertilisation, was calculated as pollinator failure = 100 × (1 - emasculated/supplemented) (cf Kalisz and Vogler, 2003).

Statistical analyses were carried out in Genstat 11th edition (Genstat, 2008). Seeds per flower data (fruit set × seeds per fruit) were non-normally distributed as there were many flowers that did not set fruit, resulting in zero inflation of the data. Fruit set and seeds per fruit were therefore analysed separately. Replication for seeds per fruit was always lower than replication for fruit set as not all flowers set fruit. Fruit set was analysed as a binomial response variable (occurred or did not occur) in generalised linear models incorporating a logit-link function. Separate analyses of the effects of emasculation and pollen supplementation were carried out for each year, as most populations were used in only one year. Fruit set did not need to be analysed for the supplementation experiment in 2007 as there was 100% fruit set in both treatments. Significance was assessed from quasi-F statistics in sequential analysis of deviance, analogous to F-statistics in ANOVA (with type I sums of squares). Models included floral manipulation (emasculation or supplementation) as a fixed factor, population as a random factor, $\log_{10}$ population size and $\log_{10}$ population isolation as covariates and population size-by-floral manipulation and population isolation-by-floral manipulation interactions. A type I approach was used because of the hierarchical structure of the data, with replicates occurring within populations and population size and isolation measured at the population level. Terms were entered in the same order as they appear in Tables 1 and 2. Population size and isolation were tested against population, and other terms were tested against the residual, assuming a dispersion parameter of one. Where models were not overdispersed (ie residual deviance ≤ residual df) we assumed residual MS = 1 for the purposes of calculation of quasi-F ratios and when models were overdispersed we used the model-calculated residual MS (Payne 2009). Model validation consisted of checking residual plots of residuals against fitted values for patterns and in the case of REML analysis of variance, checking normality of residuals (Zuur et al. 2009).

Seeds per fruit was analysed in restricted maximum likelihood (REML) analysis of variance to accommodate differences in sample size between populations. REML analysis of variance used the same statistical design as the generalised linear model for fruit set except they also included the population-by-floral manipulation interaction as a random effect. Significance was evaluated using Wald F-statistics for the fixed terms. For random terms, the change in deviance in the models when a term was dropped was compared to a chi-squared distribution with one degree of freedom (Payne, 2009). Residual plots were examined to check whether assumptions were met.

The effect of emasculation on scale deposition was assessed in a generalised linear model for binomial data with a logit link function, including emasculation and population as factors. Significance was evaluated using analysis of deviance.

Results

Emasculation significantly reduced fruit set and number of seeds per fruit in all three years, with reduction in total fecundity (RA) 67% on average: 90% in 2005, 45% in 2006 and 66% in 2007 (Tables 1, B1-6; Fig. 1a, b). The effect of emasculation was not greater in smaller or more isolated populations except that there was a greater effect of emasculation in more isolated populations for fruit set in 2005 (Tables 1, B1; Fig 3a). In other cases where there were significant population size-by-emasculation and isolation-by-emasculation interactions, these did not arise because fruit set or seeds per fruit declined more for emasculated than control flowers as population size decreased or isolation increased (Tables 1, B1-6; Figs 2, 3).

Pollen supplementation had little effect on fecundity in all three years (Tables 2, B7-B11; Figs 1c, d). Supplementation was only significant as a main effect for seeds per fruit in 2005, increasing total fecundity by 16% (ie pollen limitation = 16%) (Tables 2, B8; Fig 1d). Trends for higher numbers of seeds per fruit in supplemented flowers gave pollen limitation values of 11% in 2006 and 10% in 2007. In 2005, supplementation increased fruit set only in smaller populations as indicated by a significant supplementation-by-population size interaction (Tables 2, B7; Fig. B1). There was no evidence for any effect of population isolation on pollen limitation as the interaction between population isolation and pollen supplementation was never significant (Table 2, B7-B11; Fig B2). Pollen limitation was thus low or absent in all years, and unrelated to plant abundance. Pollinator failure, the pollen limitation that *L. formosanum* would have experienced had it been self-incompatible, was estimated as 92% in 2005, 48% in 2006 and 72% in 2007.

There was no difference between scale deposition on emasculated versus intact flowers (Quasi-$F_{1, 153} = 0.32$, $p = 0.454$), although scale deposition did vary among populations (Quasi-$F_{6, 153} = 39.70$, $p = < 0.001$). The lack of difference between scale deposition in emasculated and intact flowers strongly suggests that results were not biased by an effect of emasculation on visitation and effects of emasculation are due to inadequate visitation rather than avoidance of emasculated flowers by hawkmoths.

Discussion

Lilium formosanum depended on self-pollination for 67% of its fecundity, on average, as shown by the proportional reduction in fruit set and seeds per fruit in emasculated compared to unmanipulated flowers (Fig. 1a, b). This indicates that, despite the presence of an effective hawkmoth pollinator *Agrius convolvuli* (Rodger et al. 2010, Chapter 5), its visitation was generally inadequate, accounting for only 33% of fecundity. However, this was compensated for by high autofertility. Progeny trials have revealed almost no evidence for inbreeding depression in *L. formosanum* in South Africa (Rodger et al. 2010, Chapter 6), so reproductive assurance through self-fertilisation potentially contributes to its invasiveness.

Pollen supplementation increased fecundity by only 12% on average and was significant in only one of three years. Thus, due to high autofertility, *L. formosanum* achieved an estimated 88% of potential fecundity set by resource availability (Fig. 1c, d). Comparison of the fecundity of emasculated and pollen supplemented flowers indicates that had *L. formosanum* been self-incompatible, it would have been highly pollen limited, achieving only 29% possible fecundity, as a result of pollinator failure (pollinator failure = 71%). In fact pollen limitation under self-incompatibility could be even higher, because in populations of self-incompatibile plants, there may be pollen limitation due to cross-incompatibility between related individuals (Fischer et al. 2003, Elam et al. 2007), in addition to pollinator failure.

Pollinator failure appears to have varied substantially among years in this study, with reproductive assurance values of 90%, 45% and 66% and pollinator failure of 92%, 48% and 72% in the three years of the study. Differences between years might have arisen because different sets of populations, with different pollinator abundances, were studied in the three years. However, analysis of the effects of emasculation in the eight populations for which data was available for multiple years indicates that, at least for these populations, differences between years are probably genuine (Appendix D). Results from this study are thus consistent with previous findings that abundance of insect pollinators, including hawkmoths, often fluctuates dramatically from year to year (Herrera 1988, Campbell et al. 1997, Brunet 2009).

For plant species that lack autofertility and have specialised pollination systems, fecundity is vulnerable to fluctuations in abundance of particular pollinator species. However, pollination generalists should be buffered against such effects (Waser et al. 1996, Perez et al. 2009). Results for *L. formosanum* and another specialised hawkmoth-pollinated invasive species, *Datura stramonium*, are consistent with this idea. *Datura stramonium* depended on autonomous self-pollination for 83% of its fecundity in its invasive range in South Africa, although it was unclear whether this was due to inadequate hawkmoth visitation or extensive theft of pollen by honeybees before hawkmoth visitation could occur (van Kleunen et al. 2007).

In contrast, the autonomously self-fertilising shrub *Nicotiana glauca* experienced high rates of visitation by hummingbirds, and there was no evidence of pollinator failure in the introduced range (Schueller 2004). Further studies on reproductive assurance in invasive species with both specialised and generalised pollination systems are thus needed before general conclusions can be drawn on the effects of self-pollination and pollination specialisation on fecundity of invasive plants.

Although the reproductive assurance benefit from selfing in *L. formosanum* was substantial, it was not generally greater at low plant abundance. In 2006 and 2007, smaller and more isolated populations did not have greater reproductive assurance from selfing, and were not more pollen limited (Tables 1, 2). Low plant abundance was only supported as a cause of pollinator failure in 2005, when there was greater reproductive assurance in more isolated populations (Table B3, Fig. 3a). Although fecundity in smaller populations was more pollen-limited in 2005, this is unlikely to be due to lower pollinator visitation because reproductive assurance was not greater in smaller populations in that year (Tables 1, 2, B1, B7). Hawkmoth-scale deposition on stigmas, an indication of pollinator visitation, was scored in 2006 and 2007 and was also not related to population size or isolation (Appendix C). Thus pollinator failure occurred more as a general phenomenon in the introduced range than as an effect of low abundance of *L. formosanum*. These results provide little support for the idea that reproductive assurance from self-fertilisation is most important during bottlenecks of low abundance in invasive plant species.

It is intriguing that population size did not affect reproductive assurance in *L. formosanum*, as decreased pollinator visitation and increased pollen limitation in small populations have been reported in many other plant species (Sih and Baltus 1987, Feinsinger et al. 1991, Kunin 1993, Ågren 1996, Groom 1998, Ward and Johnson 2005, but see van Kleunen and Johnson 2005). The plants in which these Allee effects on fecundity have been detected all have small display sizes and are pollinated by bees, flies and birds. Interestingly, as found here for *L. formosanum*, other hawkmoth-pollinated plants seem relatively resilient to Allee effects. Population size did not affect reproductive assurance through selfing in *D. stramonium* (van Kleunen et al. 2007) or pollinator visitation and fecundity in the native orchid *Satyrium longicaudum*. This may be because hawkmoths are more nomadic in their movements and opportunistic in their foraging than other pollinators (as suggested by Johnson et al. 2009) or because foraging primarily by olfactory rather than visual cues renders them less capable of assessing population size prior to arrival in populations. Given that fecundity in small, isolated populations should have a large impact on the invasion process (Kot et al. 1996, Clark et al. 2001), a greater understanding of how different pollinators respond to plant abundance would allow more refined predictions about the effects of reproductive assurance and pollen limitation on invasiveness.

Although outcrossing rate has yet to be directly estimated in *Lilium formosanum*, available evidence suggests a mixed mating system, weighted towards selfing. Emasculation reduced fecundity by two thirds, on average, in results presented here. Therefore average outcrossing rate could not have exceeded 0.33, although variation in reproductive assurance between populations and years is likely to be mirrored by variation in outcrossing rate. Average outcrossing rate would probably have been lower than 0.33 because autonomous selfing and outcrossing occur simultaneously, making seed discounting likely and because emasculation did not prevent geitonogamous selfing. A molecular-marker study in the native range showed that fixation indices (F_{is}) of populations range from 0.032 to 0.901, also suggesting a mixed mating system with variation between populations (Hiramatsu et al, 2001).

The high level of reproductive assurance observed in this study; the high levels of selfing inferred and the almost complete lack of inbreeding depression detected in progeny trials (Chapter 6) suggest that *L. formosanum* populations in South Africa should be under selection for increased selfing (Lande and Schemske, 1985). In South Africa, there is variation in stigma-anther separation, an important trait associated with selfing ability (Sicard and Lenhard, 2011), both among and within populations (J.G. Rodger, unpublished results). This suggests that these populations can respond to selection for increased selfing or outcrossing and that this may have occurred. However, *L. formosanum* continues to display traits adaptive for cross-pollination by hawkmoths – large pale perianths, abundant nectar and emission of scent at night. Similarly in, *Collinsia verna*, selfing provides reproductive assurance and inbreeding depression is very low, yet complete selfing has not evolved (Kalisz and Vogler 2003, Kalisz et. al, 2004). Investigation of selection and variation in selfing rate in South African populations of *L. formosanum* may yield important insights into maintenance of mixed mating when inbreeding depression is low and selfing high.

Our results are consistent with the ideas that reproductive assurance through autonomous self-pollination contributes to invasiveness, and that pollen limitation is likely to inhibit invasiveness in self-incompatible plants with specialised pollination systems. To evaluate these hypotheses further, demographic modelling should be employed to assess whether population growth and rate of spread would be reduced in the absence of autofertility. Studies on different species will also be needed to assess in general whether reproductive assurance through self-fertilisation promotes invasiveness, and how this is affected by pollination specialisation. Attempts to identify the traits associated with invasiveness contribute to the broader goals of predicting invasiveness of species before they are introduced (Pysek et al. 2007). If reproductive assurance through self-fertilisation is important for invasion, it will be important to compare reproductive assurance and ability to self-fertilise in the native and introduced ranges. If increased ability to self-fertilise commonly evolves in the invasive range (eg Davis 2005), this will need to be taken into account when using self-compatibility and autofertility as predictors of invasiveness.

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Tables

Table 1 Significance levels from generalised linear models for fruit set and REML analysis for seeds per fruit in emasculation experiments across a range of populations differing in size and isolation. Full tables are in Appendix B.

	Fruit set			Seeds per fruit		
Effect	2005	2006	2007	2005	2006	2007
Population size	ns	ns	·	*	ns	ns
Population isolation	ns	·	**	ns	ns	ns
Population	ns	ns	ns	·	***	***
Emasculation	**	*	*	***	*	***
PS × E	ns	ns	·	*	ns	ns
PI × E	*	*	·	·	ns	ns
P × E†				ns	ns	*

· $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$; † Population-by-emasculation interaction not included in fruit set analyses as these were conducted on binomial totals for each treatment in each population.

Table 2 Significance levels from generalised linear models for fruit set and REML analysis for seeds per fruit in pollen supplementation experiments across a range of populations differing in size and isolation. Full tables are in Appendix B.

	Fruit set‡		Seeds per fruit		
Effect	2005	2006	2005	2006	2007
Population size	*	ns	ns	ns	ns
Population isolation	ns	ns	ns	ns	ns
Population†	**	ns	**	***	***
Supplementation	ns	ns	*	ns	ns
PS × S	*	ns	ns	ns	ns
PI × S	ns	ns	ns	ns	ns
P × S†			ns	ns	ns

* p < 0.05; ** p < 0.01; ***p < 0.001; †Population-by-supplementation interaction not included in fruit set analyses as these were conducted on binomial totals for each treatment in each population. ‡2007 analysis not conducted due to 100% fruit set

Figures

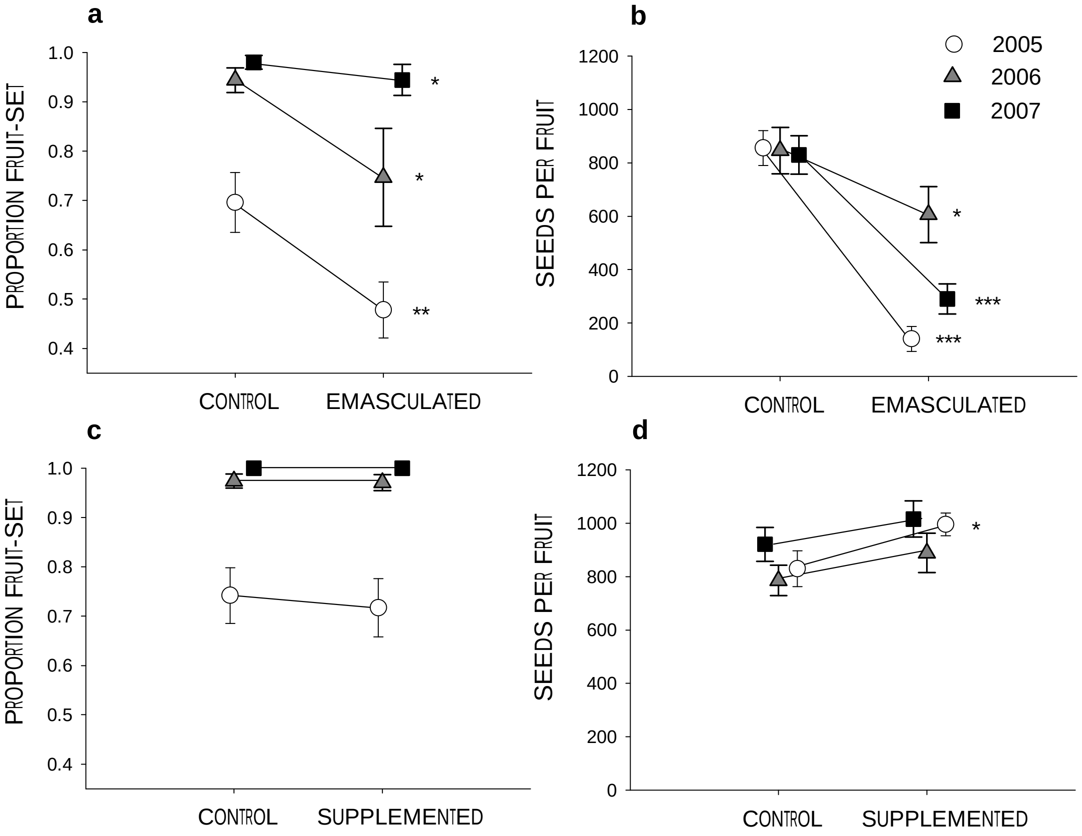

Figure 1 Effect of emasculation (a, b) and pollen supplementation (c, d) on fruit set and seeds per fruit in *Lilium formosanum* in KwaZulu-Natal, South Africa in 2005, 2006 and 2007. Means and standard errors of population proportion fruit set and mean seeds per fruit are shown. Significant effects of emasculation and supplementation indicated as * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

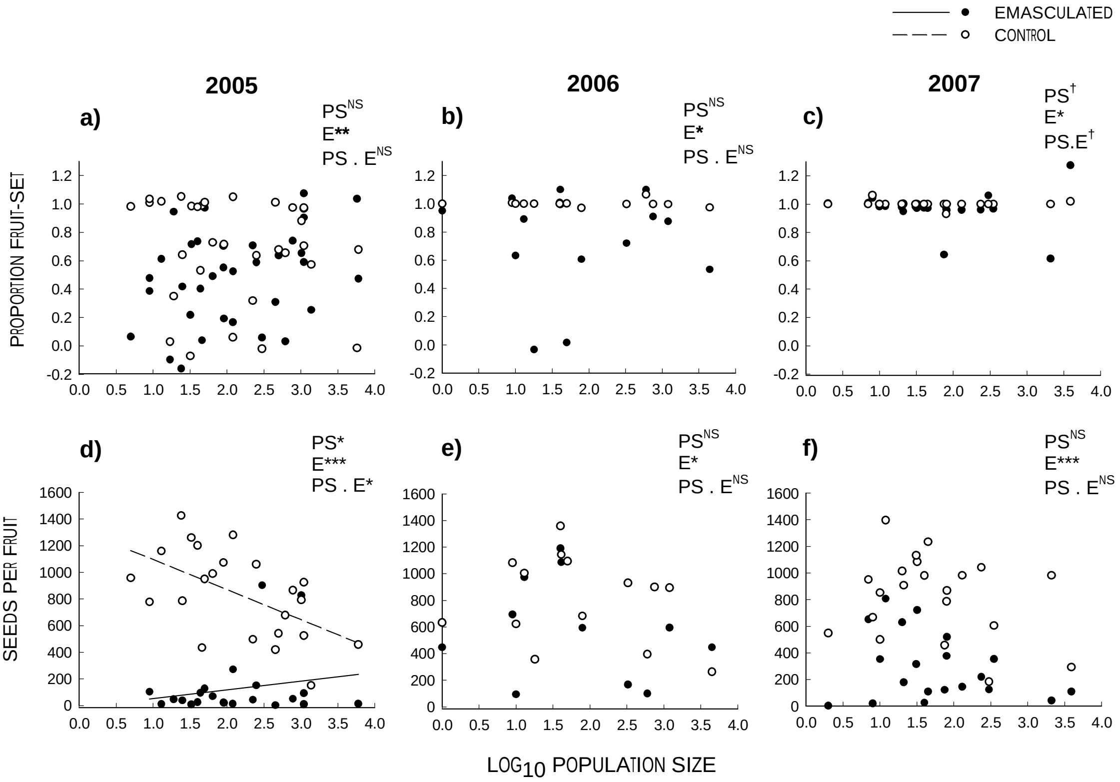

Figure 2 Fruit set (a-c) and seeds per fruit (d-f) of emasculated and intact, naturally pollinated plants in relation to population size for three years. PS = population size, E = emasculation; ns = non-significant; † = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Circles represent predicted values for populations for fruit-set (adjusted for population isolation) and mean populations values for seeds per fruit. Curves for fruit set fitted in generalised linear model using logit transformed data and backtransformed. Regression lines shown for seeds per fruit.

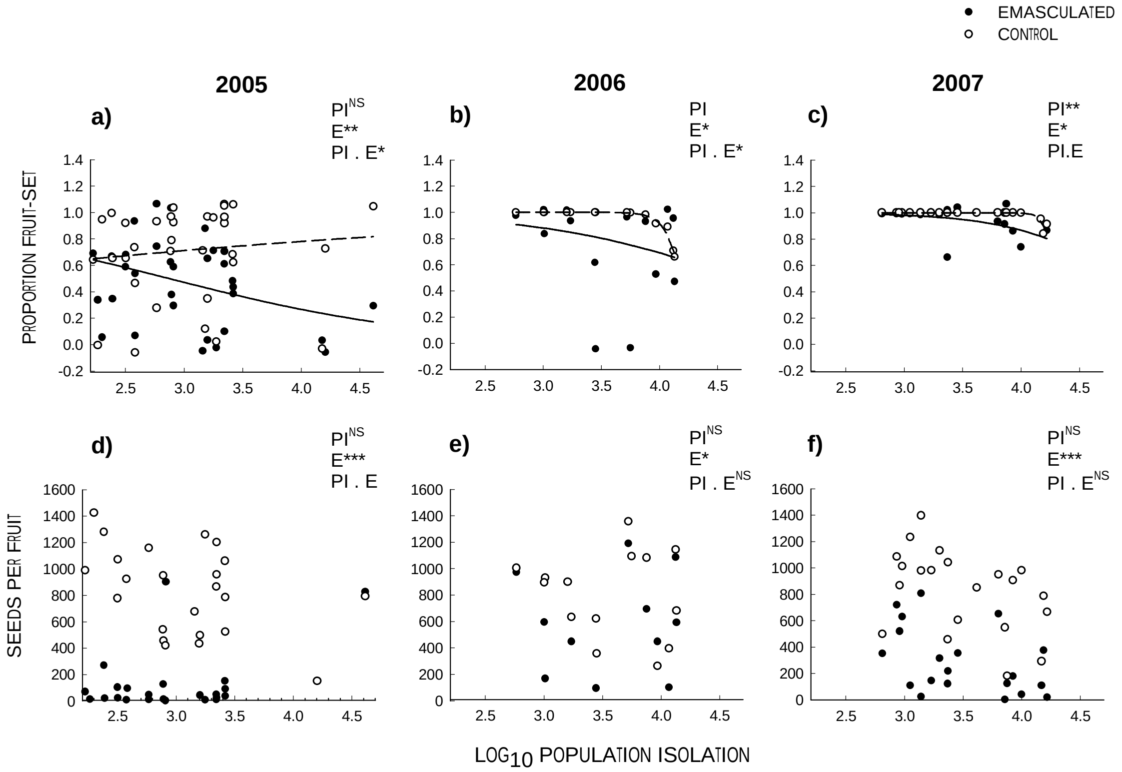

Figure 3 Fruit set (a-c) and seeds per fruit (d-f) of emasculated and intact naturally pollinated plants in relation to population isolation for three years. PI = population isolation (mean distance (m) to nearest three populations), E = emasculation; ns = non-significant; † = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Circles represent predicted values for populations for fruit-set (adjusted for population size) and mean populations values for seeds per fruit. Curves for fruit set fitted in generalised linear model using logit transformed data and backtransformed.

Appendix A

Table A1 Details of populations and sample sizes used in floral emasculation and pollen supplementation experiments and measurements of scale deposition from 2005 to 2007. Pop. = population; Isol. = Isolation; EC = controls for emasculated flowers; EE = emasculated flowers; SC = controls for pollen supplemented flowers; SS = pollen supplemented flowers; scales = flowers scored for lepidopteran scale deposition. * Approximate value. Not used in population size and isolation analyses. † Used in multi-year analyses.

Year	Pop	Latitude	Longitude	Pop size	Pop Isol	Sample sizes								
						EC		EE		SC		SS		Scales
						Fruit	Seeds	Fruit	Seeds	Fruit	Seeds	Fruit	Seeds	
2005	3†	29 45.736	30 45.398	46	1937	3	3	3		3	2	3	1	
2005	4	29 45.276	30 44.837	5802	1583	1		1		1		1		
2005	5	29 45.225	30 43.533	299	1019	1		1	1	1	1	1	1	
2005	6	29 45.292	30 43.591	499	965	3	1	3		3		3		
2005	12†	29 36.536	30 8.089	32	10940	1		3		2		1		
2005	13	29 3.406	29 24.09	9	700	1		2		3		3		
2005	14	29 3.149	29 24.505	17	465	1		1		2		2		
2005	17	29 2.944	29 24.826	19	495	3		2	1	2		3		
2005	18	29 3.049	29 24.715	44	364	2		2	1	3		3		
2005	19†	29 44.312	30 22.78	25	3265	3	2	3	1	2	1	2	1	
2005	20	29 51.803	30 18.156	5	1781	1	1	1		1	1	1	1	
2005	21	29 51.897	30 17.891	33	1485	3	3	3	1	3	1	3		
2005	24†	29 52.624	30 16.777	6000	1057	3	2	2	1	3	3	3	3	
2005	26†	29 45.151	30 21.448	1100	2994	3	3	2	1	3	3	3	3	
2005	27	29 45.159	30 21.480	250	2967	3	2	2	1	3	3	3	2	
2005	29	29 48.699	30 46.098	298	1825	3		3		3	1	3	1	
2005	30	29 49.351	30 47.007	50	774	3	3	2	2	1	1	1	1	
2005	34	30 16.155	30 45.260	1017	26598	1	1	3	1	1	1	2	1	
2005	36	30 42.206	30 26.773	615	2071	3	2	3		3	3	3	3	
2005	37	30 42.602	30 26.633	1100	2071	1		1	1					
2005	40	30 49.361	30 23.390	4	5353					2	1	1		
2005	41	30 47.023	30 24.742	225	1558	3	1	3	2	3	3	3	3	
2005	42	30 52.714	30 20.793	13	468					3	1	3	3	
2005	43	30 52.794	30 20.744	64	434	3	2	3	2	2	1	3	3	
2005	47	30 46.392	30 25.171	1100	658	3	2	3	3	3	3	3	1	
2005	48	30 46.289	30 25.264	121	551	3	3	3	2	3	2	3	3	
2005	51	30 45.634	30 25.512	454	791	3	3	3	1	3	2	3	3	
2005	52	30 45.65	30 25.878	13	544	3	3	3	2	2	2	2	2	
2005	54	30 45.325	30 26.009	20	319					3	2	3	1	
2005	55	30 45.275	30 26.011	90	379	3	2	3	2	3	1	3	3	
2005	57	30 44.466	30 26.096	9	660	2	2	2	1	2	1	2	2	
2005	58	30 44.381	30 26.107	120	668	3		3	1	3		3		
2005	59	30 41.49	30 27.130	780	3032	3	3	3	2	3	2	3	2	
2005	61	29 35.093	30 20.934	40	2232	3	3	3	2	2	2	3	2	
2005	63	29 34.249	30 18.981	24	243	2	2	2		3	1	2		

2005	66	29 34.498	30 19.733	91	521	3		3	1	2		3	2	
2005	69	29 31.618	30 30.124	1375	12401	3	2	3		3	3	3	2	
2006	3	9 45 793	30 45.366	27	7928									9
2006	12†	29 36.598	30 07.990	100-200*	17087	4	4	2	2	4	4	4	4	
2006	19†	29 44.312	30 22.780	41	13305	6	5	5	5	4	4	7	7	10
2006	21	29 53.495	30 17.264	23	10235									6
2006	24†	29 52.624	30 16.777	4500	9341	10	8	11	5	6	5	9	8	9
2006	26†	29 45.151	30 21.448	600	11729	6	5	6	6	9	8	10	10	12
2006	28†	29 49.530	30 11.253	79	13591	8	6	6	3	9	8	8	7	23
2006	70	29 47.254	30 43.667	12	3434					1	1	1	1	
2006	71	29 45.588	30 43.258	260	2294									10
2006	74	30 40.522	30 28.342	82	3623					9	9	8	8	8
2006	76	30 44.681	30 26.058	19	710					2	2	2	2	
2006	77	30 49.461	30 23.182	50	5629	2	2	2		5	5	4	4	8
2006	78	30 54.647	30 18.927	9	7534	2	2	2	2					
2006	79	30 44.997	30 25.986	327	1028	6	6	6	5	10	10	10	10	9
2006	80	30 44.284	30 26.110	43	719					5	5	5	4	9
2006	81	30 42.474	30 26.533	1200	1009	8	8	8	8	8	8	9	9	24
2006	85	29 34.087	30 18.986	13	582	1	1	1	1					8
2006	87	29 32.587	30 18.061	18	2827	1	1	1		3	3	3	3	7
2006	91	29 21.848	30 15.607	10	2787	1	1	3	2	1	1	2	2	10
2006	92	29 20.903	30 15.759	1	1722	1	1	1	1					3
2006	94†	29 27.014	30 07.113	40	5276	1	1	4	4	3	3	2	2	
2006	96	29 19.814	30 17.900	750	1600	2	2	1	1	4	4	2	2	9
2006	97†	29 32.383	30 17.087	3000*	545	8	8	8	8	9	9	9	9	
2007	3†	29 45 793	30 45 366	32	859	3	3	2	2	3	3	3	3	20
2007	12†	29 36.598	30 07.990	80	15388	6	6	7	6	8	8	8	8	31
2007	19†	29 44.312	30 22.780	21	8441	10	10	13	12	7	7	11	11	7
2007	24†	29 52.624	30 16.777	3899	14788	8	8	1	1	9	9	9	9	10
2007	26†	29 45.151	30 21.448	2100	9930	12	12	9	4	9	9	10	10	
2007	28†	29 49.530	30 11.253	8	16490	4	3	1	1					5
2007	90	29 24 503	30 14 868	7	6317	1	1	1	1	2	2	2	2	4
2007	94†	29 27.014	30 07.113	236	2349	7	7	7	7	9	9	6	6	20
2007	97†	29 32.510	30 17.511	1000*	4921	2	2	2	1	4	3	4	4	
2007	103	29 20 817	30 15.942	20	952	2	2	2	2	3	3	2	2	2
2007	105	29 20 190	30 16.669	45	1117	1	1	1	1	1	1	1	1	20
2007	106	29 19 700	30 17.118	40	1379	3	2	3	3	2	2	2	2	19
2007	109	29 37 .533	30 25.215	2	7186	1	1	1	1					
2007	110	29 47 778	30 50.977	10	4141	1	1			1	1	1	1	5
2007	111	29 47 508	30 48.569	31	1985	2	2	3	3	3	3	3	3	20
2007	113	29 47 981	30 47.745	350	2857	2	2	2	2	3	3	3	3	
2007	117	29 39 460	30 24.800	300	7495	3	3	3	3	3	3	3	3	21
2007	118	29 36.775	30 19.869	75	2332	3	3	3	2	6	6	3	3	20
2007	120	29 34.988	30 19.839	12	1381	3	3	3	3	2	2	2	2	18
2007	121	29 27.835	30 08.123	130	1687	3	3	4	4	5	5	3	3	19
2007	122	29 28.180	30 07.328	10	646	3	3	2	2	2	2	2	2	8
2007	123	29 28.387	30 06.656	81	905	3	3	3	3	2	2	1	1	19

Appendix B

Table B1 Generalised linear model of fruit set in emasculation experiment in 2005.

Effect	Change in deviance	df	Mean change in deviance	Quasi-F	p
Population size	0.05	1	0.05	0.03	0.861
Population isolation	1.96	1	1.96	1.23	0.276
Population	47.89	30	1.60	1.60	0.103
Emasculation	11.45	1	11.45	11.45	**0.002**
PS × E	0.76	1	0.76	0.64	0.432
PI × E	5.72	1	5.72	4.76	**0.037**
Residual	36.01	30	1.20		

Table B2 REML analysis of seeds per fruit in emasculation experiment in 2005.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Population size	6.19	1	18.4	6.19	**0.023**
Population isolation	0.49	1	21.7	0.49	0.49
Emasculation	113.13	1	16.1	113.13	< **0.001**
PS × E	8.31	1	16.4	8.31	**0.011**
PI × E	4.29	1	20.3	4.29	0.051

Random effects	Deviance change	df	p
Population	3.14	1	0.076
P × E	0.18	1	0.674

Table B3 Generalised linear model of fruit set in emasculation experiment in 2006.

Effect	Change in deviance	df	Mean change in deviance	Quasi-F	p
Population size	0.72	1	0.72	0.44	0.521
Population isolation	6.90	1	6.90	4.21	0.065
Population	18.04	11	1.64	1.64	0.212
Emasculation	5.00	1	5.00	5.00	**0.046**
PS × E	0.03	1	0.03	0.03	0.857
PI × E	7.14	1	7.14	7.14	**0.022**
Residual	4.75	11	0.43		

Table B4 REML analysis of seeds per fruit in emasculation experiment in 2006.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Population size	0.6	1	10.3	0.6	0.456
Population isolation	0.01	1	9.6	0.01	0.93
Emasculation	7.33	1	7.2	7.33	**0.030**
PS × E	0.29	1	9.9	0.29	0.601
PI × E	2.76	1	6.3	2.76	0.145
Random effects	**Deviance change**		**df**		**p**
Population	27.97		1		< **0.001**
P × E	1.94		1		0.164

Table B5 Generalised linear models of fruit set in emasculation experiment in 2007.

Effect	Change in deviance	df	Mean change in deviance	Quasi-F	p
Population size	2.86	1	2.86	4.24	0.054
Population isolation	5.90	1	5.90	8.75	**0.008**
Population	12.14	18	0.67	0.67	0.793
Emasculation	6.81	1	6.81	6.81	**0.018**
PS × E	3.94	1	3.94	3.94	0.064
PI × E	3.22	1	3.22	3.22	0.091
Residual	3.6×10^{-4}	17	2.1×10^{-5}		

Table B6 REML analysis of seeds per fruit in emasculation experiment in 2007.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Population size	2.61	1	18.7	2.61	0.123
Population isolation	2.85	1	17.5	2.85	0.109
Emasculation	54.26	1	16.1	54.26	< **0.001**
PS × E	0.02	1	18.7	0.02	0.903
PI × E	0.02	1	16.7	0.02	0.895
Random effects	**Deviance change**		**df**		**p**
Population	15.79		1		< **0.001**
P × E	5.17		1		**0.023**

Table B7 Generalised linear models of fruit set in pollen supplementation experiment in 2005**.**

Effect	Change in deviance	df	Mean change in deviance	Quasi-F	p
Population size	9.93	1	9.93	4.19	**0.049**
Population isolation	0.57	1	0.57	0.24	0.627
Population	75.83	32	2.37	2.37	**0.008**
Supplementation	0.03	1	0.03	0.03	0.859
PS × S	4.33	1	4.33	4.33	**0.046**
PI × S	1.21	1	1.21	1.21	0.279
Residual	20.29	32	1.63		

Table B8 REML analysis of seeds per fruit in pollen supplementation experiment in 2005.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Population size	0.77	1	24.7	0.77	0.389
Population isolation	2.01	1	27.4	2.01	0.167
Supplementation	5.86	1	73.6	5.86	**0.018**
PS × S	2.04	1	75.2	2.04	0.158
PI × S	0.02	1	76.2	0.02	0.895
Random effects	**Deviance change**		**df**		**p**
Population	7.70		1		**0.005**
P × S	0.00		1		1.000

Table B9 Generalised linear model for fruit set in pollen supplementation experiment in 2006.

Effect	Change in deviance	df	Mean change in deviance	Quasi-F	p
Population size	1.07	1	1.07	1.37	0.264
Population isolation	2.42	1	2.42	3.11	0.103
Population	9.33	12	0.78	0.78	0.665
Supplementation	0.02	1	0.02	0.02	0.886
PS × S	0.77	1	0.77	0.77	0.397
PI × S	2.36	1	2.36	2.36	0.150
Residual	4.4×10^{-3}	12	3.7×10^{-4}		

Table B10 REML analysis of seeds per fruit in pollen supplementation experiment in 2006.

Fixed effects	Wald	ndf	ddf	Wald-F	P
Population size	1.21	1	11.7	1.21	0.294
Population isolation	0.54	1	10.1	0.54	0.478
Supplementation	1.71	1	138.3	1.71	0.194
PS × S	2.13	1	138.8	2.13	0.147
PI × S	0.48	1	137.9	0.48	0.488

Random effects	Deviance change	df	P
Population	19.18	1	**< 0.001**
P × S	0.00	1	1.000

Table B11 REML analysis of seeds per fruit in pollen supplementation experiment in 2007.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Population size	0.21	1	14.3	0.21	0.655
Population isolation	2.87	1	15.1	2.87	0.111
Supplementation	0.26	1	134	0.26	0.613
PS × S	0.03	1	133.9	0.03	0.873
PI × S	0.98	1	134.2	0.98	0.324

Random effects	Deviance change	df	p
Population	28.09	1	< **0.001**
P × S	0.00	1	1.000

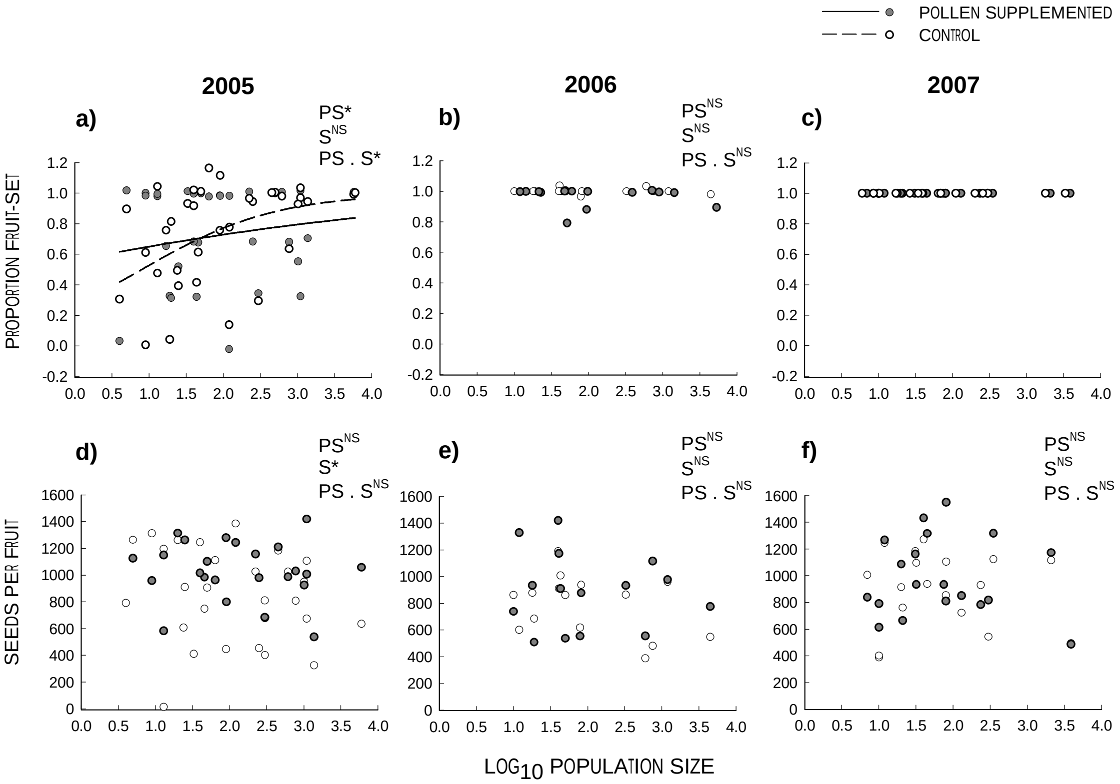

Figure B1 Fruit set (a-c) and seeds per fruit (d-f) of pollen supplemented and naturally pollinated control plants in relation to population size for three years. PS = population size, S = pollen supplementation; ns = non-significant; * $p< 0.05$. Circles represent predicted values for populations for fruit-set (adjusted for population isolation) and mean populations values for seeds per fruit. Curves for fruit set fitted in generalised linear model using logit transformed data in Genstat 11 and backtransformed. Fruit set for 2007 not analysed due to 100% fruit set. A significant population size by supplementation interaction occurred for fruit set in 2005 when only smaller populations experienced slight pollen limitation (a).

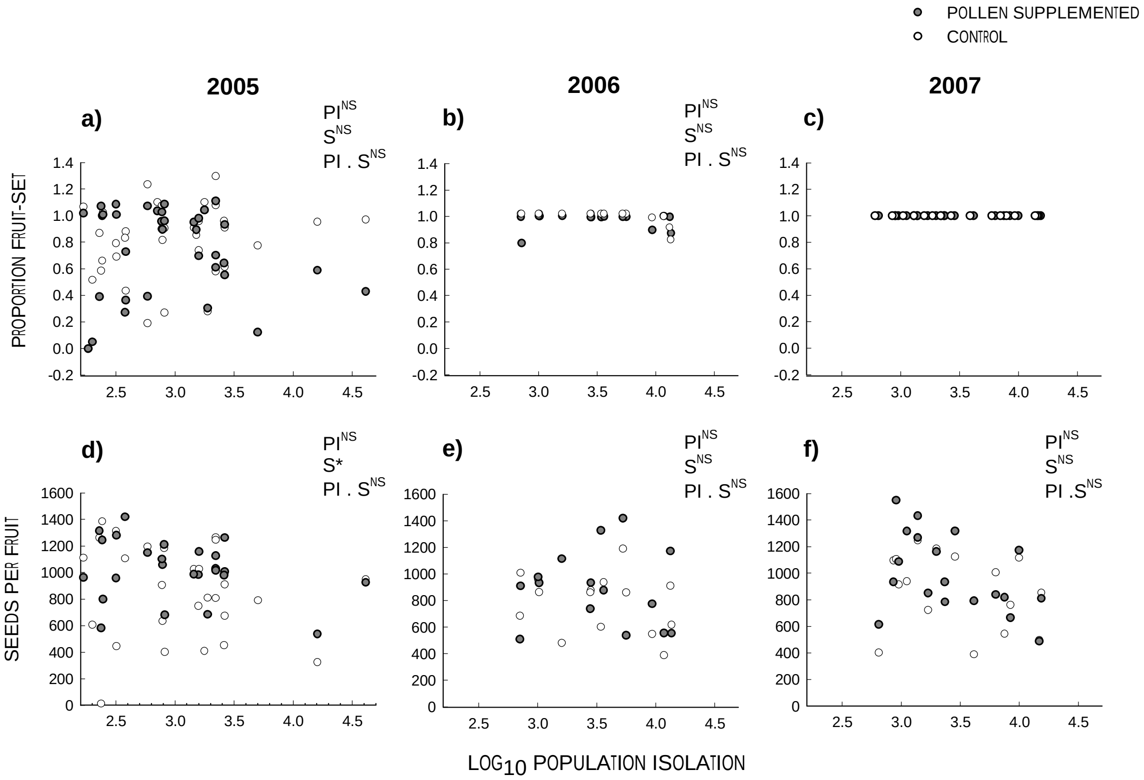

Figure B2 Fruit set (a-c) and seeds per fruit (d-f) of supplemented and naturally pollinated plants in relation to population isolation for three years. PI = population isolation (mean distance to nearest three populations), S = pollen supplementation; ns = non-significant; * p< 0.05. Circles represent predicted values for populations for fruit-set (adjusted for population size) and mean populations values for seeds per fruit. Fruit set for 2007 not analysed due to 100% fruit set.

Appendix C

Lepidopteran-scale deposition

To test whether reproductive success of emasculated flowers was related to rates of visitation, and whether visitation was related to population size and isolation, we scored *Lilium formosanum* flowers for presence/absence of Lepidopteran-scale deposition in a subset of the populations (Appendix A) used for emasculation and supplementation in 2006 (17 populations) and 2007 (18 populations). Scales were generally consistent with those of the hawkmoth *Agrius convolvuli*, the principal pollinator, but in some instances may have come from other species- one non-sphingid moth species has also been observed to visit *L. formosanum* occasionally (Rodger et al, 2010). Only flowers that had been open for at least one night were used and only one flower was sampled per plant, except in population 92 in 2006 and 120 in 2007 (Appendix A). In 2006 we attempted to sample 10 flowers and in 2007 20 flowers per population except in populations with insufficient plants with open flowers (Table S1).

Generalised linear models for binomial data were used to assess whether scale deposition was affected by population size and isolation in 2006 and 2007 separately, with population as a random factor. To test whether scale deposition (proportion of sampled flowers with scales present, arcsine-square root transformed) was related to fruit set of emasculated flowers in the same populations, we also used generalised linear models for binomial data, including population as a random factor. Similarly, we tested whether scale deposition was related to seeds per fruit for emasculated flowers in REML analysis of variance with population included as a random factor.

Averaged across populations, lepidopteran scales were observed on 33.1 ± 5.0% (Mean ± SE) of flowers in 2006 and 30.0 ± 6.7% of flowers in 2007. Although there was significant variation among populations in both 2006 ($p = 0.007$) and 2007 ($p < 0.001$), neither population size nor isolation had a significant effect on scale deposition in either year ($p > 0.05$) (Tables C1, C2; Fig. C1). Fruit set and seeds per fruit were also not significantly related to scale deposition in either year ($p > 0.3$, Tables C4-C6). This may be because the same flowers were not always used for scale deposition measurements and emasculations in these analyses, which would have been a source of error. Scale measurements also may not reflect visitation over the lifetime of the emasculated flowers because scale presence was only scored on a single occasion during or before anthesis of emasculated flowers, while flowers remain open for 4-7 days (median 6) (J.G. Rodger, unpublished results). Thus, although we remain confident that testing scale deposition against population size and isolation is reasonable, relating scale deposition to visitation on particular flowers probably requires more control for temporal variation in visitation.

Table C1 Generalised linear models for scale deposition in 2006.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Population size	0.09	1	14	0.09	0.03	0.860
Population isolation	5.44	1	14	5.44	2.009	0.180
Population	38.03	14	157	2.72	2.29	**0.007**
Residual	186.41	157		1.19		

Table C2 Generalised linear model for scale deposition in 2007.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Population size	7.62	1	15	7.62	1.67	0.216
Population isolation	2.64	1	15	2.64	0.58	0.460
Population	68.59	15	250	4.57	4.95	< **0.001**
Residual	231.04	250		0.92		

Table C3 Generalised linear model for effect of scale deposition on fruit set for emasculated flowers in 2006.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Scale deposition	1.07	1	10	1.07	0.41	0.537
Population	26.17	10	39	2.62	3.12	**0.005**
Residual	32.70	39		0.84		

Table C4 REML analysis for effect of scale deposition on seeds per fruit for emasculated flowers in 2006.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Scale depostion	0.03	1	6.5	0.03	0.864
Random effects	Deviance change		df		p
Population	14.52		1		< 0.001

Table C5 Generalised linear model for effect of scale deposition on fruit set for emasculated flowers in 2007.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Scale deposition	0.10	1	15	0.10	0.22	0.644
Population	7.00	15	42	0.47	1.18	0.323
Residual	16.61	42		0.40		

Table C6 REML analysis for effect of scale deposition on seeds per fruit for emasculated flowers in 2007.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Scale depostion	0.94	1	18.5	0.94	0.344
Random effects	Deviance change		df		p
Population	0.21		1		0.647

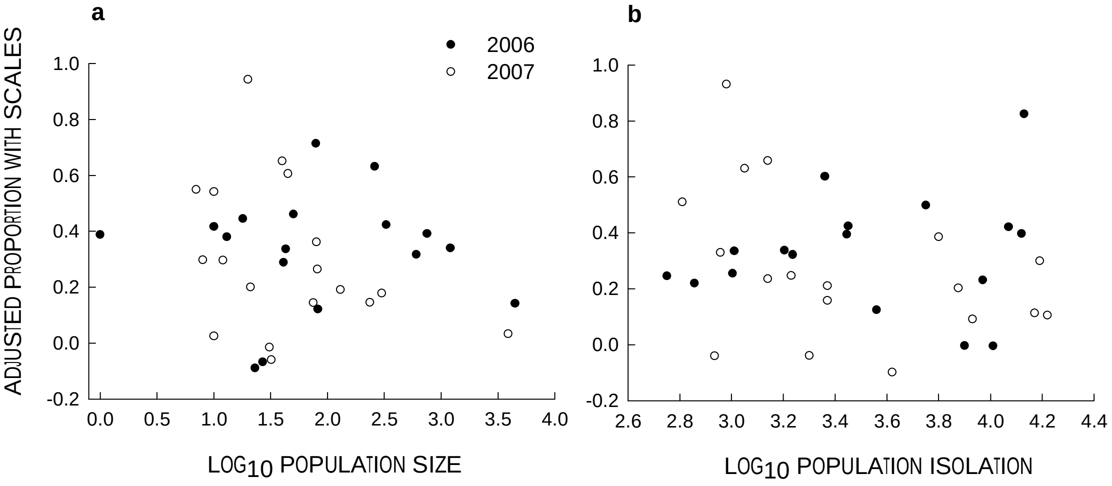

Figure C1. Lepidopteran scale deposition on flowers across a range of *Lilium formosanum* population size (a) and isolation values (b) in 2006 and 2007.

Appendix D

Analysis of emasculation and pollen supplementation experiments in populations for which data was available for more than one year

To test whether dependence on selfing and pollen limitation of fecundity varied between years in *L. formosanum*, we analysed fruit set and seeds per fruit for eight populations for which data was available for more than one year (Appendix A), using generalised linear models for fruit set and REML analysis for seeds per fruit. Year and floral manipulation were included as fixed factors, population as a random factor and all interactions between these factors were included.

Multiple-year analyses for emasculation (Tables D1, D2, Fig. D1) gave similar results to single year analyses. Dependence on selfing for fecundity varied among years as indicated by a significant year-by-emasculation interaction for seeds per fruit (Tables D2; Fig. D1b); the effect of emasculation was greatest in 2005 (Fig. D1a, b). Multiple-year analysis of pollen supplementation showed that supplementation also had the greatest effect in 2005 (Tables D3, D4, Fig. D1d).

Table D1 Generalised linear model of fruit set in emasculation experiments for populations used in more than one year between 2005 and 2007.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Population	16.62	7	165	2.37	3.81	**0.001**
Year	15.70	2	10	7.85	3.10	0.089
Emasculation	13.96	1	7	13.96	12.65	**0.009**
P × Y	25.30	10	165	2.53	4.06	< **0.001**
P × E	7.72	7	165	1.10	1.77	0.096
Y × E	2.07	2	10	1.04	1.09	0.372
P × Y × E	9.47	10	165	0.95	1.52	< **0.001**
Residual	102.73	165		0.95		

Table D2 REML analysis of seeds per fruit in emasculation experiments for populations used in more than one year between 2005 and 2007.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Year	2.75	2	11.2	1.37	0.293
Emasculation	23.86	1	6.5	23.86	**0.002**
Y × E	40.65	2	145.3	20.32	< **0.001**
Random effects	Deviance change		df		p
Population	27.95		1		< **0.001**
P × Y	25.81		1		< **0.001**
P × E	2.66		1		0.103
P × E × Y	0		1		1.000

Table D3 Generalised linear models of fruit set in pollen supplementation experiments for populations used in more than one year between 2005 and 2007.

Effect	Change in deviance	ndf	ddf	Mean change in deviance	Quasi-F	p
Population	8.87	7	179	1.27	4.73	**< 0.001**
Year	6.13	2	8	3.06	1.66	0.249
Supplementation	0.72	1	7	0.72	2.05	0.195
P × Y	14.75	8	179	1.84	6.88	**< 0.001**
P × S	2.47	7	179	0.35	1.31	0.246
Y × S	0.00	2	8	0.00	0.00	1.000
P × Y × S	0.00	8	179	0.00	0.00	0.991
Residual	47.96	179		0.38		

Table D4 REML analysis of seeds per fruit in pollen supplementation experiments for populations used in more than one year between 2005 and 2007.

Fixed effects	Wald	ndf	ddf	Wald-F	p
Year	0.2	2	16.9	0.1	0.904
Supplementation	4.17	1	184.7	4.17	**0.043**
Y × S	7.8	2	185	3.9	0.022

Random effects	Deviance change	df	p
Population	7.75	1	**0.005**
P × Y	33.62	1	**< 0.001**
P × S	0	1	1.000
P × S × Y	0	1	1.000

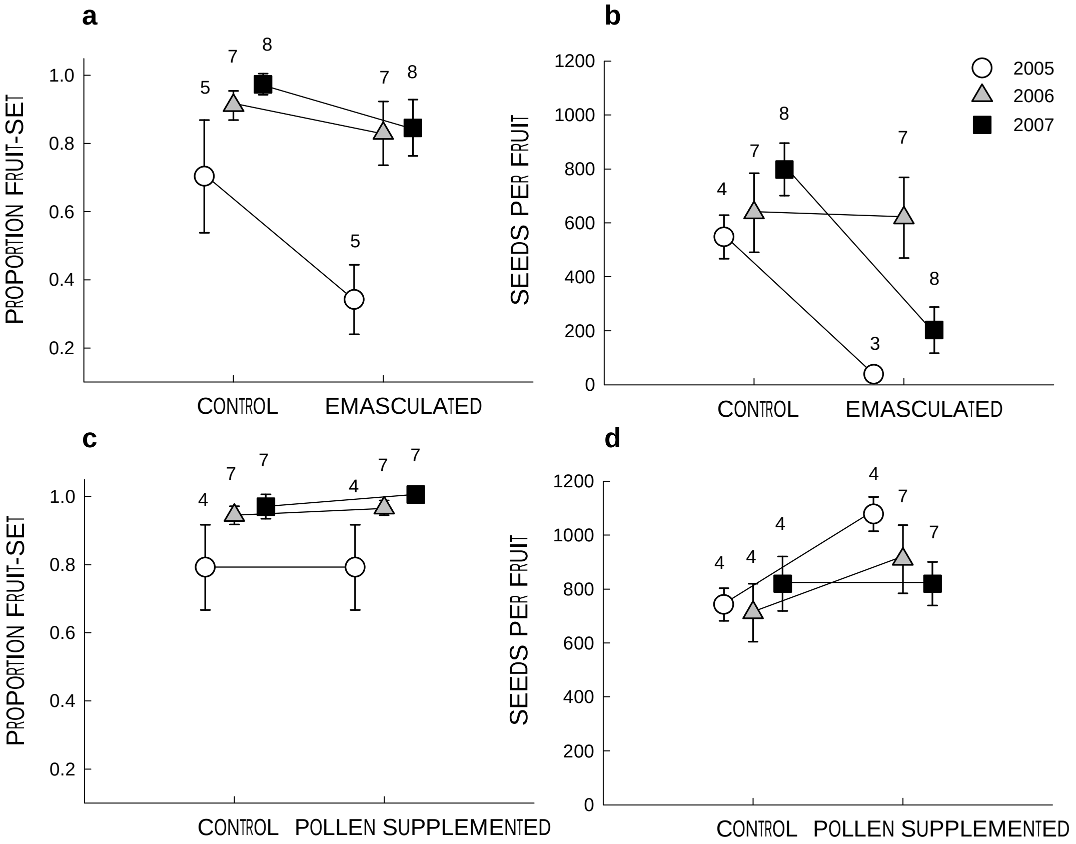

Figure D1 Fruit set and seeds per fruit for emasculation (a, b) and pollen-supplementation (c, d) experiments on *Lilium formosanum* in populations for which data was available for more than one year. Means and standard errors of population mean values shown; numbers above symbols indicate number of populations.

Chapter 8

Self-pollination alleviates reduced mate availability in isolated individuals of the invasive *LILIUM FORMOSANUM*

James G. Rodger, Mark van Kleunen and Steven D. Johnson

Abstract

It has been proposed that self-compatible plants are, on average, more invasive than self-incompatible ones because self-fertilisation provides reproductive assurance, facilitating expansion from bottlenecks of low abundance during invasion. We addressed this idea by testing whether reproductive assurance through self-fertilisation mitigated reduced cross-pollen receipt under low density in the hawkmoth pollinated and autonomously self-pollinating invasive Asian lily *Lilium formosanum*. To do this we placed emasculated and intact plants inside natural continuous patches or in pairs isolated (3-702m) from patches in two naturalised populations in KwaZulu-Natal, South Africa. In one population, emasculation reduced fecundity of isolated plants more than that of plants placed inside patches, showing that reproductive assurance compensated for reduced cross-pollen receipt caused by low abundance. As isolated emasculated plants placed in pairs with intact plants, which could act as pollen donors, had higher fecundity than emasculated plants placed in pairs with a second emasculated plant, we inferred that isolation reduced mate availability rather than pollinator visitation. This is supported by observations of lower deposition of pollen on stigmas of isolated plants in one population but no effect of isolation on deposition of lepidopteran scales, an indicator of pollinator visitation, in either population. These results suggest that reproductive assurance through selfing can contribute to invasiveness by mitigating both reduced mate availability at low plant abundance and generally inadequate pollinator visitation irrespective of abundance.

Introduction

The ability to self-fertilise is positively correlated with invasive status and size of invaded range among introduced species in several floras (van Kleunen and Johnson 2007, van Kleunen et al. 2008, Hao et al. 2011, Pyšek et al. 2011). This is consistent with the hypothesis that species with the ability to self-fertilise should be better colonisers because their reproduction is assured (Baker 1955). In self-incompatible plants, fecundity is often reduced at low abundance due to reductions in amount or suitability of cross-pollen received, because of less frequent visitation by pollinators or shortages of suitable mates – ie pollen-limitation Allee effects (e.g. Groom 1998, Elam et al. 2007). Self-fertilisation mitigates inadequate cross-pollen receipt, providing reproductive assurance, which may be defined quantitatively as the proportion of fecundity for which plants rely on self-fertilisation (Eckert et al. 2006). As populations of introduced plants will often be small initially and spread usually involves the formation of a low density leading edge (Taylor and Hastings 2005), reproductive assurance through selfing may promote invasion by alleviating pollen-limitation Allee effects (Taylor and Hastings 2005, van Kleunen et al. 2007).

Few studies have tested for pollen-limitation Allee effects in invasive plants (Davis et al. 2004, van Kleunen and Johnson 2005, Elam et al. 2007), although fecundity of self-incompatible invasive plants is often pollen-limited (Burns et al. 2011). In the self-incompatible invasive grass *Spartina alterniflora*, reduced outcross-pollen receipt in isolated plants at the leading edge of the invasion resulted in lower fecundity, substantially limiting rate of spread (Davis et al. 2004, Taylor et al. 2004). There have also been few investigations of whether self-fertilisation results in reproductive assurance in self-compatible invasive species (Schueller 2004, van Kleunen et al. 2007, Chapter 7), and where these have found evidence for reproductive assurance, it was not related to abundance (van Kleunen and Johnson 2007, Chapter 7).

Previously we showed that in the autonomously self-pollinating and hawkmoth pollinated Asian geophyte *Lilium formosanum* (Chapter 5), reproductive assurance through selfing accounted for on average 67% of fecundity, across multiple populations and in three years, in its introduced range in KwaZulu-Natal, South Africa (Chapter 7). However reproductive assurance was not related to population size and only increased with increasing population isolation in one of the three study years. We concluded that selfing provided reproductive assurance more because of generally inadequate pollinator visitation than because of reduced pollinator visitation at low abundance. However, that study included few populations consisting of single plants. Single plants may experience decreased cross-pollen receipt compared to plants in continuous populations not only due to lower visitation (Kunin 1993) but also due to reduced availability of mates in their vicinity to donate pollen (Kunin 1993, Duncan et al. 2004).

In this study we created arrays of emasculated and intact plants inside and isolated from naturalised population to investigate the following questions: (i) Does reproductive assurance alleviate decreased cross-pollen receipt in isolated plants in *L. formosanum* – ie is reproductive assurance higher in isolated plants? (ii) Does reproductive assurance increase with distance from continuous patches? (iii) Is any increase in reproductive assurance with isolation attributable to reduced pollinator visitation or mate availability?

Methods and materials

Study system

Lilium formosanum bulbs produce single erect, unbranched stems 0.3-2.5m in height, with terminal inforescences of 1-8 large, trumpet shaped flowers (Rodger et al. 2010, Chapter 5). Two populations with discrete patches of *L. formosanum* in open habitat (mainly natural grassland) were selected for experiments in February and March 2009. At Baynesfield (29 45.162S, 30 21.377E, Alt. 810m) there was a population consisting of a single large patch of 748 plants. In the Karkloof population (29 20.229, 30 17.527, Alt. 1100m) four patches of 67-610 plants were used. Reproductive assurance was assessed by comparing fecundity of intact plants, which could self-pollinate and receive pollen from pollinators, with that of emasculated plants, which depended entirely on pollinators (Eckert et al. 2006). As *L. formosanum* is pollinated principally by hawkmoths (Rodger et al. 2010, Chapter 5), which do not forage for pollen, it is unlikely that emasculation affected pollinator visitation. In fact, hawkmoths have been observed to readily visit emasculated flowers and deposition of lepidopteran scales, and indication of hawkmoth visitation, did not differ between emasculated and intact flowers observed in other populations (Chapter 7).

To test whether reproductive assurance was greater for plants isolated from continuous patches and, if so, whether this was due to decreased visitation or mate availability, we created arrays of emasculated and intact plants transplanted either into central patches or similar grassland habitat that was isolated from the patches. We obtained data from 87 plants at Baynesfield and 59 at Karkloof. Plants used were sourced from the same populations. Emasculated and intact plants were place singly inside continuous patches or isolated outside of these patches. Isolated plants were placed in pairs, consisting either of two emasculated plants or an emasculated plant and an intact plant, 1m apart (Fig. 1). Distances between successive pairs were chosen randomly from increasing intervals of the $\log_2$ scale (2-4, 4-8, 8-16...), so that as distance away from the central patch increased, density decreased. We treated isolation and density as naturally confounded variables (Duncan et al. 2004). The range of distances from central patches was 3-702m at Baynesfield and 3-561m at Karkloof. After flowering, all transplanted individuals were re-excavated and brought back to the University of KwaZulu-Natal Pietermaritzburg campus and maintained in plant pots until fruit were mature. The array at Baynesfield was set up from the 31st January to the 3rd of February and plants were recovered on the 14th of February 2009. The array at Karkloof was set up from the 28th of February to the 3rd of March and plants were recovered on the 10th of March 2009. All ovaries that contained at least one seed were scored as fruits and seeds per fruit was estimated for all fruits. The number of seeds in each fruit (which could be over 1000) was estimated from the mass of the entire contents of the fruit and the mass and number of seeds in a random sub-sample containing approximately 50 seeds. All seeds were counted in fruits containing fewer than 50 seeds.

We mainly present separate analyses for the two populations because analysing them together required inclusion of three-way interactions (eg population-by-isolation-by-emasculation) and because sample sizes were small (Figs 2-3), power to detect these interactions would have been low. Nevertheless, we also ran analyses including both populations, and we report their results when they differ from those of separate analyses of the two populations, and when significant interactions between experimental treatment and population occurred.

The effect of isolation on reproductive assurance

To test whether reproductive assurance compensated for reduced cross-pollen receipt in isolated plants, we compared fecundity in emasculated and intact plants placed inside central patches and in isolated pairs (Fig. 1: plants represented by I_1 and E_1). Reproductive assurance indices were calculated for plants inside patches and isolated from them in each population using average fecundity (fruit set × seeds per flower), with the formula RA = 1-Emasculated/Intact (Eckert et al. 2006). However statistical analyses of fruit set and seeds per fruit were conducted separately as many emasculated plants failed to set fruit, resulting in zero-inflation of the data.

Fruit set was analysed in generalised linear models for binomial response with a logit link function and number of flowers (on plants) as the binomial total. Significance was evaluated from quasi-F values obtained by sequential analysis of deviance, analogous to a type I ANOVA (Payne, 2009). This allowed us to test for the effect of distance from central patch after evaluating the effect of isolation (inside versus outside patches) per se. As isolation was entered first, distance accounted only for variation not explained by isolation. The following terms were included, entered in this order: isolation, distance from patch ($\log_{10}$ transformed), emasculation (intact versus emasculated), emasculation-by-isolation, emasculation-by-distance. Distance was scored as zero for plants inside patches. For seeds per fruit, mean values were calculated for each plant, $\log_{10}$ transformed to improve homogeneity of variance, and analysed in REML analysis of variance as sample sizes were unbalanced. The same model was used as described for the generalised linear model for fruit set. Terms were sequentially added to a model and the significance of these terms was evaluated from Wald F-statistics.

Increased mate versus pollinator limitation in isolated plants

If emasculation reduced fruit set or seeds per fruit in patches, this would almost certainly be due to insufficient visitation by pollinators (pollinator limitation), as patches contained numerous intact plants that could donate pollen to emasculated ones. However, an increase in the effect of emasculation outside of patches might occur either through decreased pollinator visitation or because pollinators carried less pollen due to a lack of nearby plants to donate pollen (mate limitation). To gain insight into the cause of any increase in reproductive assurance in isolated plants, we compared fecundity of isolated emasculated plants, paired either with another emasculated plant or with an intact plant (Fig 1: plants represented by E_2). If the effects of isolation were a result of mate limitation, then emasculated plants placed next to intact plants should have higher fruit set and seeds per fruit than those placed next to another emasculated plant. Fruit set and seeds per fruit were analysed in generalised linear models and REML analysis of variance as described above. Analyses included mate presence as a fixed factor, distance as a continuous variable and the mate presence-by-isolation distance interaction.

We also addressed the question of whether isolated plants experienced decreased pollinator visitation or mate availability by scoring emasculated flowers for the presence of lepidopteran scales, an indication of visitation, and presence of pollen on stigmas, an indication of successful pollination, using a 20X hand lens (plants represented by E_1 in Fig. 1). Each plant was scored once, three to four days after transplanting, for all flowers that had been open for at least one night. Scale and pollen deposition were analysed in general linear models for binomial data with a logit link function including isolation as a fixed factor and distance as a covariate (this data was only collected for emasculated plants). All statistical analyses were performed in Genstat 12 (Genstat 2009).

Results

Fruit set and seeds per fruit were significantly lower in emasculated than in intact plants for both Baynesfield and Karkloof (Tables 1, S1-S4; Fig. 2), indicating that selfing provided reproductive assurance. Indices of reproductive assurance were 0.75 for plants inside central patches and 0.96 for isolated plants at Baynesfield and 0.80 inside patches and 0.84 for isolated plants at Karkloof. At Baynesfield there was a significant interaction between isolation and emasculation for seeds per fruit due to a greater effect of emasculation in isolated plants (Tables 1, S2; Fig. 2b), with a non-significant trend in the same direction for fruit set (Tables 1, S1; Fig. 2a). However at Karkloof the effect of emasculation on fruit set and seeds per fruit was not related to isolation (Tables 1, S3, S4; Fig. 2c, d). The effect of emasculation on fruit set and seeds per fruit did not increase with distance in either population (Tables 1, S1-S4; Fig. S1). Although the distance-by-emasculation interaction was significant for seeds per fruit at Baynesfield, the effect of emasculation actually increased with distance. This was due to an outlier, however (Fig. S1b). When the data from the two populations were analysed together results were similar, but no longer supported a greater effect of emasculation in isolated plants for seeds per fruit (isolation-by-emasculation and population-by-isolation-by-emasculation were not significant: $p = 0.062$ and $p = 0.373$ respectively).

Isolated emasculated plants paired with potential pollen donors (intact plants) outperformed those with another emasculated plant as a partner at Baynesfield in terms of fruit set (Tables 2, S5; Fig. 3 a) and at Karkloof in terms of seeds per fruit (Tables 2, S8; Fig. 3d). The effect of pollen donor presence was not related to distance in either population (Tables 2, S5-S8; Fig. S2). When the data from the two populations were analysed together results were very similar but indicated that the effect of pollen donor presence on fruit set was significant ($p = 0.003$) but did not differ between populations (population-by-mate presence interaction, $p = 0.513$). The joint analysis for seeds per fruit also indicated a significant effect of pollen donor presence ($p = 0.002$) which differed between populations (population-by-mate presence interaction, $p = 0.001$).

Scale deposition was not related to isolation or distance at either Baynesfield or Karkloof (Tables 3, S9, S10). When data from both populations were analysed together scale deposition did not differ between population and population-by-isolation and population-by-distance interactions were not significant ($p > 0.5$). Isolated plants had significantly lower pollen receipt than those in the main patch at Baynesfield (Tables 3, S11) but not at Karkloof (Tables 3, S12). When data from both populations were analysed together, there was a significant isolation-by-population interaction ($p = 0.002$).

Discussion

Emasculation reduced fecundity significantly more in isolated plants than ones inside patches at Baynesfield (Fig. 2b), which is consistent with the hypothesis that selfing facilitates invasion by mitigating Allee effects. However, the effect of emasculation was not significantly related to isolation at Karkloof (Fig. 2c, d). The substantial reduction in fecundity following emasculation for plants inside patches in both populations (Fig. 2) indicates that pollinator visitation was generally inadequate, as we documented previously for *L. formosanum* in the study region (Chapter 7). Isolation exacerbated generally inadequate pollinator visitation at Baynesfield, resulting in isolated intact plants there depending almost entirely on selfing for their fecundity (RA = 0.96; Fig. 2a, b). Isolated emasculated plants placed next to intact plants that could act as pollen donors had higher fruit set (Baynesfield, Fig. 3a) or seeds per fruit (Karkloof, Fig. 3d) than those placed next to another emasculated plant, indicating that the effect of isolation was partly or entirely due to mate limitation. The finding that pollen deposition was lower outside than inside patches at Baynesfield while lepidopteran scale deposition was not related to isolation at either population also suggest that the effect of isolation on reproductive assurance is due to mate limitation, rather than increased pollinator limitation.

In our previous study on *L. formosanum*, conducted in three years and in multiple natural populations, RA was on average 0.67 but was not related to population size and was only greater in more isolated populations in one of the three years (Chapter 7). Both that study and this one indicate that the contribution of selfing to seed production in the introduced range arises mainly from generally inadequate pollinator visitation. Over and above this, reproductive assurance also mitigated reduced visitation in more isolated populations in one of three years in the previous multiple population study (Chapter 7) and mitigated reduced cross-pollen supply in plants isolated from continuous populations (patches) in one of two populations in this study (Fig. 2b). Although the effects of isolation of populations (Chapter 7) and individual plants (Fig. 2b) on reproductive assurance in *L. formosanum* are smaller and less consistent than the effect of low visitation in general (Chapter 7), rate of invasive spread is disproportionately sensitive to fecundity in isolated populations and individuals (Taylor and Hastings 2005). It will therefore be interesting to explore the relative contributions of selfing to invasiveness arising from general pollinator failure versus the exacerbating effects of isolation.

Further investigations are needed to assess whether self-fertilisation generally mitigates pollen-limitation Allee effects in invasive species, although this seems likely, at least for herbaceous plants, where pollen-limitation Allee effects are common (eg Groom 1998, Elam et al. 2007). As was the case for one population of *L. formosanum* in this study, several other experiments which subjected plants to low density showed steep declines in cross-pollen receipt (Kwak et al. 1998, Duncan et al. 2004) and fecundity (Kunin 1993, Taylor et al. 1999, Elam et al. 2007, Albrecht et al. 2009). These studies also inferred, as we did for *L. formosanum*, that lower mate availability was a more important cause of reduced cross-pollen receipt in isolated plants than was lower visitation (Kunin 1993, Duncan et al. 2004, Albrecht et al. 2009). Effects of isolation on pollen receipt and fecundity are, however, generally smaller in trees than they are in herbaceous plants, probably because their larger display size ensures that even isolated individuals are well visited and receive cross-pollen (e.g. Chapter 5, reviewed in Lowe et al. 2005, Kramer et al. 2008). Alleviation of pollen-limitation Allee effects by reproductive assurance is thus more likely to be important in invasive herbaceous plants than in trees (Chapter 5).

In conclusion, results presented here show that self-fertilisation mitigates both reduced mate availability at low plant abundance and generally inadequate pollinator visitation, irrespective of abundance, in *L. formosanum*. Future work will use demographic modelling techniques to assess whether reproductive assurance does contribute to invasiveness of *L. formosanum* as these results suggest.

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Table 1 Significance levels from generalised linear models for fruit set and REML analyses for seeds per fruit assessing whether the effect of emasculation is greater for plants isolated from continuous patches and, for isolated plants, whether the effect of isolation increases with distance of isolation in *Lilium formosanum*.

Effect	Baynesfield		Karkloof	
	Fruit set	Seeds per fruit	Fruit set	Seeds per fruit
Isolation	*	ns	ns	ns
Distance	*	ns	ns	ns
Emasculation	***	***	***	***
E × I	ns	*	ns	ns
E × D	ns	*	ns	ns

Table 2 Significance levels from generalised linear models for fruit set and REML analyses for seeds per fruit testing whether isolated emasculated plants have higher reproductive success in the presence of a potential mate (an intact plant) and whether this is related to distance from continuous patches in *Lilium formosanum*.

Effect	Baynesfield		Karkloof	
	Fruit set	Seeds per fruit	Fruit set	Seeds per fruit
Distance	ns	*	ns	ns
Donor presence	*	ns	ns	**
DP × D	ns	ns	ns	ns

Table 3 Significance levels from generalised linear models for scale and pollen deposition testing for effects of isolation from continuous patches and, for isolated plants, distance from continuous patches in *Lilium formosanum*.

Effect	Baynesfield		Karkloof	
	Scales	Pollen	Scales	Pollen
Isolation	ns	***	ns	ns
Distance	ns	ns	ns	ns

Figures

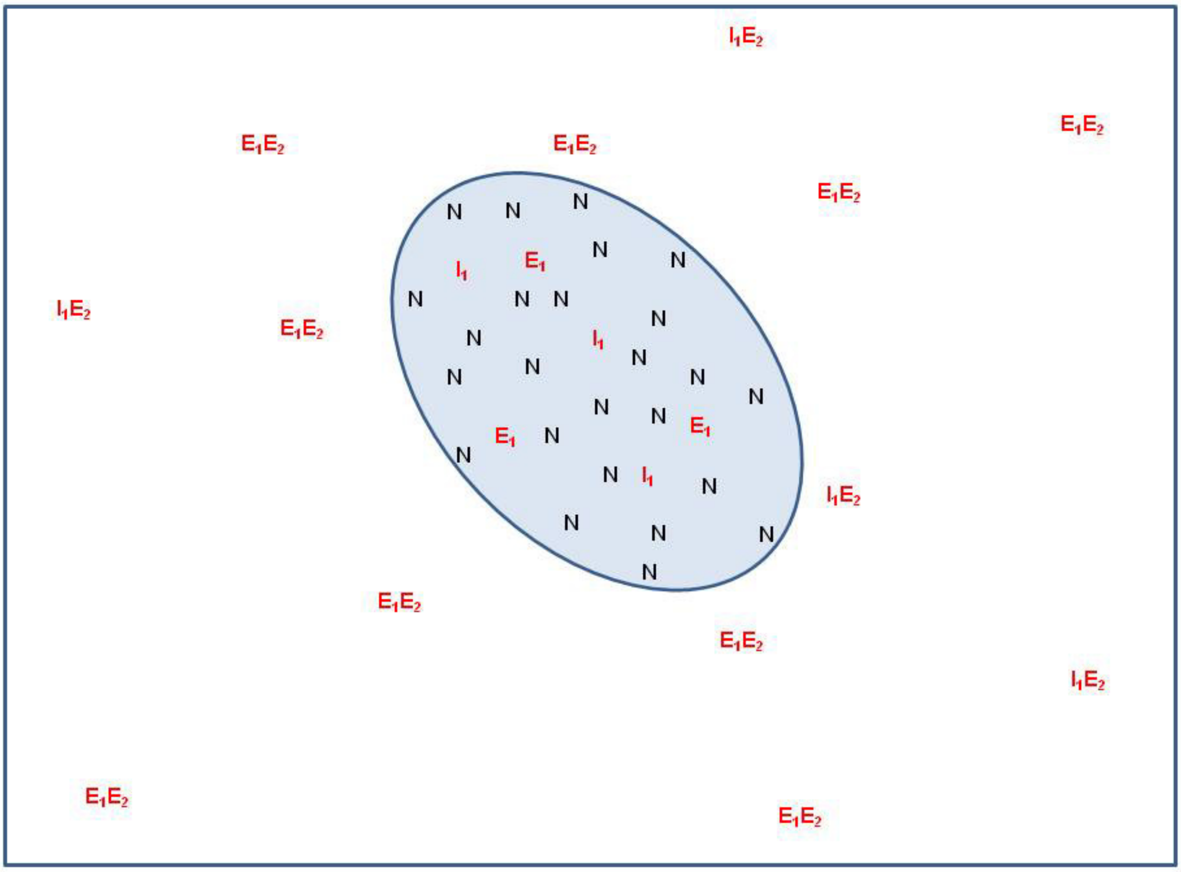

Figure 1 Schematic diagram of experimental layout, I = transplanted intact plants, E = transplanted emasculated plants, N = untransplanted, intact plants in a continuous patch. Subscript 1 denotes plants used in analysis of the effects of isolation, subscript 2 denotes plants used in the analysis of the effect of mate presence.

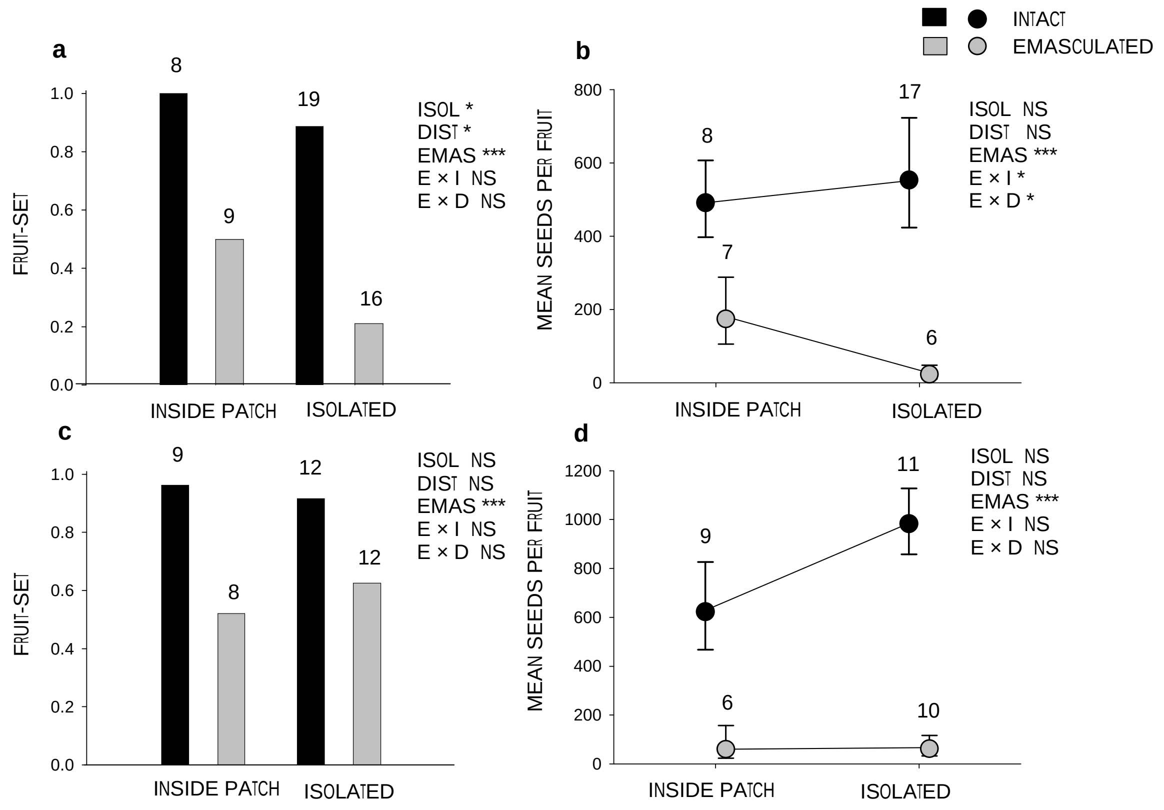

Figure 2

Fruit set and seeds per fruit for emasculated and intact plants inside and outside patches at Baynesfield (a, b) and Karkloof (c, d). For fruit set (a, c) bars represent means of fruit set values for individual plants. For seeds per fruit (b, d), back transformed means and error bars are plotted. Numbers above bars are numbers of plants.

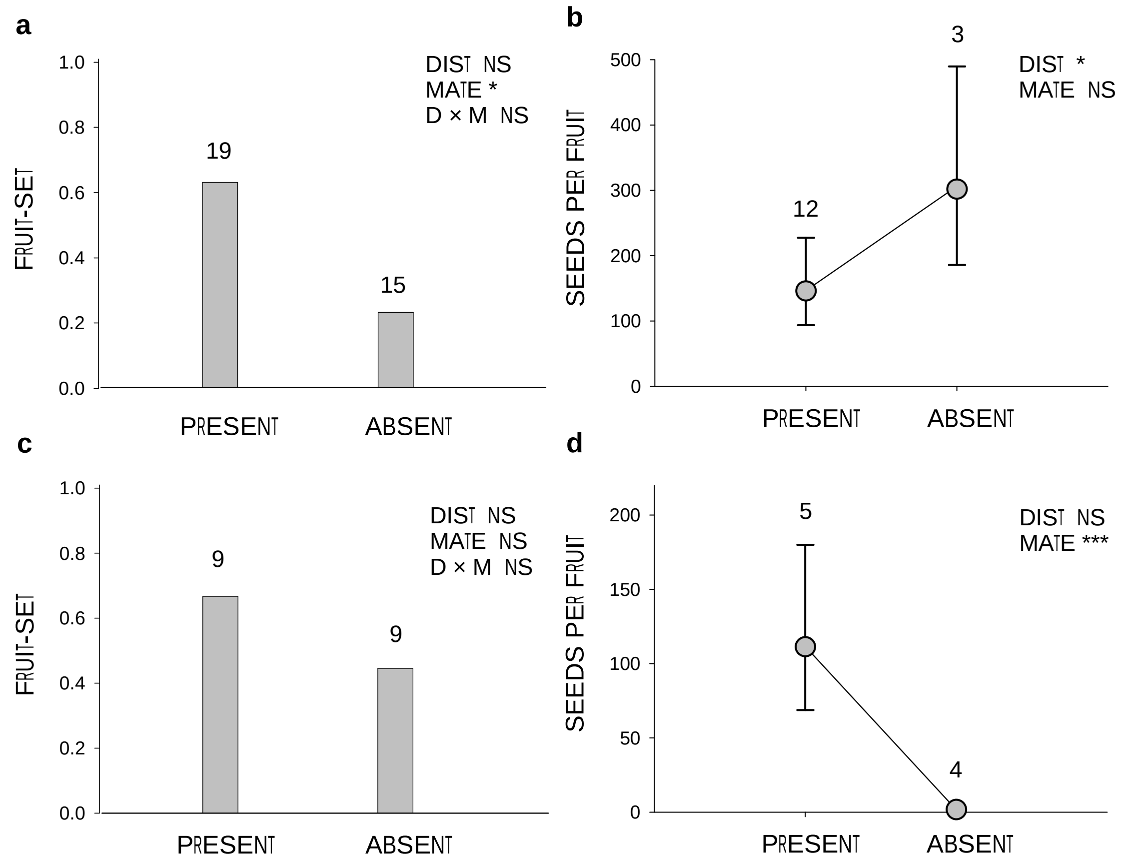

Figure 3

Fruit set (a, c) and seeds per fruit (b, d) of isolated emasculated plants placed next to an intact plant (mate present) or placed next another emasculated plant (mate absent) at Baynesfield (a, b) and Karkloof (c, d). For fruit set, bars represent means of fruit set values for individual plants. For seeds per fruit, backtransformed means and standard errors of plant means on $\log_2$ scale shown. Numbers above bars are numbers of plants. For seeds per fruit all fruit had at least one seed.

Appendix

Table S1 Generalised linear model for effects of isolation and emasculation on fruit set in array experiment in Baynesfield.

Effect	Deviance	df	Mean deviance	Quasi-F	p
Isolation	4.57	1	4.57	4.49	**0.039**
Distance	5.02	1	5.02	4.93	**0.031**
Emasculation	81.80	1	81.80	80.41	< **0.001**
E × I	1.38	1	1.38	1.36	0.251
E × D	0.02	1	0.02	0.02	0.893
Residual	46.79	46	1.02		

Table S2 REML analysis of variance for effects of isolation and emasculation on seeds per fruit in array experiment in Baynesfield.

Effect	Wald	ndf	ddf	Wald-F	p
Isolation	0.37	1	32	0.37	0.547
Distance	0.3	1	32	0.3	0.589
Emasculation	31.07	1	32	31.07	< **0.001**
E × I	7.31	1	32	7.31	**0.011**
E × D	6.54	1	32	6.54	**0.015**

Table S3 Generalised linear model for effects of isolation and emasculation on fruit set in array experiment in Karkloof.

Effect	Deviance	df	Mean deviance	Quasi-F	p
Isolation	0.25	1	0.25	0.23	0.634
Distance	0.27	1	0.27	0.24	0.625
Emasculation	18.02	1	18.02	16.37	< **0.001**
E × I	0.04	1	0.04	0.04	0.841
E × D	0.34	1	0.34	0.305	0.584
Residual	38.54	35	1.10		

Table S4 REML analysis of variance for effects of isolation and emasculation on seeds per fruit in array experiment in Karkloof.

Effect	Wald	ndf	ddf	Wald-F	p
Isolation	0.02	1	30	0.02	0.888
Distance	1.34	1	30	1.34	0.257
Emasculation	23.32	1	30	23.32	**< 0.001**
E × I	0.15	1	30	0.15	0.702
E × D	0.01	1	30	0.01	0.928

Table S5 Generalised linear model for effects of mate presence and isolation distance on fruit set of isolated plants in array experiment in Baynesfield.

Effect	Deviance	df	Mean deviance	Quasi-F	p
Distance	0.13	1	0.13	0.08	0.775
Donor presence	8.20	1	8.20	5.36	**0.028**
DP × D	0.01	1	0.01	0.01	0.942
Residual	45.92	30	1.53		

Table S6 REML analysis of variance for effects of mate presence and isolation distance on seeds per fruit of isolated plants in array experiment in Baynesfield.

Effect	Wald	ndf	ddf	Wald-F	p
Distance	7.1	1	11	7.1	**0.027**
Donor presence	0.09	1	11	0.09	0.768
DP × D	0.03	1	11	0.03	0.874

Table S7 Generalised linear model for effects of mate presence and isolation distance on fruit set of isolated plants in array experiment in Karkloof.

Effect	Deviance	df	Mean deviance	Quasi-F	p
Distance	0.77	1	0.77	0.77	0.380
Donor presence	1.02	1	1.02	1.02	0.312
DP × D	0.90	1	0.90	0.90	0.342
Residual	22.04	14	1.57		

Table S8: REML analysis of variance for effects of mate presence and isolation on seeds per fruit of isolated plants in array experiment in Karkloof.

Effect	Wald	ndf	ddf	Wald-F	p
Distance	3.45	1	5	3.45	0.122
Donor presence	37.82	1	5	37.82	**0.002**
DP × D	0.56	1	5	0.56	0.488

Table S9 Generalised linear model for effects of isolation on scale deposition in Baynesfield.

Effect	Deviance		Mean deviance	Quasi-F	p
Isolation	0.02	1	0.02	0.01	0.922
Distance	0.44	1	0.04	0.19	0.667
Residual	61.62	26	0.88		

Table S10 Generalised linear model for effects of isolation on scale deposition in Karkloof.

Effect	Deviance	df	Mean deviance	Quasi-F	p
Isolation	1.60	1	1.59	1.08	0.309
Distance	0.03	1	0.03	0.02	0.881
Residual	32.36	22	1.47		

Table S11 Generalised linear model for effects of isolation on pollen deposition in Baynesfield.

Effect	Deviance	df	Mean deviance	Quasi-F	p
Isolation	16.93	1	16.92	25.88	< **0.001**
Distance	0.00	1	0.00	0.00	1.000
Residual	17.00	26	0.65		

Table S12 Generalised linear model for effects of isolation on pollen deposition in Karkloof.

Effect	Deviance	df	Mean deviance	Quasi-F	p
Isolation	0.01	1	0.01	0.01	0.932
Distance	2.31	1	2.31	1.305	0.129
Residual	38.95	22		3.209	

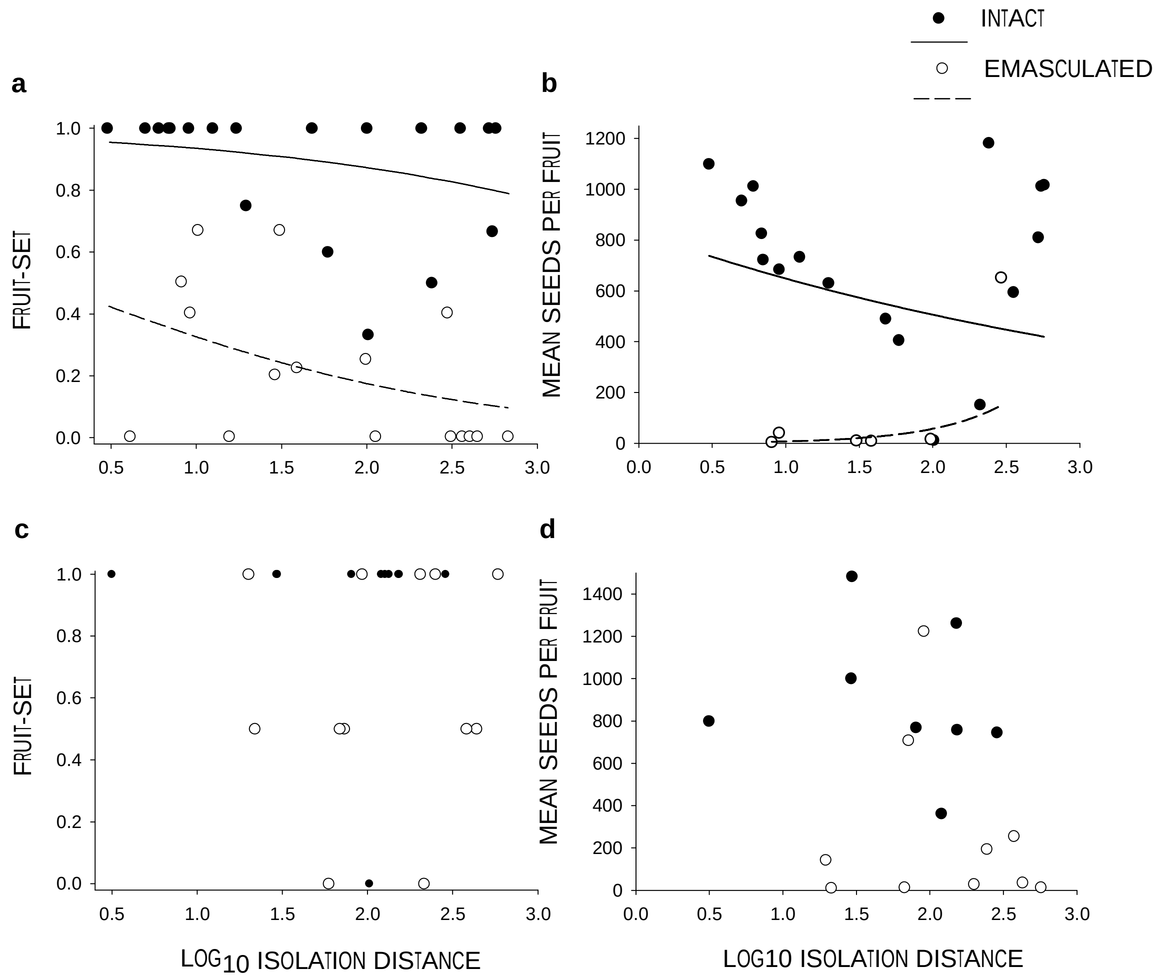

Fig S1

Fruit set (a, c) and seeds per fruit (b, d) of intact and emasculated isolated plants against distance of isolation from patches in Baynesfield (a, b) and Karkloof (c, d). Points represent proportion fruit set and mean seeds per fruit for plants. Regression lines fitted on Log_{10} transformed data and backtransformed.

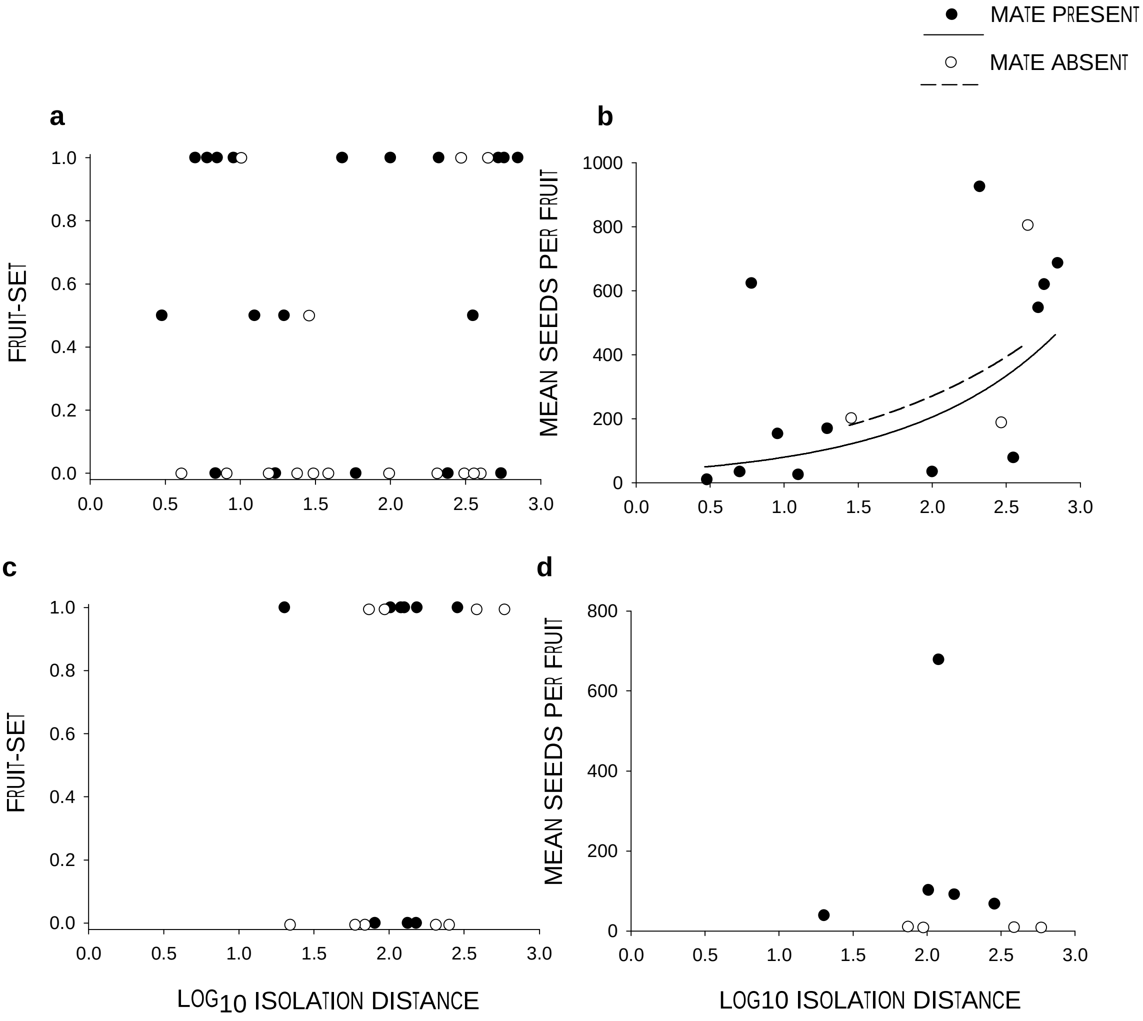

Fig. S2

Fruit set (a, c) and seeds per fruit (b, d) of emasculated isolated plants with a potential mate (an intact plant) nearby (mate present) or with another emasculated plant nearby (mate absent) at Baynesfield (a, b) and Karkloof (c, d). Points represent proportion fruit set and mean seeds per fruit for plants. Regression lines fitted on Log_{10} transformed data and backtransformed.

Chapter 9

Conclusions

In this thesis I have investigated the mechanistic basis for the correlation between ability to self-fertilise and invasiveness in plants. Specifically, I have tested whether autonomous self-fertilisation provides reproductive assurance for selected invasive plant species in the introduced range, as proposed by Baker's Law. Although the advantage proposed in Baker's original formulation was that uniparental reproduction would allow plants to reproduce in the absence of mates and pollinators, I have assessed more broadly whether self-fertilisation increases fecundity of invasive species, and whether it does so to a greater extent at low plant abundance. Further, I conducted progeny trials to assess inbreeding depression, as this cost of selfing potentially negates reproductive assurance benefits. I also conducted observations and experiments to identify the principle pollinators of my study species as reproductive assurance and its relationship to plant abundance depend on pollinator visitation.

Pollination relationships

The honeybee *Apis mellifera scutellata* was found to be the principal pollinator of the introduced *Acacia* species *A. mearnsii*, *A. dealbata* and *A. decurrens* (Chapter 2) while the hawkmoth *Agrius convolvuli* was found to be the main pollinator of *Lilium formosanum* (Chapter 5). Each of these two pollination investigations illustrates a trend emerging from the growing body of work on the pollination of invasive species in the introduced range.

Previous investigations had identified the native honeybee *Apis mellifera scutellata* as the main pollinator of *Acacia mearnsii* in its introduced range in South Africa (WRI 1950, Rambuda, 2001). I confirmed this finding and extended it to *Acacia dealbata* and *A. decurrens* (Chapter 2). It is likely that *Apis mellifera* plays a particularly important role in facilitating invasion of Australian *Acacia* species, as it has been recorded from all those examined, both in their native and introduced ranges (Bernhardt 1987, 1989, Sornsathapornkul and Owens 1998, Alves and Marins-Corder 2009, Gibson et al, 2011), and is itself either native or introduced to every continent except Antarctica. Moreover, honeybees are frequent visitors to many other invasive plant species in addition to *Acacia* (Goulson 2003, Morales and Aizen 2006, Kaiser-Bunbury et al. 2011), suggesting that *A. mellifera* plays an important role in facilitating plant invasions globally (Goulson 2003).

My investigation of *Lilium formosanum* showed that it has a highly specialised pollination system in South Africa and identified the large hawkmoth *Agrius convolvuli* as its principal pollinator in this region (Chapter 5). Fascinatingly, *A.convolvuli* is a cosmopolitan species indigenous to Taiwan, the native range of *L. formosanum*, as well as its introduced range in South Africa, Australia and Japan. It is therefore likely that *L. formosanum* is also pollinated by *A. convolvuli* in its native range and has re-established this specialised relationship upon introduction. Although it is exceptional for a specialised relationship with a specific pollinator species to be re-established following introduction, there are several examples where plants specialised to particular pollinator functional groups have established relationships with other members of those functional groups upon introduction. Examples include plants pollinated by carrion flies (Herrera and Nassar 2009), vespid wasps (Forster 1994, Coombs et al. 2009), nectar feeding birds (Schueller 2004, Geerts and Pauw 2009) and large buzz-pollinating bees (van Kleunen and Johnson 2005).

Although plants with specialised pollination systems commonly find pollinators in the introduced range, pollination specialisation may still hinder invasion. Self-incompatible plants with specialised pollination systems tend to suffer greater pollen limitation than those with more generalised pollination systems (Knight et al. 2005), presumably because they are more vulnerable to fluctuations in abundance of particular pollinator species (Waser et al. 1996). For introduced plants without autofertility, generalised pollination may thus provide a fecundity advantage, not because specialised plants lack pollinators in the novel range, as envisioned by Baker (1955), but because of unreliability of pollen receipt (Chrobock et al. unpublished manuscript, although see Gardner and Early 1996, Pemberton and Liu 2008)). Indeed, Chrobock et al. found that among plant species with specialised pollination systems, self-compatible species had spread more successfully (in more regions) than self-incompatible species. Nevertheless, invasions by self-incompatible species with specialized pollination systems are not generally precluded.

Inbreeding depression and breeding systems

Controlled pollination experiments on *A. dealbata* (Chapter 3) and *L. formosanum* (Chapter 5) showed that both species were self-compatible and autonomously self-fertilising. Progeny trials indicated inbreeding depression was high in *Acacia dealbata* (Chapter 3) but absent to very low in *Lilium formosanum* (Chapter 6).

A controlled pollination experiment showed that *A. dealbata* was self-compatible and autonomously self-pollinating, with inbreeding depression during seed development, in its introduced range in KwaZulu-Natal (Chapter 3). In subsequent progeny trials this species displayed lower rates of survival and growth of progeny from self- compared to cross-pollination, indicating inbreeding depression. Performance of progeny from open-pollination was intermediate, suggesting a mixed mating system (Chapter 3, Figs 1, 2). This inference was supported by expression of abnormalities in leaf and stem colouration that were associated with reduced performance. These abnormalities were always significantly more frequent in progeny arising from self- than in those from cross-pollination and intermediate in those from open pollination (Chapter 3). These results suggest that *A. dealbata* may suffer qualitative pollen limitation due to seed discounting, detracting substantially from any reproductive assurance benefit from selfing. In contrast, I found almost no evidence for inbreeding depression in *L. formosanum*. I assessed performance in seed development; in germination, growth, survival and flowering in a common shade-house environment and germination, growth and survival in the field. Evidence of inbreeding depression was only detected in one of five populations grown in the shade-house and only for flowering in the first of two years in which flowering took place (Chapter 6). Therefore, reproductive assurance from self-fertilization (Chapters 7, 8) can be interpreted as beneficial.

Inbreeding depression in my study species was within the range previously reported for self-compatible plants of the same life-forms, although *L. formosanum* represents the low extreme for herbaceous perennials (Hardner and Potts 1995, Husband and Schemske 1996, Ishida 2011, Robertson et al. 2011, Angeloni et al. 2011). In general herbaceous plants are far more frequently self-compatible (Barrett et al. 1996) and have lower levels of inbreeding depression than woody plants (Duminil et al. 2009). However, inbreeding depression has been examined in few invasive plants and even then seldom over the entire life-cycle. Nevertheless, apart from *Acacia* (Chapter 3), the few studies available, conducted on herbaceous plants and shrubs, indicate low or absent inbreeding depression following self-fertilisation (Nunez-Farfan et al. 1996, Suehs et al. 2004, Stout 2007, Sloop et al. 2009, Vervoort et al. 2011), consistent with promotion of invasion by selfing.

Reproductive assurance and the mitigation of pollen-limitation Allee effects

Across both the *Acacia* and *Lilium formosanum* systems, I found much less evidence for the mitigation of Allee effects by reproductive assurance than I expected. In *Acacia dealbata* I found no evidence for any effect of abundance on reproductive assurance (Chapter 4) while in *Lilium formosanum* I found reproductive assurance was sometimes higher in more isolated populations and in isolated individuals compared to those in continuous population, but was not related to population size at all (Chapters 7, 8).

I did not successfully assess reproductive assurance in *Acacia dealbata*. I had hoped that the extent of reproductive assurance through self-pollination in this species could be assessed indirectly by comparing pollen limitation between it and the self-incompatible *A. mearnsii.* However, results of pollen supplementation experiments were ambiguous about the presence of pollen limitation of fecundity in both species (Chapter 4). It is also not possible to assess reproductive assurance directly in *Acacia* species as floral emasculation is not practical for these flowers. Nevertheless, results for *A. mearnsii* showed that whether or not fruit set was pollen limited, there was no difference in pollen limitation between isolated and non-isolated trees. This strongly suggests that there was no difference in outcross pollen receipt between isolated and non-isolated trees and that for *A. dealbata*, isolation would not have affected reproductive assurance, if it had occurred (Chapter 4).

The probable reason that isolation did not affect pollen limitation of initial fruit set in *A. mearnsii* (Chapter 4) is that even isolated plants remain highly profitable for honeybee pollinators due to the large display size of individual trees. Plants in which severe Allee effects via pollen limitation have been documented are herbs or occasionally shrubs with small display size (Sih and Baltus 1987, Feinsinger et al. 1991, Kunin 1993, Ågren 1996, Groom 1998, Ward and Johnson 2005). The distances of isolation examined for *A. mearnsii* (up to 450m) are well within honeybees' routine foraging range and this species is known for its ability to integrate information on relative profitability of available resources (e.g. Visscher and Seeley 1982). Similarly, in the invasive, self-compatible but pollinator dependent shrub *Senna didymobotrya*, which is pollinated by large carpenter bees, pollen-receipt and fruit set were not related to population size (van Kleunen and Johnson 2005). This was also attributed to large display size and mobility of pollinators (van Kleunen and Johnson 2005). Studies of the effects of isolation over hundreds to thousands of metres on forest trees in fragmented landscapes have shown negative effects of isolation on outcrossing rates, fecundity and progeny performance. However these effects are relatively weak and isolated trees still make a demographic and genetic contribution to population persistence (Lowe et al. 2005, Kramer et al. 2008). My findings (Chapter 4) and those of van Kleunen and Johnson (2005) allow this inference to be extended to invasive woody plants, suggesting that relatively isolated individuals may make an important contribution to invasion through cross-pollination.

Emasculation and supplementation experiments conducted in natural populations of *L. formosanum* demonstrated substantial reproductive assurance and low or absent pollen limitation (Chapter 7). This generally high reproductive assurance, with variation among populations and years, is consistent with the idea that plants with more specialised pollination systems experience greater variation in pollen receipt (Waser et al. 1996, Knight et al. 2005, Fenster and Marten-Rodriguez 2007). Although no relationship was found between population size and reproductive assurance (Chapter 7), reproductive assurance did mitigate reduced visitation in more isolated populations in one of three years in the population size and isolation study (Chapter 7) and reduced mate availability in plants isolated from continuous populations (patches) at one of two sites in the plant isolation study (Chapter 8). Reproductive assurance through selfing thus arose mainly from generally inadequate pollinator visitation, rather than effects of low abundance. While these effects are less consistent than the effect of low visitation in general, they do provide the first support for the hypothesis that reproductive assurance mitigates potential pollen-limitation Allee effects.

Interestingly, as found here for *L. formosanum*, other hawkmoth-pollinated plants seem relatively resilient to Allee effects – population size did not affect reproductive assurance through selfing in *D. stramonium* (van Kleunen et al. 2007) or pollinator visitation and fecundity in the native orchid *Satyrium longicauda* (Johnson et al. 2009). The weak or absent effects of population size on fecundity in hawkmoth-pollinated plants (van Kleunen et al. 2007, Johnson et al. 2009, Chapters 7) contrasts to the situation in plants with similar display size pollinated by bees and birds, which tend to show dramatic decreases in both visitation and outcross-pollen receipt in small populations (e.g. Sih and Baltus 1987, Lamont et al. 1993, Ågren 1996, Groom 1998). This may be because hawkmoths forage more opportunistically than other pollinators (Johnson et al. 2009) or perhaps because use of scent as a cue for finding flowers does not allow them to accurately assess size of populations prior to arrival.

The occurrence of mixed mating

Progeny trials suggested mixed mating in *A. dealbata* while reproductive assurance estimates indicated mixed mating in *L. formosanum* (Chapters 3, 7). Mixed mating occurs frequently in nature but its adaptive basis is poorly understood (Goodwillie et al. 2005). Models of mating system evolution predict mixed mating under only very restricted conditions but this topic is complex and all of the models make simplifying assumptions that restrict their generality (Goodwillie et al. 2005). When inbreeding depression is very high, it is expected that plants will evolve towards complete outcrossing unless there is substantial reproductive assurance

(Herlihy and Eckert 2002, Goodwillie et al. 2005, Eckert et al. 2006). High inbreeding depression measured to three months in *A. dealbata* suggested that mixed mating may not be advantageous in this species, although inbreeding depression was only estimated for part of the life cycle and reproductive assurance remains unknown (Chapter 4). When reproductive assurance is high and inbreeding depression low or absent, as in *L. formosanum*, evolution should favour complete selfing (Kalisz et al. 2004, Goodwillie et al. 2005, Eckert et al. 2006). For such cases, only explanations involving the male fitness advantages of mixed mating have been invoked to explain why complete selfing does not evolve, although these models may not be applicable to animal pollinated plants (Holsinger 1991). We await more general models to explain the observed prevalence of mixed mating in plants.

Conclusions and directions

This work has improved our understanding of the relationship between self-fertilisation and invasiveness in plants. To the best of my knowledge, *L. formosanum* is the only invasive species for which both reproductive assurance and inbreeding depression have been measured in the same invasive range, giving a complete test of the benefit of selfing (Chapters 6-8). The generally high level of reproductive assurance and almost complete absence of inbreeding depression in this species makes the strongest case yet case for the role of reproductive assurance in promoting invasion. My investigations also provide the first support for the hypothesis that reproductive assurance promotes invasion specifically by alleviating pollen limitation at low abundance (Chapters 7, 8), which has very seldom been investigated (although see also van Kleunen et al. 2007).

These findings should be extended by molecular analyses of the present and historical frequency of self-pollination. Direct estimates of selfing rates based on isozymes or microsatellites will be needed for any attempt to understand the evolution of mixed mating in *L. formosanum* (Goodwillie et al. 2005). Selfing rate estimates should also be used to confirm the occurrence of mixed mating inferred from progeny performance in *A. dealbata*. In *L. formosanum*, as pollinator visitation is generally inadequate and mainly not attributable to low abundance, selfing rates have probably been high at least since introduction to South Africa. Populations that have high selfing rates generally display low genetic diversity (Glemin et al. 2006), high differentiation between populations (F_{ST}) and high inbreeding coefficients (F_{IS}) (Duminil et al. 2009). Population genetics statistics should be estimated for *L. formosanum* and examined for consistency with a history of mixed mating during invasion.

Ideally these statistics should also be assessed for source populations in the native range, although further detailed molecular work would be needed to identify likely source populations (Dormontt et al. 2011).

The high inbreeding depression displayed by *A. dealbata* to the age of three months in the shade-house suggests that selfing may not contribute to invasiveness as selfed progeny are unlikely to survive to reproduce (Chapter 3). However, inbreeding depression needs to be estimated over the entire lifespan to address the possibility that selfing contributes to invasiveness in this species. This could be accomplished by growth trials of selfed and crossed progeny to maturity in the field or molecular marker studies assessing change in inbreeding coefficient (F_{IS}) over the lifespan (Ritland 1990). Population genetics statistics measured from adults would also give an indication of historical selfing rates. Nevertheless, even if it can be shown that selfed progeny survive to reproduce, because floral morphology prevents emasculation and hence direct measurement of reproductive assurance – ie actual dependence on self-fertilisation for fecundity – it may not be possible to reach a definitive conclusion on the importance of selfing for invasion in this species.

By showing that for *Lilium formosanum* reproductive assurance was high (Chapters 7, 8) and that this benefit of selfing was not outweighed by inbreeding depression (Chapter 6) I have built a more compelling case for the role of reproductive assurance in invasion than has previously been made. However, even this does not conclusively show that selfing contributes to invasiveness. Plants are invasive when they experience population growth and spread in the novel range. Reproductive assurance will only affect invasiveness if these processes are seed limited. I am currently engaged in a demographic study to assess whether reproductive assurance increases rates of population growth and spread, and hence invasiveness, in *L. formosanum*. This involves building demographic models, parameterised with measurements of germination, growth, survival and fecundity in the field and assessing whether population growth and rate of spread would be lower without reproductive assurance. As rate of invasive spread is disproportionately sensitive to fecundity in isolated populations and individuals (Taylor and Hastings 2005), spatially explicit models should be used to explore the relative contributions of selfing to invasiveness arising from general pollinator failure versus the exacerbating effects of isolation. Modelling is likely to be less useful for answering these questions in *A. dealbata*, where reproductive assurance can not be measured directly.

While the case studies presented here require extension to conclusively assess whether that self-fertilisation contributes to invasion in these particular species, the approaches I have followed here and advocated for future work will need to be applied to other invasive species to evaluate the importance of selfing for invasion in general. A more refined understanding of the factors determining the occurrence and intensity of pollen-limitation Allee effects is also desirable to improve the conservation of rare species and the control of invasive species (Taylor and Hastings 2005). There may already be enough published material on this topic to support a meta-analysis, testing whether pollinator functional groups respond differently to flower abundance and examining how response to plant abundance is related to display size.

The questions posed in my thesis are ecological in nature and contribute to our understanding of how species traits, in this case ability to self-fertilise, affect invasiveness. However, species have their traits as a result of evolution. Naturally, if the traits responsible for invasiveness evolve in the introduced range this must be taken into account if we are to use traits to predict future invasions (Pysek and Richardson 2007). The extent to which evolution in the introduced range contributes to invasiveness is currently of great interest in invasion biology for this reason as well as the insight it can give into evolution of native species (Dormontt et al. 2011). However, few studies have yet compared reproductive biology of invasive species between the native and introduced ranges (although see for example Colautti et al. 2010a, b). Unfortunately, information on the reproductive biology of my study species in the native range is scarce and it was not possible to directly compare breeding systems or pollinators in the native and introduced range except for *A. mearnsii* (Bernhardt 1987, 1989, Moncur et al. 1991). Comparisons between the introduced and native range should be undertaken in these species and in general, to allow us to assess the extent to which ability to self-fertilise is a pre-adaptation versus it being a trait that evolves after introduction.

In conclusion, I have advanced the hypothesis that selfing promotes invasiveness in plants by showing that in the invasive *L. formosanum*, inadequate pollinator visitation and effective autonomous self-pollination result in high levels of reproductive assurance across a wide range of population size and isolation levels and that inbreeding depression is almost completely absent. Future demographic work on this species will assess whether increased fecundity results in increased rates of population growth and spread and hence invasiveness. Work on invasive *Acacia* species was less conclusive and this system is less useful for assessing the importance of selfing for invasion as reproductive assurance can not be measured directly. Nevertheless, the possibility that selfing contributes to invasiveness in *A. dealbata* and other tree species despite inbreeding depression has not been eliminated and future work should focus first on testing whether selfed progeny are able to reproduce. If this is the case, then the possibility that this increases invasion via increased fecundity and more successful long distance dispersal should be considered. Similar studies to those presented here should be initiated on other invasive species to establish the general basis of the relationship between ability to self-fertilise and invasiveness.

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Appendix 1

Reproductive biology of Australian acacias: important mediator of invasiveness?

Gibson, M. R., D. M. Richardson, E. Marchante, H. Marchante, J. G. Rodger, G. N. Stone, M. Byrne, A. Fuentes-Ramírez, N. George, C. Harris, S. D. Johnson, J. J. Le Roux, J. T. Miller, A. Pauw, M. N. Prescott, E. M. Wandrag, and J. R. U. Wilson.

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BIODIVERSITY REVIEW

Reproductive biology of Australian acacias: important mediator of invasiveness?

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ABSTRACT

Aim Reproductive traits are important mediators of establishment and spread of introduced species, both directly and through interactions with other life-history traits and extrinsic factors. We identify features of the reproductive biology of Australian acacias associated with invasiveness.

Location Global.

Methods We reviewed the pollination biology, seed biology and alternative modes of reproduction of Australian acacias using primary literature, online searches and unpublished data. We used comparative analyses incorporating an *Acacia* phylogeny to test for associations between invasiveness and eight reproductive traits in a group of introduced and invasive (23) and non-invasive (129) species. We also explore the distribution of groups of trait 'syndromes' between invasive and non-invasive species.

Results Reproductive trait data were only available for 126 of 152 introduced species in our data set, representing 23/23 invasive and 103/129 non-invasive species. These data suggest that invasives reach reproductive maturity earlier (10/13 within 2 years vs. 7/26 for non-invasives) and are more commonly able to resprout (11/21 vs. 13/54), although only time to reproductive maturity was significant when phylogenetic relationships were controlled for. Our qualitative survey of the literature suggests that invasive species in general tend to have generalist pollination systems, prolific seed production, efficient seed dispersal and the accumulation of large and persistent seed banks that often have fire-, heat- or disturbance-triggered germination cues.

Conclusions Invasive species respond quicker to disturbance than non-invasive taxa. Traits found to be significant in our study require more in-depth analysis involving data for a broader array of species given how little is known of the reproductive biology of so many taxa in this species-rich genus. Sets of reproductive traits characteristic of invasive species and a general ability to reproduce effectively in new locations are widespread in Australian acacias. Unless there is substantial evidence to the contrary, care should be taken with all introductions.

Keywords

Biological invasions, breeding system, invasive alien species, pollination, reproductive syndromes, reproductive traits, seed dispersal

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INTRODUCTION

A predictive understanding of invasiveness is needed to manage existing invasive species and for objective screening of new introductions. Elucidating the determinants of invasiveness and understanding how these interact with environmental features and extrinsic factors to mediate invasion success are fundamental questions in invasion ecology (Richardson & Pysek, 2006). Anthropogenic and environmental factors and various life-history traits, particularly features associated with reproduction and dispersal (Rejmánek *et al.*, 2005; Thuiller *et al.*, 2006; Pysek & Richardson, 2007), are often associated with invasion success (or lack thereof). Previous studies comparing life-history traits of invasive species have found several reproductive traits including seed mass, fecundity (number of seeds produced), dispersal mode and dispersal ability to be important for overcoming barriers to invasion in a new environment (Hamilton *et al.*, 2005; Pysek & Richardson, 2007; Moravcová *et al.*, 2010; Castro-Díez *et al.*, 2011). There has, however, been no comprehensive analysis of the roles of such traits in invasiveness in Australian acacias, a speciose group of plants containing several invasive species.

This study assesses the current state of knowledge regarding associations between reproductive traits and invasiveness in this group, which here refers to the *ca.*1012 taxa in the genus *Acacia* (hereafter referred to as 'Australian acacias' or *Acacia,* formerly placed in *Acacia* subgenus *Phyllodineae* and synonymous with *Racosperma*) that have Australia as at least part of their native range; see Miller *et al.* (2011) for a more recent phylogenetic treatment of this and related groups. To do this, we present an analysis in two parts: (1) a quantitative comparative analysis of specific reproductive traits for which appropriate data were available; and (2) a qualitative literature review of reproductive traits for which we could not find quantitative data, but which may be important in predicting invasiveness. We conclude with the implications for management.

Australian acacias are an excellent group for exploring determinants of invasiveness and are likely to become a model system against which other invasive plant groups are compared (Richardson *et al.*, 2011). They comprise a phylogenetically and geographically distinct group (natural distributions virtually confined to the Australian continental landmass) with 1012 described species (Richardson *et al.*, 2011), of which at least a third have been introduced and 23 are invasive in different parts of the world (Richardson & Rejmánek, 2011; Richardson *et al.*, 2011). Their well-documented introduction histories (e.g. Le Roux *et al.*, 2011) and records of invasiveness in different introduced ranges make comparative studies possible on continental and global scales. Australian acacias appear to possess a suite of reproductive and other life-history traits that have been suggested as instrumental in their success as invasive species (Milton & Hall, 1981; Richardson & Kluge, 2008). Unfortunately, invasive taxa among Australian acacias are far better studied than are non-invasive taxa; this is in line with a general bias in invasion ecology whereby invasive species that exert greater impacts on invaded environment are better studied (Pysek *et al.*, 2008). This complicates statistical analysis of associations between species character traits and invasiveness.

Little is known in general about such associations (Gallagher *et al.*, 2011), and to date, no multi-species, multi-regional study has explored how reproductive traits influence invasiveness of Australian acacias. In this study, we review available published and unpublished information on their reproductive traits and trait 'syndromes' (sets of reproductive traits that repeatedly favour a particular group of pollinators, method of reproduction, agent of seed dispersal or germination system) and compare trait values between (1) rare and common Australian acacias; (2) invasive Australian species in their native and introduced ranges; and (3) introduced invasive species and introduced non-invasive Australian acacias. Our aim is to identify those traits associated with invasiveness. Our approach has been dictated by the availability of data. For those traits for which data are available (Table S1), we use phylogenetically controlled comparative analyses to ask which reproductive traits, alone or in combination, are significant correlates of invasiveness. For those traits we were unable to analyse quantitatively, we qualitatively review all available information to address the questions: (1) Are there distinct reproductive syndromes that differ between invasive and non-invasive species? and (2) does pollinator-mediated seed production reduce or enhance naturalization or invasion in any regions? Such an approach has the potential to yield insights that are of value to plant invasion ecology in general and for refining screening protocols (e.g. Gordon *et al.*, 2010) for assessing the risk of further introductions of *Acacia* species that may lead to invasions.

Methods

Species list

We used the classification scheme of Richardson & Rejmánek (2011) to define which species are considered invasive (n = 23). The objective criteria used in their study (following Pysek *et al.*, 2004) are more conservative than those applied by others (e.g. Randall, 2002), and only species that have spread considerable distances from parent populations are considered 'invasive'. However, the criteria are not as strict as in other studies, such as Castro-Díez *et al.* (2011), who regarded species as 'invasive' (sensu Pysek *et al.*, 2004) only when supported by at least two different sources of information from different countries. Species were defined as having been introduced (n = 152) only if a herbarium record for that species has been collected from outside Australia (Richardson *et al.*, 2011).

We compiled data on at least one of eight reproductive traits for 450 of the 1012 species in the Australian *Acacia* group. Of the 860 non-introduced species, data were available for six of the traits for 324 species (Table S2). Of the 152 introduced species, data were available for all eight traits for 126 species

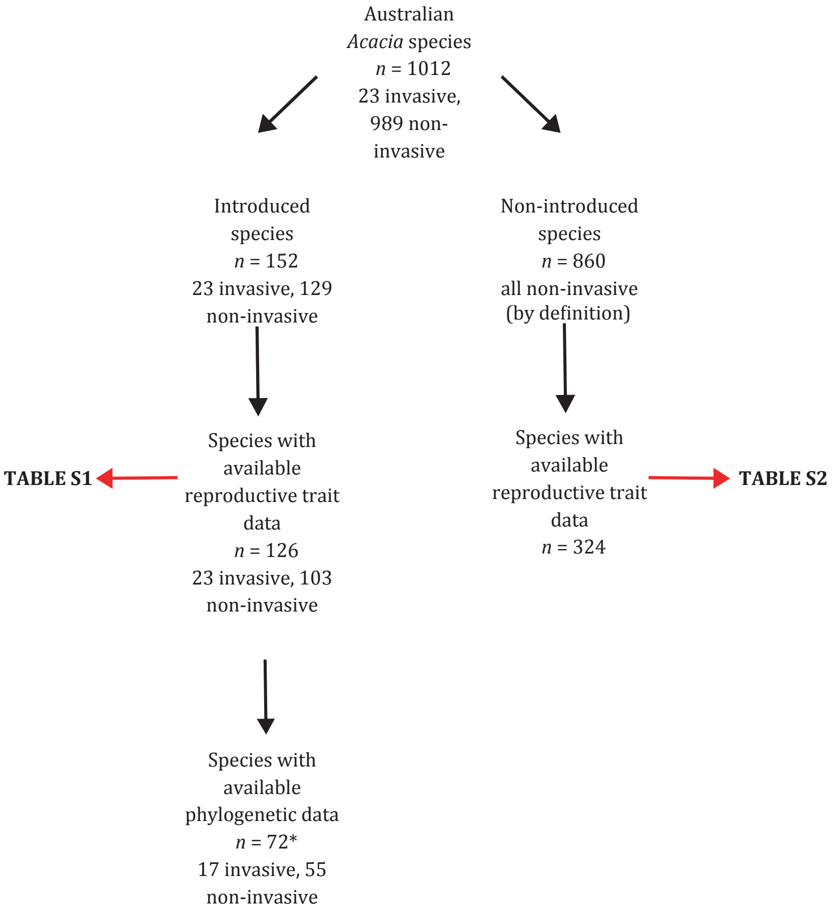

Figure 1 Breakdown of Australian *Acacia* species used in this study. *One of the species for which there was phylogenetic data had no available reproductive trait data.

(23 invasive, 103 non-invasive; see Table S1) – see Fig. 1 for a breakdown of species used in this study. We analysed data on reproductive traits using only introduced species to reduce biases caused during the introduction process.

Statistical analysis

We used R for all statistical analyses (R Development Core Team, 2011). Reproductive traits were used as explanatory variables, and invasive status (invasive and non-invasive) was used as the response variable. Explanatory variables used in quantitative analyses comprised: time to reproductive maturity; index of self-incompatibility (ISI) (number of infructescences/inflorescence); ISI (number of pods/inflorescence); combined measure of breeding system; dispersal agent (ant- or bird-dispersed seed); seed mass; resprouting ability; and length of flowering period (see Appendix 1 for details and references). Seed mass was log transformed to reduce skewness in the data. Seeds were considered to be dispersed by birds either if this was conclusively reported in the literature or, based on seed morphological traits, if the arils/funicles or elaiosomes were specifically described as being orange, yellow or red. Species were considered to be 'not bird dispersed' if they were reported to be dispersed by ants in the literature and where dispersal by birds was not mentioned. Species for which clear data were not available were omitted from the analysis. A combined measure of breeding system was inferred from multi-locus outcrossing rate (t_m), both ISI measurements, and breeding system (t_m and breeding system not used in final analyses; see Appendix 1 and Table S1). We considered a species as outcrossing if $t_m ÷ 0.8$ or ISI ≤ 0.5; otherwise, species were considered to have mixed mating systems.

Because species do not represent independent data points in comparative studies (Hadfield & Nakagawa, 2010; Stone *et al.*, 2011), we incorporated phylogenetic relationships among sampled species into our analyses using a generalized least-squares (gls) framework in the nlme package (Pinheiro *et al.*, 2009). This approach assumes a Brownian model of character evolution in which trait covariance between a pair of species decreases linearly since their time of divergence from a shared common ancestor. The phylogenetic relationship between taxa was inferred using Bayesian methods incorporated in the software MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). Our analysis incorporates sequence data for two nuclear genes (nuclear ribosomal DNA internal (ITS) and external (ETS) transcribed spacers) and four chloroplast regions (*psb*A-*trn*H intergenic spacer, *trn*L-F intron and intergenic spacer, *rpl*32-*trn*L intergenic spacer and a portion of the matK introns), comprising a tandem alignment of 5912 base pairs. Contiguous sequences were edited using Sequencher v.3.0 (Gene Codes Corporation) and manually aligned in BioEdit sequence alignment editor v.4.8.6 (Hall, 1999). Appropriate models of molecular evolution for implementation in MrBayes were identified using the programme Modeltest v.1.1 (Posada & Crandall, 1998), which identified the GTR + I + G model (general time reversible model incorporating a proportion of invariant sites and gamma-distributed rate variation in variable sites) for both the plastid and nuclear partitions of our data set. The Markov chain Monte Carlo search in MrBayes was run for two million generations with trees sampled every 1000 generations. MrBayes performed two simultaneous analyses starting from different random trees (Nruns = 2), each with four Markov chains (Nchains = 4). The first 200 sampled trees were discarded from each run as burn-in. We used the 50% majority rule consensus phylogram as our working phylogeny, with node support expressed in terms of posterior probability values. All trees were rooted using *Pararchidendron pruinosum* as an outgroup taxon.

The resultant phylogeny incorporated 72 species of the 126 species (see Miller *et al.*, 2011), and only data for these species were incorporated into phylogenetically controlled analyses (17 invasive, 55 non-invasive; see Fig. 2 for phylogenetic tree and Appendix S1 for species accession numbers). Because our analytical approach to determine phylogenetic independence requires a fully resolved phylogeny, polytomies were broken by inserting very small non-zero branch lengths. Reanalysis with such instances pruned from the data gave near-identical results (not shown). To assess the impact of phylogenetic patterns in our trait data, we compared analyses incorporating phylogenetic information for this subset of 72 species with phylogeny-free analyses for the same species set. To illustrate patterns in the full data set, we also carried out phylogeny-free analyses across the full set of 126 species. For both data sets (n = 72 and n = 126), phylogeny-free tests of trait differences between invasive and non-invasive species involved Pearson's chi-square tests for binary explanatory variables and generalized linear models for individual continuous explanatory variables.

Results

Of the eight reproductive traits we assessed, only two showed significant differences between invasive and non-invasive species in phylogeny-free analyses (Table 1A,B; see Appendix S2 for actual parameter estimates, results were similar when using either all 126 species or the subset of 72 species for which we have a phylogeny). The proportion of species that reach reproductive maturity within two years was significantly higher for invasive acacias (χ^2 = 6.90, d.f. = 1, P = 0.009). Invasive species also had a significantly higher probability of being resprouters (χ^2 = 4.34, d.f. = 1, P = 0.037) than non-invasive species. Incorporation of phylogenetic relationships into the analysis for 72 species removed the significance of resprout ability, but supported our results from the phylogeny-free analyses that invasive species reach reproductive maturity earlier (gls: coefficient = −0.553, t = −3.18, P = 0.004; Table 1B, Appendix S3).

LITERATURE REVIEW: REPRODUCTIVE BIOLOGY OF AUSTRALIAN ACACIAS

Pollination biology

As a broad generalization, we expect successful invasive species to share at least some of the following floral traits (Baker, 1955; Chittka & Schürkens, 2001; Brown *et al.*, 2002; Ghazoul, 2002; Gross *et al.*, 2010):

1. High attractiveness to available flower visitors and floral morphologies allowing pollination by many different organisms.

2. Production of very large numbers of long-lived flowers allowing seed-set even when visitation rates are low; and/or an ability to self-pollinate or reproduce vegetatively.

3. Floral induction cues match those triggering flowering in native species and emergence of native flower visitors.

Worldwide, taxa classified in the polyphyletic group *Acacia sensu lato* (genera *Acaciella*, *Mariosousa*, *Senegalia*, *Vachellia*; McNeill *et al.*, 2006) share many of these morphological traits but differ in their global distributions, pollinator assemblages and specific aspects of floral biology (Stone *et al.*, 2003). All have small tubular flowers collected together into spherical or elongated flower heads, with pollen presented on the inflorescence surface (Stone *et al.*, 2003; Raine *et al.*, 2007). Clustering of the pollen grains into a composite unit, termed a 'polyad', is a key component of the pollination efficiency of all acacias, providing an efficient means of dispersal via pollinators (Kenrick & Knox, 1982). There are always fewer ovules per ovary than pollen grains per polyad, so one polyad from a single pollination event can potentially fertilize all the ovules (Kenrick & Knox, 1982). The stigmas of the flowers are also distributed over the surface of the flower heads and are freely accessible, so that any insect that travels from one tree to another is a potential pollinator. Recruitment of insects is often enhanced by the release of floral scent just before pollen release, and visual advertisement is often maximized by synchronized opening of flowers, both within a single tree and often within a local species' population (Stone *et al.*, 2003). Floral morphology is a conserved trait across the genus and does not distinguish invasive from non-invasive Australian acacias. Such generalized morphology may facilitate invasion as it reduces the risk of pollinator limitation for introduced plants (Richardson *et al.*, 2000*a*). See Fig. 3 for photographs of pollination biology traits associated with invasiveness in Australian acacias.

Floral biology

The fundamental floral morphology shared by all Australian acacias identifies a generalist entomophilous pollination syndrome as it provides accessible floral rewards to almost any insect visitor (Bernhardt, 1989). A second pollination

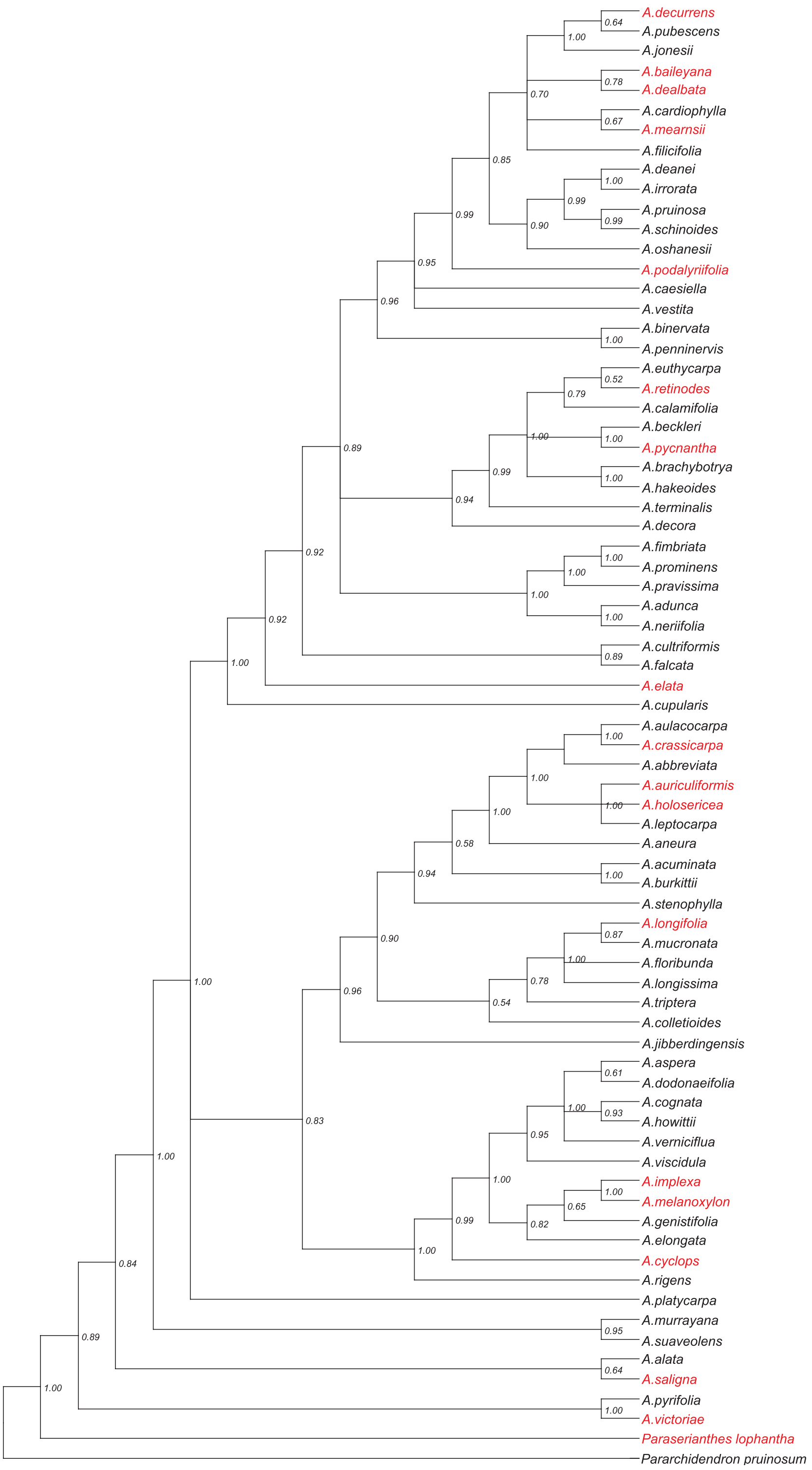

Figure 2 Bayesian phylogenetic tree depicting relationships among taxa included in the phylogenetic generalized least-squares analysis. Numbers at nodes indicate the Bayesian posterior probability (PP). Invasive taxa are shown in red. *No reproductive trait data were available for *A. vestita*.

syndrome involves pollination by nectar-feeding birds and is associated with the location of a large extrafloral nectary near the inflorescence. Pollen collected on the bird's head is transferred while it feeds on the gland's nectar (Knox *et al.*, 1985). Some species display both insect and bird pollination syndromes (e.g. *A. terminalis*, Kenrick *et al.*, 1987). As with morphology, having a generalized pollination system reduces pollinator limitation of seed set and is thus likely to contribute to the invasive success of Australian acacias (Richardson *et al.*, 2000*a*).

Australian acacias show two features in their floral biology that together distinguish them from all other related taxa (Stone *et al.*, 2003). First, no Australian acacias are recorded to secrete floral nectar, although some produce extrafloral nectar

Table 1A Phylogeny-free analyses of correlations between reproductive traits and invasiveness of 126 introduced Australian *Acacia* species (23 invasive/103 non-invasive Table S1).

Explanatory variables	Response variables			
Reproductive traits	Invasive	Not invasive	Test	Relationship
Continuous	Summary (*n*; mean, μ; range)			
Index of self-incompatibility (ISI) (infructescence/ inflorescence)	$n = 6$ $\mu = 0.425$ range = 0.02–0.86	$n = 3$ $\mu = 0.42$ range = 0.13–0.96	GLM (negative binomial errors): z = 0.010, $P = 0.992$	No effect No effect
ISI (pods/inflorescence)	$n = 7$ $\mu = 0.339$ range = 0.008–0.79	$n = 3$ $\mu = 0.447$ range = 0.07–1.1	GLM (negative binomial errors): $z = -0.212$, $P = 0.832$	No effect No effect
Seed mass (mg)	$n = 23$ $\mu = 20.3$ range = 5.7–47.8	$n = 99$ $\mu = 21.1$ range = 2.72–219	GLM (binomial errors; response var. log10 transformed): $z = 1.14$, $P = 0.254$	No effect No effect
Length of flowering (months)	$n = 22$ $\mu = 4.909$ range = 2–10	$n = 59$ $\mu = 4.890$ range = 2–12	GLM (binomial errors): $z = 0.042$, $P = 0.966$	No effect No effect
Binary	Summary ((*n*, number of total for each factor level); mean, μ; confidence interval (CI; 97.5%))			
Time to reproductive maturity (>2 years or <2 years)	$n = 13$ (10 < 2 years, 3 > 2 years) $\mu = 77\%$ <2 years CI = 54–100%	$n = 26$ (7, <2 years, 19, >2 years) $\mu = 27\%$ <2 years CI = 12–46%	Chi-square: $\chi^2 = 6.90$, d.f. = 1, $P = 0.0086$	Invasive species reach reproductive maturity earlier than non-invasive species
Combined measure of breeding system ('mixed'' or 'outcrossing')	$n = 10$ (2 mixed, 8 outcross) $\mu = 20\%$ mixed CI = 0–50%	$n = 3$ (1 mixed, 2 outcross) $\mu = 50\%$ mixed CI = 0–100%	Chi-square: $\chi^2 = 0.0903$, d.f. = 1, $P = 0.764$	No effect
Seed dispersal ('bird' or 'not bird')	$n = 15$ (8 bird, 7 not bird) $\mu = 53\%$ bird CI = 27–80%	$n = 12$ (4 bird, 8 not bird) $\mu = 33\%$ bird CI = 8–58%	Chi-square: $\chi^2 = 0.422$, d.f. = 1, $P = 0.516$	No effect
Ability to resprout (True/False)	$n = 21$ (11 can resprout, 10 cannot) $\mu = 52\%$ CI = 33–71%	$n = 54$ (13 can resprout, 41 cannot) $\mu = 24\%$ CI = 13–35%	Chi-square: $\chi^2 = 4.34$, d.f. = 1, $P = 0.037$	Ability to resprout significantly positively related to species being invasive

Details of actual parameter estimates are given in Appendix S2.

to attract insect and bird pollinators (Knox *et al.*, 1985; Vanstone & Paton, 1988). There are also other acacia (*Acacia* s.l.) species that lack nectar, including *A. nilotica* (Stone *et al.*, 1998) – the most invasive African acacia in Australia (Radford *et al.*, 2002). A second distinctive feature is that individual flowers and flower heads are relatively long-lived in Australian acacias (Prescott, 2005) compared with other acacias. Flowers on a single flower head open over a series of days, and each flower head can last for up to two weeks (Stone *et al.*, 2003; George *et al.*, 2009). Intuitively, floral longevity should contribute to the success of Australian acacias as invaders, because long-lived flowers are tolerant of competition and have a higher probability of pollination when pollination events are rare because of pollinator or mate limitation.

The ability of introduced Australian acacias to tolerate competition for pollination is likely to facilitate invasion, as introduced species enter an environment where all pollinators have established relationships with other plant species (Pysek *et al.*, 2011). Flower heads of Australian acacias open gradually and asynchronously, which favours foraging by small bees that can gather resources in small packets (Stone *et al.*, 2003). *Acacia* flowers can be either male-only or hermaphrodite (Kenrick, 2003; George *et al.*, 2009). Australian *Acacia* species have strictly protogynous flowers where the stigma is receptive

Table 1B Comparison of phylogeny-controlled and phylogeny-free analyses of relationships between reproductive traits and invasiveness for 72 introduced Australian *Acacia* species (cf. 126 species in Table 1A), comprising 17 invasive and 55 non-invasive species.

Explanatory variables	Response variables				
Reproductive traits	Invasive	Not invasive	Test	Phylogenetic generalized least squares	Relationship
Continuous	Summary (n; mean, μ; range)				
ISI (Index of self-incompatibility) (infructescence/inflorescence)	$n = 5$ $\mu = 0.34$ range = 0.02–0.78	$n = 1$ $\mu = 0.13$ range = 0.13	GLM (binomial errors): $z = 0.522$, $P = 0.602$	$t = 0.107$, $P = 0.920$	No effect with or without phylogeny
ISI (pods/inflorescence)	$n = 6$ $\mu = 0.26$ range = 0.008–0.77	$n = 1$ $\mu = 0.07$ range = 0.07	GLM (binomial errors): $z = 0.475$, $P = 0.635$	$t = 0.139$, $P = 0.895$	No effect with or without phylogeny No effect with or without phylogeny
Seed mass (mg)	$n = 17$ $\mu = 20.34$ range = 7.52–40.55	$n = 53$ $\mu = 23.16$ range = 5.21–219.77	GLM (binomial errors); response var. log10 transformed): $z = 0.777$, $P = 0.437$	$t = 0.1.01$, $P = 0.315$	No effect with or without phylogeny No effect with or without phylogeny
Length of flowering (months)	$n = 16$ $\mu = 4.63$ range = 2–10	$n = 39$ $\mu = 4.80$ range = 2–12	GLM (binomial errors): $z = -0.330$, $P = 0.741$	$t = -0.077$, $P = 0.939$	No effect with or without phylogeny
Binary	Summary ((n, number of total for each factor level); mean, μ; confidence interval (CI; 97.5%))				
Time to reproductive maturity	$n = 10$ (8 < 2 years, 2 > 2 years) $\mu = 75\%$ <2 years CI = 50–100%	$n = 16$ (7, <2 years, 19, >2 years) $\mu = 48\%$ <2 years CI = 19–69%	Chi-square: $\chi^2 = 5.44$, d.f. = 1, $P = 0.02$	$t = -3.18$, $P = 0.004$	Invasive species reach reproductive maturity earlier than non-invasive species with and without phylogeny
Combined measure of breeding system	$n = 9$ (1 mixed, 8 outcross) $\mu = 17\%$ mixed CI = 0–33%	$n = 1$ (1 outcross) $\mu = 100\%$ mixed CI = 100%	Chi-square: $\chi^2 = 1.98$, d.f. = 1, $P = 0.16$	$t = -0.103$, $P = 0.920$	No effect with or without phylogeny
Seed dispersal	$n = 12$ (6 bird, 6 not bird) $\mu = 49\%$ bird CI = 23–75%	$n = 5$ (2 bird, 3 not bird) $\mu = 40\%$ bird CI = 0–80%	Chi-square: $\chi^2 = 0.02$, d.f. = 1, $P = 0.88$	$t = -0.024$, $P = 0.981$	No effect with or without phylogeny
Ability to resprout	$n = 15$ (9 can resprout, 6 cannot) $\mu = 60\%$ CI = 33–87%	$n = 34$ (7 can resprout, 27 cannot) $\mu = 23\%$ CI = 9–35%	Chi-square: $\chi^2 = 5.67$, d.f. = 1, $P = 0.0.02$	$t = 1.08$, $P = 0.287$	Ability to resprout significantly positively related to species being invasive, but significance lost when phylogeny considered

Phylogenetic relationships among species were incorporated as a covariate in a generalized least-squares analysis (see Methods). Actual parameter estimates are given in Appendix S3.

before the anthers produce pollen (Stone *et al.*, 2003; George *et al.*, 2009). In contrast, the flower heads of African and American acacias are protandrous and release pollen synchronously, which makes them attractive to larger native bee species because all the resource is presented at once (Stone *et al.*, 2003; Raine *et al.*, 2007). To exploit this larger food resource effectively, the larger African bees, which are important pollinators of African acacias, time their arrival at each species to coincide with its daily pollen release (Stone *et al.*, 1998). This foraging behaviour would be ineffective for the exploitation of Australian acacia flowers, and it is not surprising that the most prominent visitors to introduced

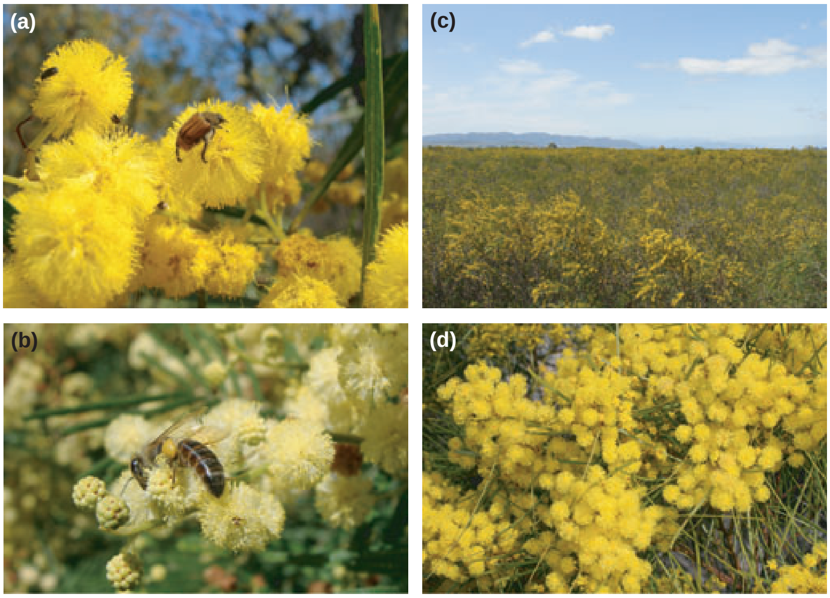

Figure 3 Important pollination biology traits associated with invasiveness in Australian acacias. These species share a generalist pollination syndrome as illustrated in South Africa where (a) *Acacia saligna* is being visited by native beetles (photograph: M.R. Gibson) and (b) *A. mearnsii* is being visited by the native honeybee, *Apis mellifera capensis* (photograph: A.M. Rogers). (c) Mass flowering in a field invaded by *A. saligna* in South Africa during its flowering peak in September (photograph: A.M. Rogers). (d) The dense flowers of *A. adunca* form an eye-catching, bright yellow floral display in Queensland (photograph: T. Low).

Australia acacias are often honeybees (*Apis mellifera*) (Bernhardt, 1987; Sedgley *et al.*, 1992; Sornsathapornkul & Owens, 1998; Alves & Marins-Corder, 2009), whose sensitivity to resource availability and ability to learn are both exceptional among bees (Willmer & Stone, 2004).

Other floral traits that may contribute to the invasive success of Australian acacias are precocity (early reproductive maturity) and longevity. Morgan *et al.* (2002) found that low final pod set (pods/inflorescence) in *A. baileyana*, as is seen in many acacias (Kenrick, 2003), was offset by precocious flowering and high flower numbers, which resulted in high seed production, probably partly facilitating its invasiveness. Early reproductive maturity is seen in many invasive acacias with some commencing flowering at just two years of age (see Table S1). In this study, both phylogeny-free and phylogenetic analyses suggested that short juvenile period was a significant factor distinguishing invasive acacias from non-invasive species. This result makes intuitive sense, because shorter juvenile periods enhance invasiveness by ensuring that seeds are produced sooner and thus confer an overall high seed production and allow for rapid accumulation of a soil seed bank. On a coarse level, floral biology appears essentially similar for all Australian acacias. Consequently, specific traits such as time of pollen release and inflorescence longevity are unlikely to distinguish invasive and non-invasive Australian acacias. However, subtle variations in combinations of sexual receptiveness and longevity (e.g. age-dependent floral colour variation; M.N. Prescott, unpublished data) could be important in this regard and require proper studies before being fully ruled out.

Pollination and pollen vectors

Pollinator assemblages vary on an annual, seasonal and geographic basis so that a diverse spectrum of floral foragers visit *Acacia* species in a given location, but the dominance of specific vectors can vary inter- and intraspecifically (Bernhardt, 1989). In their native range, *Acacia* species are visited by a variety of flower foragers, but the most important pollinators are usually bees and wasps (*Apoidea*), followed by flies, beetles and birds (Kenrick *et al.*, 1987; Vanstone & Paton, 1988; Bernhardt, 1989; Stone *et al.*, 2003; Prescott, 2005). Social bees are relatively scarce in Australia, and most of the dominant native bees are small-bodied polylectic solitary species in the families Anthophoridae, Colletidae and Halictidae. The introduced honeybee is also an important and abundant pollinator of Australian acacias in both their native and introduced ranges (Bernhardt, 1987; Thorp & Sugden, 1990; Sedgley *et al.*, 1992; Prescott, 2005). Existing studies of introduced Australian acacias in South Africa show that native honeybees (*Apis mellifera capensis* and *A. mellifera scutellata*) are dominant pollen vectors followed to a lesser extent by flies and bees (M.R. Gibson, unpublished data; J.G. Rodger unpublished data) (see Table S3 for a comprehensive list of flower visitors). In other parts of the introduced range of Australian acacias, honeybees tend to be the most abundant and effective floral visitor in terms of visitation frequency and pollen-carrying load (Sornsathapornkul & Owens, 1998), although their distribution may be restricted to areas with sufficient availability of nectar flowers (Alves & Marins-Corder, 2009). Honeybees may be especially important for pollination in the context of *Acacia* invasions as they can learn to exploit new floral resources in a matter of hours (Willmer & Stone, 2004).

The importance of biotic pollination for reproductive success depends on whether abiotic pollination occurs. Although it remains to be tested and although pollen has been collected downwind of flowering *A. mearnsii* (Wattle Research Institute, 1952; Moncur *et al.*, 1989), *Acacia* inflorescences show no apparent adaptations for capture of wind-borne pollen. In contrast to typical wind-pollinated species, which have feathery stigmas and aerodynamic features that aid in capture of pollen grains (Niklas, 1985, 1987), *Acacia* flowers have a very small cup-shaped stigma into which only one polyad can fit and lack any obvious aerodynamic structures. Wind-pollinated species have relatively high pollen to ovule ratios (median 22 150: 1) relative to animal-pollinated species (median 3450:1), although pollen-transfer efficiencies (proportion of removed pollen that is captured by stigmas) are similar (Friedman & Barrett, 2009). Typical of plants with aggregated (i.e. polyad-like) pollen (Harder & Johnson, 2008), the pollen to ovule ratio in *Acacia* is very low (53–360 for *A. mearnsii* based on measurements in Kenrick & Knox, 1982; Moncur *et al.*, 1991), compatible with dependence on animal pollen vectors. While it thus seems unlikely that wind pollination would make an appreciable contribution to fecundity, the possibility cannot yet be rejected. In the only test for wind pollination that we are aware of, fruit set of *A. mearnsii* was reduced but not eliminated in inflorescences enclosed in cages of wire and nylon mesh. However, bags reduced wind-borne pollen supply, and some flowers may have protruded through the cages and been pollinated by bees (Wattle Research Institute, 1952, 1961) so decisive experiments are still required to assess whether wind pollination is at all important for *Acacia*.

Because acacias are pollinated by generalist pollinators (such as the widely introduced honeybee), pollinator limitation seems an unlikely constraint to the spread of introduced Australian acacias relative to non-invasive taxa (Richardson *et al.*, 2000*a*) but this has not yet been studied. If pollination by *A. mellifera* enhances seed production of Australian acacias, then honeybees could facilitate *Acacia* invasions (and the facilitation could be reciprocal where both species are introduced, as in South America) (Barthell *et al.*, 2001; Morales & Aizen, 2002). We conclude that generalist pollination facilitates invasion, but there is no evidence to suggest that this factor alone explains the relative success of different Australian acacias as invasive and introduced non-invasive species both possess generalist pollination systems.

Phenology

Most Australian acacias tend to flower in massive displays from late winter to mid-spring (Bernhardt, 1989; Costermans, 2007) and have long-lived (and so competition tolerant) inflorescences (Stone *et al.*, 2003; Prescott, 2005), although the number of flower heads in bloom can fluctuate greatly depending on environmental conditions and resource availability (Sedgley, 1985; Gaol & Fox, 2002; Yates & Broadhurst, 2002). Pollen release often occurs in the middle of the day when insect abundance is greatest, which likely confers an advantage when it comes to adapting to new habitats in the initial stages of invasion (M.N. Prescott, unpublished data). Where Australian acacias are invasive in Mediterranean-type climate regions, their flowering occurs earlier than, and overlaps with, most native species whose peak flowering occurs in spring (Henderson, 2001; Godoy *et al.*, 2009). Various studies have shown early and extended flowering phenologies of invasive versus native plants to be correlated with invasive potential (Cadotte & Lovett-Doust, 2001; Pysek & Richardson, 2007; Pysek *et al.*, 2009), thus conferring a fitness advantage by reduced competition for pollinators (Stone *et al.*, 1998; Raine *et al.*, 2007). However, while this may be true in general, differences in overall length of flowering period between invasive and non-invasive Australian acacias were found to be non-significant (P. Castro-Díez, unpublished data; see discussion in Castro-Díez *et al.*, 2011).

Peak flowering prior to and during spring, while not unique to invasive Australian acacias, may contribute indirectly to invasiveness in some environments as early and prolonged flowering in *Acacia* species during peak flowering of native species in exotic ecosystems may help mitigate pollen and pollinator limitation. Again, this alone is not likely to contribute to invasiveness but may do so when it is combined with other invasion-enhancing reproductive traits that are not present in non-invasive species.

Breeding system and seed set

Completely self-incompatible species depend entirely on pollinators and mate availability, but self-compatibility and the ability to self-pollinate autonomously assure reproduction against inadequate pollinator visitation and/or mate availability (Eckert *et al.*, 2006). Australian *Acacia* species range from highly self-incompatible to completely self-compatible and autogamous (Table S1) (Moffett, 1956; Bernhardt *et al.*, 1984; Kenrick & Knox, 1989; Morgan *et al.*, 2002), and so probably vary greatly in their dependence on pollinators for realized fecundity. Realized outcrossing rates tend to be high (multilocus outcrossing rate (t_m) > 0.9 in most species: Table S1) indicating that pollinators do play an important role in their reproduction. Partial self-compatibility and intraspecific variation in self-compatibility seem relatively common in Australian *Acacia* species (Philp & Sherry, 1946; Moffett & Nixon, 1974) with some ability to reproduce by selfing known for six species, five of which are invasive (see Table S1: *Acacia dealbata, A. decurrens, A. mearnsii, A. paradoxa, A. saligna*) (J.G. Rodger, unpublished data; George *et al.*, 2008; Millar *et al.*, 2011).

The apparently high prevalence of at least some level of self-compatibility in Australian *Acacia* species is significant given the rarity of uniparental reproduction in woody plants (Barrett *et al.*, 1996). This is consistent with the observation by Rambuda & Johnson (2004) that all 13 woody species investigated in a survey of breeding systems of invasive plants in South Africa were capable of uniparental reproduction. Investigation of Australian *Acacia* species could reveal further details about the evolution of breeding systems and their role in invasiveness in woody species in general. Comparisons between invasive and non-invasive *Acacia* species are hindered by insufficient data here, as elsewhere, but available information suggest that invasive taxa tend to have higher levels of self-compatibility, suggesting ability to self-fertilize may predispose *Acacia* species to invasiveness. However, in shade house trials, selfed progeny of *A. mearnsii*, *A. decurrens* (Moffett & Nixon, 1974) and *A. dealbata* (J.G. Rodger, unpublished data) have reduced growth and survival, which would erode the reproductive assurance benefits of selfing (Herlihy & Eckert, 2002). Other self-compatible tree species have such high levels of inbreeding depression that it is unlikely that progeny arising from self-pollination ever reach reproductive maturity (Hardner & Potts, 1997; Ishida, 2006; Robertson *et al.*, 2011). A comparison of fixation index for trees from germination to reproduction (e.g. Ishida, 2006) would reveal whether selfed progeny reach reproductive maturity and therefore whether self-compatibility potentially enhances invasiveness.

Even a low capacity for reproduction by self-fertilization could be important in alleviating pollinator and mate limitation, which are likely to occur in the early stages of naturalization and invasion owing to small size or low density of populations (Baker, 1955; Davis *et al.*, 2004). Such factors have been shown to influence seed set in *Acacia* in the native range (Broadhurst & Young, 2006). However, extensive pollen dispersal may maintain outcrossing rates in small patches or isolated plants (Millar *et al.*, 2008, 2011). While ability to self-fertilize may make species more likely to become invasive or to spread at greater rates, it is not essential for invasiveness – there are prominent examples of invasive self-incompatible species in *Acacia* (e.g. *A. auriculiformis, A. pycnantha* – see Table S1) and other groups (e.g. Barthell *et al.*, 2001). Our study found no differences in indices of self-compatibility (ISI) nor breeding system strategy (mixed versus outcrossing) between invasive and non-invasive species (Table 1A,B), though data for these traits were extremely limited (see Table S1).

Seed biology

Seed biology seems to be one of the most important factors contributing to the invasion success of Australian acacias (Milton & Hall, 1981; Richardson & Kluge, 2008). Seed biology syndromes in many *Acacia* species are largely shaped by fire-driven ecosystems that are present throughout much of Australia and introduced Mediterranean-type climate regions. Fire-adaptive traits include: production of large quantities of hard-coated, heat-tolerant and long-lived seeds with the capacity for long dormancy; stimulation of germination by heat and/or smoke; seed dispersal and burial by ants; and the ability to resprout (Berg, 1975; Bell *et al.*, 1993; Specht & Specht, 1999), all of which are likely essential for the persistence and invasive success of Australian acacias (see Fig. 4 for photographs of seed biology traits associated with invasiveness).

Dispersal

Dispersal is a crucial aspect of progression from 'naturalized' to 'invasive' status when recruitment occurs at considerable distances from parent plants (Richardson *et al.*, 2000*a,b*). Australian acacias possess seed adaptations for dispersal by birds and ants (Davidson & Morton, 1984; O'Dowd & Gill, 1986), although passive dispersal via water, wind and gravity is also common.

Broadly, biotic seed dispersal in *Acacia* falls into two syndromes based on features of arils: a 'bird-dispersal syndrome' and an 'ant-dispersal syndrome' (O'Dowd & Gill, 1986). The fleshy arillate appendages (in bird-dispersed seeds) and an elaiosome (in ant-dispersed seeds) attach the seed to the seed pod lining and make them accessible to a range of bird and ant species across multiple foraging types. Such generalization of morphological traits associated with dispersal makes limitation of a seed dispersal agent in the introduced range unlikely (see Glyphis *et al.*, 1981; Holmes, 1990*a*; Richardson *et al.*, 2000*a*; Underhill & Hofmeyr, 2007). Furthermore, these traits may be evolutionarily labile since *A. ligulata* reportedly displays both syndromes (Davidson & Morton, 1984), each of which has its own advantages. Birds are important agents in that they aid in longer distance dispersal (Holmes, 1990*a*) and, through ingesting the seeds, are able to aid in the germination of *Acacia* species requiring chemical scarification (e.g. *A. cyclops*, *A. melanoxylon*) (Glyphis *et al.*, 1981; Richardson & Kluge, 2008). Ants rapidly remove and bury *Acacia* seeds in subterranean nests and so contribute to dispersal on a local scale (Holmes, 1990*a*). Species noted as having a 'bird-dispersal syndrome' are likely also dispersed vertically by ants, as myrmecochory accounts for much of the movement of seed from the litter layer into the seed bank (Richardson & Kluge, 2008). Dispersal by birds of an 'ant-dispersal syndrome' species appears less likely (O'Dowd & Gill, 1986).

Importantly, seed morphology and dispersal agents in the native range of Australian acacias are not always accurate predictors of dispersal agents in introduced ranges. For example, in Portugal, South Africa and Florida, invasive *Acacia* seeds are effectively dispersed by a wide range of opportunistic agents besides those that one would consider functional equivalents of dispersal agents in the native range. These include baboons, domestic and wild ungulates and humans (Ridley & Moss, 1930; Middlemiss, 1963; Kull & Rangan, 2008). In the Western Cape of South Africa, primarily insectivorous barn swallows ingest seeds and act as effective dispersal agents of *A. cyclops* (Underhill & Hofmeyr, 2007), and other granivorous, ground-dwelling birds disperse *Acacia* seeds (Duckworth & Richardson, 1988; Knight & Macdonald, 1991). In New Zealand, most native avian seed dispersers are now extinct (Anderson *et al.*, 2006), and the ant fauna is relatively depauperate and limited in distribution (Don, 2007), with only three ant species including seeds in their diet. Despite these limitations, at least eight Australian *Acacia* species have become invasive in New Zealand (Richardson & Rejmánek, 2011) with *A. baileyana* showing evidence of long-distance dispersal although the dispersal agent is not known (E.M. Wandrag, unpublished data). Furthermore, in many human-dominated systems, long-distance dispersal of introduced species is mostly human mediated (Trakhtenbrot *et al.*, 2005), so this distinction is likely less important in determining spread rates than may be predicted.

Abiotic dispersal in water and soil is important in many regions (Milton & Hall, 1981). There is a strong association

Figure 4 Important seed biology traits associated with invasiveness in Australian acacias. (a) Seed production of *Acacia saligna* in South Africa during the early 1980s, prior to the introduction of the rust fungus *Uromycladium uromyces,* which has since greatly reduced seed production (photograph: D.M. Richardson). (b) Seed production of *A. longifolia* in its native range in Australia (photograph: C. Harris). Seeds that fall to the ground can remain viable for 50+ years, making their eradication nearly impossible. (c) *A. cyclops* seeds remain in the tree canopy longer than those of species that are typically ant-dispersed; the bright red aril attracts birds that disperse the seeds (photograph: A.M. Rogers). (d) *A. longifolia* seeds are typically ant-dispersed in the native range, although bird-dispersal is predicted based on aril attributes; they are attached to the seed pod by an elaiosome that attracts ants (photograph: C. Harris). (e) Invasive species, such as *A. saligna* pictured here, have a greater tendency to resprout following a disturbance event than non-invasive species (photograph: D.M. Richardson). (f) The mass germination of *Acacia* seeds after fire, as in *A. pycnantha* in South Africa shown here, is a major hurdle to control efforts (photograph: D.M. Richardson).

between *A. dealbata* invasions and watercourses in Chile and Portugal (H. Marchante, unpublished data; Pauchard *et al.*, 2008). Movement of soil for road building is also a major dispersal route of *A. dealbata* and *A. longifolia* in Portugal (H. Marchante, unpublished data). Similarly in South Africa, rivers and soil movement aid in the dispersal of acacias that invade riparian areas, such as *A. mearnsii* (de Wit *et al.*, 2001).

Seed mass in *Acacia* was found to be positively correlated with invasiveness in a recent study (Castro-Díez *et al.*, 2011) but did not consistently differ in our study nor in a multi-species study comparing seed mass between native and introduced ranges (C. Harris *et al.*, unpublished data). These results contradict findings for *Pinus* where smaller seed size is positively associated with invasiveness, as small seeds are more suitable for long-distance dispersal by wind (Richardson, 2006). The difference between pines and acacias in this regard is not surprising. Unlike pines, most acacias are animal dispersed, and dispersal by wind is of trivial importance. Factors other than size contribute to dispersibility, and seed size plays an entirely different role as mediator of colonization and establishment success.

Dispersal traits associated with a bird-dispersed syndrome in Australian acacias clearly predispose these species to spread rapidly in a new environment (see discussion of this for *A. cyclops* in South African fynbos by Higgins *et al.*, 2001) because of the importance of long-distance dispersal events in driving invasions (Trakhtenbrot *et al.*, 2005). However, of the 23 species of Australian *Acacia* considered invasive (*sensu* Pysek *et al.*, 2004; Richardson & Rejmánek, 2011), only eight species are known to be bird-dispersed or possess typical bird-dispersed seed traits (Davidson & Morton, 1984; O'Dowd & Gill, 1986; Langeland & Burks, 1998; Stanley & Lill, 2002): *Acacia auriculiformis, A. cyclops, A. holosericea, A. implexa, A. longifolia, A. mangium, A. melanoxylon and A. salicina* (see Table S1). Additionally, our analysis found that seed dispersal by birds was not significantly correlated with invasiveness. In Portugal, two of the most invasive and widespread *Acacia* species (*A. dealbata* and *A. longifolia*) are ant-dispersed (Marchante *et al.*, 2010), as are *A. saligna* and *A. mearnsii* in South Africa (French & Major, 2001; Richardson & Kluge, 2008). Thus, the contribution of different dispersal agents to invasiveness remains unclear but further suggests a role of human-mediated dispersal and interactions with environmental factors.

Seed bank dynamics

A reproductive trait that strongly influences invasiveness of Australian acacias is their capacity to form extensive and persistent soil seed banks (Richardson & Kluge, 2008). Accumulation times differ depending on the species (see

Table 2 of Richardson & Kluge, 2008), and the average shortest time frame is roughly eight years. The seeds of some *Acacia* species that have become invasive can remain dormant for 50–100 years or more (Farrell & Ashton, 1978; New, 1984). Richardson & Kluge (2008) list four main factors that contribute to the size of soil-stored seed banks in Australian acacias in South Africa: the annual seed rain; the age of the stand; stand density or canopy cover; and distance from the canopy. Additional factors include level of granivory, decay and germination (Marchante *et al.*, 2010). Biological control agents that negatively affect flower, flower bud or pod production, such as *Melanterius* weevils (Dennill & Donnelly, 1991; Impson *et al.*, 2004) that directly feed on acacia seeds, can reduce annual seed rain. The rate of seed accumulation in the soil increases until the stand is about 30 years old, and denser stands produce more seeds, so control efforts to reduce seed production should focus on younger, denser *Acacia* stands (Milton & Hall, 1981; Holmes, 1990*b*). Seed density in the soil is highest under the tree canopy and decreases sharply with distance (see Zenni *et al.*, 2009; Marchante *et al.*, 2010), although Marchante *et al.* (2010) found a few seeds of *A. longifolia* up to 7 m from the edge of invaded stands.

The main drivers of seed bank persistence and maintenance appear to be ants, although gravity and water may be the dominant drivers where ants are absent. Once seeds have dropped to the ground, ants bury many of them in their nests to allow them to exploit arils (Milton & Hall, 1981). In doing so, they often account for the majority of vertical seed movement into the upper seed bank. *Acacia* seeds gain a threefold advantage through protection from above-ground seed predators, protection from fire and incorporation into the seed bank (Gill, 1985; Holmes, 1990*a*). In South Africa, ants may play a critical role in accumulating seed banks of Australian acacias and aiding in their invasiveness (Holmes, 1990*c*; Richardson *et al.*, 2000*a*).

The role of seed bank density in *Acacia* invasiveness is unclear. Both higher and lower seed bank densities have been recorded in the introduced range of various *Acacia* species when compared to that in the native range (Milton & Hall, 1981; Richardson & Kluge, 2008; Marchante *et al.*, 2010). Additionally, methods of measuring seed bank and seed rain vary widely, making comparisons between introduced and native ranges problematic (see Table 2 for a summary of Australian *Acacia* seed data from various introduced and native regions). Prolific seed production and large accumulations of seeds in the seed bank certainly contribute to a species' ability to invade an ecosystem but these qualities alone do not guarantee invasiveness. Buist (2003) found that closely related pairs of rare and widespread *Acacia* species produced similar numbers of seeds and similar-sized, persistent soil seed reserves, indicating that level of seed production does not necessarily determine abundance of a species. These traits likely need to work in concert with certain physiological and morphological traits, such as germination ability, resource utilization, rapid growth of seedlings and dispersal investment, to contribute to invasiveness.

Germination

The majority of invasive *Acacia* species possess seeds whose germination is stimulated by fire, but some invasive species, notably bird-dispersed taxa, may be stimulated to germinate through chemical scarification via ingestion by an appropriate dispersal agent (Glyphis *et al.*, 1981; Fraser, 1990; Richardson & Kluge, 2008). These stimuli are required to break physical dormancy of the hard, water impermeable seed coat and allow germination of *Acacia* seeds, which have consistently high viability and low germinability over time. However, in Portugal, total viability and germinability were found to be significantly higher (and dormancy lower) in seeds from recently invaded soils for *A. longifolia* (Marchante *et al.*, 2010).

Invasive Australian acacias tend to germinate after disturbance, although disturbance is not essential. *Acacia dealbata* shows high survival within native forest and in open areas in Chile where it can endure long periods of drought and shade under canopies of native trees (Fuentes-Ramírez *et al.*, 2011). Moreover, mutualistic relationships with nitrogen-fixing bacteria are important for successful establishment of leguminous species, so the presence of compatible rhizobia is also essential for determining the colonization ability of introduced species (Parker *et al.*, 2006; Rodríguez-Echeverría *et al.*, 2011). Interestingly, Rodríguez-Echeverría *et al.* (2011) found that these bacterial symbionts are often cointroduced with their *Acacia* hosts from Australia, suggesting the presence of suitable soil symbionts in the introduced range may not be an important limiting factor in *Acacia* invasions *per se*.

Studies from the introduced ranges of Australian acacias report that a considerable number of seeds produced and allocated to seed rain are lost to factors such as early germination, granivory or decay (Marchante *et al.*, 2010). However, the consistently high seed viability found in many species of *Acacia* appears to be fundamental to their ability to invade (see Table 2) (Richardson & Kluge, 2008; Marchante *et al.*, 2010). Germination characters *per se* do not appear to be characteristic of invasiveness as invasive Australian *Acacia* species in South Africa can show opposing characteristics of either high dormancy, low germination and decay rates and rapid seed bank accumulation, or low dormancy, high germination and decay rates and gradual seed bank accumulation (Richardson & Kluge, 2008).

Comparisons of rare and widespread species show some association with factors that influence seed germination. The burial depth and heat-stimulation requirements of a species are important factors affecting germination that can determine how rare or widespread it is (Brown *et al.*, 2003). Comparisons of reproductive traits in two rare acacias and their common relatives showed differences in the germination (reduced range of temperature for germination in rare species) and higher rates of predation of fruit and seed in the rare species (Buist, 2003). Seed viability and dormancy levels between invasive and non-invasive species have not been compared. It may be predicted that, because such traits are adaptations to

Table 2 Seed rain density (SRD), seed bank density (SBD) and seed viability (SV) for Australian acacias in native and introduced ranges.

Acacia species	Seed rain density per m^{2}per year (SRD)	Seed bank density per m^2 (SBD)	Seed viability (SV)	Region	References	Observations
A. baileyana	19559	–	–	Australia (native range)	17	SRD – maximum #seed/tree
A. baileyana	1824 (3010)	–	–	New Zealand	26	SRD – average # seeds per m^2 averaged over 7-day period
A. cyclops	–	1430–5140 (142 –281)	46–95.3%	South Africa	10	
A. cyclops	–	2832–7792 (402–1019)	99.2%	South Africa	8	SBD – range of four different blocks
A. cyclops	1197 [1373–3019*]	2031	87%	South Africa	15	SRD – *estimated #seed per m^2 projected canopy
A. cyclops	540 (710)	–	–	Australia (introduced range)	6	SRD – estimated from reproductive output data (determined by dividing total mass of seeds removed from pods by mass per individual seed)
A. cyclops	1900 (1930)	–	–	Australia (native range)	6	
A. dealbata	–	10000	90%	Chile	25	
A. dealbata	2553 (3244)	–	–	New Zealand	26	SRD – average # seeds per m^2 averaged over 7-day period
A. dealbata	–	*ca.* 22500	30%	Portugal	13	SV: probably underestimated (seeds heated to 50 C without scarification)
A. elata	–	–	50%	–	22	SV – final germination after scarification
A. holosericea	–	–	>95%	Australia (native range)	7	
A. longifolia	2000–12000	500–1500	>85%	Portugal	14	SRD – 2000: smaller trees next to the ocean (windward); 12000: bigger trees leeward
A. longifolia	–	–	>88%	Portugal	16	
A. longifolia	11500	34000	–	South Africa	19	SRD – maximum number
A. longifolia	–	2078–3473 (488–498)	99%	South Africa	21	
A. longifolia	2923	7646	97%	South Africa	15	
A. longifolia	–	4528 (1075)	99%	South Africa	4	After introduction of biological control agent, max numbers
A. longifolia	2530 (3430)	–	–	Australia (introduced range)	6	SRD – estimated from reproductive output data (determined by dividing total mass of seeds removed from pods by mass per individual seed)
A. longifolia	810 (1180)	–	–	Australia (native range)	6	
A. mangium	410	–	–	Indonesia	23	SRD – estimated from seed production in kg per ha per year
A. mearnsii	–	5314/696	–	South Africa	20	SBD- maximum number/average

Table 2 Continued.

Acacia species	Seed rain density per m^{2}per year (SRD)	Seed bank density per m^2 (SBD)	Seed viability (SV)	Region	References	Observations
A. mearnsii	–	38340	–	South Africa	15	
A. mearnsii	–	–	>83.4%	South Africa	12	
A. melanoxylon	3218	48739	70%	South Africa	15	SRD & SBD: Donald, 1959 cited by Milton & Hall, 1981
A. melanoxylon	–	–	85–91%	Australia (native range)	2	
A. melanoxylon	740 (800)	–	–	Australia (introduced range)	6	SRD – estimated from reproductive output data (determined by dividing total mass of seeds removed from pods by mass per individual seed)
A. melanoxylon	1160 (1810)	–	–	Australia (native range)	6	
A. paradoxa	–	1000	–	South Africa	28	
A. paradoxa	58#	–	–	Australia (native range)	1	SRD – #firm seed production per plant
A. pycnantha	31#	–	99%	Australia (native range)	1	
A. saligna	–	7920–45800 (560–3220)	>86%	South Africa	10	
A. saligna	2645–13472	–	–	South Africa	27	SRD – measured in 1989, *ca.* 2 years after introduction of biocontrol agent
A. saligna	446–3035	–	–	South Africa	27	SRD – measured in 2004, *ca.* 18 years after introduction of biocontrol agent
A. saligna	5443 [10562*]	11920	83%	South Africa	15	SRD – #seed/tree based on few trees; * estimated seed per m^2 projected canopy
A. saligna	–	715–8097	–	South Africa	9	SBD – after introduction of biological control agent; values estimated from 4 places and 3 depths
A. saligna	–	–	>90%	Israel	3	
A. saligna	–	2000–189000 (53333)	–	South Africa	18	After introduction of biological control agent; average from 8 sites, samplings during 6 years
A. saligna	–	1389–3600 (207–279)	–	Australia, New South Wales (introduced range)	24	
A. saligna	–	–	73%	–	22	SV – final germination after scarification
A. saligna	–	3158–38714 (1194–4006)	>65%	South Africa	11	SBD – range of 4 sites, at 0–15 cm
A. saligna	760 (750)	–	–	Australia (introduced range)	6	SRD – estimated from reproductive output data (determined by dividing total mass of seeds removed from pods by mass per individual seed)
A. saligna	540 (650)	–	–	Australia (native range)	6	

Table 2 Continued.

Acacia species	Seed rain density per m^{2}per year (SRD)	Seed bank density per m^2 (SBD)	Seed viability (SV)	Region	References	Observations
A. salicina	–	–	77%	–	22	SV – final germination after scarification
A. victoriae	–	50–3900	80%	Australia (native range)	5	

Values refer to mean values unless otherwise specified (standard deviation in parentheses where available).

1: Brown *et al.* (2003); 2: Burrows *et al.* (2009); 3: Cohen *et al.* (2008); 4: Fourie (2008); 5: Grice & Westoby (1987); 6: C. Harris *et al.* (unpublished data); 7: Hellum (1990); 8: Holmes (1989); 9: Holmes (2002); 10: Holmes *et al.* (1987); 11: Jasson (2005); 12: Kulkarni *et al.* (2007); 13: H. Marchante, unpublished data; 14: Marchante *et al.* (2010); 15: Milton & Hall (1981); 16: M. Morais, unpublished data; 17: Morgan (2003); 18: Morris (1997); 19: Pieterse (1987); 20: Pieterse (1997); 21: Pieterse & Cairns (1986); 22: Rehman *et al.* (2000); 23: Saharjo & Watanabe (2000); 24: Tozer (1998); 25: G. Valencia, unpublished data; 26: E.M. Wandrag, unpublished data; 27: Wood & Morris (2007); 28: Zenni *et al.* (2009).

fire-driven ecosystems, other *Acacia* species originating from similar regions also likely possess such germination traits.

Alternative modes of reproduction and persistence

Acacia displays a variety of regeneration strategies besides germination from seed, including root suckering, and basal resprouting (Bell *et al.*, 1993; Reid & Murphy, 2008), which predispose them to weediness and can occur following disturbance such as fire and mechanical removal (Reid & Murphy, 2006). In South Africa, for example, species such as *A. cyclops*, which lack the ability to resprout after fire, have high demographic dependence on seeds, while species such as *A. saligna*, which resprouts vigorously, depend less on seeds for population persistence. Spooner (2005) found that disturbance by road works in Australia triggered a range of responses, such as a combination of basal resprouting, root suckering and seedling emergence, which led to a population increase for three *Acacia* species. Similarly, resprouting is a major reproductive mechanism in *A. dealbata* in Chile and Europe and may facilitate its rapid invasion of new environments (Marchante *et al.*, 2008; Lorenzo *et al.*, 2010; Fuentes-Ramírez *et al.*, 2011). Our study also found that resprout ability was greater for invasive species than for non-invasive species where they are introduced globally. Long-lived seed banks and ability to resprout are key determinants of persistence; together with the ability to disperse, these traits are hugely influential ingredients of invasive success since they ensure persistence and effectively permanent occupancy of invaded sites (e.g. Richardson & Cowling, 1992).

DISCUSSION

Our literature review found that traits including generalist pollination systems, prolific seed production, efficient seed dispersal and the accumulation of large and persistent seed banks, which often have fire-, heat- or disturbance-triggered germination cues, are characteristic of Australian acacias in general. We did not find distinct reproductive syndromes that differed between invasive and non-invasive species, although this may be both because trait data were not available for all species, and those species for which data are available might not be representative.

Pollinator-mediated seed production is likely to facilitate invasion of *Acacia* species where they are introduced but should not differ for introduced non-invasive species as Australian acacias possess similar floral morphology and attract similar (generalist) pollinator groups (e.g. *Apis mellifera*). Flowering and seed production are clearly important for invasion success and account for the massive number of propagules that accumulate to create a long-lived soil seed bank that is the largest hurdle to effective control (Wilson *et al.*, 2011). We found that invasive species reach reproductive maturity earlier, and this could certainly contribute to a faster accumulation of a seed bank, which is a vital requirement for ensuring persistence in regularly disturbed environments, such as those in which most Australian acacias are invasive (Richardson *et al.*, 1990, p. 362). These results are supported in other studies that have also documented the important role of a short juvenile interval to seed production (in *A. baileyana*, see Morgan *et al.*, 2002) and spread rate (in *Pinus*, see Higgins *et al.*, 1996; Higgins & Richardson, 1999). Time to reproductive maturity was also found to be shorter for invasive than non-invasive species when phylogeny was accounted for. This trait has not been discovered to have phylogenetic signal, and in an analysis using the most recent phylogeny for Australian *Acacia*, Miller *et al.* (2011) found that invasive species were phylogenetically over-dispersed (i.e. there was no phylogenetic signal for invasiveness). However, our results suggest that certain traits, which may be related to evolutionary history, can affect invasiveness and indicate that phenological precocity may be important for future consideration in phylogenetic studies.

Seed dispersal is critical for the spread of introduced Australian acacias, and although biotic dispersal agents are important, the majority of dispersal is likely human-mediated and focussed on economically important species. The ability to resprout undoubtedly aids in persistence during initial establishment as it makes a population less susceptible to stochastic events. This is supported by the results of our study that show resprout ability to be significantly greater for invasive species. Our results are similar to those of Pysek & Richardson (2007) who found that vegetative reproduction is positively associated with invasiveness in vascular plants across multiple comparative studies. However, resprouting ability should not directly aid in the ability of plants to spread.

There is much room to improve our knowledge of the reproductive biology in this genus. The role of pollinator-mediated seed production, especially by *Apis mellifera*, appears to be important to reproductive success of *Acacia* where they are introduced, and this needs to be formally tested. In addition, self-compatibility has the potential to facilitate the invasion process by enabling seed production when mate and pollinator availability is low, but formal tests are needed to see whether effects of inbreeding depression cancel out such benefits. Whether the reproductive traits that we tested are related to evolutionary history is unknowable at this point. The lack of clear phylogenetic signal in *Acacia* is probably due to the lack of data both in the value of the reproductive traits and in the sampling of the phylogenetic tree. That our results suggest reproductive traits are related to evolutionary history is an important issue that will need further research. Thus, we recommend that future analyses incorporate variable and phylogenetic data for a wider array of invasive and non-invasive species (see Box 1 for a list of research priorities).

The finding that certain reproductive traits show no obvious correlation with invasiveness in Australian acacias may be attributable to a number of factors. First and foremost is the shortage of data for many Australian acacias, both invasive and non-invasive, and consequent small sample sizes (see Table 1-A,B for sample sizes). This makes detection of more subtle correlations between reproductive traits and invasiveness difficult, resulting in an incomplete picture for understanding such relationships. Secondly, there is clearly no single 'ideal' reproductive syndrome that equips certain species in this group particularly well to establish, undergo rapid population growth (often from small founder populations), and to persist across the full range of habitats to which they have been introduced. Thirdly, if much of the reproductive trait data for invasive and non-invasive introduced species comes from studies within the native range, they may not incorporate differences in measurements because of region-specific factors of the introduced range. Such disparities in data highlight the need for measuring reproductive performance of individual invasive *Acacia* species in the introduced and native range. A fourth possibility is that all Australian acacias possess inherent reproductive and/or other life-history traits that facilitate invasiveness, and thus, all Australian acacias have the capacity to become invasive. Specific features of reproductive biology may be less important than a range of human-mediated factors that influence the abundance and distribution of species across potentially invasible sites, such as facets of the introduction history, propagule pressure, residence time and country-specific utilization or treatment of particular species via economic, environmental and social avenues.

Key stages for invasiveness of the reproductive life cycle of Australian acacias are useful to identify to determine options for the intervention to reduce success and achieve management objectives (Wilson *et al.*, 2011). Control efforts should aim, in the first instance, to prevent the accumulation of massive seed banks (Richardson & Kluge, 2008) as once a seed bank is established, the population is practically impossible to eradicate. Biological control provides the most cost efficient, long-term control method and should be the foundation of effective integrated control operations. The upper seed bank is where the majority of *Acacia* seeds are able to successfully germinate and so should be the target area for control measures of which burning is the most effective. However, the applicability in practice of such useful additional measures as burning, mechanical control and herbicide application is context specific. To reduce human-mediated dispersal, planting Australian acacias near points of dispersal pathways (e.g. near

Box 1 Priorities for future research on the reproductive ecology of Australian acacias

To elucidate determinants of invasiveness, a variety of approaches are necessary to establish a complete profile for identifying reproductive traits consistently associated with invasion success in novel environments. This includes conducting multi-species studies encompassing native and multiple introduced ranges and comparative studies that contrast invasive *Acacia* species with co-occurring native species, as well as with non-invasive *Acacia* species or closely related taxa. Data for these comparisons regarding reproductive traits are widely lacking, and further studies are needed to gather information on reproductive biology.

Very little research has been carried out on the pollination biology of Australian acacias. Given its fundamental role in reproductive success and therefore invasion, further research is needed to determine the relative contributions of different insect visitors and wind pollination to outcrossing and seed set in the introduced range for invasive species and non-invasive species as well as for invasive species in exotic and native ranges. This information could be used to determine whether pollination efficiency contributes to a species' invasiveness.

Both breeding system data, based on controlled pollinations that indicate potential for selfing, and mating system data, based on molecular markers that give the rates of outcrossing, are needed. Breeding system data are lacking for some invasive *Acacia* species and for almost all non-invasive species in their introduced ranges. Comparisons are needed between both groups to determine how breeding system links to invasiveness and also between invasive species in the native range and in the introduced range to examine the extent of interspecific breeding system plasticity. Findings have implications for management protocols regarding genetic modifications and expected seed yields following self-pollination.

Thorough documentation of seed dispersal syndromes in the group is needed, for example, to determine whether the bird-dispersal syndrome is overrepresented in taxa that have become invasive. Insights from such work will provide useful information for improving the management of already invasive Australian acacias and help to refine tools for more effective screening of new introductions.

rivers, along roads) should be prohibited (Wilson *et al.*, 2011). Although the significant association of resprouting ability with invasiveness in the phylogeny-free analyses may be misleading in evolutionary terms, it is still useful from a management perspective. Thus, wherever Australian *Acacia* species that attain reproductive maturity early or have a strong capacity for resprouting are planted, proactive measures should be implemented to manage invasiveness.

Despite our attempts to test for individual reproductive traits that contribute to invasiveness, larger sample sizes facilitated by greater data availability are necessary before any firm conclusions can be drawn in this regard. Because there is still a depauperate knowledge surrounding this group of globally important invasive plants, reproductive traits of invasive Australian acacias and their distinguishing characteristics need to be the focus of future research directives (see Box 1). Hence, until there is substantial evidence to the contrary, caution should be exercised concerning introductions of all Australian acacias given their general ability to reproduce effectively in new locations.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 The complete set of reproductive traits for introduced Australian acacias (n = 126).

Table S2 The complete set of reproductive traits for non-introduced Australian acacias (n = 324).

Table S3 List of Australian *Acacia* flower visitors.

Appendix S1 Accession numbers for those species used in phylogenetic analyses.

Appendix S2 Phylogeny-free analyses of relationships between individual reproductive traits in Australian *Acacia* species and invasive status (invasive versus non-invasive).

Appendix S3 The effect of individual reproductive traits on Australian *Acacia* species' invasive status (invasive versus non-invasive) using phylogeny as a covariate.

BIOSKETCH

All co-authors are actively involved in research on the ecology of Australian *Acacia* species. **M. G. is a Master's** student and S.D.J., J.J.L.R., D.M.R and J.R.U.W are core team members at the DST-NRF Centre of Excellence for Invasion Biology (http://academic.sun.ac.za/cib/). M.G.'s thesis at Stellenbosch University deals with the effects of *Acacia saligna* on native plant–pollinator communities. Her research interests lie in invasion biology, novel ecosystem interactions and restoration and conservation research.

Author contributions: M.R.G. and D.M.R. conceived the ideas; M.R.G., E.M. and H.M. collected most of the new data; M.B., N.G., M.R.G., C.H., E.M., H.M., J.T.M., D.J.M., M.N.P., J.G.R. and E.M.W. contributed additional data; J.J.L.R, J.T.M. and G.N.S. wrote the phylogenetic methods section; J.J.L.R. and J.T.M. reconstructed the phylogeny; M.R.G. and J.R.U.W. analysed the data; E.M. and H.M. created Table 2; J.G.R. and G.N.S. contributed to the 'Pollination biology' section; M.B., S.D.J. and J.G.R. contributed to the 'Breeding system' section; A.F.-R. contributed to 'Germination' section; A.P. provided conceptual insight and revision support. M.R.G. led the writing with support from D.M.R.

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APPENDIX 1

Description of variables, abbreviations and levels used in statistical analyses and Table S1. T = True, F = False, NA = not applicable.

Variable type	Abbreviation	No. species for which data are available	Levels (and range of values if continuous)	References
Explanatory				
Reproductive trait				
Age to reproductive maturity	Mature	39	Categorical, binary: '1' ≤ 2 years; '2' ÷ 2 years	1–6
Multi-locus outcrossing rate (t_m)	Outcross	8	Continuous: 0.65–0.97	7–15
Index of self-incompatibility (ISI) (infructescence per inflorescence)	Compatible1	9	Continuous: 0.02–0.96	16–19
ISI (pods per inflorescence)	Compatible2	10	Continuous: 0.008–1.1	16;17;19;20
Breeding system*	Breed	13	Categorical: 'apomictic'; 'SI' = self-incompatible; 'pSC' = partially self-compatible; 'SC' = self-compatible	9; 12; 16; 17; 19–22
Combined measure of breeding system†	Combined	13	Categorical, binary: 'Mixed' or 'Outcross'	see footnote †
Seed dispersed by ants	Ant	16	Categorical: T/NA‡	5; 20; 23-25; 26
Seed dispersed by birds	Bird	13	Categorical: T/NA	6; 23; 24; 26–30
Biotic seed dispersal	Dispers (combination of previous two columns in Table S1)	27	Categorical, binary: 'not bird' dispersed if ant = T & bird = NA; 'bird' dispersed if bird = T	
Seed mass	Seed mass	122	Continuous: 2.72–219.77 (mg)	1; 24; 31
Resprout ability	Resprout	75	Categorical, binary: T/F	5; 31; 32
Duration of flowering season	Flower duration	81	Continuous: 2–12 (months)	5; 31–33
Response				
Invasive or not invasive	Invasive		Binary: 0/1	34

1: J.T. Miller, unpublished data; 2: Australian Native Plants Society, http://anpsa.org.au/a-pod.html, October 2010; 3: Global Invasive Species Database, http://interface.creative.auckland.ac.nz/database/species/ecology.asp?si=1662&fr=1&sts=sss&lang=EN, 1 October 2010; 4: Kerala Agricultural University, 2002; 5: World Wide Wattle, http://www.worldwidewattle.com, February 2011; 6: Zenni *et al.* (2009); 7: Broadhurst *et al.* (2008); 8: Butcher *et al.* (1999); 9: George *et al.* (2008); 10: Millar *et al.* (2008); 11: Moffett (1956); 12: Moran *et al.* (1989*b*); 13: Muona *et al.* (1991); 14: Philp & Sherry (1946); 15: Coates *et al.* (2006); 16: M. R. Gibson, unpublished data; 17: Kenrick & Knox (1989); 18: Moncur *et al.* (1991); 19: J. G. Rodger, unpublished data; 20: Morgan *et al.* (2002); 21: Andrew *et al.* (2003); 22: Moffett & Nixon (1974); 23: Davidson & Morton (1984); 24: Kew Gardens Seed Information Database, http://data.kew.org/sid/sidsearch.html, February 2011; 25: Lorenzo *et al.* (2010); 26: O'Dowd & Gill (1986); 27: Langeland & Burks (1998); 28: Moran *et al.* (1989*a*); 29: Stanley & Lill (2002); 30: Starr *et al.* (2003); 31: Castro-Díez *et al.* (2011); 32: D. J. Murphy, unpublished data; 33: Arbres et arbustes de La Réunion, http://arbres-reunion.cirad.fr/especes/fabaceae/acacia_heterophylla_willd, February 2011; 34: Richardson & Rejmánek (2011).

*When only t_m was available, we used the criteria: SI is t_m ÷ 0.8.

†Inference from t_m, ISI and breeding system for which species are classified as either outcrossing (if t_m ÷ 0.8 or ISI ≤ 0.5 a species is classified as outcrossing) and otherwise as mixed mating.

‡References could only confirm (and not refute) that an ant or bird dispersed seed of a given species, and thus, criteria for 'not bird' dispersed were required (see Biotic seed dispersal (above) and Methods section of main article).

Appendix 2

The implications of uniparental reproduction for colonization and invasion: Baker’s Law still rules

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Abstract

The idea that organisms capable of uniparental reproduction are more likely than obligate outcrossers to establish populations after long-distance dispersal was suggested by Herbert G. Baker, and has since become known as 'Baker's Law'. It was important conceptually because it showed how breeding systems could have consequences for population ecology, evolution and biogeography. Although it has been largely associated with studies of long-distance colonization, Baker's Law has also been applied in studies of local colonization, meta-populations and species invasiveness. There is evidence for an association between self-compatibility and island colonization in hermaphroditic plants, although the relatively high frequency of dioecious species on islands is often considered a violation of Baker's Law. The ability of uniparental reproduction also appears to benefit local colonization by conferring reproductive assurance in the absence of mating partners at the range margins. Moreover, the ability of uniparental reproduction is frequently associated with invasive species. Finally, the ability of uniparental reproduction promotes population establishment by conferring a general resilience to Allee effects. Despite the broad empirical support, the mechanisms by which uniparental reproduction promotes population establishment are not yet well understood. Therefore, we foresee increased interest in Baker's Law as an explanation for biogeographic and evolutionary patterns.

Introduction

Colonization, alongside extinction, is one of the major drivers of global patterns in the geographic distribution of species (Levin 2006). Because reproduction is an obvious prerequisite for successful colonization, the reproductive systems of species are very likely a major determinant of colonization success. Outcrossing results in recombination and promotes genetic variation, and consequently the ability to adapt rapidly (e.g. Stebbins 1957; Barrett et al. 2008; Moran et al. 2009). At the other end of the spectrum of reproductive systems, uniparental reproduction (e.g. self-fertilization, agamospermy, parthenogenesis and vegetative reproduction; Table 1; Fig. 1) provides reproductive assurance (Table 1) when there is little or no opportunity for outcrossing (Darwin 1867; Eckert et al. 2006). Uniparental reproduction should therefore become more prevalent when opportunities for outcrossing are more restricted.

Opportunities for outcrossing may frequently be absent after long-distance dispersal (Table 1) because it can result in single individuals being completely isolated. Therefore, in environments that have to be reached by long-distance dispersal, taxa with uniparental reproduction should be more common (Baker 1955). In his work on plant species of the Plumbaginaceae, the botanist Herbert G. Baker observed that obligately outcrossing species occurred in the centre of the family's distribution, whereas facultatively self-fertilizing species occurred on the periphery, and were often disjunct from the centre (Baker 1953). Similar patterns were reported for other plant taxonomic groups, such as the genera *Linus*, *Oxalis* and *Primula* (Baker 1959), suggesting that this might be a general pattern for plants. Also, hermaphroditic genotypes of normally unisexual freshwater shrimp species (Notostraca) were reported to be most prevalent at northern latitudes and in peripheral populations (Longhurst 1955). This suggested to Baker (1955) that the pattern of uniparental reproduction at range margins may apply to all groups of organisms.

Inspired by these observations, Baker (1955) proposed the rule that "*With self-compatible individuals a single propagule is sufficient to start a sexually-reproducing colony, making its establishment much more likely than if the chance growth of two self-incompatible yet cross-compatible individuals sufficiently close together spatially and temporally is required*". In addition, he wrote that *"Self-compatible flowering plants are usually able to form some seed in the absence of visits from specialized pollinating insects, which may be absent from the new situation"*. He also explicitly stated that other modes of uniparental reproduction, including apomixis (i.e. agamospermy; Table 1) and vegetative reproduction, would, like self-fertilization, provide reproductive assurance (Fig. 1). Thus, uniparental reproduction should increase the likelihood of successful colonization when potential mating partners are absent. Moreover, for plants, autonomous reproduction (Table 1) would be of additional benefit when pollinators are scarce or absent in the new range, even when potential mating partners are present. Because Baker's hypothesis is a simple and attractive idea that might explain many distributional and evolutionary patterns, Stebbins (1957) declared it "Baker's Law", although many authors refer to it as "Baker's Rule".

The promotion of Baker's hypothesis to a Law may have been premature because at that time the evidence was fairly limited, both in terms of the generality of the patterns observed and the mechanism proposed to explain them. In support stood Baker's (1955), Longhurst's (1955) and Stebbins' (1957) examples of taxonomic groups (genera and families) with outcrossing species at the centre of the distribution and species with uniparental reproduction on the periphery. In addition, Baker (1955) noted that weeds, which must frequently colonize new habitats, are typically self-compatible or have vegetative reproduction. Despite Stebbins' (1957) assertion of generality, counter examples were quickly provided. Surveys of the flora of the Hawaiian Islands revealed exceptionally high frequencies of dioecious (Table 1) plant species, which are by definition obligate outcrossers (Carlquist 1966; Bawa 1982). This led Carlquist (1966) to conclude, "*If dioecious stocks immigrated to the Islands, "Baker's Law" must, in part, be abandoned*". Baker's (1955) hypothesis did not, however, preclude colonization by obligate outcrossers but emphasized that species with uniparental reproduction should have a higher colonization success (Baker 1955, 1967). Tests of Bakers's Law, therefore, must be based on comparative multi-taxa studies conducted in a statistical framework and not from case-by-case studies of individual taxa.

Baker's Law has inspired biologists across the fields of biogeography, reproductive biology, conservation biology, invasion biology and molecular genetics, but remains controversial. In this comprehensive review, we begin by making explicit the predictions of Baker's Law, as these have not always been fully appreciated or understood (e.g. Carlquist 1974). We then discuss the support for these predictions provided by different research fields, and discuss some of the biological processes that may underlie the patterns identified. In particular we consider the possibility that the advantages of uniparental reproduction for colonizing species are due, not only to their ability to reproduce as a single founding individual (Baker's original emphasis), but also to their resilience to Allee effects, which are more general obstacles to population establishment and persistence (Stephens et al. 1999). The controversial idea that uniparental reproduction could evolve through natural selection due to its positive effects on colonization ability is also discussed. We conclude the review with promising directions for future research.

Predictions arising from Baker's Law

The following predictions can be made from Baker's Law. (1) Taxa with uniparental reproduction should colonize suitable habitat more rapidly when the opportunity arises, unless they produce fewer propagules with lower dispersability than taxa dependent on biparental reproduction (Pannell and Barrett 1998). For instance, they should have been among the first plants to colonize previously glaciated areas after the retreat of the ice sheets. Moreover, we expect that species with uniparental reproduction might shift their ranges more rapidly in response to current climate change than species not capable of uniparental reproduction. (2) Taxa with uniparental reproduction should establish after dispersal across oceans more frequently than taxa that require partners. Therefore in groups with distributions that are disjunct due to dispersal across oceans there should be a higher frequency of self-compatible taxa in the colonized than the source parts of their distributions. A specific prediction would be higher frequencies of taxa with uniparental reproduction on oceanic islands than on comparable mainland areas. (3) There should be a higher frequency of uniparental reproduction among naturalized alien taxa than among the source biota of those aliens. Moreover, among alien taxa, those with uniparental reproduction should spread more rapidly and occupy larger ranges. (4) There should be a higher frequency of taxa with uniparental reproduction in habitats where high levels of disturbance favor frequent cycles of extinction and recolonization (i.e. meta-population dynamics), as found in many anthropogenic habitats. (5) Species with uniparental reproduction should be capable of reproducing successfully when mating partners are either absent or scarce, and should therefore exhibit a general resilience to Allee effects.

The geographic distribution of breeding systems on continents

Baker (1955) inferred that uniparental reproduction aids in colonization from the observation that taxa with this mode of reproduction tend to have distribution ranges consistent with long-distance dispersal. In line with this, Randle et al. (2009) showed recently that species of *Collinsia* capable of autonomous seed production (Table 1) have larger ranges in North

America than closely related sister species that do not have this capacity. For many of the taxonomic groups for which Baker's Law appears to hold (Baker 1953; Stebbins 1957; Baker 1959), the current distributional pattern arose after colonization of previously glaciated areas from glacial refugia. A phylogeographic study reported that autogamous homostylous species have a wider distribution in previously glaciated areas than allogamous heterostylous species in *Primula* sect. *Aleuritia* (Guggisberg et al. 2006). Overall, phylogeographic studies suggest that uniparental reproduction conferred a strong advantage for post-glacial colonization, as would be predicted by Baker's Law.

While most of the examples provided by Baker (1953, 1959), Longhurst (1955) and Stebbins (1957) in support of Baker's Law referred to variation in breeding systems (Table 1) among species within families and genera, breeding systems also frequently vary within species. For example, the occurrence of self-compatible populations of the normally self-incompatible *Arabidopsis lyrata* in the Great Lakes region of North America is likely to be a consequence of the evolution of self-compatibility during post-glacial colonization of this species (Mable and Adam 2007). Furthermore, the apomictic, polyploid plant *Townsendia hookeri*, which recolonized previously glaciated areas in western North America, appears to have recurrently evolved from non-apomictic, diploid lineages south and north of the ice sheets (Thompson and Whitton 2006). Self-compatible or self-fertilizing morphs of species are found mainly at the distributional range margins (Rick et al. 1979; Schoen 1982; Wyatt 1986; Barrett and Shore 1987; Barrett et al. 1989; Huff and Wu 1992; Busch 2005; Herlihy and Eckert 2005; Moeller and Geber 2005; Oritz et al. 2006; Darling et al. 2008; Fig. 2). A recent meta-analysis also showed that the degree of asexual reproduction is larger at the range margins of clonal plant species (Silvertown 2008; also see Richards 2003). For many species, but not for all (Fig. 2), this capacity for uniparental reproduction is particularly pronounced at the arctic range margins, where pollination might be less reliable as a consequence of the harsh climate. These distributional patterns of breeding systems within species are in line with the predictions of Baker's Law. However, a non-exclusive alternative for these distributional patterns of breeding systems within species is offered by Peck et al. (1998). They propose that because migration from the centre to the range margins is likely to exceed reverse migration, particularly so for margins with shorter growing seasons than the centre, uniparental reproduction might provide a mechanism that isolates the resident genotypes from the migrants that might otherwise offset local adaptation through interbreeding. Quantifying how progeny from experimental crosses between plants from central and peripheral populations perform in the peripheral habitats would be a way of testing this hypothesis.

Among the studies on intraspecific variation in breeding systems, few have included tests of whether the mating system (i.e. the realized outcrossing rate; Table 1) differs between central and peripheral populations, and whether the self-compatible morphs have an advantage over the outcrossing morphs at the range margins. Plants of *Aquilegia canadensis* from peripheral populations have floral characteristics, such as low herkogamy (i.e. anther-stigma separation), that suggest a high capacity for autogamy, but the actual outcrossing rates did not differ between central and peripheral populations (Herlihy and Eckert 2005). Reciprocal transplants of self-compatible plants from peripheral populations and self-incompatible individuals from the centres of distribution in both *Arenaria uniflora* (Wyatt 1986) and *Leavenworthia alabamica* (Busch 2005) showed that the benefit of self-compatibility, in terms of seed production, was not restricted to peripheral populations. Possibly, selfing morphs have an advantage in setting seeds, but due to inbreeding depression are gradually outcompeted by outcross offspring in the centre of the distribution. Future studies of this kind should therefore not only assess seed production but also the quality of the offspring.

Although few studies on animals refer to Baker's Law, many studies on clonal animals, including sea anemones, earthworms, snails, slugs, woodlice, millipedes, grasshoppers, moths, flies, beetles and lizards reveal a preponderance of clonality at range margins (Hughes 1989). This phenomenon in clonal animals is usually referred to as geographic parthenogenesis, and was already described by Vandel (1928) before Baker formulated his hypothesis for the phenomenon. For example, *Daphnia pulex* reproduces predominantly sexually by cyclical parthenogenesis, in southern Scandinavia, close to the centre of its European range, whereas populations in northern Scandinavia reproduce predominantly asexually by obligate parthenogenesis (Ward et al. 1994). In summary, the continental distributions of uniparentally reproducing organisms, both plants and animals, are generally consistent with Baker's Law.

Breeding systems on oceanic islands

The high incidence of dioecious plant species on oceanic islands, particularly on the Hawaiian Islands (Carlquist 1966; Bawa 1982), resulted in intense debate about the importance of Baker's Law as an explanation for species distributional patterns. It led Carlquist (1966) to conclude that the number of propagules arriving at a site was seldom a severe barrier to colonization. He also pointed out that Baker's own data on the genus *Limonium* showed that dimorphic breeding systems were more frequent in species confined to small oceanic islands (94.5%) compared to the entire genus (79.6%; Carlquist 1974, p. 532). In a response, Baker and Cox (1984) showed that the percentage of dioecious species on 22 island floras was high, but did not generally deviate from mainland areas at comparable latitudes. However, Baker and Cox (1984) only compared the percentages of dioecious species on islands and mainlands in temperate regions, and it remains to be seen whether their findings also apply to subtropical and tropical regions.

Baker (1967) further countered Carlquist's (1966) conclusion by pointing out that self-incompatible species are infrequent on islands. While there are some highly self-incompatible genera of Asteraceae on the Hawaiian Islands, many other genera appear to be self-compatible (Carr et al. 1986). The Galapagos Islands are dominated by species that are self-compatible with a capacity for autogamy (Rick 1966; McMullen 1987). And, although both New Zealand (Godley 1979; Webb and Kelly 1993) and the Juan Fernández Islands (Anderson et al. 2001) have high proportions of dioecious species, self-incompatible hermaphroditic species are relatively rare on these islands. Furthermore, for some species with intraspecific variation in the breeding system, the self-fertilizing genotypes appear to have been more successful as colonizers. For example, colonization of Caribbean islands and Central America from Brazil by the normally tristylous *Eichornia paniculata* was accompanied by the loss of morphs and the recurrent evolution of self-fertilization (Barrett et al. 1989). Therefore, in contrast to the patterns of dioecy, Baker's Law appears to hold for oceanic islands when considering self-incompatibility.

There is some evidence that the high frequency of dioecious plants on some oceanic islands does not reflect high colonization success of dioecious species, but instead reflects the evolution of dioecious species from self-compatible hermaphroditic colonizers, and subsequent radiation of the dioecious species on the islands (Baker 1967; Baker and Cox 1984). Moreover, it could be that the few dioecious species that successfully colonized had higher radiation rates than the self-compatible hermaphroditic colonizers. Indeed, while 27.5% of the species on the Hawaiian Islands are dioecious, these are confined to just 14.9% of the genera (Carlquist 1966). This percentage of dioecy is still considerably higher than the estimated 7% of angiosperm genera with dioecy (Renner and Ricklefs 1995), but it suggests a rate of radiation in dioecious genera in Hawaii that is above average. Interestingly, this would contradict the finding of Heilbuth (2000) that species richness is usually lower in dioecious taxa than in hermaphroditic or monocious sister taxa. One reason that dioecious species might evolve on islands from self-fertilizing colonizers is that once a species has established, inbreeding depressing might impose selection for mechanisms that avoid self-fertilization. Self-incompatibility could be an alternative outcome of such selection, but the evolution of dioecy involves fewer evolutionary steps than that of self-incompatibility (Thomson and Barrett 1981).

Phylogenies derived from molecular genetic analysis shed some light on the origin of dioecious and self-incompatible taxa on oceanic islands. A recent study indicated that *Deinandra* species on the California Islands probably originated by adaptive radiation from a self-incompatible ancestor from western North America, which was interpreted as a violation of Baker's Law (Baldwin 2007). Another recent study on self-compatible and self-incompatible species of *Tolpis* on the Canary Islands suggested that the colonizing ancestor had most likely a leaky self-incompatibility system (Crawford et al. 2008). Curiously, this was interpreted as an exception to Baker's Law despite the fact that leaky self-compatibility is a form of potential uniparental reproduction (Fig. 1). On the other hand, Sakai (1995) found that in at least 11 plant lineages on Hawaii, dimorphism evolved from hermaphroditism in colonists. However, she also found that among all successful colonists 8.6% were already dioecious. This percentage of dioecious colonists is considerably lower than the 27.5% and 14.9% dioecious species and genera, respectively, in the current Hawaiian flora (Carlquist 1966), and indicates that, as suggested by Baker (Baker 1967; Baker and Cox 1984), most dioecious species on the islands evolved *in situ*. Nevertheless, the 8.6% dioecious species among the initial colonizers is still higher than the global average of 6% (Renner and Ricklefs 1995), which suggests that these species were not completely limited by the absence of partners or pollinators during colonization of the Hawaiian Islands.

There are several potential reasons why dioecious species could, contrary to expectation, be good colonizers. First, colonization by long-distance dispersal of multiple individuals may be frequent in dioecious plants as they tend to be long-lived woody perennials (Renner and Ricklefs 1995), which would allow individuals to survive until a partner arrives (Cox 1989; Lloyd and Schoen 1992; Bond 1994). Indeed, numerous predominantly herbaceous angiosperm genera are represented on islands by woody shrubs or trees (Böhle et al. 1996). Many dioecious species also have multi-seeded fleshy animal-dispersed fruits, which in contrast to single seeded or wind-dispersed fruits increase the likelihood of simultaneous long-distance dispersal of several individuals (Bawa 1982; Baker and Cox 1984; Cox 1989; Vamosi et al. 2003; Renner and Ricklefs 1995). Second, colonization by single individuals of dioecious species may be possible if dioecy is leaky (Table 1; Baker and Cox 1984). Evidence for the contribution of leaky dioecy (Table 1) to successful island colonization was recently found in the genus *Diospyros* on Mauritius (Venkatasamy et al. 2007). In addition, it has been suggested that dioecious species frequently have generalist pollinators (Bawa 1982; Charlesworth 1993), which reduces the likelihood that dioecious species will suffer from pollinator limitation after long-distance dispersal.

The few studies on breeding systems of animals on islands are in support of Baker's Law. Jordal et al. (2001) found that across 45 Pacific and Old World tropical islands, numbers of outbreeding taxa of wood- and bark-boring beetles in the curculionid subfamily Scolytinae decreased more rapidly with island size than did those of inbreeders (Fig. 3). Inbreeding species have a haplo-diploid genetic system in which females can produce haploid sons by parthenogenesis, and subsequently mate with these sons. The inbreeders are also generally more widespread than the outbreeders, suggesting a higher colonization ability of inbreeders. A study of Scolytinae beetles on Cocos Island found that 74% of the taxa are inbreeders compared with 40-50% on the mainland (Kirkendall and Jordal 2006). In summary, with the exception of the unexpectedly high frequencies of dioecious plant taxa, oceanic islands are dominated by taxa capable of uniparental reproduction, which is consistent with Baker's Law.

Invasiveness of alien species

The increase of human traffic around the world has weakened most geographic barriers and resulted in long-distance dispersal of many alien organisms (Richardson et al. 2000). Therefore, biological invasions offer a unique opportunity for testing the importance of Baker's Law as an explanation of distributional and evolutionary patterns. Long before Herbert Baker formulated his hypothesis, George Henslow (1879) emphasized the immense advantage of introduced self-fertilizers over introduced cross-fertilizers in the absence of visitation by native insects. Henslow's hypothesis could therefore be seen as a predecessor of Baker's Law (in accordance with Stigler's (1999) Law of Eponymy that virtually no scientific discovery is named after its original discover).

Henslow's hypothesis was directly countered by Asa Gray (1879), who argued that most European weeds in North America are likely to be outcrossers. Although Gray also admitted that most of these species are capable of self-fertilization, which would be in line with Henslow's hypothesis and Baker's Law, he wrote, "*We cannot avoid the conclusion that self-fertilization is neither the cause, nor a perceptible cause of the prepotency of the European plants which are weeds in North America".* Thus, to a remarkable degree, the disagreement between Henslow and Gray foreshadowed later controversies around Baker's Law.

Some of the world's most aggressive invasive plant species, such as *Alternanthera philoxeroides*, *Chromolaena odorata* and *Fallopia japonica,* have predominantly uniparental reproduction. On the other hand, some other highly invasive species, such as *Centaurea solstitialis* in North America (Sun and Ritland 1998) and *Senecio inaequidens* in Europe (Lafuma and Maurice 2007) are self-incompatible, which has been regarded as evidence against Baker's Law. However, to test which characteristics contribute to invasiveness of alien species, one should compare multiple alien species differing in their degree of invasiveness (Muth and Pigliucci 2005). Although a number of studies have attempted to identify predictors of invasiveness in this way (reviewed in Pyšek and Richardson 2007), few have included sufficiently detailed information on breeding systems to test Baker's Law. In a study involving 142 alien species in the United States, Sutherland (2004) found, in direct opposition to the predictions of Baker's Law, that invasive alien weeds are less frequently self-compatible than non-invasive ones. However, that study was biased towards inclusion of invasive species that are perennials, particularly shrubs and trees, which are more frequently self-incompatible than annuals (Lloyd and Schoen 1992; Barrett et al. 1996). A recent analysis, controlling for life form, of 361 European species naturalized in the USA found, in contrast to the study of Sutherland (2004) and in support of Baker's Law, that species capable of autonomous seed production had larger naturalized ranges than those not capable of autonomous seed production (van Kleunen and Johnson 2007).

The study by van Kleunen and Johnson (2007) included only species that had become established (i.e. naturalized) in the USA. Ideally one should also test whether alien species that establish differ from related ones that failed to establish after introduction (Pyšek and Richardson 2007). Such comparisons are difficult to conduct in the introduced range because non-established species are no longer present. In a recent study, van Kleunen et al. (2008) circumvented this problem by studying the capacity for autonomous seed production of ten pairs of congeneric naturalized and non-naturalized species of Iridaceae, all introduced to other continents, in their native range in South Africa. This study demonstrated that the naturalized species have on average a higher capacity for autonomous seed production than their congeners that failed to naturalize (Fig. 4). More evidence in favour of Baker's Law was provided by Rambuda and Johnson (2004), who found that among 17 perennial plants invasive in South Africa, 100% were capable of uniparental reproduction, thus contrasting significantly with the world's general flora in which approximately 50% of species are self-incompatible (Fryxell 1957). Moreover, recent studies reported that among alien plant species on islands in the Mediterranean (Lambdon et al. 2008) and in Germany (Küster et al. 2008), the ones capable of uniparental reproduction are more widespread than the ones without this ability. Overall, these results suggest that, in accordance with Baker's Law, breeding systems are useful for predicting establishment of alien plant species.

For species with intraspecific variation in their breeding system, one would expect that genotypes capable of uniparental reproduction should have a selective advantage during invasion. Fryxell (1957) refers to a study of Gustafson (1946/1947) reporting that Scandinavian biotypes of *Dechampsia caespitosa* that are fully sexual in their native habitat reproduce by "facultative vivipary" (i.e. apomixis) in California. Similarly, Darwin (1876) demonstrated that offspring of plants of *Escholtzia californica* that Fritz Muller showed to be self-sterile in Brazil were self-fertile when grown in England. Interestingly, offspring of these plants sent back to Brazil by Darwin were initially self-fertile but became self-sterile again after a few years, indicating that the change in breeding system between the native and the introduced range is most likely a plastic response to the change of environment. The importance of plasticity in the breeding system for colonization success has, however, to best of our knowledge not been explored yet.

Besides plasticity of the breeding system, there might also be genetic variation and thus potential for evolution of the breeding system. This could be an alternative explanation for the findings of Gustafson mentioned above. For the usually self-incompatible *Spartina alternifolia*, self-compatible genotypes have been found in its invasive range along the North American Pacific coast (Daehler 1998). Recently, it was also suggested that invasive hybrids of the alien *S. alternifolia* and the native *S. foliosa* in San Francisco Bay have evolved self-fertility, and that this has contributed to their rapid spread (Sloop et al. 2009). Similarly, *Rubus alceifolius*, which reproduces sexually in its native range in Southeastern Asia, reproduces by agamospermy (Table 1) in its introduced ranges on Madagascar and Reunion (Amsellem et al. 2001). Although vegetative reproduction of plants is frequently associated with invasiveness (reviewed in Kolar and Lodge 2001, Pyšek 1997 and Silvertown 2008), we are not aware of any study that has explicitly tested whether apomictic seed production is associated with invasiveness.

Among the naturalized insects in the United States, orders with many haplo-diploid taxa, in which males are produced from unfertilized eggs, are overrepresented (Simberloff 1986). Many of these haplo-diploid species also practice extreme inbreeding (Wrench and Ebbert 1993). Niemelä and Mattson (1996) suggested that the much higher numbers of European phytophagous insects invading North American forests than North American phytophagous insects invading European forests is in part due to the higher frequency of uniparental reproduction among the European species. Furthermore, a recent study reports that three naturalized beetles in old-growth tropical forests are all highly inbreeding (Kirkendall and Ødegaard 2007). In summary, the high frequency of taxa and genotypes capable of uniparental reproduction among invasive organisms is consistent with Baker's Law.

Short-distance colonization: meta-population dynamics and pest species

While Baker used many examples of long-distance (e.g. trans-oceanic) dispersal, the principles behind Baker's Law (i.e. partner limitation, and in the case of plants also pollinator limitation) should to some extent also apply to colonization at a local scale (Pannell and Barrett 1998). Indeed, among three fern species that colonize forests on abandoned agricultural fields in New York, the best colonizer has the highest capacity for self-fertilization (Flinn 2006). This implies that the principle of Baker's Law should also apply to meta-population dynamics, where the effect of local extinctions may be compensated by re-colonization events (Pannell and Barrett 1998; Pannell and Dorken 2006).

Meta-populations are not only affected by the breeding system through the higher colonization ability of self-fertilizing taxa and genotypes but also by the reduced levels of gene flow between uniparentally reproducing populations (Antonovics 1968; Lowry and Lester 2006). Meta-population models have shown that the benefits of uniparental reproduction during colonization can explain the maintenance of co-sexuality and self-compatibility in species in which dioecy or self-incompatibility would otherwise be stable in long-lived populations (Pannell 1997a, Pannell and Barrett 1998). In particular, this could explain the evolution of hermaphroditism from a dioecious breeding system (Pannell 1997b, 2002). Indeed, in the androdioecious plant *Mercurialis annua*, outcrossing rapidly decreases with distance away from the closest partner, and as a consequence hermaphrodites have an advantage over male plants during colonization (Eppley and Pannell 2007). Similarly, an experimental study on the gynodioecious *Silene vulgaris* showed that, although females had higher fitness within populations, self-compatible hermaphrodites had higher fitness than females when isolated by over 40 m from the nearest population (Taylor et al. 1999). Thus, Baker's Law could explain the evolution and/or the maintenance of breeding systems that allow for uniparental reproduction in species that follow meta-population dynamics.

Baker (1955) pointed out that some of the best evidence in support of Baker's Law comes from studies on the reproductive biology of weeds, which usually rapidly (re-)colonize habitats disturbed by humans. In a comparative work on weedy and non-weedy taxa of the genera *Eupatorium* and *Ageratum*, Baker (1965) showed that weedy species indeed tend to be self-compatible and autogamous, whereas the non-weedy species are generally self-incompatible. Although the data of Baker (1965) suggested a positive association between weediness and self-fertilization, which had already been predicted by Henslow (1879), general agreement on the principle requires data on a larger number of species. In a pollinator-exclusion experiment on 65 weeds in Canada, all 33 annual species and 21 of 23 biennials and caespitose perennials were capable of autogamy, while the eight species with vegetative reproduction were not capable of autogamy (Mulligan and Findlay 1970). Whereas the study of Mulligan and Findlay (1970) lacked a control group of non-weedy species, Price and Jain (1981) found that among 400 species in the British Isles, the ones classified as weeds or colonizers (i.e. species occurring in disturbed habitats) were more likely to be self-compatible or autogamous than the ones not classified as such. Similarly, among agricultural invertebrate pest species of genera in which parthenogenesis occurs, 45% are parthenogenic in North America and 48% in Italy, while the overall incidence of parthenogenesis in these genera is much lower (10% and 16% in North America and Italy, respectively; Hoffmann et al. 2008). None of these studies on pest species were restricted to native taxa, and as a consequence the results might partly reflect the high frequency of uniparental reproduction among invasive alien species. A notable exception is a study of Stebbins (1965), which found that among native Californian plants there was no indication that annuals with predominant self-fertilization were more likely to be weeds than perennials with predominant outcrossing. Clearly, more studies comparing native pest species and non-native pest species are required to draw any general conclusion on whether Baker's Law applies to pest status independently of invasiveness.

Allee effects

Baker (1955) emphasized the importance of uniparental reproduction for the avoidance of reproductive failure by isolated colonizing individuals. From another perspective, Baker's Law can be viewed as a hypothesis about traits that render organisms resilient to extreme Allee effects (i.e. diminished reproductive success of individuals in small populations; Stephens et al. 1999). Studies of Allee effects, which have mainly focused on declining rather than on expanding species, have often implicated partner limitation, and for plants also pollinator limitation (Ghazoul 2005). These are the same biological factors underlying Baker's Law (Baker 1955). Consistently, a recent meta-analysis indicated that self-compatible plants in contrast to self-incompatible ones are less likely to experience Allee effects in populations with low genetic variation (Leimu et al. 2006).

During colonization, newly founded populations will tend to be small (Baker 1955). Furthermore, populations along an invasion front will also tend to be small and dispersed (Barrett *et al.* 2008). Thus species that are resilient to Allee effects might be more likely to successfully colonize and become invasive. This is supported by theoretical models showing that Allee effects can cause abrupt species range limits and colonization to fail (Keitt et al. 2001). Since reproductive assurance in general appears to play a role in resilience of species to Allee effects (Leimu et al. 2006), we need to consider the possibility that the mechanism behind Baker's Law might lie in the consequences of reproductive assurance for individual fitness and dynamics of small founder populations.

Despite much theoretical interest in the importance of Allee effects for colonization and particularly invasions by alien organisms, and the implications for their management (Liebhold and Bascompte 2003; Taylor and Hastings 2005; Jerde et al. 2009), only a few empirical studies have tested for Allee effects in colonizing organisms. A recent re-introduction experiment of the dioecious copepod *Hesperodiaptomus shoshone* in Californian lakes demonstrated that Allee effects may limit colonization success of this species (Kramer et al. 2008). Field studies on the self-compatible, though not autogamous, shrub *Senna didymobotrya* (van Kleunen and Johnson 2005), and the predominantly autogamous annual herb *Datura stramonium* (van Kleunen et al. 2007) revealed no evidence for Allee effects in the invasive range of these species in South Africa. On the other hand, Elam et al. (2007) showed that fruit set increases with population size and genetic diversity of the founder population in experimental populations of the self-incompatible alien of hybrid origin *Raphanus sativus* (Fig. 5). These results indicate that *R. sativus* suffers from partner limitation, possibly in combination with pollinator limitation, in small founder populations. This species may have become invasive despite Allee effects because it can overcome them by having multi-seeded dispersal units (Elam et al. 2007). A modeling study based on empirical data for the invasive gypsy moth *Lymantria dispar* also indicates the importance of Allee effects for population establishment and eradication of the species (Liebhold and Bascompte 2003). Therefore, although obligately outcrossing species may suffer from Allee effects, which is in line with the principle of Baker's Law, this does not necessarily prevent them from becoming invasive.

Intuitively, one would expect that animal-pollinated plant species would suffer more from Allee effects than abiotically pollinated species, and therefore benefit disproportionately from uniparental reproduction. However, a study of 361 European species naturalized in North America showed that the positive effect of uniparental reproduction on the spread of these species was larger for abiotically-pollinated than for animal-pollinated species (van Kleunen and Johnson 2007). This is congruent with other recent studies indicating that pollen limitation in wind-pollinated plants may be larger than expected (reviewed in Koenig and Ashley 2003). Consistent with this finding and with Baker's hypothesis of limitation of fecundity by partner availability, plants of the largely self-incompatible, wind-pollinated grass *Spartina alterniflora* suffer from reduced seed set due to pollen limitation in low density patches in its invasive range in western North America (Davis et al. 2004a, 2004b). A subsequent modeling study showed that this Allee effect is likely to have retarded the invasion of *Spartina alterniflora* (Taylor et al. 2004). Overall, these studies show that self-compatible alien species seldom suffer from Allee effects, and thus might spread faster than self-incompatible alien species once established.

Conclusions and directions for future research

In formulating his hypothesis, Baker (1955) made an important new connection between the hitherto separate fields of reproductive biology, biogeography and population ecology. The evidence that has accrued since then is largely in support of his idea that uniparental breeding systems promote establishment of populations. Nevertheless, a limitation of most biogeographic studies that have addressed this issue is that they are correlative, and thus do not necessarily indicate the causes of the observed patterns. The focus on future work will thus need to be on establishing the mechanisms that underlie Baker's Law. For example, no studies have experimentally assessed whether breeding systems can be a driver of geographic distributions by introducing species or genotypes with different breeding systems into new locations and monitoring the consequences for population establishment and expansion.

The basic assumptions underlying Baker's Law are that colonizing or introduced alien species are more partner-limited, and, for plants, pollinator-limited than native species. Curiously, these assumptions have rarely been tested explicitly. Some studies have shown that alien plant species, at least the ones that have successfully established, are generally well integrated in plant-pollinator visitation webs (e.g. Memmot and Waser 2002; Aizen et al. 2008), but these studies did not assess the effectiveness of the pollinators. A notable exception is a recent study of Harmon-Threatt et al. (2009), which showed that some naturalized alien species are more pollen-limited than closely related native species. Future studies should, however, also test whether species that successfully established are less limited by these factors than the ones that were introduced but did not establish.

If plants generally suffer from mate and/or pollinator limitation when introduced to new locations, species with mixed mating systems should have higher self-fertilization rates in invasive than in native populations. A comparison between seven native North American and three alien Scottish and four alien New Zealand populations of the herbaceous plant *Mimulus guttatus* did not reveal a difference in the actual outcrossing rates, suggesting that this species is well served by pollinators in its non-native ranges (van Kleunen and Fischer 2008). Clearly, to assess the generality of this pattern, future studies should assess self-fertilization rates of native and invasive populations of a larger number of species.

Although breeding systems appear to be associated with establishment success, it could be that establishment is ultimately not determined by the breeding system but by characteristics that are frequently associated with it. Some studies have explicitly corrected for potential confounding species characteristics, such as life span and life form, but others remain unexplored. For example, uniparental reproduction, in both plants and animals, is frequently associated with polyploidy (Bierzychudek 1985; Levin 2002; Kearney 2005). Polyploid taxa and genotypes are thought to have higher environmental tolerances than diploid taxa, and as a consequence might have higher colonization abilities. Therefore, future studies should disentangle the roles of different correlated characteristics in the establishment of populations.

The evolutionary transition from outcrossing to self-fertilization has occurred frequently in plants (Schoen et al. 1997; Goodwillie 1999). Biogeographic studies of Baker (1955) and others suggest that the evolution of self-fertilization has been an important step during range expansion and establishment of new populations after long-distance dispersal. However, few studies have tested explicitly for selection on self-fertilization during colonization. A notable exception is a study by Moeller and Geber (2005) who established experimental populations of *Clarkia xantiana*, and showed strong selection for reduced anther-stigma separation, which increases the capacity for autogamy, in small populations but not in large populations. Clearly, future studies should involve similar experiments with other species to assess how general this pattern is, and whether selection reverses once populations have established and acquired sufficient genetic variation (i.e. mates) and pollinators.

One potential drawback of uniparental reproduction, either by self-fertilization or asexual means, is that it makes plants more vulnerable to pathogens and other enemies (Stevens 1948 as cited in Fryxell 1957; Schmid 1994). Pathogens are therefore likely to impose selection for outcrossing mechanisms in plants, i.e. against uniparental reproduction (Levin 1975; Busch et al. 2004). If colonizing plants are released from their pathogens, as posited by the enemy-release hypothesis (Elton 1958; Crawley et al. 1987; but see van Kleunen and Fischer 2009), this would imply that the selective force against uniparental reproduction by pathogens will have decreased in the novel range. To the best of our knowledge (see also overview in Eckert et al. 2006), this aspect of colonization has not been considered in models of breeding system evolution. Therefore, future modeling and empirical studies should address whether the potential benefit of uniparental reproduction during population establishment is increased in the absence of pathogens.

A new development that is likely to lead to increased interest in Baker’s Law is that many recent studies have identified long-distance dispersal, rather than vicariance, as the main explanation for current-day global patterns of distributions of higher plant taxa (Renner 2005). The evidence behind this insight comes largely from dated phylogenies, and shows that many plant families and genera with distributions across the southern continents arose well after the break-up of Gondwanaland (McGlone 2005; Cowie and Holland 2006; Wallis and Trewick 2009). We find it tantalizing that many of the plant families and genera that span continents and yet evolved too recently for this to be a result of vicariance consist of species that are self-compatible or at least have leaky self-incompatibility (e.g. *Eryrthrina*, *Lycium*; Waters and Craw 2006). There are tremendous opportunities in future studies for mapping breeding systems onto dated phylogenies in order to test whether trans-continental colonists were more likely to be capable of uniparental reproduction, as predicted by Baker’s Law.

In conclusion, the formulation of Baker's Law has been an important stimulus for research in many different biological disciplines. So far, explicit tests of Baker's Law have been largely restricted to plants, and we urgently need studies on other groups of organism to gain insight in the general importance of Baker's Law as an explanation of species distributions and evolution of reproductive systems. Importantly, Baker's Law is not only of theoretical interest, but has direct application for the management of invasive alien species and pest species in general and for conservation of rare species. There are, however, still many open questions, and it is therefore likely that Baker's Law will continue to guide research related to biogeography and reproductive biology of organisms.

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Tables

Table 1 Glossary of the reproductive terminology used in this review

Term	Definition
Agamospermy	See apomixes
Apomixis	The asexual production of seed (also termed agamospermy). Equivalent to parthenogenesis in animals. We distinguish between autonomous apomixis when fertilization of the seed endosperm is not required, and pseudogamous apomixis when fertilization of the seed endosperm is required. Some species of animals have sperm-dependent parthenogenesis in which asexual embryo formation requires the presence of sperm.
Autogamy	Fertilization of ovules with pollen from the same flower. Autogamy is autonomous when self-fertilization does not require the aid of a pollinating agent.
Autonomous seed production	Uniparental reproduction through seeds that does not require mating with another individual or the action of a pollinating agent. This excludes pollinator-facilitated self-fertilization, pseudogamous apomixis and sperm-dependent parthenogenesis.
Breeding system	The potential to reproduce by cross-fertilization, self-fertilization and asexual means in individuals, populations and species, encompassing physiological characters, such as self-incompatibility and morphological characters, such as anther-stigma separation, which determine this potential.
Dioecy	The production of male and female gametes by different individuals.
Leaky dioecy	The occasional production of hermaphrodite flowers and/or individuals in otherwise dioecious populations.
Leaky self-incompatibility	The occasional production of self-compatible flowers and/or individuals in otherwise self-incompatible populations.

Mating system	The realized breeding system in terms of the rates at which outcrossing, selfing and clonal reproduction occur in populations and species. In a sexually reproducing population, the outcrossing rate is a measure of the mating system.
Parthenogenesis	The asexual production of offspring. Mainly used to describe asexual reproduction in animals.
Reproductive assurance	The ability to assure reproduction, usually by means of uniparental reproduction, when reproduction would otherwise be limited by opportunities for outcrossing. Opportunities for outcrossing may be limited by availability of partners and, for plants, pollinators.
Self-fertilization	Fertilization of a female gamete with a male gamete from the same genetic individual.
Self-incompatibility	Inability of male gametes to fertilize female gametes of the same genetic individual.
Uniparental reproduction	Reproduction involving only one parent: it takes place in both animals and plants either sexually by self-fertilzation or asexually by apomixis (parthenogenesis) or vegetative reproduction.
Vegetative reproduction	Means of asexual reproduction other than seed formation through apomixis (e.g. formation of asexual offspring along stolons and rhizomes).

Figures

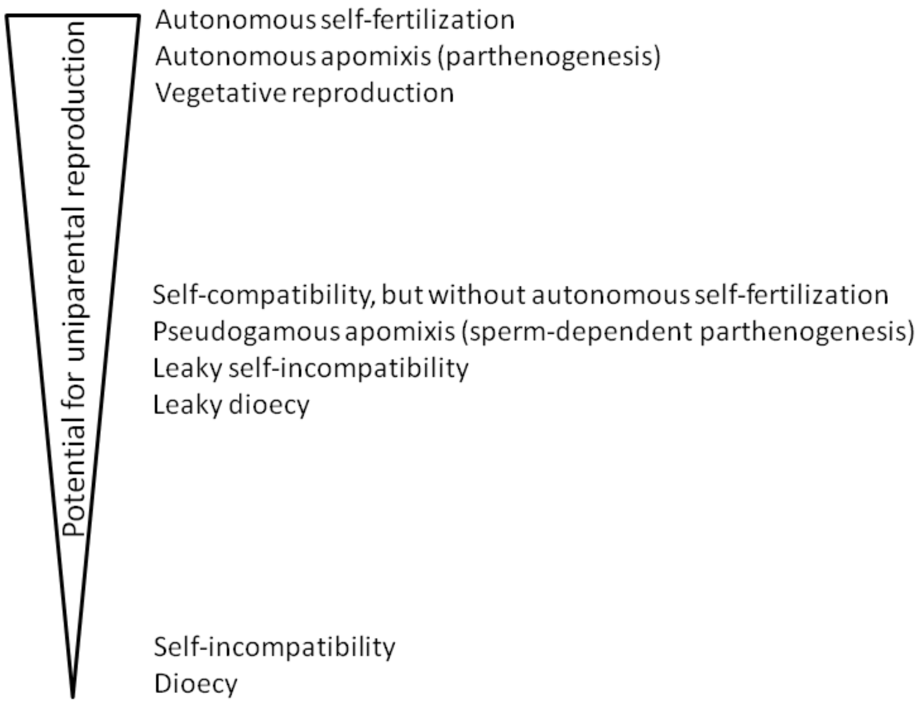

Figure 1 The potential for uniparental reproduction depends on the breeding system of the organism. Dioecy and self-incompatibility do not provide any potential for uniparental reproduction while autonomous self-fertilization, autonomous apomixis (parthenogenesis) and vegetative reproduction provide the full potential for uniparental reproduction. Under certain conditions, leaky self-incompatibility, leaky dioecy, self-compatibility and pseudogamous apomixis (sperm-dependent parthenogenesis) may also allow for uniparental reproduction.

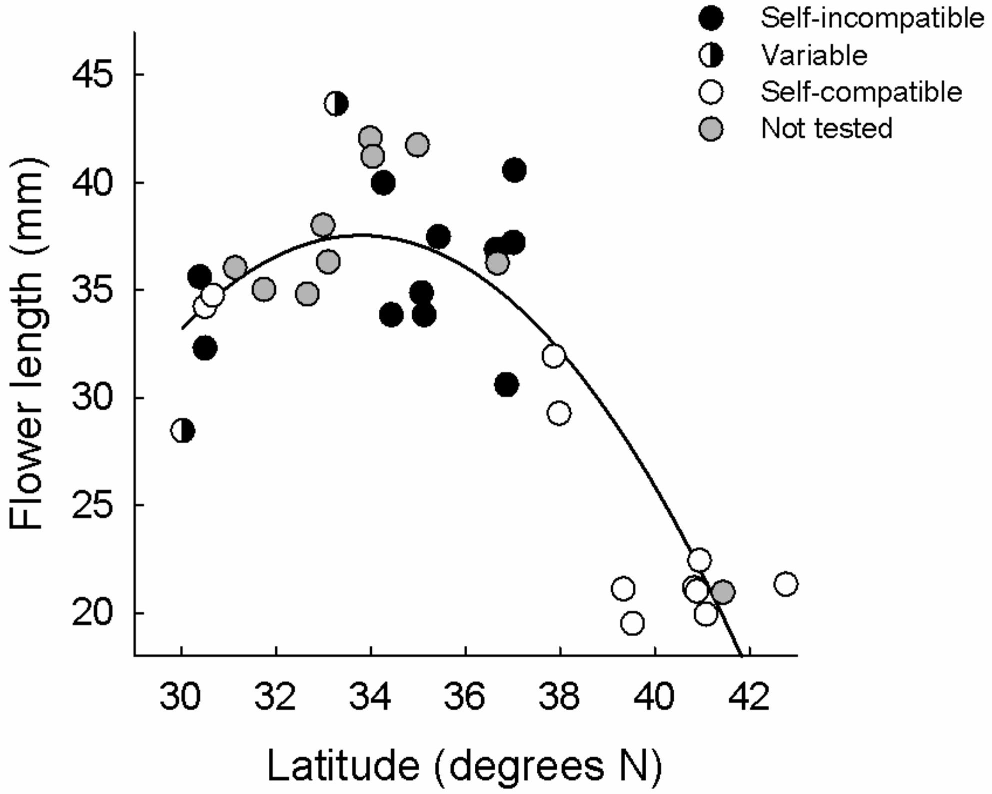

Figure 2 Geographical variation in breeding systems among 34 populations of Abronia umbellata across the full extent of the species' range. The graph shows the quadratic relationship between flower length and latitude ($r^2 = 0.751$, $P < 0.0001$), but of most interest here is that the populations with self-compatible morphs are found at the margins of the latitudinal distribution of the species. Whether the plants from each population were self-incompatible or self-compatible, as determined by crosses in the glasshouse, is indicated with different symbols. Both incompatibility types were found in the two 'variable' populations (redrawn from Darling et al. 2008, New Phytologist 178:424-435).

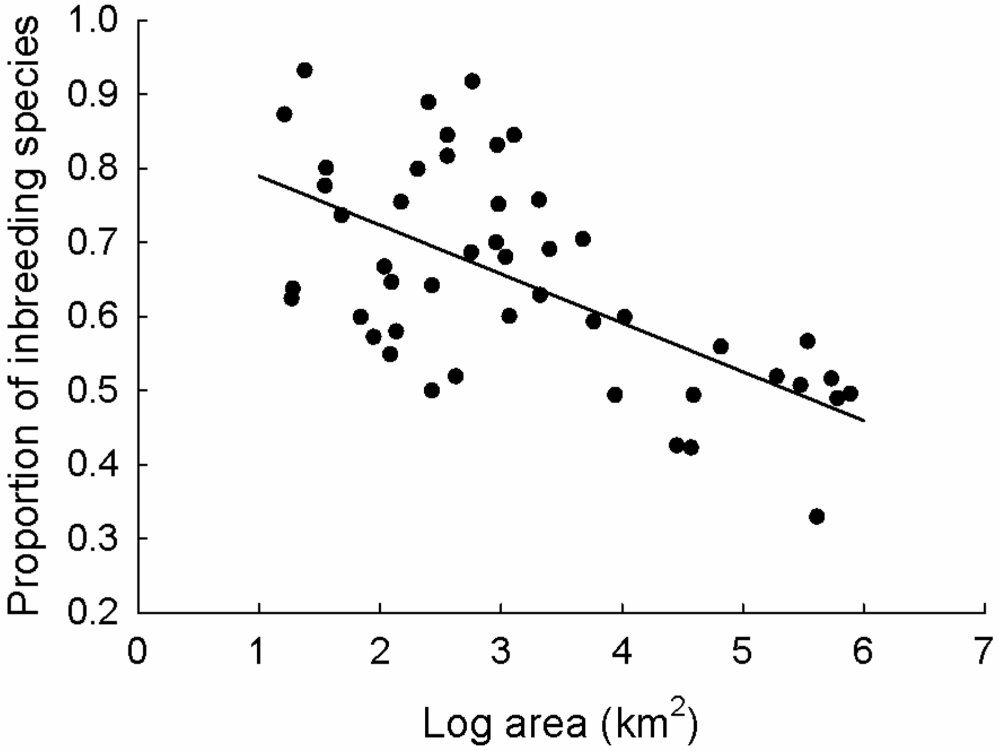

Figure 3 Proportion of inbreeding species of Scolytinae and Platypodidae in relation to island area. The slope is highly significant ($t = -5.41$, $P > 0.01$) (redrawn from Jordal et al. 2001, Global Ecology and Biogeography 10:345-357).

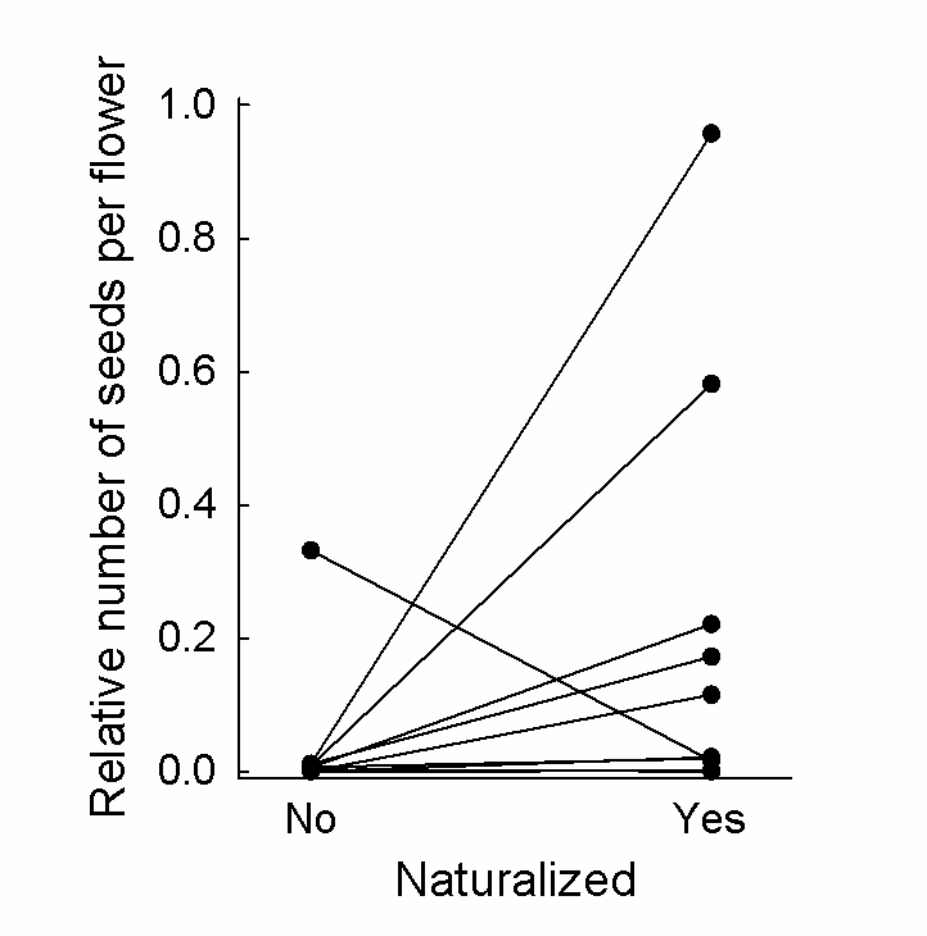

Figure 4 Naturalization within congeneric pairs of South African Iridaceae that have been introduced elsewhere for horticultural purposes is positively associated with the capacity for autonomous reproduction. The graph shows the number of seeds per flower when pollinators have been excluded relative to the number of seeds per flower after supplemental hand-pollination. Each line corresponds to a congeneric pair of naturalized and non-naturalized species (redrawn from van Kleunen et al. 2008, American Naturalist 171:195-201).

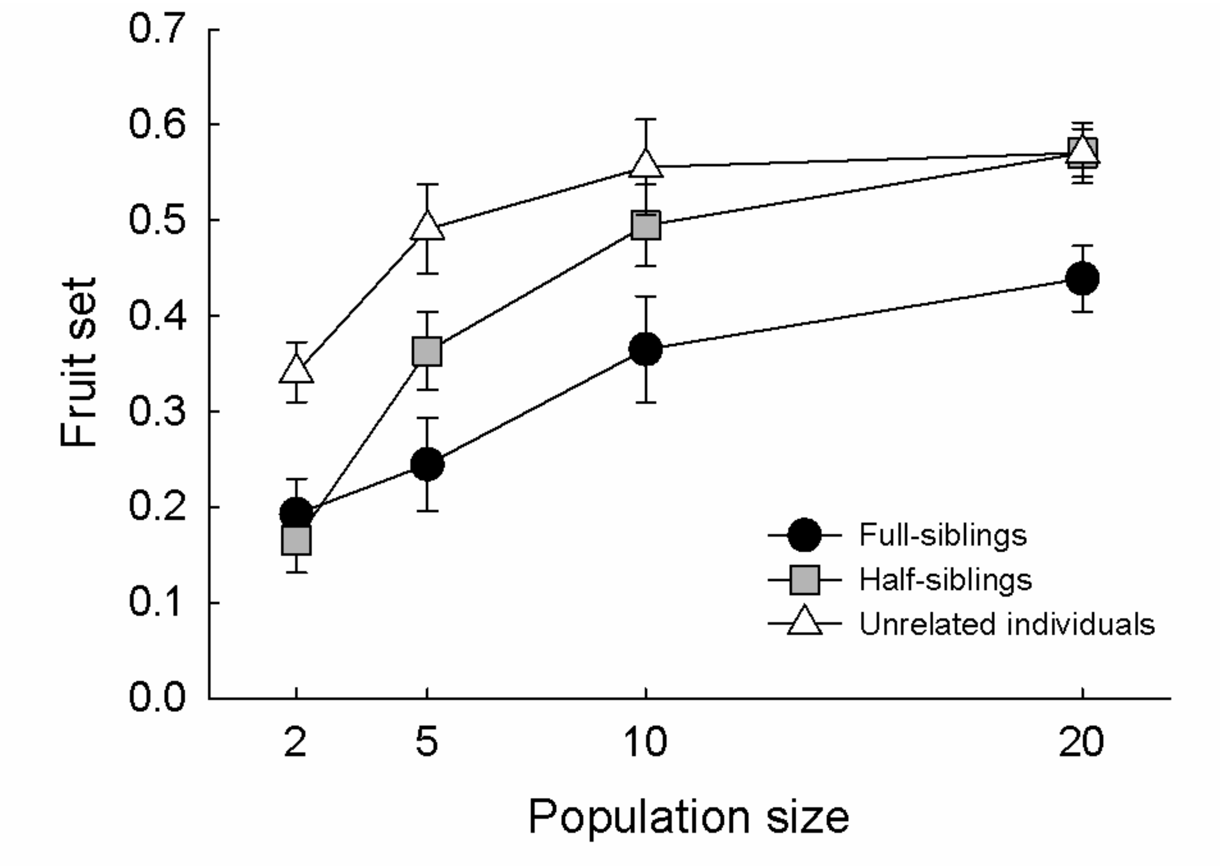

Figure 5 The effects of population size and genetic relatedness on fruit set (number of fruits/number of flowers). Symbols represent means across populations +/- SEM (redrawn from Elam et al. 2007, PNAS 104:549-552).