

**Assembly and disassembly of bird pollination
communities at the Cape of Africa**

by

Sjirk Geerts

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Department of Botany and Zoology

Faculty of Natural Sciences

Supervisor: Dr. A. Pauw

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Declaration

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Abstract

With the current global decline in pollinators, and the concurrent decline in plant species, pollination research is becoming increasingly important. However, studies outside Europe and North-America and on groups other than insects are needed to make generalisations possible. In this thesis I study how pollination structures plant and bird communities in a biodiversity hotspot, the Cape Floristic Region of South Africa. I show that bird-plant pollination mutualisms are an important ecological factor structuring ornithophilous Proteaceae and nectar-feeding bird communities. This close association between plant and bird communities suggests an important role for community wide pollination mutualisms.

How these mutualisms disassemble in reaction to a range of anthropogenic impacts is determined. Firstly, I use experimental manipulation of honeybee density to test whether honeybee farming affects nectar-feeding birds. Hive addition increased honeybee abundance far above natural levels but nectar-feeding bird pollinators were not consistently affected. Secondly, I document the impact of a two lane tar road on the bird pollination community. The two-fold decline found in pollination along roadsides, should have important implications for the way we view and manage road verges for ecological processes. Thirdly, I investigated how fragmentation affects bird-pollination communities by assessing an endangered, bird-pollinated plant, *Brunsvigia litoralis*. The only flower visitor at the urban sites, the shorter billed Greater Double-collared Sunbird is unable to access the nectar due to a long perianth tube. The longer billed Malachite Sunbird was the sole pollinator of *B. litoralis* at the rural site, significantly increased seed set. The lack of ecological analogs in these urban fragments might place pollinator specialist plants, such as *B. litoralis*, at risk. Fourthly, fire is a frequent disturbance in communities of bird-pollinated plants. In a before/after fire observation study and a burnt/unburnt transplant study, birds visited flowers in the “before fire” and “unburnt” areas only. The results are surprising given the large number of bird-pollinated plants flowering in the early post-fire vegetation. Lastly, I find that alien invasive plant species are incorporated into the native pollination community in a spectacular way; sunbirds adapt to a hummingbird-like, hovering lifestyle to obtain nectar. Alien invasive plants greatly increase nectar-feeding bird abundance; in turn, birds enhance seed set in these alien plants.

I conclude by asking whether the disassembling of bird pollination communities really matters. To answer this question I report on a decade of demographic data on the geophytic bird-pollinated *Brunsvigia orientalis*. In the demographic analysis, the elasticity component

for reproduction was more important than expected for a long lived plant. Reduced population growth in the shade and a large investment in a winged inflorescence, suggest *B. orientalis* is a light demanding, well dispersed, gap colonising species. The link between pollination and seed has been made before, but I take this one step further and show that pollination intensity predicts population growth rate. By linking plant demography and pollination, I was able to predict the future of plant populations under variable pollination conditions. The disassembly of bird pollination communities only becomes important for population persistence once the mutualism has almost entirely broken down.

Abstrak

Met die huidige globale afname in bestuiwers en die gelyktydige afname in plant spesies, word bestuwing navorsing toenemend belangrik. Studies buite Europa en Noord-Amerika en op groepe anders dan insekte is nodig om veralgemenings moontlik te maak. In hierdie tesis bestudeer ek hoe bestuwing struktuur gee and plant en voël gemeenskappe in 'n biodiversiteit hotspot, die Kaapse Floristiese Ryk van Suid-Afrika. Ek wys dat voël-plant bestuings mutualismes 'n belangrike ekologiese faktor is in die strukturering van voël bestuifde Proteaceae gemeenskappe en nektar-etende voël gemeenskappe. Hierdie noue assosiasie tussen plant en voël gemeenskappe impliseer 'n belangrike rol vir gemeenskapwye bestuings meganismes.

Ek bepaal hoe hierdie mutualismes aftakel in reaksie op 'n verskeidenheid van antropogeniese impakte. Eerstens gebruik ek 'n eksperimentele manipulasie van heuningby getalle om te toets of bye boerdery nektar-etende voëls affekteer. Byekorf toevoeging het heuningby getalle laat toeneem tot ver bo natuurlike vlakke maar nektar-etende voël bestuiwers is nie konsekwent beïnvloed nie. Tweedens dokumenteer ek die impakte van 'n twee baan teerpad op die voël bestuings gemeenskap. Die twee-malige afname in bestuwing langs paaie sal belangrike implikasies hê vir die manier hoe ons pad reserwes sien en bestuur met betrekking tot ekologiese prosesse. Derdens bestudeer ek hoe fragmentasie die voël-plant gemeenskappe affekteer deur die bedreigde voël-bestuifde *Brunsvigia litoralis* te assessee. Die enigste besoeker in die meer stedelike area, die Groot-rooibandsuikerbekkie, wat 'n korter snawel het, is nie in staat om die nektar te bereik nie, weens 'n te lang blombuis. Die Jangroentjie suikerbekkie met sy langer snawel is die enigste bestuier van *B. litoralis* in die meer landelike area, met 'n betekenisvolle vermeerdering in saad vorming. Die gebrek aan ekologies analogiese spesies in die stedelike fragmente kan 'n risiko inhou vir bestuier gespesialiseerde plante soos *B. litoralis*. Vierdens, vuur is 'n gereelde versteuring van voël-plant gemeenskappe. In 'n voor/na vuur observasie studie en 'n brand/nie-brand verplasing studie, het voëls blomme net in die “voor brand” en “nie-brand” areas besoek. Hierdie resultate is verrassend siende die groot hoeveelheid voël-bestuifde plante wat blom direk na brande. Laastens het ek gevind dat uitheemse indringer plante geïnkorporeer word in die inheemse bestuiwers gemeenskappe op 'n skouspelagtige manier; suikerbekkies pas aan tot 'n kolibri-tipe, fladderende lewenswyse om nektar te bekom. Uitheemse indringer plante het nektar-etende voël hoeveelhede laat toeneem; in reaksie het voëls saad opbrengs vermeerder.

In konklusie vra ek of hierdie aftakeling van die voël bestuiwers gemeenskap belangrik is. Om hierdie vraag te antwoord assessee ek 'n dekade van demografiese data van die geofietiese, voël-bestuifde plant, *Brunsvigia orientalis*. In die demografiese analises was die elasticiteit komponent van reproduksie belangriker as verwag vir 'n langlewende plant. Verminderde populasie groei in die skaduwee en 'n hoë investering in 'n gevlerkte bloeiwyse suggereer dat *B. orientalis* 'n lig afhanklike, goed verspreide, gaping koloniserende spesie is. Die skakel tussen bestuiwing en saadvorming is voorheen gemaak, maar ek neem dit een stap verder en wys dat bestuiwings intensiteit populasie groeikoers voorspel. Deur plant demografie en bestuiwing te koppel was ek in staat om die toekoms van populasies onder variërende bestuiwings kondisies te voorspel. Die aftakeling van voël bestuiwings gemeenskappe word slegs belangrik vir populasies se voortbestaan wanneer die mutualisme amper heeltemal verdwyn het.

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1 Introduction

Most extant angiosperms rely on a biotic vector for pollen transfer. Animal-mediated sexual reproduction is therefore a key component in maintaining global plant diversity and providing vital ecosystem services. And yet, it appears that pollinators are globally threatened by human activities (Bond 1994; Allen-Wardell *et al.* 1998; Kearns *et al.* 1998; Lindberg and Olesen 2001). These threats are diverse and range from fragmentation, climate change, pathogens, alien invasive species, increased fire frequencies and the interactions between them (Potts *et al.* 2010). The recent declines in wild pollinators can result in loss of pollination services with important negative ecological consequences. The evidence for these pollinator declines are from studies on insects, mainly from Europe and North America. Studies on other pollinator groups and continents are therefore an urgent necessity.

Much of the extensive literature on pollination deals with the evolutionary aspects of specialization and adaptation. The adaptations of organisms, in particular those of plants and their animal pollinators, are well known, with some of the clearest examples displayed by flowers adapted to specialist nectar-feeding birds (Castellanos *et al.* 2004; Hingston *et al.* 2004; Anderson *et al.* 2005; Medan and Montaldo 2005; Goldblatt and Manning 2006; Micheneau *et al.* 2006; Geerts and Pauw 2009). Over 920 species of birds pollinate plants, including the well known hummingbirds (Trochilidae) from the Americas, the sunbirds (Nectarinidae) and sugarbirds (Promeropidae) from Africa and the honeyeaters in Australia (Stiles 1981). Bird flowers are typically robust, reddish in colour, have large volumes of dilute nectar and lack scent (van der Pijl 1961). The debate on specialisation and evolutionary aspects provide excellent insight into these bird-plant mutualisms and together with the wealth of general bird data from atlas databases, a good platform for studies on the assembly and disassembly of nectar-feeding bird and plant communities is presented.

The assembly and disassembly of bird pollination communities is an important and ideal study system for the following reasons. Firstly, birds and especially sunbirds with their rapid movements, fluorescent colours, and intricate associations with flowers, appeal to all who has observed them and should be better utilised in creating public interest in plant-pollinator conservation. Secondly, plant-bird pollination systems are sensitive to anthropogenic alterations and should serve as an indicator system in areas of the world where they contribute considerably to plant reproduction (Paton 1993). Lastly, the assembly and disassembly of nectar-feeding bird communities is important knowledge in a biodiversity hotspot like the Cape Floristic Region (CFR) of South-Africa where these birds are disproportionately important as pollinating agents (Rebelo 1987).

In this thesis I describe the assembly and disassembly of the bird pollination community in the CFR at the tip of the African continent. This area with its great diversity of plant species, and high levels of endemism, is one of the 34 conservation hotspots of the world (Myers *et al.* 2000). Approximately 75% of the ornithophilous plant species from southern Africa occur in the Cape and they make up almost 4% of the Cape flora (Rebelo 1987). This is in contrast with other regions where ornithophilous plant species typically comprises 5% of a region's flora, or 10% in the case of certain islands (reviewed in Whelan *et al.* 2008). Of the approximately 323 CFR bird species only nine (2.8%) are specialist nectar-feeding birds. In contrast, the proportion of nectar-feeding birds in the avifauna is 4% in Brazil and Peru, 6% in tropical dry forest and 10% in the montane forests of Costa Rica (reviewed in Whelan *et al.* 2008). Therefore when matching plants and birds, the CFR has an extremely high ratio of more than 35 plant species per nectar-feeding bird species (Rebelo 1987). This number is higher than other temperate communities like the temperate forests of Southern South America, where ca. 20 ornithophilous woody plant species depend on a single hummingbird species (Aizen and Ezcurra 1998). Thus, the depauperate nectarivorous bird fauna plays a disproportionately important role in maintaining the very large number of bird-pollinated plant species found at the Cape. Furthermore, such a simple community with so few interacting species, is an ideal study system since its simplicity might aid in understanding the processes that govern its assembly and disassembly (Johnson 1992).

I begin this thesis by exploring the assembly of the nectar-feeding bird community and their dependant plant communities and ask: are bird-plant pollination mutualisms an important ecological factor structuring ornithophilous Proteaceae and nectar-feeding bird communities (Chapter 2)? I try to get at the underlying mechanism for plant-pollinator diversity correlations (Potts *et al.* 2010) and combine this with an analysis of Proteaceae distributions in relation to their predicted dependence on birds for population persistence (Bond 1994).

Once the importance of bird pollination communities as an ecological factor structuring ornithophilous plants and nectar-feeding bird communities was known, the disassembly thereof through anthropogenic influences is considered. I start of by considering the high levels of human enhanced honeybee densities through beekeeping in the CFR (Chapter 3). The utilisation of natural habitat for beekeeping is classified as a anthropogenic disturbance worldwide (reviewed in Huryñ 1997; Goulson 2003; Paini 2004). Although honey bees, *Apis mellifera*, are native to South African fynbos, managed hives potentially elevate the abundance of honeybees far above natural levels, but impacts on other floral resource-dependent species have not been considered. Currently beekeeping is excluded from South African nature reserves as a precautionary measure. The validity of this for pollinators and in particularly for the bird-plant pollination mutualisms is unknown and these studies are long overdue (Chapter 3).

One of the more pervasive habitat disturbances of the current era are roads (Forman and Alexander 1998). Road effects have been thoroughly studied in first world countries (reviewed by Trombulak and Frissell 2000), but the possible disruption of plant-pollinator mutualisms has not been considered before. Therefore, I use the bird pollination community to assess road impacts on pollination systems (Chapter 4).

Together with roads come urbanisation and the fragmentation of once continuous habitat. Although an extensive fragmentation literature exists, nectar-feeding bird pollination is rarely considered (Saunders *et al.* 1991; Aguilar *et al.* 2006). Where nectar-feeding birds

have been considered, fewer nectar-feeding birds (Daily *et al.* 2001) and lower abundance of bird-pollinated plants in fragments were found (Mayfield *et al.* 2006), but neither study examined pollination. Where it has been considered there was no impact on nectar-feeding birds since the species considered preferred open disturbed habitat (Aizen & Feinsinger 1994). By using the endangered pollinator specialist, *Brunsvigia litoralis*, I assess how the bird pollination community is affected in fragmented habitats. Furthermore, I consider the extent to which nectar-feeding birds are redundant in the sense that other parts of the community can act as an alternative source of pollen transport (Chapter 5).

Pollen transport in post-fire habitats are known to be low (Cowling 1992). Given the large number of bird-pollinated plants that flower shortly after fire, I assess the important question of how the nectar-feeding bird community disassembles in a post-fire habitat. With a rarely done before-after fire approach, nectar-feeding bird community response to fire is assessed. By transplanting post-fire ornithophilous flowering species between burnt and unburnt areas I determine to what extent post-fire flowering plants are pollen limited (Chapter 6).

An anthropogenic impact that is felt across pollinator communities and ecosystems are alien invasive plants. Alien invasive plants are the result of human settlement whether unintentional (Chapter 7) or intentionally (Chapter 8). Studies on alien invasive plant species have increased exponentially over the past few decades, particularly in South Africa where work is driven by projects such as Working for Water and Working for Fire (Olckers 2004). However, how these invasive plants integrate and alter community mutualisms, particular in a South African context has received relatively little attention (Richardson *et al.* 2000). Therefore, by using the bird-plant mutualistic community I consider the ability of alien invasive plants to be incorporated and alter nectar-feeding communities and vice versa. From a controlling and legislation perspective this is vital information for recent emerging invasive species. Chapter 8 was therefore done in conjunction with the Early Detection and Rapid Response (EDRR) program in South

Africa to assist in management prescriptions for the eradication of early invasive species which have the potential to spread and become serious invaders (Olckers 2004).

An important question in pollination ecology is if declines in the one partner can lead to linked declines in the other and whether mutualism disruption affects population persistence in the long term. Once the assembly (Chapter 2) and anthropogenic impacts potentially disassembling the bird pollination community mutualisms have been considered (Chapter 3-8), I test whether pollen limitation, due to reduced pollination, will cause population extirpation (Chapter 9 and 10). In the first of these two chapters I explore the demography and life history of a bird pollinated plant, *Brunsvigia orientalis*. Once the basic life history was known, population projections were done for populations across a fragmented and pollinator variable landscape. With these models I could evaluate the impacts on plant population persistence with the disassembly of the bird pollination community.

References

- Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* 9:968-980
- Aizen MA, Ezcurra C 1998. High incidence of plant–animal mutualisms in the woody flora of the temperate forest of southern South America: biogeographical origin and present ecological significance. *Ecologia Austral* 8: 217–236.
- Aizen, MA, Feinsinger P 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology* 75:330-351.
- Allen-Wardell G et al. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conserv. Biol.* 12:8-17
- Anderson BW, Spencer WW, Barrett CH (2005) Specialized bird perch aids cross-pollination: A plant scores by providing an access point for visiting sunbirds to feed on its nectar. *Nature* 435:41

- Bond WJ (1994) Do mutualisms matter - assessing the impact of pollinator and disperser disruption on plant extinction. *Philos. Trans. R. Soc. Lond. Ser. B-Biol. Sci.* 344:83-90
- Castellanos MC, Wilson P, Thomson JD (2004) 'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology* 17:876-885
- Cowling RM (1992) *The ecology of fynbos: nutrients, fire and diversity*. Oxford University Press, Cape Town.
- Daily GC, Ehrlich PR, Sanchez-Azofeifa GA (2001) Countryside biogeography: Use of human dominated habitats by the avifauna of southern Costa Rica. *Ecological Applications* 11:1-13
- Forman RTT, Alexander LE (1998) Roads and their major ecological effects. *Annu. Rev. Ecol. Syst.* 29:207-231
- Geerts S, Pauw A (2009) Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *S. Afr. J. Bot.* 75:699-706
- Goldblatt P, Manning JC (2006) Radiation of pollination systems in the Iridaceae of sub-Saharan Africa. *Annals of Botany* 97:317-344
- Goulson D (2003) Effects of introduced bees on native ecosystems. *Annual Review of Ecology Evolution and Systematics* 34:1-26
- Hingston AB, Gartrell BD, Pinchbeck G (2004) How specialized is the plant-pollinator association between *Eucalyptus globulus* ssp *globulus* and the swift parrot *Lathamus discolor*? *Austral Ecol.* 29:624-630
- Huryn VMB (1997) Ecological impacts of introduced honey bees. *Quarterly Review of Biology* 72:275-297
- Johnson SD (1992) Plant-animal relationships. In: Cowling RM (ed) *The ecology of fynbos: nutrients, fire and diversity*. Oxford University Press, Cape Town, pp. 175-205.
- Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: The conservation of plant-pollinator interactions. *Annu. Rev. Ecol. Syst.* 29:83-112

- Lindberg A, Olesen JM (2001) The fragility of extreme specialization: *Passiflora mixta* and its pollinating hummingbird *Ensifera ensifera*. *Journal of Tropical Ecology* 17:323-329
- Mayfield MM, Ackerly D, Daily GC (2006) The diversity and conservation of plant reproductive and dispersal functional traits in human-dominated tropical landscapes. *Journal of Ecology* 94:522-536
- Medan D, Montaldo NH (2005) Ornithophily in the Rhamnaceae: the pollination of the Chilean endemic *Colletia ulicina*. *Flora* 200:339-344
- Micheneau C, Fournel J, Paillet T (2006) Bird pollination in an angraecoid orchid on Reunion Island (Mascarene Archipelago, Indian Ocean). *Annals of Botany* 97:965-974
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403:853-858
- Olckers T (2004) Targeting emerging weeds for biological control in South Africa: The benefits of halting the spread of alien plants at an early stage of their invasion. *Journal of Applied Ecology* 100:64-68
- Paini DR (2004) Impact of the introduced honey bee (*Apis mellifera*) (Hymenoptera: Apidae) on native bees: A review. *Austral Ecol.* 29:399-407
- Paton DC (1993) Honeybees in the Australian Environment. *Bioscience* 43:95-103
- Potts SG, Biesmeijer JCS, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* 25:345-353
- Rebelo AG (1987) Bird pollination in the Cape Flora. In: Rebelo AG (ed) A preliminary synthesis of pollination biology in the Cape flora, 141 edn. CSIR, Pretoria, pp 83-108
- Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmanek M (2000) Plant invasions - the role of mutualisms. *Biol Rev* 75:65-93
- Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation - a review. *Conserv. Biol.* 5:18-32
- Stiles FG (1981) Geographical aspects of bird-flower coevolution, with particular reference to Central America. *Ann. Mo. Bot. Gard.* 68:323-351

- Trombulak SC, Frissell CA (2000) Review of ecological effects of roads on terrestrial and aquatic communities. *Conserv. Biol.* 14:18-30
- van der Pijl L (1961) Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution* 15:44-59
- Whelan CJ, Wenny DG, Marquise RJ (2008) Ecosystem services provided by birds. In: *Year in Ecology and Conservation Biology 2008*, vol 1134. Blackwell Publishing, Oxford, pp 25-60

2 Pollination structures plant and bird communities in Cape Fynbos, South Africa

With the current global decline in pollinators, relationships between species interactions and diversity are becoming more important. If pollinator communities depend strongly on the diversity of flowering plants and vice versa, declines in the one will cause linked declines in the other. This topic of pollination communities has hardly been touched on, particularly so in Africa. Here I study how pollination structures plant and bird communities in the Cape fynbos of South Africa. I study the nectar-feeding bird pollinator community since the few consisting species aid in understanding the processes governing its assembly and disassembly. For plants I use the diverse Proteaceae family with its many bird dependant plants. I sampled 34 one-hectare plots across the Cape Floristic Region for vegetation type, vegetation age, nectar volume, nectar-feeding bird abundance and species richness. On a larger scale, I am the first to correlate two extensive datasets namely the South African Bird Atlas and Protea Atlas. To determine the dependence of Proteaceae communities on nectar-feeding birds, a vulnerability analysis was conducted. On a small scale, nectar rather than vegetation structure or vegetation type is the most important predictor of the nectar-feeding bird community. On a larger scale ornithophilous Proteaceae abundance and species richness correlated significantly with the abundance and species richness of the nectar-feeding bird community. The addition of more than five ornithophilous Proteaceae species for each new nectar-feeding bird species, demonstrates an asymmetrical plant-pollinator relationship. The close association between plant and bird communities found in this study suggests an important role for community wide pollination mutualisms. In the vulnerability calculations, range-restricted ornithophilous Proteaceae species turn out to be more vulnerable to changes in the nectar-feeding bird community than more widespread species. The most vulnerable species only occur in the most species rich Proteaceae communities, highlighting the fact that conservation of the entire plant-bird pollination web is essential.

Introduction

The focus of plant-pollinator interactions has recently shifted from species specific pair-wise interactions towards a more inclusive community-wide approach (Stanton 2003; Sargent and Ackerly 2008; Stanway 2009). By using a community-wide approach, impacts of change in fire frequencies (Ne'eman et al. 2000; Potts et al. 2001; Potts et al. 2003), alien invasive species (Traveset and Richardson 2006; Lopezaraiza-Mikel *et al.* 2007; Bartomeus *et al.* 2008; Morales and Traveset 2009; Valdovinos *et al.* 2009), climate change (Hegland et al. 2009; Phillips et al. 2010) and fragmentation (Aguilar et al. 2006) can be better evaluated since more connections in the plant-pollinator community are considered. Recent reviews have highlighted the probable vulnerability of relationships in these plant-pollinator communities and pointed to the importance – but scarcity – of large scale studies (Kearns and Inouye 1997; Hobbs and Yates 2003) predominantly for continents other than Europe and North America (Potts *et al.* 2010).

The topic of pollination communities has barely been touched on in the Cape Floristic Region (CFR) of South Africa (Roger et al. 2004). Pollinators are thought to be important in the origin and maintenance of the Cape's floral diversity, and yet, it appears that pollinators and in particular nectar-feeding birds are globally threatened by human activities (Bond 1994; Allen-Wardell *et al.* 1998; Kearns *et al.* 1998; Lindberg and Olesen 2001). Therefore, it is surprising that even the more charismatic pollinators such as nectar-feeding birds, have barely been studied on a community level and little is known about the factors that determine their community composition (but see Cameron 1999; Fox and Hockey 2007; Fleming and Muchhala 2008; Symes *et al.* 2008).

Specialist nectar-feeding birds in the CFR are within the families Nectarinidae (sunbirds) and the Promeropidae (sugarbirds). This specialist nectar-feeding bird community is a relatively simplistic one with only six important nectar feeding bird species of which only four occur throughout the CFR. They therefore play a disproportionately important role in maintaining the large number of bird-pollinated plant species at the Cape, which comprises about four percent of the total plant species (Rebelo 1987). In view of the fact that this system can become very specialised, it is potentially more vulnerable than bird-pollination systems in other parts of the

world which involve many more bird species (Feinsinger 1978; Brown and Bowers 1985; Bond 1994; Geerts and Pauw 2009). For a community pollination study the nectar-feeding bird community is an ideal system, since extensive information is available in the form of bird atlas data. From the plant community perspective the Proteaceae is an ideal study system since it contains many bird pollinated plants and one of the most detailed distribution datasets from anywhere in the world is available for this group (Rebelo 2006).

Proteaceae is one of the most diverse plant groups in South Africa, with about 360 species, of which 330 are restricted to the CFR (Rebelo 2001). Of these, approximately 80 species are potentially pollinated by nectar-feeding birds, with the Cape Sugarbird particularly important (Skead 1967; Mostert *et al.* 1980; Collins 1983b; Fraser 1989; Martin and Mortimer 1991; Fraser and McMahon 1992). Most ornithophilous Proteaceae retain seeds on the plant in serotinous infructescences until the whole plant is killed by fire. The next generation then grows from seeds, resulting in similar aged stands of plants. Proteaceae are relatively slow to mature and only flower abundantly 5 to 6 years after a fire (Cowling 1992). With Proteaceae such an important component of the CFR vegetation, amazingly few comprehensive pollination studies have been conducted on avian pollination in this family (Collins 1983b; Coetzee and Giliomee 1985; Collins and Rebelo 1987; Whitehead *et al.* 1987; Wright *et al.* 1991; Wright 1994; Hargreaves *et al.* 2004). Proteaceae species with copious amounts of nectar, large red-coloured terminal inflorescences and long pollen presenters are assumed to be bird-pollinated (Vogts 1982; Rebelo *et al.* 1984). However, in some of these species long-proboscid flies might play an important role (Devoto *et al.* 2006; Manning 2004). Nectar-feeding birds visit Proteaceae inflorescences for nectar rather than protein rich insects (Tjorve *et al.* 2005). Many Proteaceae are dependant on pollen vectors since they are self-incompatible and set no viable seed when bagged (Horn 1962). Therefore, being obligate outcrossers many Proteaceae species might be particularly vulnerable to declines in pollination services.

Few studies have explored the link between reduced pollinator services, pollen limitation and plant community composition (Hegland and Totland 2008). It has been found however that obligate outcrossing plant species decline in parallel with flower visitors (Biesmeijer *et al.* 2006). Indeed, a large part of the variation in plant richness can be explained by pollinators,

with a positive correlation between flowering plant species richness and pollinators on a community level reported from a number of countries (Steffan-Dewenter and Tschardtke 2001; Potts 2003; Kleijn *et al.* 2004; Fründ *et al.* 2010). In general, these plant–pollinator networks have a robust structure (Memmott *et al.* 2004; Fortuna and Bascompte 2006). It has been suggested that under very high disturbance pressures, these networks could reach a tipping point (Potts *et al.* 2010) but most likely plant–pollinator communities will gradually change over time as pressures increase. Alterations in these plant–pollinator communities can only be determined if historic plant-pollinator community data is available (Pauw and Hawkins 2010). Alternatively, current plant-pollinator communities can be compared across a gradient of transformed landscapes. Large scale atlas database correlations supply a wealth of data and can be particularly useful in these correlations between plants and their pollinators. These correlative approaches should ideally be supported by some mechanistic evidence which could include amongst others, climate, topography, or micro site determinants such as vegetation type and structure (Potts *et al.* 2010). These smaller scale variables might be important for pollinator protection, nest sites and food supplies.

Plant species richness will change with a change in particular components of the pollinator community. The amount of change will depend on individual plants traits which can be summarised as follow: 1) degree of dependence on pollinators for seed set, 2) degree of pollinator specificity and 3) degree of dependence on seeds for population persistence (Bond 1994). These can be combined in an index of vulnerability (*VI*) where $VI = BS \times PS \times SD$ with *BS* the breeding system, *PS* the pollinator specificity and *SD* the demographic dependence on seeds. Plant species are ranked for each term from zero to one. Plant species with a high value are expected to be absent from areas of lower pollinator abundance, since they are more dependant on pollination and have fewer reproductive backup mechanism, whilst species with low vulnerability values are less dependent on specific pollinators or on seeds for persistence.

Therefore, in this study I ask whether nectar-feeding birds, and sugarbirds in particular, are important predictors of the Proteaceae community assembly and vice versa. The strength of this mutualism is important from both a plant and a pollinator perspective since Proteaceae species are strongly affected by anthropogenic fires and alien plant invasions, with nectar-

feeding birds affected by anthropogenic habitat fragmentation, increased fire frequencies, diseases such as avian malaria, poisoning and climate change. Therefore, are bird-plant pollination mutualisms an important ecological factor structuring ornithophilous Proteaceae and nectar-feeding bird communities? To answer this question the study is divided into three components. (1) Extensive field sampling at a small scale across vegetation types and ages by using a chrono-sequence approach (post-fire habitats in different regenerative stages). (2) A larger scale correlative approach that utilises two extensive datasets, the South African Bird Atlas Project and the Protea Atlas Project. (3) An analysis of bird-pollinated Proteaceae assemblage composition along a nectarivorous bird abundance and species richness gradient, to test for a shift in favour of species with alternative pollinators, or low seed dependence, with a decline in nectar-feeding birds.

Methods

Plant-pollinator communities on a one hectare plot scale

Proteaceae abundance and species richness can be expected to act on bird communities via nectar availability. However, correlations between ornithophilous Proteaceae species richness and abundance and nectarivorous bird richness and abundance could also result from structural changes to the plant community brought about by the presence of large Proteaceae shrubs. Hence, I tested the relative ability of two measures of vegetation structure (post-fire age and vegetation type) and nectar availability in explaining variation in bird diversity and abundance. I selected 34 one hectare plots across the South-Western Cape and determined vegetation age, vegetation type, nectar availability and nectar-feeding bird richness and abundance on the same day for each site (Appendix 1).

Vegetation of different ages, known as a chrono-sequence, were sampled (Foster and Tilman 2000) and post-fire age estimated by counting the sequence of lateral branching (new lateral branches form annually) of a non sprouting Proteaceae species, with the mode accepted as the true age (van der Merwe 1969; Lamont 1985). In order to determine the effect of nectar availability in different vegetation types, areas containing Proteaceae, Ericaceae and Restionaceae communities of different vegetation ages, were sampled. In this part of the study only representatives of the genus *Protea* were included, since genus *Leucospermum* is not

flowering in winter when fieldwork was conducted, and genus *Mimetes* did not occur in any one of our sampling plots. To improve sample size for statistical analysis, vegetation types were divided into *Protea*-dominated and non-*Protea* vegetation only.

Ten minute point counts were conducted by standing on a ladder and recording all nectar-feeding birds heard or seen within a 25 meter radius (Bibby *et al* 2000). During a pilot study, counts were conducted for 1 hour each at 3 sites. Nectar-feeding bird species richness was found to reach a plateau after 10 minutes of observation, so subsequent censuses were only 10 minutes in duration. Although short, this was sufficient to detect most nectar-feeding birds, since they are territorial, conspicuous and have a clearly distinguishable call. Point counts were done early in the morning when avian nectarivores are most active, with rainy and very windy days avoided (Fry 2000).

For the calculation of nectar availability all bird-pollinated plants in the plot were identified. Nectar in individual *Erica* flowers could be measured and the total number of flowers counted to estimate nectar availability. For *Proteas* a means of accurately quantifying nectar needed to be developed first. This method is developed in the section below and is used throughout the study (see ‘A method to accurately determine nectar in *Protea* inflorescences’). Within bird sampling plots, inflorescences in a randomly selected 5 by 5 meter plot were identified and counted and nectar measured. In the open cup shaded *Proteas*, like *Protea repens*, nectar that spilt out between the flowers was measured before the inflorescence was sectioned. Nectar was converted to milligram of sucrose and scaled to amount of sucrose per hectare. Bird occurrence and nectar measurements were conducted at different scales, but this is consistently done across all sites. No inflorescences were bagged since this might change nectar volumes and concentration (Calf *et al.* 2001), and I was interested in nectar standing crop.

The significance of these variables was assessed in a general regression model (GRM), with nectar-feeding bird richness, or nectar-feeding bird abundance as the response variable and (1) vegetation age [continuous, years since fire], (2) vegetation type [categorical, *Protea* / non-*Protea*] and (3) nectar [continuous, sucrose per hectare (mg), log-transformed] as predictor variables. The assumptions of the GRM were investigated using probability plots; of which the

residuals were normally distributed. The minimum age at which *Protea* vegetation attracts most nectar-feeding birds was estimated by visually assessing the correlations between vegetation age and the nectar-feeding bird community.

A method to accurately determine nectar in Protea inflorescences

Nectar varies hugely between and within *Protea* inflorescences (pers. obs). In order to accurately determine nectar for a specific species and plot, the amount of variation between flowers, inflorescences, bushes and sites needed to be quantified. *Protea neriifolia* was used as a model species as it is common, bears many inflorescences per plant and is regularly visited by sugarbirds and sunbirds and is therefore assumed to be representative of other bird pollinated species within the same genus.

Protea inflorescences almost always open from the outside to the middle of an inflorescence (Rebello 2001). As a consequence, flowers in the middle are younger and have less nectar than those on the outside of the inflorescences. Measuring a single flower per inflorescences is therefore not sufficient (Calf 1999). In this study the nectar for a row of flowers ($n = \pm 14$) across the middle of inflorescence was measured and the average nectar volume and concentration per flower calculated. The total number of flowers in the inflorescence is then multiplied by the average amount of nectar per flower. 3-7 inflorescences per plant for 5 plants were measured at two sites (Jonkershoek 33°59'15.4"S 18°57'11.1"E and Gordonsbay 34°10'23.4"S 18°50'23.2"E). Inflorescences were chosen to be intermediate in age, as these are the inflorescences mostly visited by sugarbirds (pers. obs). Inflorescences were removed early morning and nectar was extracted in the laboratory using either a 5 μ l or a 40 μ l capillary tube (Drummond Scientific Company, Broomall, Pennsylvania, USA). Nectar concentrations were determined with a 0–50% field handheld refractometer (Bellingham and Stanley, Tunbridge Wells, UK). To account for dilution effects by rain, all nectar measurements were transformed to milligrams (mg) of sugar.

Variation between plants and inflorescences was compared with a nested ANOVA with sugar (mg) per flower as the dependant factor, bushes the fixed factor and inflorescences and flowers

random factors. To compare differences between the two sites a Mann-Whitney *U*-test was used.

Nectar standing crop per flower averaged 8.87 μ l (range 0 μ l – 75 μ l) and sucrose concentration averaged 24% (range 1% - 39%). Sucrose per flower averaged 2.62 mg (range 0 mg - 16.2 mg) and per inflorescence 861.1 mg (range 247.8 mg - 2266.4 mg). At both sites variation between inflorescences on a plant was high (Gordonsbay: ANOVA, $F_{23, 351} = 4.25$, $P < 0.01$ Jonkershoek: ANOVA, $F_{19, 267} = 1.93$, $P = 0.01$). Variation between plants at both sites was only significant at the Gordonsbay population (ANOVA, $F_{4, 23} = 4.13$, $P < 0.05$), probably because Jonkershoek plants were similarly aged, whilst Gordonsbay plants differed in age. There was no significant difference between nectar per plant between the two sites ($N_{\text{Jonkershoek}} = 5$, $N_{\text{Gordonsbay}} = 5$, $Z = 0.31$, $P = 0.75$, Mann-Whitney *U*-Test).

These results show that in *P. neriifolia* most variation is between inflorescences rather than between plants or sites. Therefore, fewer inflorescences per plant, and rather more plants are sampled in the remainder of this study.

Protea Atlas data

From the extensive Protea Atlas Project database (<http://protea.worldonline.co.za>), distribution range data for all ornithophilous and non-ornithophilous Proteaceae species in the Cape Floristic Region (CFR) were extracted. Species were only assessed for the CFR because it is a biodiversity hotspot, contains one of the most complete abundance and distribution records of species for any region and contains many more ornithophilous Proteaceae species than other parts of South Africa. The Protea Atlas Project was conducted between 1991-2002, with the aim of recording distribution and ecological data for Proteaceae across Southern Africa (Rebelo 1991; Rebelo 2001). In addition to presence and absence data, each protea population and species had their abundance data recorded according to Table 2.1. Distributional data were collected at 500 m diameter localities, with coordinates accurate to within 100 m. A total of just more than 250 000 species counts at some 60 000 localities have been recorded, this includes null sites. The Protea atlas data was scaled to QDGC (quarter-degree-grid-cell) level. Although the Protea data is available on a much finer scale, the bird atlas data is currently only

available at a QDGC scale. Excluding coastal QDGC, grids with more than half of the grid being ocean, made no significant difference in any calculation; therefore they are included. All other QDGC grids with more than half the land area within the CFR are included for a total of 180 QDGC. Species richness and abundance for ornithophilous and non-ornithophilous Proteaceae species was calculated for each of the 180 QDGC. Each Proteaceae individual is treated as an adult plant.

I compiled a list of all Proteaceae species conforming to the bird pollination syndrome according to morphological features, bird sightings, atlas database and various other sources (Rebello unpublished data, pers. obs., Rebello 2001; Stanway 2009). Hereafter referred to as ornithophilous Proteaceae species (n = 82 species) and included members from the genera *Protea*, *Mimetes* and *Leucospermum* (Appendix 2). Accurate pollination studies in the Proteaceae family are uncommon; therefore our species inventory might be incomplete.

Table 2.1. Population conversion factors for Protea Atlas population data used in calculating Proteaceae population numbers.

Population code	Population count or estimate
Blank (not recorded)	0
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
Frequent (10-100)	70
Common (100-10 000)	7 000
Abundant (> 10 000)	50 000

Bird Atlas data

Information on bird distribution and relative abundance was obtained from the South African Bird Atlas Project (SABAP 1, Harrison et al. 1997). All specialist nectar-feeding bird data was extracted from this database at the finest possible scale, QDGC, which is approximately 27 km long (north-south) and 24 km wide (east-west). SABAP data were recorded over a period of 6 years (1987–92). Temporal resolution was one calendar month averaged over all study years with monthly data transformed to annual resolution. Reporting rates have been used in SABAP as an index of relative abundance to compare population densities in different parts of a species range (Underhill et al. 1991). Reporting rate is the number of occurrences divided by the number of checklists for a unit area. It has been suggested that abundance is relative, and should preferably be used when comparing abundances within species, or between species which are equally conspicuous and identifiable (Underhill et al. 2008). However, this relative abundance from the atlas data has been evaluated previously, and found to correspond well to other field data and considered fit for use in general population studies (Fairbanks et al. 2002). Sampling intensity in SABAP differed widely (Harrison et al. 1997). When excluding the few poorly sampled QDGC i.e. those for which fewer than ten species checklists have been received, there was no significant changes in our results, therefore the complete dataset is included (Hugo and Van Rensburg 2009).

Plant-pollinator communities on a landscape scale

By combining these two extensive datasets, exploration of the relationships between nectar-feeding bird communities and ornithophilous Proteaceae communities on a landscape scale is possible. Proteaceae abundance and species richness were correlated with the nectar-feeding bird community abundance and richness using Spearman's rank correlations. To determine the underlying mechanisms and assess whether the link between these communities is coupled by structural changes associated with Proteaceae shrubs or through the nectar produced, non-ornithophilous Proteaceae species are included with ornithophilous Proteaceae species in a general regression model. In this general regression model, the nectar-feeding bird community richness and individual bird species abundance were the response variables and both ornithophilous and non-ornithophilous Proteaceae abundance (logged) and species richness were the predictor variables.

To determine the assemblage of these communities and how they respond to species loss, nestedness analyses were conducted. Nestedness rank was used instead of species richness. Whilst the order in which plots are ranked is somewhat similar, nestedness analysis considers species community composition and not only species richness. Nestedness analysis test whether communities are assembled such that the species present in species-poor communities are real subsets of the species present in more diverse communities (Patterson and Atmar 1986; Atmar and Patterson 1993; Hansson 1998). Nestedness is considered significant if species incidences differ more than expected by chance. It was suggested that most communities are significantly nested (Wright et al. 1998) with bird communities in particular, highly nested (Hansson 1998; Martinez-Morales 2005). Methodology to quantify nestedness varies (Almeida-Neto et al. 2007) and although a number of programs were produced, all with different null models (reviewed in Ulrich et al. 2009), Nestcalc is sufficient for our purposes as I found no significant differences in results between Nestcalc and other nestedness calculators (Greve *et al.* 2005; Guimaraes and Guimaraes 2006; Rodriguez-Girones and Santamaria 2006). In the program Nestcalc nestedness is calculated from a temperature metrics. A system temperature of 0° indicates a perfectly nested system where each species is lost in turn whilst a temperature of 100° indicates total disorder. The assemblage of species found in a community is considered nested ($T = 0^\circ$) if all species present in that community is also found in all communities supporting a greater total number of species. A low temperature indicates that most species present in species-poor communities are also present in more species rich communities (i.e., a more nested distribution). Large deviations from nested distribution indicate that species distributions are more variable (higher T°).

For nectar-feeding birds and ornithophilous Proteaceae communities each QDGC was assigned a nestedness rank according to the position in a nestedness table, where the most nested grid included all species within the community. Nestedness ranks of nectar-feeding birds were correlated with ornithophilous Proteaceae abundance and species richness. Four species of the avian nectar-feeding guild occur throughout the CFR. A few other species occur in the CFR, but were excluded, since they only occur at particular times of the year or their distribution is limited to CFR fringes. The nestedness ranks of ornithophilous Proteaceae for each QDGC was

correlated with nectarivorous bird species richness and abundance. In addition Proteaceae species richness, ranked according to nestcalc, was compared in sugarbird absent and present QDGC.

For all correlations on large datasets Bonferroni corrections were used with the default case wise deletion of missing data. Arc-GIS 9.1 software is used for all spatial analysis with Projected Coordinate System: WGS 1984 UTM Zone 34S and Geographic Coordinate System: GCS WGS 1984. Vagrants, birds with reporting rates of less than one percent, had no significant effects on any results, but to increase the readability of the maps they were excluded.

Vulnerability of the plant-pollinator communities

To determine the dependence of ornithophilous Proteaceae communities on nectar-feeding birds, a vulnerability analysis was conducted (Bond 1994). Pollinator specificity (PS) values are extracted from Protea Atlas pollinator observations and various other sources and calculated as 1/number of pollinators (Appendix 2). For species killed by fire and thus entirely dependent on seeds the demographic dependence on seeds (SD) is indicated as one. For resprouters an estimated age of one hundred years was used, therefore SD of resprouters are 1/100. Breeding system (BS) data was obtained from various sources (Horn 1962; Vogts 1982; Collins 1983b; Coetzee and Giliomee 1985; Lamont 1985; Seiler and Rebelo 1987) (Appendix 2). For many species data on one or more of these variables does not exist, therefore closely resembling species or species within the same morphological group were used where appropriate (Rebelo 2001). For some species I still had no data; therefore the number of protea species in the vulnerability analysis differs from the correlative analysis. Average vulnerability [VI] for each species was calculated as $VI = BS \times PS \times SD$. To obtain an accurate representation of community vulnerability [CV] the average vulnerability was calculated for each community.

Proteaceae species distribution (per QDGC) was analysed in a nestedness analyses (Nestcalc). Widespread species are ranked higher, obtaining a lower number, than range restricted species. These nestedness ranks were correlated with the Proteaceae vulnerability [VI] scores as well as

with pollinator specificity (PS) only. Similarly, Proteaceae community vulnerabilities were correlated to ornithophilous Proteaceae community ranks according to species richness (Spearman's rank). All statistical analyses were done in STATISTICA 9.

Results

Plant-pollinator communities at a one hectare plot scale

Nectar, expressed in milligrams of sucrose, varied greatly between plots and species, from 63.5 mg sucrose per inflorescence in *Protea nitida* to 1686.8 mg sucrose per inflorescence in *Protea coronata* (Appendix 3). Nectar in *Protea* vegetation older than 4 years was significantly higher than in young non-flowering *Protea* vegetation or other vegetation types (t-test $n_{Protea\ vegetation} = 16$ $n_{young\ and\ non-Protea\ vegetation} = 18$; $t = 9.82$ $df = 32$ $P < 0.001$). Floral nectar ($\log [mg\ sucrose \cdot ha^{-1}]$) is strongly correlated with nectar-feeding bird richness (Spearman-R = 0.82, $t = 8.24$, $N = 34$, $P < 0.001$; Fig. 2.1) and abundance (Spearman-R = 0.87 $t = 10.0$, $N = 34$, $P < 0.001$). In a general regression model of bird community abundance and richness, total sugar availability per hectare is a better predictor than the structure of the vegetation (Table 2.2). Only once *Protea* vegetation is more than 4 years old, can nectar-feeding bird species richness and abundance obtain substantial levels (dotted threshold line in Fig. 2.2a and b). Sugarbirds are only present when *Protea* vegetation is at least four years of age (Mann-Whitney U -test, $U = 10$, $Z = 3.16$ $P = 0.002$, $n_{sugarbird\ presence} = 15$, $n_{sugarbird\ absence} = 8$; Fig. 2.3). This indicates that *Protea* vegetation age in turn influences nectar availability (Spearman-R = 0.57, $t = 3.19$, $N = 23$, $P = 0.004$).

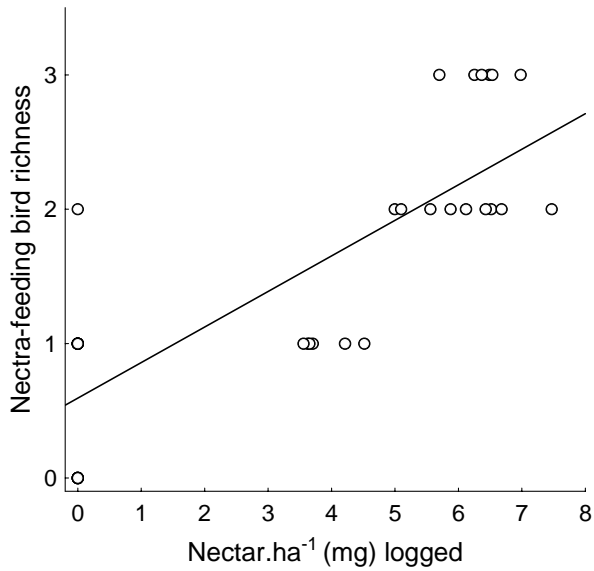


Figure 2.1. The relationship between nectar and nectar-feeding bird species richness at a one hectare plot scale (Spearman-R = 0.82, $t = 8.24$, $N = 34$, $p < 0.001$). The same pattern is observed for nectar feeding bird abundance.

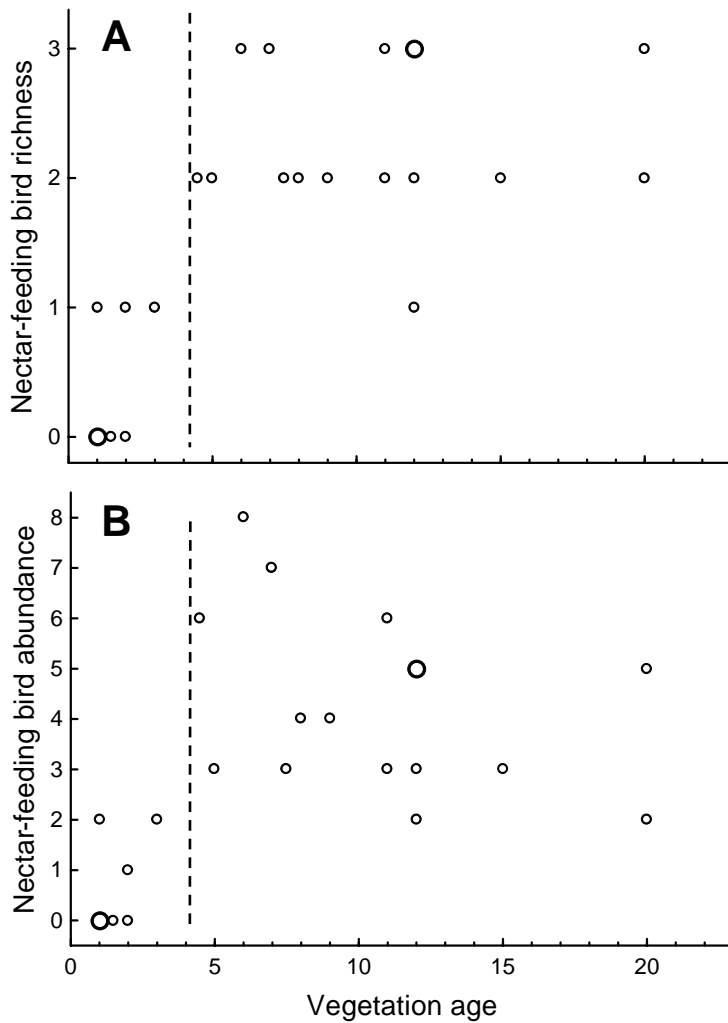


Figure 2.2. Proteaceae vegetation age versus (a) species richness and (b) abundance of nectar-feeding birds. Only once the vegetation is more than 4 years old (dotted line), can nectar feeding bird species richness and abundance obtain substantial levels. Bigger dots indicate two data points. $N = 23$, one-hectare plots.

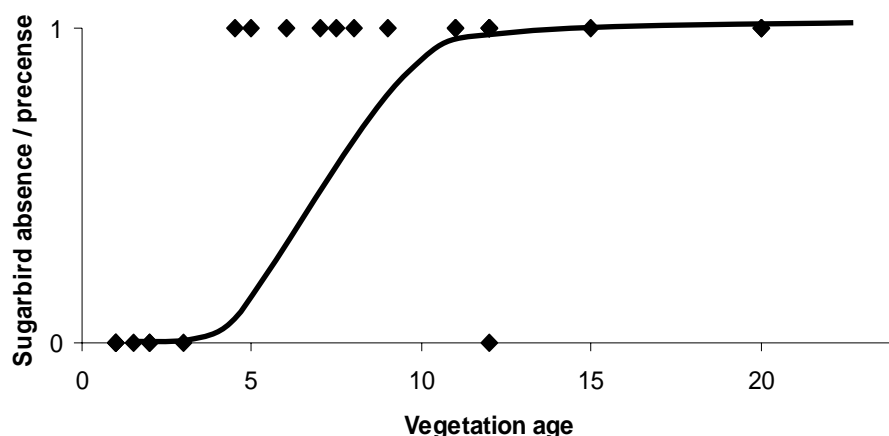


Figure 2.3. Proteaceae vegetation needs to be at least four years in age before Cape Sugarbirds are present. The line shown is from a fitted generalised linear model with binomial errors and vegetation age as an explanatory variable. Sugarbirds absent is zero, sugarbird present is one.

Table 2.2. The following predictor variables were used in a general regression model (GRM) to predict the nectar-feeding bird community on a small scale: (1) vegetation age [years since fire], (2) vegetation type [protea / non-protea] and nectar [mg sucrose per hectare, log-transformed]. Nectar-feeding bird abundance adjusted $R^2 = 0.66$, $P < 0.001$; nectar-feeding bird richness adjusted $R^2 = 0.63$, $P < 0.001$.

	Nectar-feeding bird abundance				Nectar-feeding bird richness			
	<i>df</i>	MS	F	<i>P</i>	<i>df</i>	MS	F	<i>P</i>
Intercept	1	0.04	0.03	0.870	1	0.44	1.28	0.267
Vegetation type	1	3.06	1.91	0.177	1	0.00	0.01	0.913
Vegetation age	1	3.07	1.92	0.176	1	0.77	2.23	0.146
Sucrose.ha ⁻¹ log (mg)	1	82.42	51.49	0.000	1	8.28	24.03	0.000
Error	30	1.60			30	0.34		

Plant-pollinator communities on a landscape scale

Ornithophilous Proteaceae abundance and species richness correlated significantly with the abundance and species richness of the nectar-feeding bird community (Fig. 2.4, Table 2.3). The steep slope ($y = -9.109 + 5.4515x$) of more than 5 ornithophilous Proteaceae species for each added nectar-feeding bird species demonstrates an asymmetrical plant-pollinator relationship. The most species rich ornithophilous Proteaceae communities also contained the highest number of nectar-feeding birds (Spearman-R = 0.51 $t = 7.59$, $N = 163$, $P < 0.001$; Fig. 2.5). Within this nectarivorous bird community, ornithophilous Proteaceae abundance and species richness correlate strongly with abundance (reporting rate) of Cape Sugarbirds and Orange-breasted Sunbirds and to a lesser extent with the more generalist Malachite Sunbird whilst no correlation exists with the Southern Double-collared Sunbird (Table 2.3). This very close association between sugarbirds and ornithophilous Proteaceae is shown graphically in Figure 2.6. The most species rich ornithophilous Proteaceae communities are absent from areas where no sugarbirds occur (Mann-Whitney U -Test, $n_{\text{sugarbird absent grids}} = 24$ $n_{\text{sugarbird present grids}} = 139$ $Z = 4.36$, $P < 0.001$; Fig. 2.7).

Nectar-feeding birds are dependant on the nectar supplied by Proteaceae rather than changes in vegetation structure that they induce, since ornithophilous Proteaceae abundance and species richness is a better predictor of the nectar-feeding bird community than the abundance and species richness of non-ornithophilous Proteaceae species (General Regression Model $R^2 = 0.40$, $P < 0.001$; Table 2.4).

The nectar-feeding bird community is highly nested (temperature: 8.77° ; fill: 64.2%, indicating 64.2% of the elements reflect interactions present). Nestedness rank according to species richness of the nectar-feeding bird community is not correlated with ornithophilous Proteaceae abundance (Spearman-R = 0.15 $t = 1.52$, $N = 57$, $P = 0.25$). As one continues down the ornithophilous Proteaceae species richness gradient nectar-feeding bird species are lost with a decrease in plant species richness, but this was only a near significant trend (Spearman-R = 0.24 $t = 1.88$, $N = 57$, $P = 0.066$). Sample size was less than 180 since nestcalc excludes grids where all nectar-feeders are present.

Table 2.3. Correlations between ornithophilous Proteaceae abundance and species richness with nectar-feeding bird reporting rates (QDGC level). Significance value of 0.001 after Bonferroni correction; N = 180 in all cases.

	Ornithophilous Proteaceae abundance			Ornithophilous Proteaceae species richness		
	Spearman - R	t	P	Spearman - R	t	P
Nectar-feeding bird community	0.58	9.52	0.000	0.60	9.90	0.000
Cape Sugarbird	0.72	14.03	0.000	0.68	12.41	0.000
Malachite Sunbird	0.45	6.64	0.000	0.35	5.05	0.000
Orange-breasted Sunbird	0.74	14.84	0.000	0.71	13.53	0.000
Southern Double-collared Sunbird	0.15	1.96	0.051	0.07	0.95	0.343

Table 2.4. To assess whether the link between plant and pollinator communities in the CFR is driven by changes in vegetation structure, caused by the presence of Proteaceae shrubs, or by changes in nectar availability, non-ornithophilous Proteaceae abundance and species richness is included with ornithophilous Proteaceae species in a general regression model. In this model, the nectar-feeding bird community richness and individual bird species were the response variables and both ornithophilous and non-ornithophilous Proteaceae abundance (logged) and species richness the predictor variables.

	Nectar-feeding bird community				Cape Sugarbird			
	df	MS	F	P	df	MS	F	P
Intercept	1	66.29	184.77	0.000	1	0.00	0.02	0.880
Species richness of non-ornithophilous Proteaceae	1	0.15	0.42	0.516	1	0.06	2.15	0.144
Abundance of non-ornithophilous Proteaceae (logged)	1	1.25	3.48	0.064	1	0.04	1.31	0.255
Species richness of ornithophilous Proteaceae	1	1.56	4.34	0.0389	1	0.17	6.15	0.014
Abundance of ornithophilous Proteaceae (logged)	1	0.52	1.45	0.231	1	0.12	4.31	0.039
Error	175	0.36			175	0.03		

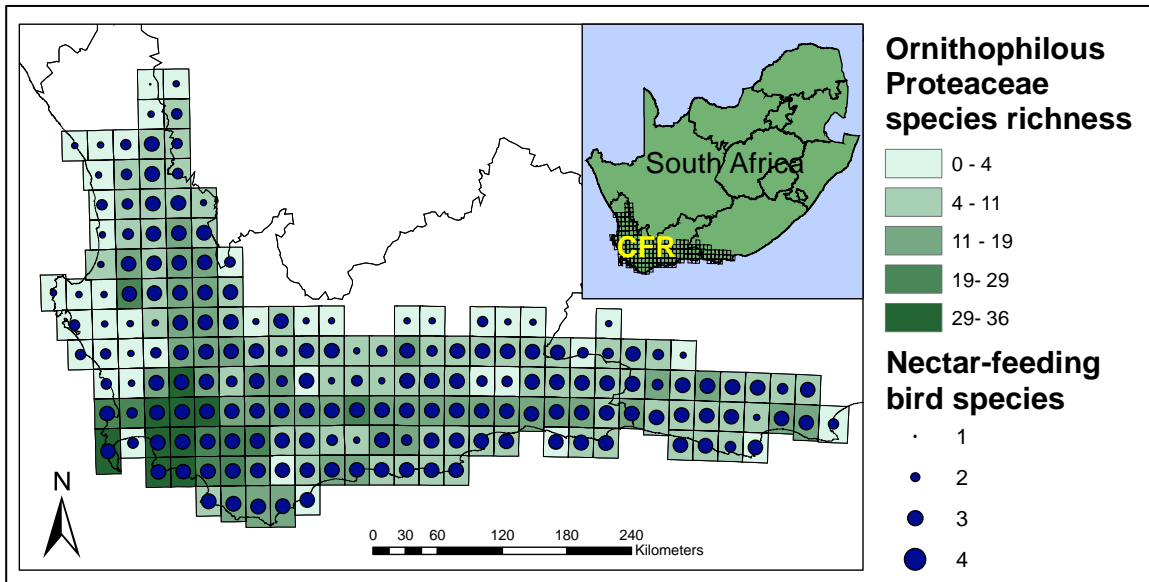


Figure 2.4. Spatial representation of ornithophilous Proteaceae species richness and the number of nectar-feeding bird species for each QDGC in the CFR. For ornithophilous Proteaceae abundance the pattern is similar, since Proteaceae abundance is correlated with species richness (reporting rates < 1% excluded in map).

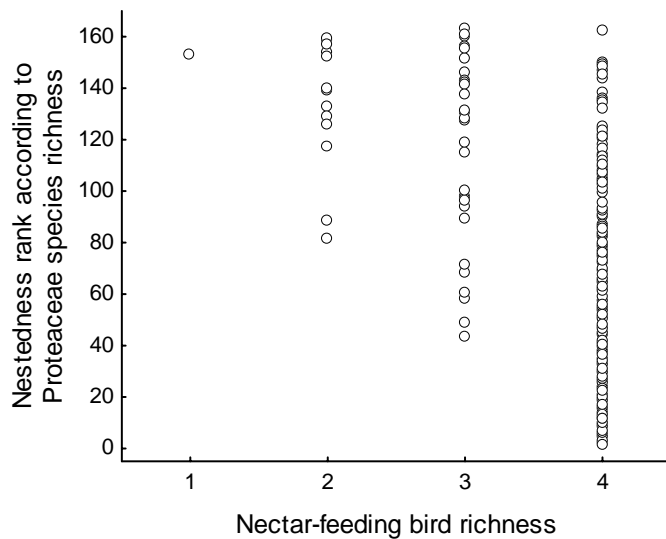


Figure 2.5. Correlation between nectar-feeding bird species richness and ornithophilous Proteaceae species richness ranked according to a nestedness calculator (Nestcalc) with the most species rich site ranked number one.

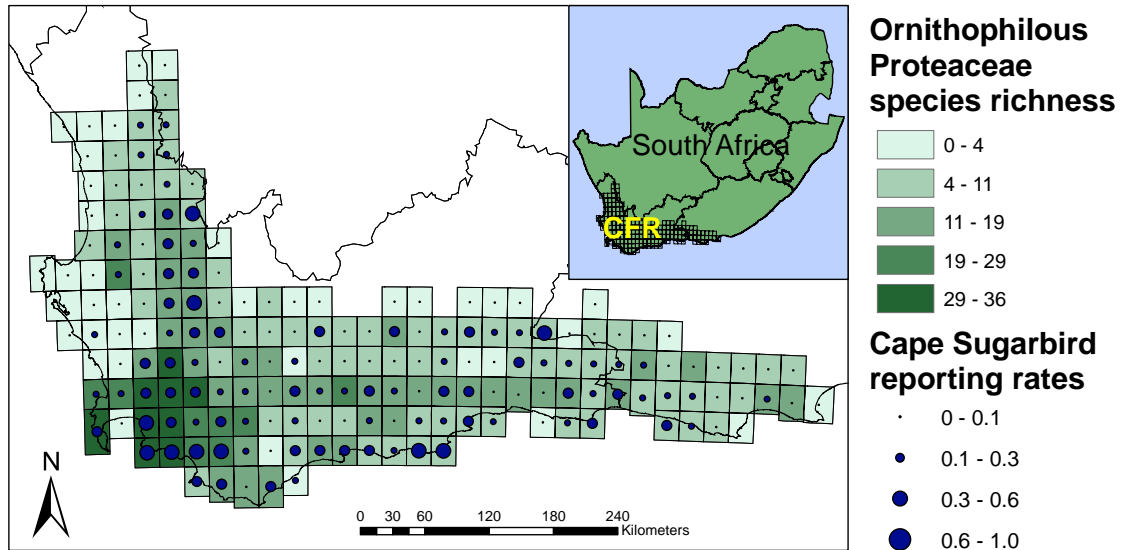


Figure 2.6. Spatial representation of ornithophilous Proteaceae species richness versus the reporting rate of Cape Sugarbirds. For Proteaceae abundance the pattern is similar.

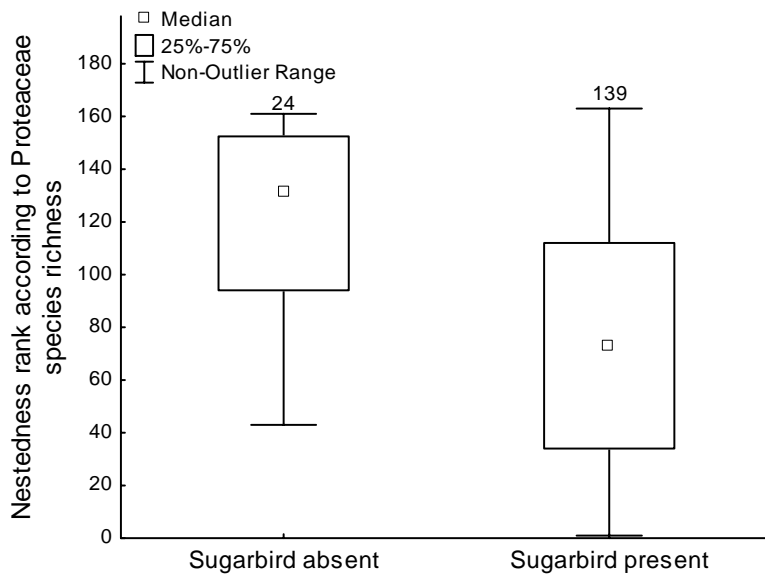


Figure 2.7. Proteaceae species richness and sugarbird absence or presence (Mann-Whitney U-Test, $N_{\text{sugarbird absent areas}} = 24$, $N_{\text{sugarbird present areas}} = 139$, $Z = 4.36$, $P < 0.001$).

Vulnerability of the plant-pollinator communities

Species within the ornithophilous Proteaceae community varied greatly in their predicted vulnerability to pollinator loss (Appendix 2). At one extreme are the invulnerable species like the resprouting *Protea nitida* Miller with many pollinators and low dependence on seeds for persistence ($VI = 0.00$). On the other extreme are species with a vulnerability value close to one such as *Mimetes saxatilis* Phill, pollinated only by one or a few pollinators, incapable of selfing and dependent on seeds alone for reproduction. The ornithophilous Proteaceae community is highly nested (temperature: 8.49° ; fill: 13.6%). The nestedness rank of Proteaceae species is significantly correlated with the vulnerability of individual Proteaceae species (Spearman-R = 0.53 $t = 4.49$, $N = 53$, $P < 0.001$; Fig. 2.8) and vulnerability to pollinator specificity (Spearman-R = 0.54 $t = 4.53$, $N = 56$, $P < 0.001$). Widespread species like *P. nitida*, *P. repens*, *P. laurifolia*, *P. neriifolia* and *P. cynaroides* have low predicted vulnerability to pollinator loss (Appendix 2).

The number of ornithophilous Proteaceae species in the pollination community varied across the 163 study grids from one to a maximum of 36 species (17 grids without any ornithophilous Proteaceae species excluded in these analyses). Average vulnerability of these communities varied from almost invulnerable to pollinator loss ($CV < 0.001$) to very vulnerable ($CV = 0.34$). Ornithophilous Proteaceae communities with a higher average vulnerability are also the more species rich communities (Spearman-R, average vulnerability = -0.61 $t = 9.77$, $N = 163$, $P < 0.001$; Spearman-R, vulnerability to pollinator specificity only = -0.76 $t = 14.78$, $N = 163$, $P < 0.001$).

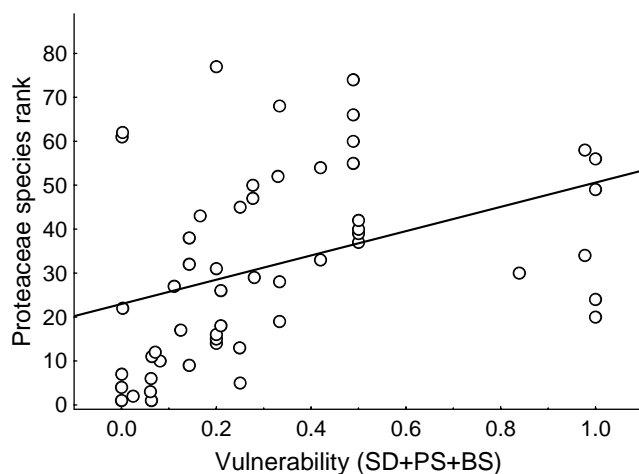


Figure 2.8. Vulnerability of individual Proteaceae correlated with the nestedness rank of each species, with the most common species, *Protea nitida* and *P. repens*, ranked number one (Spearman-R = 0.53 $t = 4.49$, $N = 53$, $P < 0.001$)

Discussion

The close association between plant and bird communities found in this study suggests an important role for community wide pollination mutualisms. These mutualisms work both ways, with Proteaceae supplying nectar for nectar-feeding birds and high numbers of nectar-feeding birds presumably enhancing seed set in Proteaceae plants. Species richness does matter indeed; an increase of either nectar-feeding bird species richness or ornithophilous Proteaceae is linked to an increase of the other. The relationship is highly asymmetric with more than 5 Proteaceae species added for each nectar-feeding bird species. Such asymmetry has been widely reported for other pollination network relationships (Memmott 1999; Jordano *et al.* 2003).

The underlying mechanism linking nectar-feeding bird communities to ornithophilous Proteaceae communities is nectar availability, rather than structural changes in vegetation associated with large Proteaceae shrubs. There is strong evidence for nectar as driver of the nectar-feeding bird community on a small and large scale. On a small scale, the step wise manner in which birds are increasing with vegetation age shows that it is not a gradual increase in vegetation size but rather a sudden increase in nectar when ornithophilous Proteaceae start flowering (Fig. 2.2). On a large scale ornithophilous Proteaceae are better correlated with the nectar-feeding bird community than non-ornithophilous Proteaceae which do not contain nectar (Table 2.4). Nectar availability is a product of Proteaceae abundance and species richness, which, in turn, is determined by post-fire vegetation age and vegetation type. Although nectar-feeding birds are known to be more dependant on ornithophilous Proteaceae than other avian guilds (De Swardt 1993) and make up the bulk of avian fauna in mature Proteaceae vegetation, other avian species like seedeaters, Cape Turtle Dove (*Streptopelia capicola* Sundevall), and predators, Fiscal (*Lania collaris* L.), are also closely associated with Proteaceae vegetation (this study, Winterbottom 1964). These species are not dependent on nectar but on vegetation structure for nest sites or prey items.

Many Proteaceae species are predicted to face range contractions with a change in climate (Midgley *et al.* 2003). However at least some species are known to be able to successfully grow outside their home range and could thus potentially move with climate change (Latimer *et al.* 2009). More importantly, frequency of fire, which is likely to increase with climate

change (IPCC 2001), will reduce the extent of mature vegetation and the nectar available to the bird community (Bond et al. 2003). Within the nectar-feeding bird community, species differ in their nectar requirements, with the large bodied Cape Sugarbird needing substantial amounts (Collins 1983a) which can only be supplied by Proteaceae vegetation of at least 4 years in age (Fig. 2.3). As a consequence specialised Proteaceae species may suffer reduced seed set and population viability in isolated populations over and above any direct climatic-induced impacts they may experience. The probability of extinction depends on the strength of the pollinator-plant mutualism, which can be partly evaluated by vulnerability analysis.

Plant communities with higher vulnerabilities consist of more species and are capable of attracting the complete nectar-feeding bird community. Highly vulnerable Proteaceae species are able to exist in these diverse Proteaceae communities. In species poor communities however, these highly vulnerable species are absent. Although the dataset is crude, widespread ornithophilous Proteaceae species within the CFR have a lower vulnerability value than those with restricted ranges. This implies that the nectar-feeding bird community plays an important role in shaping the plant community and that range change of particular nectar-feeding bird species can elicit plant extinction. Sugarbird reporting rate in particular, is positively correlated with the Proteaceae community; in the most diverse Proteaceae communities sugarbirds are always present, whilst in less diverse communities sugarbirds are usually not present (Fig. 2.7). This suggest, in concordance with Rebelo (1987), that sugarbirds are of greater significance for Proteaceae than other nectar-feeding birds. Indeed the entire life history of sugarbirds is adapted to the Proteaceae and they are reliant on Proteaceae for food resources during breeding, and leave their territories only during the dry season when flowers are absent (Rebelo *et al.* 1984; Calf *et al.* 2003).

Species poor Proteaceae communities consist of species with traits that are predicted to bring independence from birds. This may include reliance on self-pollination, alternative pollinators like insects (Wright et al. 1991) or individual longevity through resprouting. In the past, too frequent fires have been indicated as the only source of change in the Proteaceae community (le Maitre and Midgley 1992; Bond 1994). However, nectar-feeding birds potentially also shape communities through their absence or presence as pollen carriers. To test this importance

of nectar-feeding birds for Proteaceae one should correlate pollen limitation at the population and community level with nectar-feeding bird abundance and richness. Studies of pollination mechanism and basic breeding system experiments in the Proteaceae are an urgent necessity to refine our knowledge of the importance of different pollination guilds, and species within these guilds, for Proteaceae reproduction. In conclusion, patterns observed in this study might well be true for many other bird dependent plant communities as well as for insect dependent plant communities in the CFR but also in other parts of the world.

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References

- Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* 9:968-980
- Allen-Wardell G et al. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conserv. Biol.* 12:8-17
- Almeida-Neto M, Guimaraes PR, Lewinsohn TM (2007) On nestedness analyses: rethinking matrix temperature and anti-nestedness. *Oikos* 116:716-722
- Atmar W, Patterson BD (1993) The measure of order and disorder in the distribution of species in fragmented habitat. *Oecologia* 96:373-382
- Bartomeus I, Vila M, Santamaria L (2008) Contrasting effects of invasive plants in plant-pollinator networks. *Oecologia* 155:761-770
- Bibby CJ, N. D. Burgress, D. A. Hill, S. H. Mustoe. (2000) *Bird census techniques* 2nd ed. Academic Press, London.
- Biesmeijer JCS et al. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313:351-354
- Bond WJ (1994) Do mutualisms matter - assessing the impact of pollinator and disperser disruption on plant extinction. *Philos. Trans. R. Soc. Lond. Ser. B-Biol. Sci.* 344:83-90

- Bond WJ, Midgley GF, Woodward FI (2003) What controls South African vegetation - climate or fire? *S. Afr. J. Bot.* 69:79-91
- Brown JH, Bowers MA (1985) Community organization in hummingbirds: relationship between morphology and ecology. *The Auk* 102:251-269
- Calf KM (1999) The role of male territory size and quality in mating and reproductive success of Cape and Gurney's Sugarbirds, *Promerops cafer* and *Promerops gurneyi*. MSc thesis, University of Stellenbosch, Stellenbosch
- Calf KM, Downs CT, Cherry MI (2001) Territoriality and breeding success in Gurney's sugarbird, *Promerops gurneyi*. *African Zoology* 36:189-195
- Calf KM, Downs CT, Cherry MI (2003) Territoriality of Cape Sugarbirds (*Promerops cafer*) between and within breeding seasons. *Ostrich* 74:125-128
- Cameron C (1999) The effects of fragmentation of renosterveld vegetation on bird community composition. MSc mini-thesis, University of Cape Town, Cape Town
- Carlson JE, Holsinger KE (2010) Natural selection on inflorescence color polymorphisms in wild Protea populations: the role of pollinators, seed predators, and intertrait correlations. *American Journal of Botany* 97:934-944
- Coetzee JH, Giliomee JH (1985) Insects in association with the inflorescence of *Protea repens* (L.) (Proteaceae) and their role in pollination. *Journal of the Entomological Society of Southern Africa* 48:303-314
- Collins BG (1983a) A first approximation of the energetics of Cape Sugarbirds (*Promerops cafer*) and Orange-breasted Sunbirds (*Nectarinia violacea*). *South African Journal of Zoology* 18:363-369
- Collins BG (1983b) Pollination of *Mimetes hirtus* (Proteaceae) by Cape Sugarbirds and Orange-breasted Sunbirds. *S. Afr. J. Bot.* 49:125-142
- Collins BG, Rebelo T (1987) Pollination Biology of the Proteaceae in Australia and Southern-Africa. *Australian Journal of Ecology* 12:387-421
- Cowling RM (1992) The ecology of fynbos: nutrients, fire and diversity. Oxford University Press, Cape Town.
- Darlington PJ (1957) Zoogeography: the geographical distribution of animals. John Wiley, New York.

- De Swardt DH (1993) Factors affecting the densities of nectarivores in *Protea Roupelliae* Woodland. *Ostrich* 64:172-177
- Devoto M, Montaldo NH, Medan, D (2006) Mixed hummingbird: Long-proboscid-fly pollination in 'ornithophilous' *Embothrium coccineum* (Proteaceae) along a rainfall gradient in Patagonia, Argentina. *Austral Ecology* 31:512-519
- Fairbanks DHK, Kshatriya M, van Jaarsveld AS, Underhill LG (2002) Scales and consequences of human land transformation on South African avian diversity and structure. *Anim. Conserv.* 5:61-74
- Feinsinger P (1978) Ecological interactions between plants and hummingbirds in a successional tropical community. *Ecological Monographs* 48:269-287
- Fleming TH, Muchhala N (2008) Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of vertebrate pollination systems. *J. Biogeogr.* 35:764-780
- Fortuna MA, Bascompte J (2006) Habitat loss and the structure of plant-animal mutualistic networks. *Ecology Letters* 9:278-283
- Foster BL, Tilman D (2000) Dynamic and static views of succession: Testing the descriptive power of the chronosequence approach. *Plant Ecology* 146:1-10
- Fox SJC, Hockey PAR (2007) Impacts of a South African coastal golf estate on shrubland bird communities. *South African Journal of Science* 103:27-34
- Fraser M, McMahon L (1992) Habitat change by Cape Sugarbirds and Orange-breasted Sunbirds in an apparent response to fire in old mountain fynbos. *Promerops* 21:51-54
- Fraser MW (1989) Short-term responses of birds to fire in old mountain fynbos. *Ostrich* 60:172-182
- Fründ J, Linsenmair KE, Blüthgen N (2010) Pollinator diversity and specialization in relation to flower diversity. *Oikos* doi: 10.1111/j.1600-0706.2010.18450.x
- Fry CH (2000). *The Birds of Africa*. Vol. VI. Academic Press, London
- Geerts S, Pauw A (2009) Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *S. Afr. J. Bot.* 75:699-706
- Greve M, Gremmen NJM, Gaston KJ, Chown SL (2005) Nestedness of Southern Ocean island biotas: ecological perspectives on a biogeographical conundrum. *J. Biogeogr.* 32:155-168

- Guimaraes PRJ, Guimaraes PR (2006) Improving the analyses of nestedness for large sets of matrices. *Environmental Modelling & Software*:1512-1513
- Hansson L (1998) Nestedness as a conservation tool: plants and birds of oak-hazel woodland in Sweden. *Ecology Letters* 1:142-145
- Hargreaves A, Johnson S, Nol E (2004) Do floral syndromes predict specialization in plant pollination systems? An experimental test in an “ornithophilous” African *Protea*. *Oecologia* 140:295–301
- Harrison JA, Allan DG, Underhill LG, Herremans M, Tree AJ, Parker V, Brown CJ (eds) (1997) *The atlas of southern African birds*. BirdLife South Africa, Johannesburg
- Hegland SJ, Nielsen A, Lazaro A, Bjerknes AL, Totland O (2009) How does climate warming affect plant-pollinator interactions? *Ecology Letters* 12:184-195
- Hegland SJ, Totland O (2008) Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialisation levels? *Oikos* 117:883-891
- Hobbs RJ, Yates CJ (2003) Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. *Australian Journal of Botany* 51:471-488
- Horn W (1962) Breeding research on South African plants: fertility of Proteaceae. *Journal of South African Botany* 28:259-268
- Hugo S, Van Rensburg BJ (2009) Alien and native birds in South Africa: patterns, processes and conservation. *Biological Invasions* 11:2291-2302
- IPCC (2001) *Climate change: impacts, adaptation and vulnerability*. Cambridge University Press, Cambridge, UK
- Jordano P, Bascompte J, Olesen JM (2003) Invariant properties in coevolutionary networks of plant-animal interactions. *Ecology Letters* 6:69-81
- Kearns CA, Inouye DS (1997) Pollinators, flowering plants, and conservation biology - much remains to be learned about pollinators and plants. *Bioscience* 47:297-307
- Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: The conservation of plant-pollinator interactions. *Annu. Rev. Ecol. Syst.* 29:83-112
- Kleijn D, Berendse F, Smit R, Gilissen N, Smit J, Brak B, Groeneveld R (2004) Ecological effectiveness of agri-environment schemes in different agricultural landscapes in the Netherlands. *Conserv. Biol.* 18:775-786

- Lamont B (1985) The comparative reproduction biology of three *Leucospermum* species (Proteaceae) in relation to fire response and breeding system. *Australian Journal of Botany* 33:139-145
- Latimer AM, Silander JA, Rebelo AG, Midgley GF (2009) Experimental biogeography: the role of environmental gradients in high geographic diversity in Cape Proteaceae. *Oecologia* 160:151-162
- le Maitre DC, Midgley JJ (1992) Plant reproductive ecology. In: Cowling RM (ed) *The ecology of fynbos: nutrients, fire and diversity*. Oxford University Press, Cape Town
- Lindberg A, Olesen JM (2001) The fragility of extreme specialization: *Passiflora mixta* and its pollinating hummingbird *Ensifera ensifera*. *Journal of Tropical Ecology* 17:323-329
- Lopezaraiza-Mikel ME, Hayes RB, Whalley MR, Memmott J (2007) The impact of an alien plant on a native plant-pollinator network: an experimental approach. *Ecology Letters* 10:539-550
- Manning J (2004) The exciting discovery of a new pollination system in the ribbon pincushion, *Leucospermum tottum*. *Veld and Flora* 90:10-12
- Martin R, Mortimer J (1991) Fynbos: too frequent burning poses threat to Cape Sugarbirds. *Promerops* 199:6-8
- Martinez-Morales MA (2005) Nested species assemblages as a tool to detect sensitivity to forest fragmentation: the case of cloud forest birds. *Oikos* 108:634-642
- Memmott J (1999) The structure of a plant-pollinator food web. *2*:276-280
- Memmott J, Waser N, Price M (2004) Tolerance of pollination networks to species extinctions. *Proc. R. Soc. Lond. B* 271:2605–2611
- Midgley GF, Hannah L, Millar D, Thuiller W, Booth A (2003) Developing regional and species-level assessments of climate change impacts on biodiversity in the Cape Floristic Region. *Biol. Conserv.* 112:87-97
- Morales CL, Traveset A (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecology Letters* 12:716-728
- Mostert DP, Siegfried WR, Louw GN (1980) Protea nectar and satellite fauna in relation to the food- requirements and pollinating role of the cape sugarbird. *South African Journal of Science* 76:409-412

- Ne'eman G, Dafni A, Potts SG (2000) The effect of fire on flower visitation rate and fruit set in four core-species in east Mediterranean scrubland. *146:97-104*
- Patterson BD (1987) The principle of nested subsets and its implications for biological conservation. *Conserv. Biol. 1:323-334*
- Patterson BD, Atmar W (1986) Nested subsets and the structure of insular mammalian faunas and archipelagos. *Biological Journal of the Linnean Society 28:65-82*
- Pauw A, Hawkins JA (2010) Reconstruction of historical pollination rates reveals linked declines of pollinators and plants. *Oikos* doi: 10.1111/j.1600-0706.2010.19039.x
- Phillips RD, Hopper SD, Dixon KW (2010) Pollination ecology and the possible impacts of environmental change in the Southwest Australian Biodiversity Hotspot. *365:517-528*
- Potts SG (2003) Linking bees and flowers: how do floral communities structure pollinator communities? . *Ecology 84:2628-2642*
- Potts SG, Biesmeijer JCS, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol. 25:345-353*
- Potts SG, Dafni A, Ne'eman G (2001) Pollination of a core flowering shrub species in Mediterranean phrygana: variation in pollinator diversity, abundance and effectiveness in response to fire. *Oikos 92:71-80*
- Potts SG, Vulliamy B, Dafni A, Ne'eman G, O'Toole C, Roberts S, Willmer P (2003) Response of plant-pollinator communities to fire: changes in diversity, abundance and floral reward structure. *Oikos 101:103-112*
- Rebelo AG (1987) Bird pollination in the Cape Flora. In: Rebelo AG (ed) *A preliminary synthesis of pollination biology in the Cape flora*, 141 edn. CSIR, Pretoria, pp 83-108
- Rebelo AG (1991) *Protea Atlas Manual*. Protea Atlas Project, Kirstenbosch.
- Rebelo AG (2001) *Proteas: A field guide to the Proteas of southern Africa*. Fernwood Press
- Rebelo AG (2006) Protea atlas project website. <http://protea.worldonline.co.za/default.htm>.
- Rebelo AG, Siegfried WR, Crowe AA (1984) Avian pollinators and the pollination syndromes of selected mountain fynbos plants. *S. Afr. J. Bot. 3:285-296*
- Rodriguez-Girones MA, Santamaria L (2006) A new algorithm to calculate the nestedness temperature of presence-absence matrices. *J. Biogeogr. 33:924-935*
- Roger JG, Balkwill K, Gemmill B (2004) African pollination studies: where are the gaps? *International journal of tropical insect science 24:5-28*

- Sargent RD, Ackerly DD (2008) Plant-pollinator interactions and the assembly of plant communities. *Trends in Ecology and Evolution* 23:123-130
- Seiler HW, Rebelo AG (1987) A sexual difference in the Cape Sugarbirds role as a pollinator of *Protea lepidocarpodendron*? *Ostrich* 58:43-44
- Skead CJ (1967) *Sunbirds of Southern Africa*. Balkema, Cape Town
- Stanton ML (2003) Interacting guilds: moving beyond the pairwise perspective on mutualisms. *Am. Nat.* 162:S10-S23
- Stanway R (2009) *Pollination community interactions in the Cederberg, South Africa*. M.Sc. thesis, University of Stellenbosch, Stellenbosch.
- Steffan-Dewenter I, Tscharntke T (2001) Succession of bee communities on fallows. *Ecography* 24:83-93
- Symes CT, Nicolson SW, McKechnie AE (2008) Response of avian nectarivores to the flowering of *Aloe marlothii*: a nectar oasis during dry South African winters. *J Ornithol* 149:13-22
- Tjorve KMC, Geertsema GH, Underhill LG (2005) Do sugarbirds feed on arthropods inside or outside *Protea* inflorescences? *Emu* 105:293-297
- Traveset A, Richardson DM (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends Ecol. Evol.* 21:208-216
- Ulrich W, Almeida M, Gotelli NJ (2009) A consumer's guide to nestedness analysis. *Oikos* 118:3-17
- Underhill LG, Oatley TB, Harrison JA (1991) The role of large-scale data-collection projects in the study of southern African birds. *Ostrich* 62:124-148
- Underhill LG, Prys-Jones RP, Harrison JA, Martinez P (2008) Seasonal patterns of occurrence of Palearctic migrants in southern Africa using atlas data. *Ibis* 134:99-108
- Valdovinos FS, Ramos-Jiliberto R, Flores JD, Espinoza C, Lopez G (2009) Structure and dynamics of pollination networks: the role of alien plants. *Oikos* 118:1190-1200
- van der Merwe P (1969) Datering van veldbrande met behulp van *Protea mellifera* Thunb. *Suid-Afrikaanse tydskrif vir natuurwetenskap en tegnologie* 9:251-254
- Vogts M (1982) *South Africa's Proteaceae. Know them and grow them*. Struik, Cape Town

- Whitehead VB, Giliomee JH, Rebelo AG (1987) Insect pollination in the Cape flora. In:
Rebelo, A.G. (ed.). A preliminary synthesis of pollination biology in the Cape flora.
South African national scientific programmes report No. 141. CSIR, Pretoria. pp. 52-82.
- Winterbottom JM (1964) Notes on the comparative ecology of the long-tailed sugarbird and
Orange-breasted Sunbird. *Ostrich* 35:239-240
- Wright DH, Patterson BD, Mikkelsen GM, Cutler A, Atmar W (1998) A comparative analysis
of nested subset patterns of species composition. *Oecologia* 113:1-20
- Wright MG (1994) Seed production by *Protea laurifolia* (Proteaceae) after insect versus insect
and bird pollination: a quality difference. *South African Journal of Science* 90:199
- Wright MG, Visser D, Coetzee JH, Giliomee JH (1991) Insect and bird pollination of Protea
species in the Western Cape - further data. *South African Journal of Science* 87:214-
215

Appendix 1. A list of all one hectare plot field sites where the following data was collected: vegetation age and type, nectar-feeding bird abundance and species richness and total sugar per hectare (mg).

Site	Vegetation type	Estimated vegetation age	Number of nectar-feeding bird species	Nectar-feeding bird abundance	Cape Sugar-bird presence	Total sugar (g) hectare	GPS coordinates
Kogelberg 1	Protea	20	3	5	yes	9616	34°20'62.0"S 18°55'90.7"E
Kogelberg 2	Protea	7.5	2	3	yes	754	34°20'71.7"S 18°55'58.0"E
Buffelslaagte	Protea	1	0	0	no	0	34°18'83.7"S 18°49'68.0"E
Helderberg nature reserve 1	Protea	12	2	5	yes	29688	34°02'69.3"S 18°52'26.9"E
Helderberg nature reserve 2	Protea	2	0	0	no	0	34°02'74.2"S 18°52'08.0"E
Table mountain pipe track	Protea	1	0	0	no	0	33°57'10.8"S 18°3'63.7"E
Table mountain cable car	Protea	1	1	2	no	0	33°57'13.9"S 18°24'84.0"E
Kogelberg Harold Porter 1	Protea	8	2	4	yes	99	34°20'71.7"S 18°55'58.0"E
Paarl mountain, monument	Protea	12	3	5	yes	1793	33°45'56.5"S 18°56'75.2"E
Cape point	Protea	11	2	3	yes	3296	34°15'71.0"S 18°27'42.5"E
Redhill 1	Protea	11	3	6	yes	3085	34°13'12.9"S 18°24'82.5"E
Du Toitskloof pass 1	Protea	9	2	4	yes	2693	33°44'87.8"S 19°04'19.8"E
Du Toitskloof pass 2	Protea	3	1	2	no	0	33°44'86.4"S 19°04'19.4"E
Du Toitskloof pass 3	Protea	5	2	3	yes	1315	33°43'02.2"S 19°05'03.6"E
Franschoek Villiersdorp road	Protea	1.5	0	0	no	0	33°55'74.3"S 19°09'60.1"E
Jonkershoek fire break	Protea	2	1	1	no	5	33°59'16.7"S 18°57'07.7"E
Jonkershoek Swartboskloof	Protea	6	3	8	yes	3434	33°59'15.4"S 18°57'11.1"E
Jonkershoek Panorama trail	Protea	4.5	2	6	yes	4808	33°59'32.5"S 18°58'36.3"E
East of Kleinmond	Protea	6	3	7	yes	2332	34°19'45.9"S 19°01'85.9"E
Paarl mountain	Protea	12	1	2	no	4	33°44'17.9"S 18°57'19.2"E
Theewaterskloof dam 1	Protea	15	2	3	yes	361	-
Theewaterskloof dam 2	Protea	20	2	2	yes	126	-
Jonkershoek 1	Protea	12	3	3	yes	503	-
Redhill 2	Non-protea	2	1	1	no	0	34°11'29.7"S 18°23'65.2"E
Redhill 3	Non-protea	3	2	2	no	0	34°11'22.9"S 18°23'76.0"E
Redhill 4	Non-protea	5	1	1	no	0	34°11'08.7"S 18°23'86.2"E
Scarborough	Non-protea	3	1	3	no	4	34°11'84.3"S 18°22'83.6"E
Kogelberg Harold porter 2	Non-protea	8	1	6	no	33	34°20'62.0"S 18°55'90.7"E

Site	Vegetation type	Estimated vegetation age	Number of nectar-feeding bird species	Nectar-feeding bird abundance	Cape Sugar-bird presence	Total sugar (g) hectare	GPS coordinates
Kogelberg Harold porter 3	Non-protea	8	1	2	no	4	34°20'80.6"S 18°55'77.4"E
West of Kleinmond	Non-protea	5	1	3	no	16	34°20'23.6"S 18°59'75.6"E
Cape Point road Restio	Non-protea	11	1	1	no	0	34°14'31.4"S 18°25'23.2"E
Cape Point road Leucadendron	Non-protea	11	1	1	no	0	34°14'22.7"S 18°25'17.9"E
Helshoogte	Non-protea	12	1	1	no	0	33° 55'40.2"S 18°54'65.9"E

Appendix 2. A list of all Proteaceae species classified as ornithophilous according to Protea atlas data, Rebelo (unpublished data), personal observations and Rebelo (2001). Species are ranked (by Nestcalc) according to their distribution range. A vulnerability (VI) value for most species is calculated as follows: $BS \times PS \times SD$, where BS is breeding system, PS pollinator specificity and SD dependence on seeds. BS is 1 – (pollinator excluded seed set/open pollination seed set). For some species, BS data was not available and closely related species were than used were appropriate (Rebelo 2001). Seed dependence (SD) was available for all species and species are either dependent, value of 1, or independent on seeds, value of 1/100. PS is 1/(number of pollinators) and is taken from the detailed pollinator section in the Protea Atlas except where insects are known to be not important pollinators (Rebelo unpublished data), or were indicated otherwise in the reference column. This is a crude dataset and due to the lack of Proteaceae pollination studies, not all species are included in the vulnerability calculations. Species without an vulnerability value but used in the correlative analysis are listed at the bottom.

Species	Rank	Breeding system [BS]	Pollinator specificity [PS]	Seed dependence [SD]	Vulnerability Index [VI = BS x PS x SD]	Reference
<i>P. nitida</i> Miller	1	0.200	0.077	0.01	0.0002	PS: own data and Protea atlas, BS: Wright et al 1991
<i>P. repens</i> (L.) L.	2	0.886	0.071	1	0.0633	PS: own data and Protea atlas, BS: Coetsee and Giliomee 1985
<i>P. neriifolia</i> R.Br.	3	0.268	0.091	1	0.0244	BS: Wright et al 1991
<i>P. laurifolia</i> Thunb.	4	0.855	0.071	1	0.0611	BS: Wright et al 1991
<i>P. cynaroides</i> (L.) L.	5	0.010	0.200	0.01	0.0000	BS: Wright et al 1991
<i>P. eximia</i> (Salisb. ex Kn.) Four.	6	1.000	0.250	1	0.2500	BS: <i>P. longifolia</i> also spoon-bract group BS: average from other species in bearded group
<i>P. lorifolia</i> (Salisb. ex Kn.) Fourc.	7	0.498	0.125	1	0.0623	with data (<i>P. laurifolia</i> , <i>P. neriifolia</i> and <i>P. lorifolia</i>)
<i>L. cuneiforme</i> (Burm.f) Rourke	8	0.505	0.100	0.01	0.0005	BS: Lamont 1985
<i>P. punctata</i> Meisn.	9	1.000	0.143	1	0.1429	BS: <i>P. mundii</i> also white protea group
<i>M. cucullatus</i> (L.) R.Br.	10	0.977	0.167	0.5	0.0815	BS: <i>M. hirtus</i>
<i>P. magnifica</i> Link	11	0.257	0.250	1	0.0641	BS: Wright et al 1991

<i>P. coronata</i> Lam.	12	0.498	0.143	1	0.0712	BS: average from other species in bearded group with data (<i>P. laurifolia</i> , <i>P. neriifolia</i> and <i>P. lorifolia</i>)
<i>P. grandiceps</i> Tratt.	13	0.498	0.500	1	0.2491	BS: average from other species in bearded group with data (<i>P. laurifolia</i> , <i>P. neriifolia</i> and <i>P. lorifolia</i>)
<i>P. burchellii</i> Stapf	14	1.000	0.200	1	0.2000	BS: <i>P. longifolia</i> also spoon-bract group
<i>P. mundii</i> Klotzsch	15	1.000	0.200	1	0.2000	BS: Horn 1962
<i>P. susannae</i> Phill.	16	1.000	0.200	1	0.2000	BS: Horn 1962
<i>P. obtusifolia</i> Buek ex Meisn.	17	1.000	0.125	1	0.1250	BS: Horn 1962
<i>L. wittebergense</i> Compton	18	0.839	0.250	1	0.2098	BS: <i>L. cordifolium</i>
<i>P. aurea aurea</i> (Burm.f) Rourke	19	1.000	0.333	1	0.3333	BS: <i>P. mundii</i> also white protea group
<i>P. rupicola</i> Mund ex Meisn.	20	1.000	1.000	1	1.0000	BS: <i>P. glabra</i>
<i>P. speciosa</i> L.	21	0.498	0.500	0.01	0.0025	BS: average from other species in bearded group with data (<i>P. laurifolia</i> , <i>P. neriifolia</i> and <i>P. lorifolia</i>)
<i>L. reflexum</i> Rourke	22	1.000	1.000	1	1.0000	BS: Horn 1962
<i>L. vestitum</i> (Lam.) Rourke	23	0.839	0.250	1	0.2098	BS: <i>L. cordifolium</i>
<i>P. glabra</i> Thunb.	24	1.000	0.111	1	0.1111	BS and PS from Stanway 2009
<i>P. lanceolata</i> Meyer ex Meisn.	25	1.000	0.333	1	0.3333	BS: Horn 1962
<i>L. cordifolium</i> (Salisb. ex Kn.) Fourc.	26	0.839	0.333	1	0.2798	BS: Lamont 1985
<i>L. glabrum</i> Phill.	27	0.839	1.000	1	0.8393	BS: <i>L. cordifolium</i>
<i>L. praecox</i> (cf <i>truncatum</i>)	28	1.000	0.200	1	0.2000	BS: <i>L. reflexum</i>
<i>P. longifolia</i> Andrews	29	1.000	0.143	1	0.1429	BS: Horn 1962
<i>L. patersonii</i> Phill.	30	0.839	0.500	1	0.4196	BS: <i>L. cordifolium</i>
<i>M. pauciflorus</i> R.Br.	31	0.977	1.000	1	0.9775	BS: <i>M. hirtus</i>
<i>P. lacticolor</i> Salisb.	32	1.000	0.500	1	0.5000	BS: Horn 1962
<i>L. truncatum</i> (Buek. ex Meisn.) Rourke	33	1.000	0.143	1	0.1429	BS: <i>L. reflexum</i>
<i>L. formosum</i> (Andrews) Sweet	34	1.000	0.500	1	0.5000	BS: <i>L. reflexum</i>
<i>P. venusta</i> Compton	35	1.000	0.500	1	0.5000	BS: <i>P. mundii</i> also white protea group
<i>P. aristata</i> Phill.	36	1.000	0.500	1	0.5000	BS: <i>P. lanceolata</i> also true protea group PS: Seiler 1987 and Protea Atas. BS: average from other species in bearded group with data (<i>P. laurifolia</i> , <i>P. neriifolia</i> and <i>P. lorifolia</i>)
<i>P. lepidocarpodendron</i> (L.) L.	37	0.498	0.333	1	0.1659	BS: average from other species in bearded group with data (<i>P. laurifolia</i> , <i>P. neriifolia</i> and <i>P. lorifolia</i>)
<i>L. praemorsum</i> (Meisn.) Phill.	38	1.000	0.250	1	0.2500	BS: <i>L. reflexum</i>

<i>L. pluridens</i> Rourke	39	0.839	0.330	1	0.2770	BS: <i>L. cordifolium</i>
<i>L. grandiflorum</i> (Salisb.) R.Br.	40	1.000	1.000	1	1.0000	BS: <i>L. reflexum</i>
<i>L. lineare</i> R.Br.	41	0.839	0.330	1	0.2770	BS: <i>L. cordifolium</i>
<i>L. praecox</i> Rourke	42	1.000	0.330	1	0.3300	BS: <i>L. reflexum</i>
<i>L. mundii</i> Meisn.	43	0.839	0.500	1	0.4196	BS: <i>L. cordifolium</i>
<i>M. hirtus</i> (L.) Salisb. ex Kn.	44	0.977	0.500	1	0.4887	BS and PS: Collins 1983a
<i>L. gueinzii</i> Meisn.	45	1.000	1.000	1	1.0000	BS: <i>L. reflexum</i>
<i>M. saxatilis</i> Phill.	46	0.977	1.000	1	0.9775	BS: <i>M. hirtus</i>
<i>M. chrysanthus</i> Rourke	47	0.977	0.500	1	0.4887	BS: <i>M. hirtus</i>
<i>L. conocarpodendron viridum</i> Rourke	48	0.839	0.143	0.01	0.0012	BS: <i>L. cordifolium</i>
<i>M. fimbriifolius</i> Salisb. ex Kn.	49	0.977	0.200	0.01	0.0020	BS: <i>M. hirtus</i>
<i>M. hottentoticus</i> Phill & Hutch.	50	0.977	0.500	1	0.4887	BS: <i>M. hirtus</i>
<i>P. aurea potbergensis</i> (Rourke) Rourke	51	1.000	0.333	1	0.3333	BS: <i>P. mundii</i> also white protea group
<i>M. stokoei</i> Phill. & Hutch.	52	0.977	0.500	1	0.4887	BS: <i>M. hirtus</i> PS: Carlson and Holsinger (2010); BS: <i>P. mundii</i> also white protea group
<i>P. subvestita</i> NE.Br.	53	1.000	0.200	1	0.2000	also white protea group
Species without an vulnerability value						
<i>L. catherinae</i> Compton						
<i>L. conocarpodendron</i> <i>conocarpodendron</i> (L.) Buek.						
<i>L. erubescens</i> Rourke						
<i>L. fulgens</i> Rourke						
<i>L. muirii</i> Phill.						
<i>L. oleifolium</i> (Bergius) R.Br.						
<i>L. profugum</i> Rourke						
<i>L. spathulatum</i> R.Br.						
<i>L. tottum</i> (L.) R.Br.						
<i>M. arboreus</i> Rourke						
<i>M. argenteus</i> Salisb. ex Kn.						
<i>M. capitulatus</i> R.Br.						

M. palustris Salisb. ex Kn.
M. splendidus Salisb. ex Kn.
P. angustata R.Br.
P. compacta R.Br.
P. convexa Phill.
P. denticulata Rourke
P. holosericea (Salisb. ex Kn.) Rourke
P. inopina Rourke
P. longifolia minor
P. lorea R.Br.
P. nitida dwarf Miller
P. pendula R.Br.
P. pityphylla Phill.
P. pudens Rourke
P. stokoei Phill.
P. witzenbergiana Phill.

Appendix 3. Location and nectar amount for *Protea* and *Erica* species within the one hectare plots. In plots with a number of nectar producing species it was impossible to measure nectar for all species in one day and values from other sites are used.

Site	Plant Species	Nectar sugar mg per flower (<i>Erica</i>) or per inflorescence (<i>Protea</i>)
Kogelberg Harold Porter 2 and 3	<i>Erica coccinea</i>	0.02
Scarborough	<i>E. abietina</i>	0.08
West of Kleinmond	<i>E. perspicua</i>	0.49
Scarborough	<i>E. plukenetii</i>	0.05
Paarl mountain monument	<i>Protea burchelli</i>	113.25
East of Kleinmond	<i>P. compacta</i>	389
Helderberg nature reserve 1	<i>P. coronata</i>	1687
Paarl mountain	<i>P. laurifolia</i>	147
Du Toitskloof 1 and 3	<i>P. laurifolia</i>	281
Kogelberg Harold Porter 1	<i>P. lepidocarpodendron</i>	50
Cape Point	<i>P. lepidocarpodendron</i>	175
Kogelberg 1	<i>P. mundii</i>	39
Du Toitskloof pass 1	<i>P. repens</i>	314
Jonkershoek Swartboskloof	<i>P. neriifolia</i>	861
Jonkershoek fire break	<i>P. nitida</i>	64

3 Farming with native bees (*Apis mellifera* subsp. *capensis* Esch.) has varied effects on nectar-feeding bird communities in South African fynbos vegetation*

Outside their natural range, honeybees (*Apis mellifera*) are known to have detrimental effects on indigenous pollinators through exploitative or interference competition, but little is known about the effect of honeybee farming in areas where honeybees occur naturally. In the Cape Floristic Region of South Africa, where honeybees are indigenous, managed hives potentially elevate the abundance of honeybees far above natural levels, but impacts on other floral resource-dependent species have not been studied. Here I use experimental manipulation of honeybee density to test whether honeybee farming affects nectar-feeding birds. I selected the common sugarbush (*Protea repens*), utilized by both birds and bees, and analysed the time (before/after) by treatment (control/experiment) interaction to explore changes in bee abundance, nectar availability and bird abundance at three sites. Hive addition increased honeybee abundance in inflorescences of *P. repens* above expected levels. Despite experimental increase in honeybee numbers, there is no reduction in nectar sugar availability relative to the control areas. Where honeybee density was highest, Cape Sugarbird (*Promerops cafer*) numbers declined relative to expected, but sunbirds (Nectarinidae) were not affected at any of the sites. I conclude that stocking rates of more than one honey bee per *P. repens* inflorescence have detrimental effects on bird abundance due to interference, rather than resource competition.

Introduction

Honeybees (*Apis mellifera*) are invasive aliens in many parts of the world, where they disrupt native pollination mutualisms and have a negative impact on native pollinators (reviewed in Huryñ 1997; Goulson 2003; Paine 2004; Traveset and Richardson 2006). Honeybees are extreme generalist floral visitors, and their impact is felt throughout the pollination network. Many studies, however, have focused on impacts on nectar-feeding

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birds (Paton 1993). Outside their natural range, honeybees decrease flower visitation in native nectar-feeding birds through exploitative competition by reducing nectar availability, or more directly through interference competition (McDade and Kinsman 1980; Paton 1993; Gross and Mackay 1998; Hansen et al. 2002; Mallick and Driessen 2009). In contrast to the wealth of knowledge about the impacts of honeybees in areas where they are alien, little is known about the potential effect of honeybee farming on pollinators (and bird pollinators in particular) where honeybees are native (but see Brand 2009). Although nectar-feeding birds and honey bees has co-existed for a long time in the native range, the potential for impacts exists because honeybee farming elevates the traditionally low abundance of honeybees far above natural levels by supplying additional nest sites. This artificially elevated abundance is expected to negatively effect the traditional interactions with other floral visitors through exploitative (decreasing the shared resource, nectar) or interference competition.

The Cape honeybee, *Apis mellifera* subsp. *capensis* Eschscholtz, is endemic to the Cape Floristic region. In this region, its abundance might historically have been limited by the lack of nesting sites (Whitehead et al. 1987). Large parts of the Cape lack trees and rocky outcrops are scarce on the sandy coastal plain. Today, however, a large number of managed beehives (approx. 58000) are kept in the Cape Floristic Region (Hassan 2002). Beekeepers use fynbos vegetation outside reserves for honey production and as an over-wintering ground outside the fruit orchard pollination season (Hassan 2002). Many of the Cape's *Protea* species have their flowering peak in winter, and are subsequently heavily used by these managed honeybees.

In terms of food resources, Proteaceae are probably the most important plant family for nectar-feeding Cape Sugarbirds (*Promerops cafer*) and sunbirds (Nectarinidae) in fynbos vegetation. Interactions between Cape plants and nectar feeding birds can be extremely specialised (Geerts and Pauw 2009b). Similarly, some Proteaceae species are morphologically specialised for exclusive pollination by long-billed birds, but many are more generalised and have open inflorescences that allow visitation by a wide variety of birds and insects, including honeybees (Rebelo *et al.* 1984; Coetzee and Giliomee 1985; Wright *et al.* 1991; Wright 1994; Vaughton 1996; England *et al.* 2001; Tjorve *et al.* 2005; Whelan *et al.* 2009). Nectar-feeding birds, and sugarbirds in particular, track nectar resources

of the most abundant and widespread species like the generalist sugarbush, *Protea repens* (Fig. 3.1a) (Anderson *et al.* 1983; Harrison *et al.* 1997; Geerts and Pauw 2009a). This species is heavily utilised by both birds and bees and is therefore an ideal study species to assess the potential impact of managed honeybees (Burger *et al.* 1976; Anderson *et al.* 1983).

Beehives are transportable and therefore suitable for manipulative pollination experiments. I use this feature to answer the following questions in this pilot study: (1) Does the introduction of managed beehives elevate the abundance of honeybees in *P. repens* inflorescences above natural levels? (2) Does hive introduction lead to a decline in nectar availability in *P. repens* inflorescences? (3) Is nectar-feeding bird abundance affected in close proximity to beehives?

Materials and methods

Study species and study site

Protea repens (L.) occurs in dense similar-aged stands that recruit from seed after fires (Fig. 3.1b). Study sites were selected to have at least 7000 flowering *P. repens* individuals and none or few other *Protea* species inbetween (Rebelo 2006). Potential sites were excluded if sugarbirds were absent or beehives were already present. Three sites within protected areas (most natural vegetation outside reserves already had managed beehives in place) were selected: Franschoek in the Hottentots Holland Nature reserve (experimental site: 33°55'09.7"S, 19°09'43.3"E; control site: 33°54'53.1"S, 19°09'29.6"E); Jonkershoek Nature reserve (experimental site: 33°58'40.6"S, 18°56'41.5"E; control site: 33°59'23.6"S, 18°57'18.2"E) and Kogelberg Nature reserve (experimental site: 34°19'46.0"S, 18°58'47.8"E; control site: 34°20'26.8"S, 18°58'56.1"E).

Experimental design

Plant–animal mutualisms typically vary greatly in both space and time (e.g., Pauw 2006). To detect anthropogenic impacts against this background of natural variability, I use an experiment/control, before/after design (Stewart-Oaten *et al.* 1986), and test whether the experiment and control plots show different trends over time irrespective of differences in absolute levels.

At each site two 50 × 50 m plots (one control and one experimental) were laid out and five beehives were placed in the centre of each experimental plot. Honey bees firstly forage and deplete resources in close proximity to their hives (Paton 1999). With data collection done within plots, i.e. nearby the hives, fewer hives could be used than are typically used by beekeepers. Experimental and control plots were separated by at least 0.7 km (Franschhoek, 0.7 km; Jonkershoek, 1.7 km; Kogelberg, 1.3 km), because of the low number of hives, resource use would be most intense within about 0.5 km of the apiaries (Anderson et al. 1983; Wenner et al. 1991; Paton 1999). Bird and bee counts were conducted and nectar measured on 4 days before and 4 days after hive introduction. After hive introduction, I allowed a readjustment period of at least 3 days before observations were reinitiated.

Data collection was done in the morning between 9:00 and 10:30; in mid-winter this is the period avian nectarivores are most active (Fry 2000). Control and experimental plots were sampled immediately after each other in an alternating order. Rainy and windy days were avoided; therefore data collection was not on sequential days and a lengthy process (Appendix 1).

Honeybee and nectar abundance

During each of the eight observation periods at each site, honeybee numbers were estimated in the experimental and control plots by randomly selecting ten inflorescences and shaking them to release all bees (Fig. 3.1c).

On the same days, sugar availability was estimated for five inflorescences in peak flower in both the control and experimental plots. Inflorescences were sub-sampled by measuring the nectar volume and concentration in all flowers along a cross-section through the centre of the inflorescence. Nectar that spilt out between the flowers was measured before the inflorescence was sectioned. Data were scaled to the inflorescence level by multiplying the average nectar volume per flower with the number of flowers per inflorescence (range 41-180). Nectar was extracted using a 40µl capillary tube (Drummond Scientific Company, Broomall, PA, USA) and concentrations measured with a 0–50% handheld refractometer (Bellingham and Stanley, Tunbridge Wells, UK). Nectar volume was strongly affected by rain entering the open cup-shaped inflorescences; therefore, nectar volume and concentration

were converted to milligrams of sucrose per inflorescence. Numbers of inflorescences per plot were counted and multiplied with the average milligrams of sucrose per inflorescence to calculate total sugar per plot (nectar of all other *Protea* species in the plot was also measured).

Bird counts

Bird visitation to inflorescences was low. Therefore, for both the experimental and control plot all nectar-feeding birds were counted in 20-min sessions (before entering the plot for other observations). Bird counts were conducted by standing on a ladder ~ 1 m outside the plot in order to oversee the entire plot without disturbing birds and altering their movements. Individual birds were only counted once, even if they entered the plot multiple times. This was possible because the birds are few, conspicuous and do not move quickly.

Statistical analysis

The effect of beehive addition on (1) honeybee abundance in inflorescences, and (2) sunbird and sugarbird abundance was tested with generalised estimating equation (GEE) regression models (PROC GENMOD) with a log link function, a Poisson data distribution and the assumption of an exchangeable correlation matrix (SAS Version 9.1.3 Institute Inc. 2005). To test whether beehive addition caused a change in nectar, sucrose availability in inflorescences was subjected to a repeated-measures analysis of variance (RM-ANOVA) in STATISTICA 9.0 (StatSoft 2009, Tulsa, USA). Of particular importance is the significance of the interaction terms between treatment (hives/no hives) and time periods (before/after) at individual sites. A significant interaction indicates that the treatment factor does not react uniformly over all levels of the other factor namely time. The vertical bars in all figures denote 95% bootstrap confidence intervals.

Results

Bee numbers

Significance of the treatment by time interaction term indicated that the addition of managed beehives increased the abundance of honeybees in *P. repens* inflorescences relative to expected levels at two of the three sites (Table 3.1). Although not significant (probably due to large variation between days at control and experimental plots), highest bee numbers were

achieved at Franschoek, where hive addition elevated bee abundance to approximately one bee per inflorescence (Fig. 3.2). At Jonkershoek, a similar trend in the bee abundance was observed. At Kogelberg, however, the number of bees in inflorescences in the experimental plot stayed constant, while bee abundance in the control plot dropped significantly. Almost all bees were foraging for nectar rather than pollen.

Nectar

Nectar volumes per inflorescence varied from 0 to 13.58 ml (average = 3.37 ml, $n = 240$ inflorescences) while concentrations ranged from 1.5 to 43.4% sucrose (average = 15.0, $n = 240$ inflorescences). Nectar of the few co-occurring *Protea* species was also measured but was extremely low (less than 1% of total) compared with total nectar availability in *P. repens* (except for the control site at Jonkershoek). Sucrose per plot (other *Protea* species occurring in plot included) was highest at Franschoek (898.1 g) followed by Jonkershoek (578.4 g) and Kogelberg (132.4 g). The time period by treatment interaction term for sucrose content was not significant at any of the sites (Table 3.2), although a trend was observed at Franschoek (Fig. 3.2).

Bird counts

Sugarbird abundance at the Franschoek experimental site decreased significantly between before and after treatments ($P = 0.001$), with no significant effects at Kogelberg or Jonkershoek (Table 3.1; Fig. 3.2). For sunbirds (*Nectarinia famosa* and *Anthobaphes violacea*), the time by treatment interaction term was not significant for any of the three sites. Too few individuals of occasional nectar-feeding bird species (e.g. *Zosterops virens*, *Pycnonotus capensis*) were observed to warrant further analysis.

Table 3.1. Effect of experimental beehive addition on honeybee abundance and nectarivorous bird abundance in three populations of *Protea repens*.

Site	Bees	Sunbirds	Sugarbirds
Franschhoek	$Z = 0.89, P > 0.05$	$Z = -0.43, P > 0.05$	$Z = -4.48, P < 0.05$
Jonkershoek	$Z = 2.05, P < 0.05$	$Z = 1.23, P > 0.05$	$Z = -0.61, P > 0.05$
Kogelberg	$Z = 2.35, P < 0.05$	$Z = -1.27, P > 0.05$	$Z = -0.68, P > 0.05$

Values are for the interaction term (Treatment * Time) within a Generalized Estimation Equation model (GEE).

Table 3.2. Effect of experimental beehive addition on nectar availability quantified as sucrose availability in three *Protea repens* populations.

Site	<i>df</i>	MS	<i>F</i>	<i>P</i>
Franschhoek	1	415	0.04	NS
Jonkershoek	1	150827	2.12	NS
Kogelberg	1	54016	1.04	NS

Values are for the interaction term (Treatment * Time period) for the repeated-measures analysis of variance (RM-ANOVA).

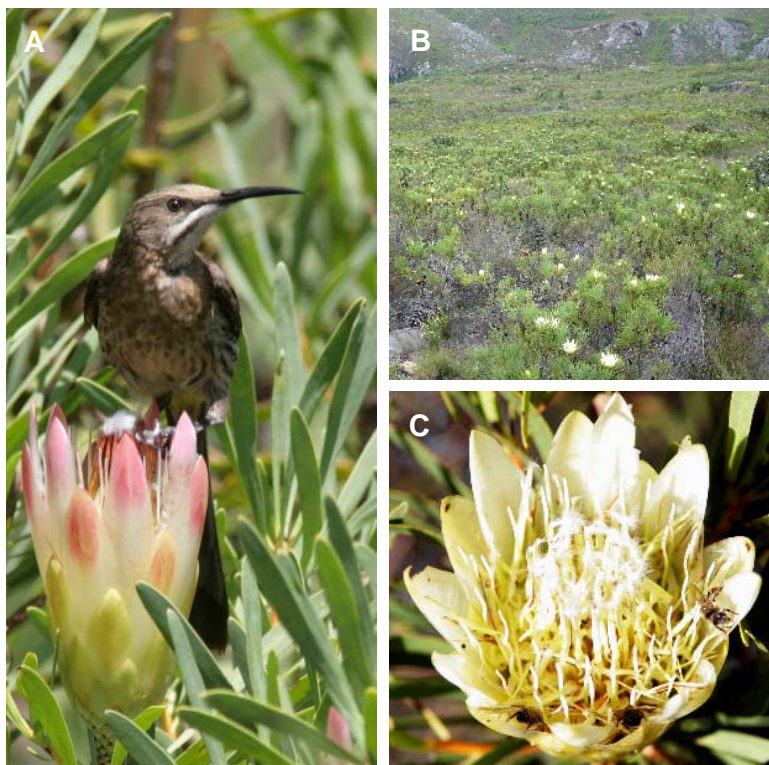


Figure 3.1 (a) Cape Sugarbird (*Promerops cafer*) visiting a *Protea repens* inflorescence. (b) Mono-dominant stand of *Protea repens* at one of the study sites. (c) *P. repens* inflorescence with honeybees. (photographs by Sjirk Geerts)

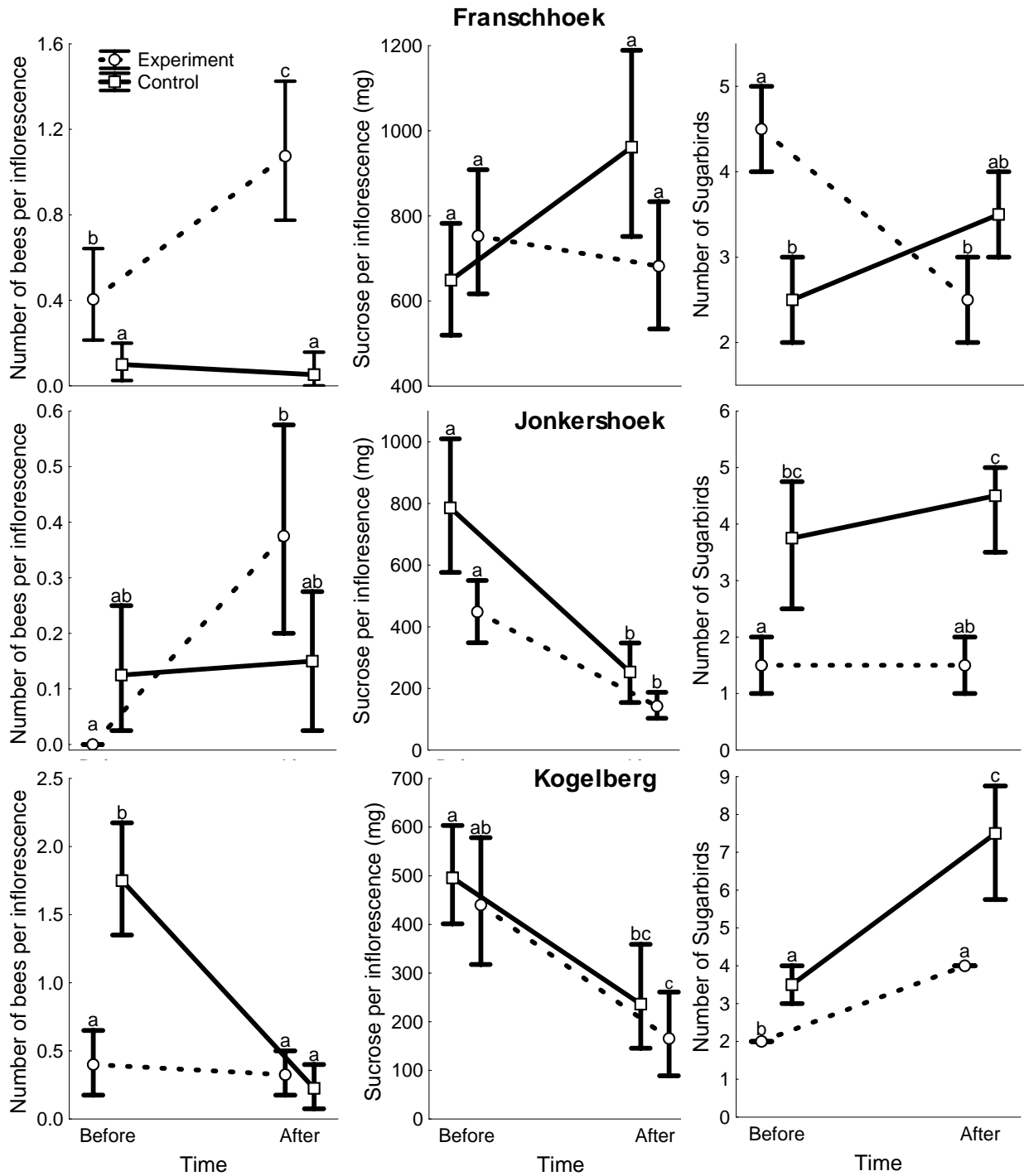


Figure 3.2. Effect of hive introduction on honeybee abundance in flowers, nectar sugar availability and the number of sugarbirds in three populations of *Protea repens* (Franschhoek, Jonkershoek and Kogelberg). Symbols above bars indicate significant differences. Vertical bars denote 95% bootstrap confidence intervals.

Discussion

Across the three study sites, beehive addition had mixed effects on native nectar-feeding birds with no significant effect on nectar. Hive addition increased honeybee abundance in inflorescences of *Protea repens* above expected levels at two of the three sites, with a non-significant trend at the third site. At the site (Franschhoek) where the highest experimental honeybee density was reached, the number of sugarbirds also declined relative to expected levels, but sunbirds were not significantly affected at any of the sites. It is worth noting that data collection at this particular site was also at the peak of the breeding season (May to July) for sugarbirds (Calf et al. 2003).

The lack of an effect of bees on *P. repens* nectar might be a consequence of the relatively small number of hives that were introduced per plot and the super-abundance of nectar in the inflorescences (Paton 1999). Nectar-feeding birds are known to cease visitation when bees become active and abundant in a resource limited environment; separating the floral resources during the day through exploitative competition (Valido et al. 2002; Dupont et al. 2004). At most, hive addition increased the abundance of honeybees by about one bee per inflorescence (Franschhoek; Fig. 3.2), while smaller increases were achieved at the other two sites. It can be expected that these small increases will not affect nectar availability (which reached almost 900 g per 2500 m²) and therefore will not lead to resource competition.

Even in the absence of competition for shared resources, bees are known to exclude nectar-feeding birds by direct interference (see, e.g., Gill et al. 1982). The density, however, at which sugarbirds start to respond to honeybee numbers is unknown (M. Allsopp, personal communication). Although not explicitly tested, there might be some support for competition via this route at Franschhoek, where a significant response was detected in sugarbird abundance with no measurable effect on nectar availability. Perhaps birds will shift to utilising different co-occurring *Protea* species, and are therefore capable of persisting in the habitat (Hansen et al. 2002) or increasing territory size with an increase in bee numbers (Paton 1993; Calf et al. 2003).

Differential exclusion experiments show that *P. repens* is self-incompatible, and insect pollination alone results in as much seed set as insect and bird pollination combined (Coetzee

and Giliomee 1985). A reduction in bird abundance with a concomitant increase in honeybee abundance is thus unlikely to result in large changes in seed set for *P. repens*, although mating patterns and gene flow might be affected. In fact, due to the relatively generalised pollination system of *P. repens*, managed honeybee colonies could potentially replace lost bird pollinators in fragmented areas (Paton 1999; Dick 2001; Goulson 2003).

Careful guidelines have been developed to regulate the exploitation of natural vegetation by livestock farmers. To the best of our knowledge, comparable stocking densities have not been suggested for honeybee farming. This study shows that modest stocking rates have little impact on nectarivorous birds that depend on *Protea repens* nectar. In addition, it would be interesting to consider plant species that have lower nectar volumes, as well as plant species that are used as a pollen source by managed honeybees.

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References

- Anderson RH, Buys B, Johannsmeier MF (1983) Beekeeping in South Africa. Bulletin No. 394, Department of Agriculture, Pretoria, South Africa
- Brand MR (2009) The short term impact of a collection of commercial Cape honeybee (*Apis mellifera capensis* Esch.) colonies on invertebrate flower visitors within a near pristine Fynbos habitat in the Cape Floristic Region. MSc Dissertation, Stellenbosch University, Stellenbosch
- Burger AE, Siegfried WR, Frost PGH (1976) Nest-site selection in the Cape Sugarbird. Zool Afr 11:127-158
- Calf KM, Downs CT, Cherry MI (2003) Territoriality of Cape Sugarbirds (*Promerops cafer*) between and within breeding seasons. Ostrich 74:125-128
- Coetzee JH, Giliomee JH (1985) Insects in association with the inflorescence of *Protea repens* (L.) (Proteaceae) and their role in pollination. Entomol Soc S Afr 48:303-314

- Dick CW (2001) Genetic rescue of remnant tropical trees by an alien pollinator. *Proc R Soc Lond B* 268:2391-2396
- Dupont YL, Hansen DM, Valido A, Olesen JM (2004) Impact of introduced honey bees on native pollination interactions of the endemic *Echium wildpretii* (Boraginaceae) on Tenerife, Canary Islands. *Biol Conserv* 118:301-311
- England PR, Beynon F, Ayre DJ, Whelan RJ (2001) A molecular genetic assessment of mating-system variation in a naturally bird-pollinated shrub: contributions from birds and introduced honeybees. *Conserv Biol* 15:1645-1655
- Fry CH, Keith S, Urban EK (2000) *The birds of Africa*, vol VI. Academic Press, London
- Geerts S, Pauw A (2009a) African sunbirds hover to pollinate an invasive hummingbird-pollinated plant. *Oikos* 118:573-579
- Geerts S, Pauw A (2009b) Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *S Afr J Bot* 75:699-706
- Gill FB, Mack AL, Ray RT (1982) Competition between hermit hummingbirds Phaethorninae and insects for nectar in a Costa Rican rain forest. *Ibis* 124:44-49
- Goulson D (2003) Effects of introduced bees on native ecosystems. *Annu Rev Ecol Evol Syst* 34:1-26
- Gross CL, Mackay D (1998) Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomataceae). *Biol Conserv* 86:169-178
- Hansen DM, Olesen JM, Jones CG (2002) Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? *J Biogeogr* 29:721-734
- Harrison JA, Allan DG, Underhill LG, Herremans M, Tree AJ, Parker V, Brown CJ (eds) (1997) *The atlas of southern African birds*. BirdLife South Africa, Johannesburg
- Hassan RME (2002) Accounting for stock and flow values of woody land resources: methods and results from South Africa. CEEPA, University of Pretoria, Pretoria
- Huryn VMB (1997) Ecological impacts of introduced honey bees. *Q Rev Biol* 72:275-297
- Mallick SA, Driessen MM (2009) Impacts of hive honeybees on Tasmanian leatherwood *Eucryphia lucida* Labill. (Eucryphiaceae). *Austral Ecol* 34:185-195
- McDade LA, Kinsman S (1980) The impact of floral parasitism in two neotropical hummingbird-pollinated plant species. *Evolution* 34:944-958
- Paini DR (2004) Impact of the introduced honey bee (*Apis mellifera*) (Hymenoptera: Apidae) on native bees: a review. *Austral Ecol* 29:399-407

- Paton DC (1993) Honeybees in the Australian environment. *Bioscience* 43:95-103
- Paton DC (1999) Impact of commercial honeybees on flora and fauna in Ngarkat Conservation Park. Rural Industries Research and Development Corporation, Canberra
- Pauw A (2006) Floral syndromes accurately predict pollination by a specialized oil-collecting bee (*Rediviva peringueyi*, Melittidae) in a guild of South African orchids (Coryciinae). *Am J Bot* 93:917-926
- Rebelo AG (2006) Protea atlas project website. <http://protea.worldonline.co.za/default.htm>.
- Rebelo AG, Siegfried WR, Crowe AA (1984) Avian pollinators and the pollination syndromes of selected mountain fynbos plants. *S Afr J Bot* 3:285-296
- Stewart-Oaten A, Murdoch WW, Parker KR (1986) Environmental-impact assessment – pseudoreplication in time. *Ecology* 67:929-940
- Tjorve KMC, Geertsema GH, Underhill LG (2005) Do sugarbirds feed on arthropods inside or outside Protea inflorescences? *Emu* 105:293-297
- Traveset A, Richardson DM (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends Ecol Evol* 21:208-216
- Valido A, Dupont YL, Hansen DM (2002) Native birds and insects, and introduced honey bees visiting *Echium wildpretii* (Boraginaceae) in the Canary Islands. *Acta Oecol* 23:413-419
- Vaughton G (1996) Pollination disruption by European honeybees in the Australian bird-pollinated shrub *Grevillea barklyana* (Proteaceae). *Plant Syst Evol* 200:89-100
- Wenner AM, Meade DE, Friesen LJ (1991) Recruitment, search behavior, and flight ranges of honey-bees. *Am Zool* 31:768-782
- Whelan RJ, Ayre DJ, Beynon FM (2009) The birds and the bees: pollinator behaviour and variation in the mating system of the rare shrub *Grevillea macleayana*. *Ann Bot London* 103:1395-1401
- Whitehead VB, Giliomee JH, Rebelo AG (1987) Insect pollination in the Cape flora. In: Rebelo, A.G. (ed.). A preliminary synthesis of pollination biology in the Cape flora. South African national scientific programmes report No. 141. CSIR, Pretoria. pp. 52-82.
- Wright MG (1994) Seed production by *Protea laurifolia* (Proteaceae) after insect versus insect and bird pollination: a quality difference. *S Afr J Sci* 90:199
- Wright MG, Visser D, Coetzee JH, Giliomee JH (1991) Insect and bird pollination of Protea species in the Western Cape – further data. *S Afr J Sci* 87:214-215

Supplementary Material Appendix 1. Data collection dates for bee and bird counts and nectar measurements and date of hive introduction at each site.

Site	Date of data collection	Treatment (bee and bird counts and nectar measurements)
Franschhoek	05-Jun-08	Before
	06-Jun-08	Before
	09-Jun-08	Before
	10-Jun-08	Before
	10-Jun-08	Bee hives introduced
	13-Jun-08	After
	14-Jun-08	After
	18-Jun-08	After
	24-Jun-08	After
Jonkershoek	27-Jun-08	Before
	30-Jun-08	Before
	01-Jul-08	Before
	02-Jul-08	Before
	10-Jul-08	Bee hives introduced
	14-Jul-08	After
	16-Jul-08	After
	18-Jul-08	After
	19-Jul-08	After
Kogelberg	25-Jul-08	Before
	30-Jul-08	Before
	04-Aug-08	Before
	06-Aug-08	Before
	13-Aug-08	Bee hives introduced
	18-Aug-08	After
	20-Aug-08	After
	22-Aug-08	After
	27-Aug-08	After

4 Easy technique for assessing pollination rates in the genus *Erica* reveals road impact on bird pollination in the Cape fynbos, South Africa *

Road traffic is one of the most pervasive forms of anthropogenic disturbance, but its impact on pollination, a potentially sensitive ecosystem process, has not been investigated. Such an assessment is needed in order to evaluate the potential for roadside verges to act as biodiversity refugia and corridors in otherwise transformed landscapes. Here, I document the impact of a two lane tar road on pollination by birds in the Cape fynbos of South-Africa. To do so, I developed a quick and widely applicable method of determining pollination rates in bird pollinated members of the large genus *Erica*. Experiments with caged birds showed that the status of the anther ring (broken/perfect) indicated a sunbird visit with 92% accuracy, while field surveys confirmed anther ring status also serves as a proxy for pollen receipt to stigmas. Using this technique I determined pollination rate in *Erica perspicua* at three distances from the road (0-10m, 20-30 and 40-50 m). After controlling for flower colour, robbing rate and plant density, significantly fewer anther rings were disturbed in close proximity to the road. The documented two-fold decline in pollination along road sides could have important implications for the way we view and manage road verges as refugia for species and ecological processes.

Introduction

Road construction is one of the most widespread forms of landscape modification (Trombulak and Frissell 2000; Laurance *et al.* 2008). Road impacts include: edge effects, changes in animal behaviour, collision with vehicles, spread of exotics and alterations in the chemical and physical environment (reviewed in Forman and Alexander 1998; Spellerberg 1998) with potential impacts, such as road avoidance by birds, extending beyond one kilometre (van der Zande *et al.* 1980; Forman and Deblinger 2000).

*Geerts, S. & Pauw, A. Austral Ecology in press, doi:10.1111/j.1442-9993.2010.02201.x

Road impacts on avian species have received much attention, in particular road kills, edge effects and breeding suppression close to roads. Research has found that responses of different species vary, but total bird captures declined significantly at road edges as most birds are reluctant to cross a road (Develey and Stouffer 2001; Laurance *et al.* 2004). Some birds however prefer roads (Dean and Milton 2003; Lambertucci *et al.* 2009) so it seems specific species (Kuitunen *et al.* 1998) or guilds (Forman and Alexander 1998; Kuitunen *et al.* 1998; Laurance *et al.* 2004) of birds are more affected. Among the various reasons proposed, including predators, visual disturbance and air pollutants, traffic noise seems to be the major cause for changes in avian community assembly (see review by Forman and Alexander 1998; Forman and Deblinger 2000; Peris and Pescador 2004).

Despite the adaptability of nectar-feeding birds (Geerts and Pauw 2009a), plant populations occurring on road sides, and dependent on birds for pollen transfer, could be at risk of pollination failure due to the disruption of foraging patterns. Few studies however have assessed this potential impact with most bird pollination studies focusing on the fragmentation caused by roads rather than the impacts of the roads themselves (see for example Cunningham 2000; Mayfield *et al.* 2006). Despite the potential for negative effects on animals, roadside environments are known to act as refugia for many plant species and are gaining recognition for their role in nature conservation and in maintaining genetic connectivity of the landscape (Pauwels and Gulinck 2000; Byrne *et al.* 2007). However, the preservation of ecological processes in road side environments and conserving the ecological processes involved has received little attention (but see for example Bhattacharya *et al.* 2003; Hopwood 2008). To truly recognize road impacts Trombulak and Frissell (2000) have advocated a broader view by rather focusing on the ecosystem and community level. Deterioration of certain processes (i.e. seed dispersal and pollination) in roadside reserves might alter the reproductive success of plant communities with a subsequent loss in biodiversity (Trombulak and Frissell 2000).

Pollination studies are often hampered by the difficulty of obtaining quantitative data in dense stands of flowering plants where flower visitors are scarce. Hence it is extremely valuable to identify easily quantifiable proxies for visitation rate as quantification of visitation rates through direct observation is time consuming. For example, flower

visitation can be easily determined in orchids (Nilsson 1992; Pauw 2007) and certain milkweeds (Ollerton and Liede 1997) by quantifying pollinaria removal. In other plant species tripped flowers (Parker 1997) or bumble bee claw marks allow rapid assessment of visitation rates (Washitani *et al.* 1995). Similarly it has been suggested that the probing beak of a visiting sunbird could disturb the anther ring of bird pollinated *Erica* flowers (Rebelo *et al.* 1985; Schumann *et al.* 1992). Before receiving a visit, the anthers of *Erica* species are fused at their pores into a perfect ring. No pollen is released until the anther ring is ruptured. Whether this is an accurate predictor of visitation rate and pollen transfer however, has not been tested before. The condition of the anther ring might be a poor indicator of bird visits if the rings rupture spontaneously during development, if they fail to rupture during legitimate bird visits, or if they rupture during visits by nectar robbers.

There are more than 860 species of *Erica* in South Africa compared to about a dozen species in Europe and the rest of Africa (Baker and Oliver 1967; Schumann *et al.* 1992). More than 400 *Erica* species occur in the South-Western Cape. Autogamy is apparently rare in most of the South-Western Cape *Erica* species (Rebelo *et al.* 1985).

Approximately 70 species are pollinated by birds, mainly by the relatively short-billed Orange-breasted Sunbird (*Anthobaphes violacea*), the only nectar feeding bird almost exclusively associated with *Erica* species (Rebelo *et al.* 1984; Rebelo *et al.* 1985; Schumann *et al.* 1992; Geerts and Pauw 2009b).

I assessed the indirect effects of roads on bird pollination through changes in bird behaviour. There are few studies of road effects in South-Africa with no studies in the fynbos vegetation at the Cape, despite the conservation importance of road verges in this region (Dean and Milton 2003; Weiermans and van Aarde 2003). Therefore in this study I test: whether disturbed anther rings are a good proxy for pollination rate (1) by testing whether a sunbird visit disturbs the anther ring and (2) by the presence or absence of pollen on stigmas of flowers with disturbed and intact anther rings. To verify the utility of this technique in a field setting I examined road impacts on flower visitation by (1) correlating number of anther rings disturbed to distance from a road and (2) excluding plant density, robbing rate and flower colour as possible predictors of flower visitation.

Methods

Study species

Erica perspicua J.C.Wendl is a bird pollinated species which grows in great profusion in marshy areas at low altitudes and which is particularly common in the study area. They grow up to 2 m tall and bear long spikes of flowers. The tubular corolla varies in length from 10 to 20 mm (Schumann *et al.* 1992). In caged bird experiments I used another typical bird pollinated plant, *E. versicolor*. This widespread species forms a sturdy bush with firm branches bearing the tubular flowers. The flowers were obtained from cultivated plants from the Jan Marais reserve (33°55'98.7"S; 18°52'38.3"E) in Stellenbosch. *E. versicolor* flowers are very similar to those of *E. perspicua* (both in section *Evanthe*). Ideally one would want to use the same plant species throughout, but *E. versicolor* was flowering in close proximity for the caged bird experiments and fresh flowers could thus be obtained daily. *E. perspicua* on the other hand occurs in a number of discrete populations along road sides, and was therefore the ideal study species for the second part of the study.

Caged bird experiment – Erica versicolor

Two Orange-breasted Sunbirds, a juvenile male and a female were captured in Jonkershoek Nature reserve (33°59'27.8"S; 18°57'12.9"E) in the Western Cape on the 19th of June 2007. The sunbirds were acclimatized in the laboratory for two days. A cage with dimensions of 1m x 1m x 0.5m was used with the bottom, top and back of the cage constructed of wood and the other sides covered with shade cloth. Sunbirds were fed *ad lib* on sugar water (25% sucrose), *Protea repens* and *Protea neriifolia* inflorescences. At commencing of the experiment all food sources were removed. To test the relationship between pollination rate and the condition of the anther ring, virgin *E. versicolor* flowers (n = 103) were exposed to caged sunbirds. Flowers with disturbed anther rings were removed from inflorescence prior to exposure. Only a few flowers (n < 5) were presented at a time to ensure accurate observation of all sunbird visits. After 5 minutes, or when all flowers were at least visited once, flowers were removed and anther rings were scored as intact or disturbed (only three awkwardly placed flowers were not visited, and were not included in the analysis). Control flowers were treated like experimental flowers, except they were not placed in, but directly adjacent to the cage.

In *E. versicolor* the stigma is exerted slightly beyond the anthers and can be removed to quantify the number of pollen grains on the stigma, without disturbing the anthers or releasing the pollen. The stigmas from visited flowers with disturbed anther rings were removed and placed on fuchsin-stained gel, heated until melting point, then placed on a glass slide for counting under a 10x magnification.

Stigma pollen loads under natural conditions

To quantify stigmatic pollen loads under natural conditions I collected 25 *E. perspicua* branches and selected paired flowers per branch (disturbed and undisturbed anther ring) for determination of stigmatic pollen loads.

Other floral visitors

To examine whether insects disturb anther rings, exclusion experiments were undertaken in natural populations of *E. versicolor* in the Jan Marais reserve. To exclude birds, floral buds were bagged with wire mesh (mesh size = 12 mm) (n = 34). Buds bagged with gauze bags excluded both birds and insects (n = 117), whilst in open flowers all floral visitors were allowed (n = 74). After 5 days when all buds were open, flowers were examined and anther rings noted as intact or disturbed (4 July 2007). Finally, a few weeks after flowering, fruit set was quantified.

Road impact on pollination rate - Erica perspicua

After testing the accuracy of the disturbed anther ring technique as a proxy for pollination rate I used this technique to test road impact on bird pollination along a two lane tar road, the R44, at Betties Bay (34°21'38.8"S; 18°52'27.6"E). According to PAWC (2004) traffic volume for this road section (TR02701) was approximately 3301 vehicles per day with 6.5% of these being heavy vehicles. The speed limit varies between 80 km/hour and 100 km/hour. The road cuts through the hyper-diverse Kogelberg Biosphere Reserve. Large areas of natural vegetation (approximately 8 years old) still line this road including patchily distributed populations of *Erica perspicua*.

In April 2008, eight *E. perspicua* populations were sampled on each side of the road at three distances. The populations were distributed along approximately 15 km of the road. Since road effects on birds are known to be strongest within the first 50 to 100 metres of the edge, 10 m by 10 m plots per population were established at 0-10m (zero

is the point where vegetation begins, as first 2 metres are regularly mowed), 20-30m and 40-50m from the road edge (Laurance *et al.* 1998; Laurance *et al.* 2002; Laurance 2004). Five flowers from 16 randomly selected plants per plot were scored for disturbed anther rings and robbing rate. Robbing was confirmed by the presence of a small slit made by robbers (mainly honeybees and carpenter bees; pers. obs.) at the base of the corolla. When there were fewer than 16 plants (6 out of 24 plots), more flowers per plant were examined.

I included in the analysis three additional variables that might influence pollination rate: (1) Plant density is well known to affect pollination rate (Kunin 1993), and *E. perspicua* density is likely to vary with distance from the road (see pages areas closer to roads were narrower). (2) Similarly, robbing rate (the number of flowers damaged by nectar thieving insects) might be correlated with distance from the road and might affect pollination rate by the legitimate pollinators if they can distinguish damaged flowers with lower rewards (reviewed in Maloof and Inouye 2000). (3) Flower colour varies from white to dark pink and birds might be attracted to a particular colour.

Statistics

For the caged bird experiments a Mann-Whitney *U*-Test was used to test differences in stigma pollen counts between visited and control flowers. Paired flowers per branch (disturbed and undisturbed anther ring) for determination of stigmatic pollen loads under natural conditions did not fit a normal distribution and were analysed with a Wilcoxon matched-pairs test. These analyses were conducted in STATISTICA 8.0 (StatSoft, Inc. 2008, Tulsa, USA). To avoid pseudo replication within plots, for the road impact on visitation rate study, a Mixed Models analysis was performed. This method estimates the various effects using a restricted maximum likelihood method (REML) and as such does not produce the OLS estimates (MS and SS). The Mixed Models procedure was used to model the necessary correlation between pink and white flowers within a plot in the presence of continuous covariates. The following effects were estimated: colour and distance from the road. Colour was considered a within-factor effect and distance from road was treated as a between-factor effect. Density and robbing rate were included as continuous covariates in the model (PROC MIXED, SAS v 9.1, Cary, North Carolina, USA).

Results

Caged bird experiment – Erica versicolor

Monitoring of sunbird behaviour at *E. versicolor* flowers revealed that the sunbird beak enters the flower with some force, which ruptures the anther ring and releases an explosive puff of pollen (Fig. 4.1a). The stigma of the next flower makes contact with the pollen on the sunbird's beak before the beak contacts the anthers (Fig. 4.1b). 92% of anther rings were disturbed in flowers ($n = 103$) that received visits from sunbirds, whereas no anther rings were disturbed in the control flowers ($n = 24$). Stigmatic pollen counts for *E. versicolor* were significantly lower for flowers with intact (Fig. 4.1c) versus flowers with disturbed (Fig. 4.1d) anther rings ($n_{\text{group 1}} = 22$, $n_{\text{group 2}} = 21$, $z = -4.37$, $p < 0.01$, Mann-Whitney *U*-Test, Fig. 4.2a).

Stigma pollen loads under natural conditions

For the *E. perspicua* flowers collected from the natural populations, flowers with disturbed anther rings had significantly more pollen on the stigma than flowers from the same branch with undisturbed anther rings (Wilcoxon matched-pairs test, $z = 4.37$, $p < 0.01$, $n = 25$, Fig. 4.2b).

Other floral visitors

Other than Orange-breasted Sunbirds and Southern Double-collared Sunbirds (*Cinnyris chalybea*) (Fig. 4.1e), honey bees (*Apis mellifera*) and solitary bees also visit *E. versicolor* flowers. In the treatment that excluded birds but allowed insects, no rings were ruptured relative to the open control, indicating that bees are apparently incapable of disturbing the anther ring. For bagged flowers only one flower ($n = 117$ flowers) had a ruptured anther ring, indicating that anther rings rarely rupture spontaneously during the development of the flower (Table 4.1). Fruit set was highest in open flowers (Table 4.1).

Road impact on pollination rate - Erica perspicua

Mean density of plants was 46 (range 1 - 212) plants per 10 m². In total 1630 flowers were examined (655 white and 975 pink). 11% of flowers were robbed. Colour had no effect on robbing rate ($U = 259$, $z = 0.58$, $p = 0.56$, Mann-Whitney *U*-Test). Density, flower colour and robbing rate had no effect on the number of flowers with disturbed anther rings (Table 4.2). Distance from the road was a significant covariate in our

analysis and had a negative impact on bird pollination (Table 4.2; Mixed Models Repeated Measures ANOVA $F_{2, 14} = 4.35$, $p = 0.03$); with a significantly lower number of flowers with ruptured anther rings at the road side edge than the plots further removed from the road (Fig.4.3; post hoc Bonferroni, $p < 0.05$).

Table 4.1. Testing the potential of floral visitors, other than sunbirds, to disturb the anther ring and result in fruit set in *Erica versicolor*. Percentage of flowers with disturbed anther rings and fruit set to flower ratio for the three treatments are presented.

	Percentage anther rings disturbed (<i>N</i> flowers)	Percentage fruit set (<i>N</i> flowers)
Open flowers	90.5 (74)	95.3 (43)
Bagged flowers	0.9 (117)	13.3 (15)
Flowers in wire cages	0 (34)	10.5 (19)

Table 4.2. Mixed Models Repeated Measures analysis for effects of distance from road, density, flower colour and robbing rate on percentage of anther rings disturbed (pollination rate) in *Erica perspicua*.

Source	df (between groups)	df (within group)	<i>F</i>	<i>P</i>
Distance from road	2	14	4.35	0.03
Plant density	1	33	2.87	0.10
Flower colour	1	7	1.89	0.21
Percentage of flowers robbed	1	33	1.34	0.26
Distance from road×colour	2	14	2.12	0.16

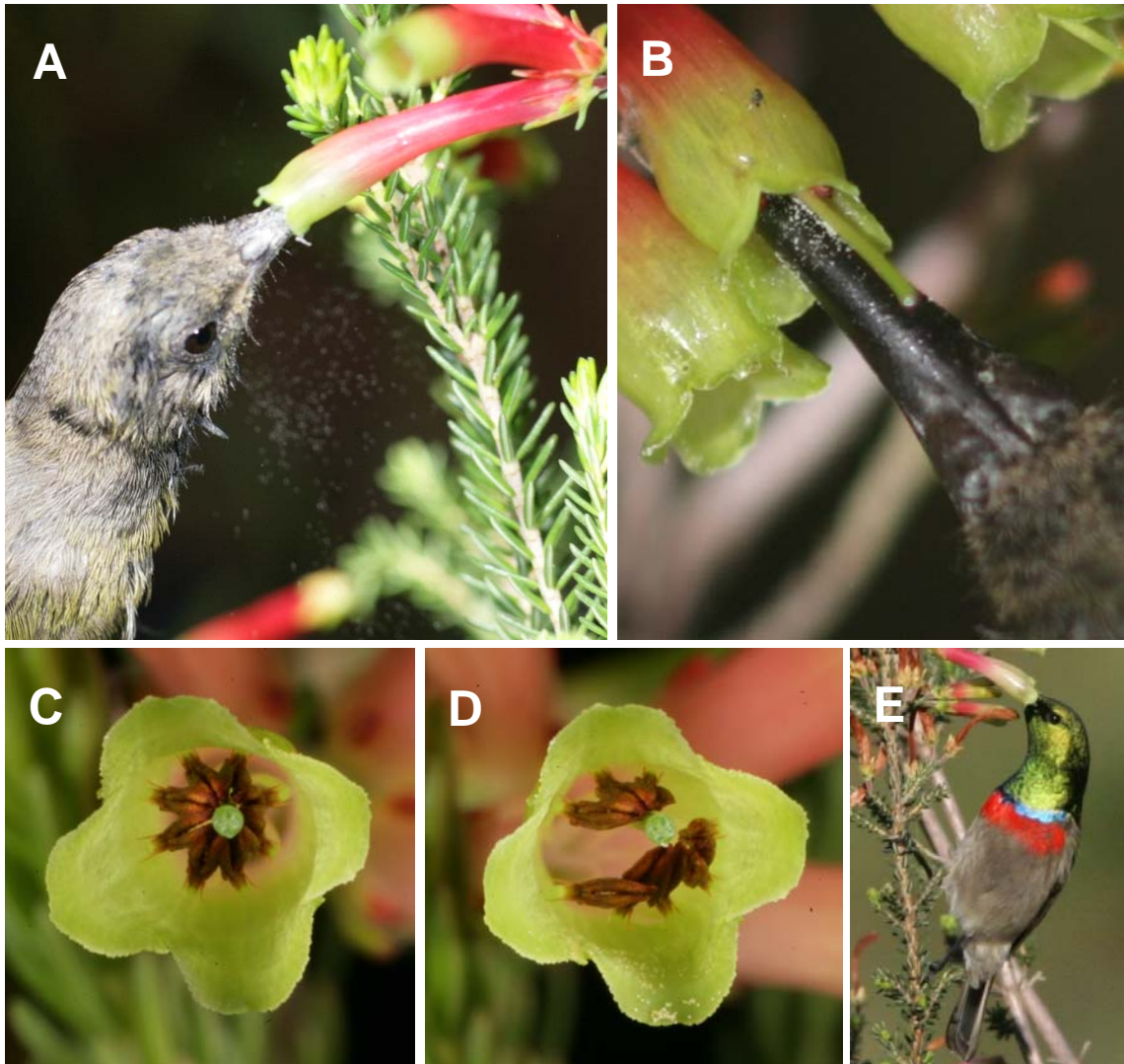


Figure 4.1 (a) A juvenile Orange-breasted Sunbird visiting a *E. versicolor* flower in the laboratory, note the pollen cloud. (b) Pollen on the beak of an Orange-breasted Sunbird contacts the exerted stigma. (c) A virgin *Erica versicolor* flower with intact anther ring. (d) A ruptured anther ring after being visited by an Orange-breasted Sunbird. (e) Other sunbirds like this Southern Double-collared Sunbird also visit *Erica versicolor*. (photographs by Sjirk Geerts)

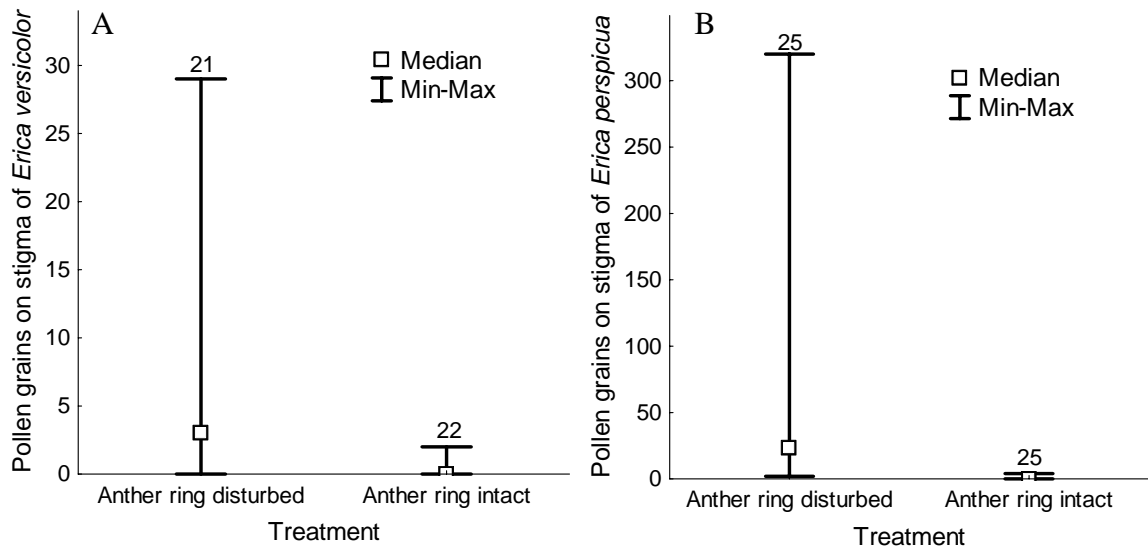


Figure 4.2 (a) Amount of pollen on stigmas of virgin *E. versicolor* flowers was significantly lower than on flowers visited by caged sunbirds. (b) Amount of pollen on stigmas of 25 pairs of field collected *E. perspicua* flowers was significantly lower on intact versus disturbed anther rings. Numbers above bars indicate number of flowers.

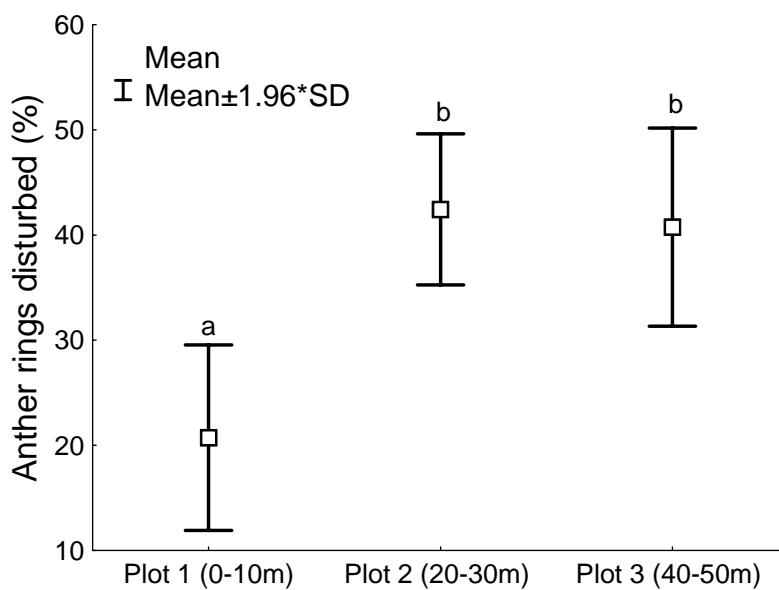


Figure 4.3. Pollination rate varies significantly with distance from the road edge (Mixed Models Repeated Measures analysis ANOVA $F_{2,14} 4.35$, $p = 0.03$) with significantly lower pollination rate closer to the road (post hoc Bonferroni, $p < 0.05$). Homogenous groups share the same letter.

Discussion

Our results suggest that a two-lane tar road, with relatively low traffic volumes, has a negative impact on bird pollination in close proximity to the road. The only similar assessment found no effect of roads on nectar feeding bird abundance or diversity, but pollination rate was not quantified (Laurance 2004). The relatively short impact distances detected in this study (10 m) could suggest that road effects on bird pollination are minor relative to effects on breeding birds, which have been reported to reach up to kilometres from highways (Reijnen *et al.* 1996; Palomino and Carrascal 2007). Furthermore, the road studied here has a relatively low traffic volume, and busier roads are likely to have a greater impact and affect a larger area (Van der Zande *et al.* 1980; Forman *et al.* 2002).

Reasons for lower pollination rates in close proximity to the road were not tested; noise being most probable (Forman and Deblinger 2000). Territory size of nectarivorous birds however varies with distance from the road and could be as much as 25% larger near road edges than in forest interiors (Ortega and Capen 1999), and larger territories would reduce visitation rate to individual flowers.

Our results highlight previous findings that the full effects of roads on ecological processes might be undetectable for many years, because despite a decline in reproduction long living plants might persist for some time (Findlay and Bourdages 2000). Although the plant community will persist for many years the pollinator dependant species might decline and the road verge community structure might eventually change. With 60% of bird species occurring at a lower density near highways this pattern could be more widespread than initially thought (Reijnen *et al.* 1995; Reijnen *et al.* 1996). Relevant questions are the degree of dependence on pollinators for seed set and dependence on seeds for population persistence (Bond 1994). Comparing current road edge biodiversity with historical data could reveal interesting insights into the long-term effects of depressed pollination on road verge biodiversity (Spooner and Lunt 2004).

From the data presented here a ruptured anther ring is a good predictor of a sunbird visitor and pollen transfer onto the stigma. This technique is therefore useful to quickly obtain a snapshot of pollinator activity, a rapid assessment for a large quantity of data.

When the anthers are not disturbed however, they will eventually part on their own (T. Oliver 2007 pers. comm.), therefore it is suggested that when determining pollination rate withered flowers are avoided. Other than an indication of a visit by a sunbird a disturbed anther ring is also indicative of pollen receipt by the stigma.

The disturbed anther ring technique allows examination along natural or anthropogenic gradients, one of which was explored here (road impact). Furthermore the geographic mosaic of pollination rates can be established, although the technique supplies no information on the pollinator fauna itself. So for example could it be useful in determining the pollination rate in rare and endangered species like the reintroduced bird pollinated *Erica verticillata* (extinct in the wild), or in one of the more than hundred *Erica* species that are currently listed as endangered or critically endangered (Raimondo et al. 2009). Potentially the disturbed anther ring technique applies to other Ericaceae members with similar floral morphology; whether hummingbird pollinated or pollinated by insects (Freitas *et al.* 2006). The genus *Vaccinium* (Ericaceae), for example, has a very similar floral morphology and contains approximately 450 species (mostly from the Northern Hemisphere) including commercially important plants. I thus hope that this technique will be useful in answering conservation related questions and assist in pollination studies of endangered and economically important plants with similar floral morphologies.

Acknowledgements

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References

Baker H. A. & Oliver E. G. H. (1967) *Ericas in Southern Africa*. Purnell Press, Cape Town

- Bhattacharya M., Primack R. B. & Gerwein J. (2003) Are roads and railroads barriers to bumblebee movement in a temperate suburban conservation area? *Biol Conserv* 109: 37-45
- Bond W. J. (1994) Do mutualisms matter - assessing the impact of pollinator and disperser disruption on plant extinction. *Philos Trans R Soc Lond Ser B-Biol Sci* 344: 83-90
- Byrne M., Elliott C. P., Yates C. & Coates D. J. (2007) Extensive pollen dispersal in a bird-pollinated shrub, *Calothamnus quadrifidus*, in a fragmented landscape. *Mol Ecol* 16: 1303-14
- Cunningham S. A. (2000) Depressed pollination in habitat fragments causes low fruit set. *Proc R Soc Lond Ser B-Biol Sci* 267: 1149-52
- Dean W. R. J. & Milton S. (2003) The importance of roads and road verges for raptors and crows in the Succulent and Nama-Karoo, South Africa. *Ostrich* 74: 181-6
- Develey P. F. & Stouffer P. C. (2001) Effects of roads on movements by understory birds in mixed-species flocks in central Amazonian Brazil. *Conserv Biol* 15: 1416-22
- Findlay C. S. & Bourdages J. (2000) Response time of wetland biodiversity to road construction on adjacent lands. *Conserv Biol* 14: 86-94
- Forman R. T. T. & Alexander L. E. (1998) Roads and their major ecological effects. *Annu Rev Ecol Syst* 29: 207-31
- Forman R. T. T. & Deblinger R. D. (2000) The ecological road-effect zone of a Massachusetts (USA) suburban highway. *Conserv Biol* 14: 36-46.
- Forman R. T. T., Reineking B. & Hersperger A. M. (2002) Road traffic and nearby grassland bird patterns in a suburbanizing landscape. *Environ Manage* 29: 782-800
- Freitas L., Galetto L. & Sazima M. (2006) Pollination by hummingbirds and bees in eight syntopic species and a putative hybrid of Ericaceae in Southeastern Brazil. *Plant Syst Evol* 258: 49-61
- Geerts S. & Pauw A. (2009a) African sunbirds hover to pollinate an invasive hummingbird-pollinated plant. *Oikos* 118: 573-9
- Geerts S. & Pauw A. (2009b) Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *S Afr J Bot* 75: 699-706

- Hopwood J. L. (2008) The contribution of roadside grassland restorations to native bee conservation. *Biol Conserv* 141: 2632-40
- Kuitunen M., Rossi E. & Stenroos A. (1998) Do highways influence density of land birds? *Environ Manage* 22: 297-302
- Kunin W. E. (1993) Sex and the single mustard - population-density and pollinator behavior effects on seed-set. *Ecology* 74: 2145-60
- Lambertucci S. A., Speziale K. L., Rogers T. E. & Morales J. M. (2009) How do roads affect the habitat use of an assemblage of scavenging raptors? *Biodivers Conserv* 18: 2063 - 74
- Laurance S. G. W. (2004) Responses of understory rain forest birds to road edges in Central Amazonia. *Ecol Appl* 14: 1344-57
- Laurance S. G. W., Stouffer P. C. & Laurance W. E. (2004) Effects of road clearings on movement patterns of understory rainforest birds in central Amazonia. *Conserv Biol* 18: 1099-109
- Laurance W. F., Croes B. M., Guissouegou N., Buij R., Dethier M. & Alonso A. (2008) Impacts of roads, hunting, and habitat alteration on nocturnal mammals in African rainforests. *Conserv Biol* 22: 721-32
- Laurance W. F., Ferreira L. V., Rankin-De Merona J. M. & Laurance S. G. (1998) Rain forest fragmentation and the dynamics of Amazonian tree communities. *Ecology* 79: 2032-40
- Laurance W. F., Lovejoy T. E., Vasconcelos H. L., Bruna E. M., Didham R. K., Stouffer P. C., Gascon C., Bierregaard R. O., Laurance S. G. & Sampaio E. (2002) Ecosystem decay of Amazonian forest fragments: A 22-year investigation. *Conserv Biol* 16: 605-18
- Maloof J. E. & Inouye D. W. (2000) Are nectar robbers cheaters or mutualists? *Ecology* 81: 2651-61
- Mayfield M. M., Ackerly D. & Daily G. C. (2006) The diversity and conservation of plant reproductive and dispersal functional traits in human-dominated tropical landscapes. *J Eco* 94: 522-36
- Nilsson L. A. (1992) Orchid pollination biology. *Trends Ecol Evol* 7: 255-9
- Ollerton J. & Liede S. (1997) Pollination systems in the Asclepiadaceae: a survey and preliminary analysis. *Biol J Linnean Soc* 62: 593-610
- Ortega Y. K. & Capen D. E. (1999) Effects of forest roads on habitat quality for ovenbirds in a forested landscape. *The Auk* 116: 937-46

- Palomino D. & Carrascal L. M. (2007) Threshold distances to nearby cities and roads influence the bird community of a mosaic landscape. *Biol Conserv* 140: 100-9
- Parker I. M. (1997) Pollinator limitation of *Cytisus scoparius* (Scotch broom), an invasive exotic shrub. *Ecology* 78: 1457-70
- Pauw A. (2007) Collapse of a pollination web in small conservation areas. *Ecology* 88: 1759-69
- Pauwels F. & Gulinck H. (2000) Changing minor rural road networks in relation to landscape sustainability and farming practices in West Europe. *Agric Ecosyst Environ* 77: 95-9
- PAWC. (2004) Provincial Government of the Western Cape. Road Network Information Reports. http://rnis.wcape.gov.za/rnis/rnis_web_reports.main
- Peris S. J. & Pescador M. (2004) Effects of traffic noise on passerine populations in Mediterranean wooded pastures. *Appl Acoust* 65: 357-66
- Raimondo D. C., L. Von Staden W. Foden J. E. Victor N. A. Helme R. C. Turner D. A. Kamundi & P. A. Manyama 2009. Red List of South African plants. *Strelitzia* 25. South African National Biodiversity Institute, Pretoria.
- Rebelo A. G., Siegfried W. R. & Crowe A. A. (1984) Avian pollinators and the pollination syndromes of selected mountain fynbos plants. *S Afr J Bot* 3: 285-96
- Rebelo A. G., Siegfried W. R. & Oliver E. G. H. (1985) Pollination syndromes of *Erica* species in the South-Western Cape *S Afr J Bot* 51: 270-80
- Reijnen R., Foppen R. & Meeuwsen H. (1996) The effects of traffic on the density of breeding birds in Dutch agricultural grasslands. *Biol Conserv* 75: 255-60
- Reijnen R., Foppen R., Terbraak C. & Thissen J. (1995) The effects of car traffic on breeding bird populations in woodland. III. Reduction of density in relation to the proximity of main roads. *J Appl Ecol* 32: 187-202
- Schumann D., Kirsten D. & Oliver E. G. H. (1992) *Ericas of South-Africa*. Fernwood Press, Vlaeberg, Cape Town.
- Spellerberg I. F. (1998) Ecological effects of roads and traffic: a literature review. *Global Ecol Biogeogr Lett* 7: 317-33
- Spooner P. G. & Lunt I. D. (2004) The influence of land-use history on roadside conservation values in an Australian agricultural landscape. *Aust J Bot* 52: 445-58
- Trombulak S. C. & Frissell C. A. (2000) Review of ecological effects of roads on terrestrial and aquatic communities. *Conserv Biol* 14: 18-30

- Van der Zande A. N., Keurs W. J. T. & Vanderweijden W. J. (1980) The impact of roads on the densities of 4 bird species in an open-field habitat - evidence of a long-distance effect. *Biol Conserv* 18: 299-321
- Washitani I., Kato M., Nishihiro J. & Suzuki K. (1995) Importance of queen bumble bees as pollinators facilitating inter-morph crossing in *Primula sieboldii*. *Plant Species Biol* 9: 169-76
- Weermans J. & van Aarde R. J. (2003) Roads as ecological edges for rehabilitating coastal dune assemblages in northern KwaZulu-Natal, South Africa. *Restor Ecol* 11: 43-9

5 The cost of being specialised: pollinator limitation in the endangered geophyte *Brunsvigia litoralis* (Amaryllidaceae) in the Cape Floristic Region of South Africa.*

Current habitat fragmentation is unprecedented and impacts on ecological processes, especially specialised inter-specific interactions, are understudied. In this study I examine pollination in rural and urban populations of *Brunsvigia litoralis*, an endangered endemic, which is a flagship species for plant conservation in South Africa. *Brunsvigia litoralis* has flowers conforming to the bird-pollination syndrome, but the only flower visitor at the urban sites, the Greater Double-collared Sunbird (*Cinnyris afra*) (1.6 visits/flower/hour), is unable to access the nectar in the usual way due to a long perianth tube (38.8 mm) and resorts to robbing. To test for pollinator limitation of seed set at the urban sites flowers were pollen supplemented. Seed set in supplemented plants increased by more than an order of magnitude relative to controls. The longer billed Malachite Sunbird (*Nectarinia famosa*) was observed as the sole pollinator of *B. litoralis* at the rural site where seed set was significantly higher. Although long lived, the absence of pollinators in these urban fragments might place populations at an extinction risk. This charismatic species could serve as a flagship species for community awareness and involvement in local conservation efforts.

Introduction

South Africa is renowned for its large number of red-listed plant species with small population sizes (Raimondo et al. 2009) and for its highly specialized pollination systems (Johnson and Steiner 2000; Johnson et al. 2009). Specialized pollination systems are predicted to be sensitive to anthropogenic disruption (Bond 1994), while small population size is a frequent cause of reduced fecundity (Lamont et al. 1993; Agren 1996; Groom 1998; Ward and Johnson 2005). Nevertheless, few studies have addressed the question of whether endangered South African plants, which occur in small populations, suffer high levels of pollinator-limited seed set. The question has important management implications: if

*Geerts, S. & Pauw, A. In review, South African Journal of Botany

pollinators are indeed limiting seed set in these endangered plants, fecundity can be enhanced by pollinator conservation or hand-pollination.

Birds are important pollinators of many rare South African plants (Rebello 1987), but are also particularly sensitive to environmental degradation, and are among the first species to be lost from human impacted ecosystems (Turner 1989; Saunders et al. 1991; Lamont et al 1993; Turner 1996; Debinski and Holt 2000). Within the Cape Floristic Region avian nectarivores are negatively affected by human structures like roads (Geerts & Pauw 2010) and demonstrate lower species richness and density in very small fragments compared to larger natural areas (Pauw 2004; Fox & Hockey 2007). Within this avian nectar feeding guild, the Malachite Sunbird, *Nectarinia famosa* (Linnaeus), is particularly sensitive to anthropogenic influences, and is seldom found in small conservation areas (when in a highly transformed matrix) or penetrating deep into human settlements (Pauw 2004). However, Malachite Sunbirds are important pollinator specialists, acting as the sole pollinator for a group of deep-flowered plant species within the Cape flora (Geerts & Pauw 2009). This high degree of specialization could potentially lead to facilitate pollination mutualism disruption in fragmented habitats (Bond 1994; Johnson & Steiner 2000).

Natural habitats in the lowlands of the Cape Floristic Region are highly fragmented by agriculture and urbanisation, but effects on ecological processes have rarely been studied (but see Donaldson et al. 2002; Pauw 2007). An important consequence of habitat fragmentation, which has received relatively little attention, is the potential erosion of biodiversity through the breakdown of pollination mutualisms (Aizen & Feinsinger 1994a, b; Steffan-Dewenter & Tschardt 1999; Murren 2002). Plants in small populations can have reduced chances of pollination, because they are less attractive to pollinators and are unable to support viable populations of pollinating animals (Collins et al. 1984; Sih & Baltus 1987; Jennersten 1988; Johnson 1992; Lamont et al. 1993; Morgan 1999). These effects are likely to be exacerbated in self-incompatible animal-pollinated plant species, because seed set depends on the presence of a pollen vector (Kearns et al. 1998; Cunningham 2000; Wilcock & Neiland 2002; Aguilar et al. 2006).

Brunsvigia litoralis R.A. Dyer is a narrow endemic to the coastal lowlands of the Eastern Cape Province of South Africa and is listed as Endangered according to the IUCN Red List categories. Most of the remaining populations occur in small fragments of coastal vegetation, often in a residential setting and are threatened by urban expansion (Raimondo et al. 2009). A spectacular inflorescence is produced in autumn (from February to April) while the plant is in a leafless state. The flowers show all the features indicative of bird pollination: flowers are scentless, red, robust and tubular, and contain large volumes of nectar with a low concentration (Faegri & van der Pijl 1979; Proctor et al. 1996). Furthermore the long floral tube of *B. litoralis* suggests membership of the Malachite Sunbird pollination guild (Geerts & Pauw 2009), but this hypothesis remains untested. In this study I test: (1) whether *B. litoralis* is pollinated by Malachite Sunbirds, and (2) whether pollinator specialization is associated with pollinator limited seed set in a small population.

Methods

Study species

Brunsvigia litoralis R.A.Dyer (Amaryllidaceae) occurs on coastal sands from Cape St. Francis to Port Elizabeth (Eastern Cape, South Africa) (Doutt 1994; Snijman 2002). Leaves are present during the wet winter (May-September) and plants are leaf-less during the dry summer (October-April). When flowering (March-April) the umbellate inflorescences are easily seen above the grasses in open fields among the low coastal shrubs of the genera *Sideroxylon*, *Searsia*, and *Cassine* (Fig. 5.1a). Flower morphology is similar to *Brunsvigia orientalis* (L.) Aiton ex Eckl. (Pauw 2004) and *Brunsvigia josephinae* (Redouté) Ker Gawl (pers. obs.). *B. litoralis* is a long-lived bulbous plant that is unable to reproduce vegetatively belowground and therefore depends entirely on seeds for reproduction (G. Duncan pers. comm.). Seed set, in turn, is dependent on cross-pollination because the plants are self-incompatible (Koopowitz 1986 cited in Doutt 1994).

Study sites

I studied fragmented sites consisting of “habitat islands” within expanding human settlements. In the flowering seasons of 2006 and 2007 three sites located in a residential area at Cape St Francis and two within the city of Port Elizabeth were studied (henceforth

“urban sites”). In 2007 I added an undisturbed site in a large area of natural vegetation (henceforth “rural site”). This is the largest known population and occurs within an untransformed area (about twenty square kilometres) at Rowallan park on the western outskirts of Port Elizabeth.

To determine the proportion of plants flowering, plants in one fragment were marked with painted sticks in two previous years. Only 16% of the marked plants flowered in 2006 (B. Logie pers. com.), and this fraction is likely to be an overestimate because small individuals are difficult to locate. Population size of flowering plants (for 2006) were 11, 42 and 10 plants for Cape St Francis, 25 and 30 for the two urban populations within Port Elizabeth, and an estimated 100-120 flowering plants for the rural population at Port Elizabeth.

Flower morphology and nectar

Tube length was measured in young flowers using a steel ruler (n = 20 flowers). Although tube length is the distance from the base of the nectaries to where the nectar chamber is sealed (Fig. 5.1b), the perianth tube experienced by flower visitors is effectively longer. Tube length was therefore measured from the top of the ovary to where the petals no longer overlap. Nectar was extracted early in the morning in the field (~9:00AM) with 40 μ l capillary tubes and the sugar concentration determined with a Bellingham and Stanley 0-50% handheld refractometer (n = 5). The sample size is low because all other flowers were robbed.

Flower visitation and robbing rate

Detailed observations of the behaviour of flower visitors were made at five urban populations (three at Cape St Francis; two at Port Elizabeth) and one larger rural population (near Port Elizabeth). This was done from a distance of ~10 m aided by close focusing 8 x 40 binoculars. Flower visitors were only recorded in the morning. At the urban sites in Cape St Francis 12 hours of observations were conducted (7 hours, 16-18 March 2006; 4 hours, 9-10 March 2007; 1 hour, 10 March 2007, relocation site). At the urban sites in Port Elizabeth 2 hours of observation were conducted (7 March 2007); while at the nearby rural site eight hours of observations (8, 9 March 2007) were made.

All flower visitors and their contact with anthers and/or stigma were recorded. Many more hours however were spent in these populations whilst doing measurements and photography, and although not formally recorded, bird behaviour conformed to observations made during the formal observation periods. A visit was scored as legitimate if there was contact with anthers and/or stigma. Visitation rates were quantified by dividing the number of visits observed per hour by the number of open flowers in the census area to obtain visits per flower per hour. To determine robbing rate 50 flowers were haphazardly selected across two urban sites in Cape St Francis and at the rural site in Port Elizabeth. Robbing was confirmed by the presence of small holes punctured in the perianth tube at the base of the corolla.

Limited observations were also conducted on two Port Elisabeth urban fragments as well as an artificial population at Cape St Francis. Local residents relocated *B. litoralis* from a site earmarked for development, thus establishing a population of ~15 individuals.

Seed set

Individuals in two urban populations were marked and randomly allocated to either a control (left unmanipulated) or a pollen supplemented (hand-pollinated) treatment in 2006. For the pollen supplemented treatment, an anther from another plant, at least 10 meters away, was brushed across the stigmatic surface of each female flower on the inflorescence. Hand pollination was repeated every second or third day during the entire flowering period for all marked plants, to ensure pollination of nearly 100% of the flowers.

The Cherry Spot moth (*Diaphone eumela* Stoll) and the Lily Leaf Miner (*Brithys crini* Fabricius) consumed most capsules in two and five inflorescences in 2006 and 2007 respectively at the urban site and these were subsequently left out of the analysis. At the rural site most inflorescences had on average twenty percent of their capsules badly damaged through herbivory; therefore to avoid further damage the inflorescences were removed and kept in buckets with water.

There was no seed set data for the two Port Elisabeth urban fragments or the artificial population at Cape St Francis population, therefore these sites are excluded in further

analysis. To exclude possible effects due to seed set comparison over different years, urban seed counts were repeated in 2007 for comparison with the rural site. Differences in seed set between hand pollinated and open naturally pollinated flowers at the urban sites (2006), and seed set between naturally pollinated flowers from the urban and rural sites (2007) included many zero values and were therefore compared with the nonparametric Mann-Whitney *U*-Test. All analyses were performed in STATISTICA 9.0 (StatSoft, Inc. 2009, Tulsa, USA).

Results

Flower morphology and nectar

Morphological matching indicates that the perianth tube of 38.8 ± 3.6 mm (mean \pm SD, $n = 20$) is too long for a commonly observed visitor, the Greater Double-collared Sunbird (*Cinnyris afra* Linnaeus), to reach the nectar in the normal way [Culmen length (mm): female range: 20.1-26.3, average 24.5; male range: 26.7-30.6, average 28.0 (Hockey et al. 2005)]. The nectar is protected in a nectar chamber (Fig. 5.1b) and a longer beak is needed to gain access to the nectar via the legitimate entrance to the flower. *B. litoralis* is a typical bird-pollinated plant with a high nectar volume ($28 \pm 9.7\mu\text{l}$) and a low sugar concentration (16%).

Flower visitation and robbing rate

At the urban sites in Cape St. Francis, a total of 190 visits by Greater Double-collared Sunbirds were observed as well as a few visits by a dipteran that was attracted to the red petals of one specific inflorescence. Greater Double-collared Sunbird visitation rates averaged 0.27 visits per flower per hour in 2006 (no visits per flower per hour for 2007). The birds failed to contact the reproductive parts during all observed visits. 95.8% of the visits consisted of primary nectar robbing (Inouye 1980); a hole is pierced at the base of the flower to gain access to the nectar (Fig. 5.1c, d). Ants, which visited the flowers subsequent to robbing, also utilize these holes. When nectar is obtained without any damage to the flower, but without contact to the reproductive parts of the flower, it is known as thieving. This happened for 4.2% of the visits. This is achieved by entering the flower from the side while perching on an adjacent flower. At the two Port Elisabeth urban fragments and the artificial

established population at Cape St Francis no flower visitors were observed. Absence of claw marks on pedicles is an indication that these plants are very rarely, if ever, visited.

At the rural site the long-billed Malachite Sunbird was the only flower visitor (0.5 visits per flower per hour) (Fig. 5.1e). No illegitimate visits were recorded and pollen was transferred between flowers on Malachite Sunbird foreheads (Fig. 5.1f).

At the urban sites robbing rate, determined as the percentage of flowers with a pierced perianth, was 100% ($N = 90$ flowers in 2006; $N = 50$ flowers in 2007) (Fig. 5.1d) whilst at the rural site there was no robbing ($N = 50$ flowers in 2007). A mean of 25.2 ± 10.1 (SD) flowers is produced per inflorescence ($N = 65$ plants).

Seed set

There was no significant difference between seed set at the two urban sites in 2006 or 2007 (4 and 7 open inflorescences in 2006, $Z = 0.47$, $P = 0.64$; 5 and 21 pollen supplemented inflorescences in 2006, $Z = 1.56$, $P = 0.12$, Mann-Whitney *U*-Test; 10 and 10 open inflorescences in 2007, $Z = 0.034$, $P = 0.96$, Mann-Whitney *U*-Test). To improve sample size these data are pooled in subsequent analysis. Seed set of 0.77 ± 0.49 (median \pm SD) seeds per capsule in Greater Double-collared Sunbird visited flowers increased to 8.77 ± 4.19 (SD) seeds per capsule in pollen-supplemented plants (11 open inflorescences, 26 pollen supplemented inflorescences, $Z = 4.72$, $P < 0.001$, Mann-Whitney *U*-Test; inflorescences had on average 23 flowers) (Fig. 5.2). Compared to open flowers at the urban sites (year 2007) seed set was significantly higher in the rural population (20 urban inflorescences, 6 rural inflorescences, $Z = 2.50$, $p = 0.01$, Mann-Whitney *U*-Test; inflorescences had on average 26 flowers) (Fig. 5.3). Seed set on rural plants averaged 1.5 ± 1.0 (SD) seeds per capsule.

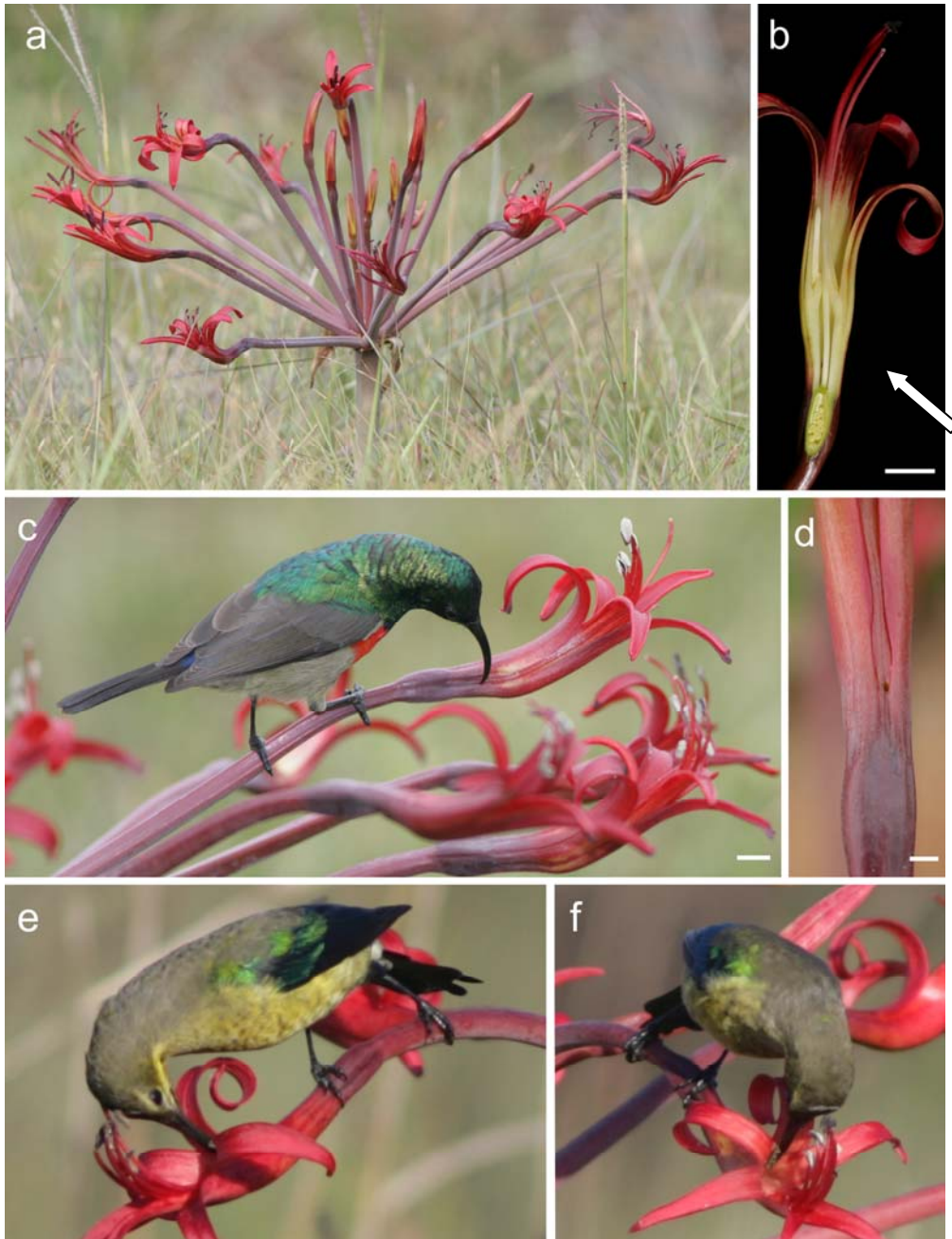


Figure 5.1 (a) A young umbellate inflorescences of *Brunsvigia litoralis*. Inflorescence height approximately 50 cm. (b) *B. litoralis* corolla tube, the arrow indicates the sealed nectar chamber. (c) A male *Cinnerys afra* (Greater Double-Collared Sunbird) robbing the nectar of a *B. litoralis* flower at a urban population (Cape St Francis). (d) Holes pierced by *C. afra* to gain access to the nectar. Scale bar = 5 mm. (e) *Nectarinia famosa* (Malachite Sunbird) pollinating a *B. litoralis* flower at the rural site. (f) Pollen is visible on the head of *N. famosa*. Scale bar = 10 mm in each case.

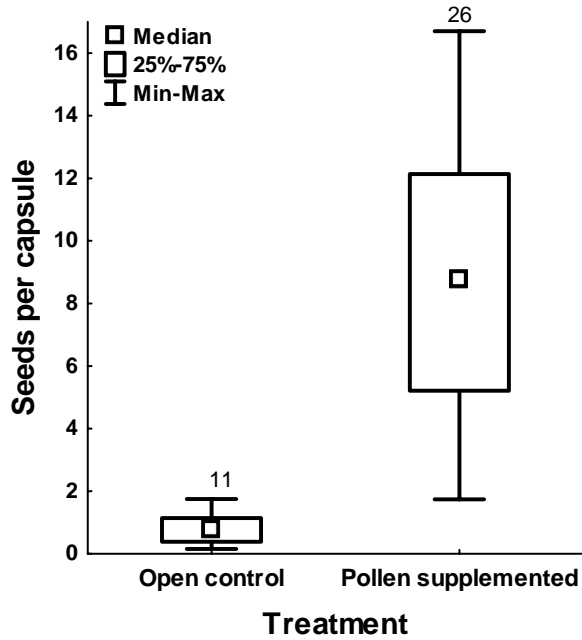


Figure 5.2. Mean number of seeds per capsule (2006) between plants with hand-pollinated and open flowers in urban *Brunsvigia littoralis* populations ($Z = 4.72$, $p < 0.001$, Mann-Whitney U -Test). Numbers above bars = number of plants.

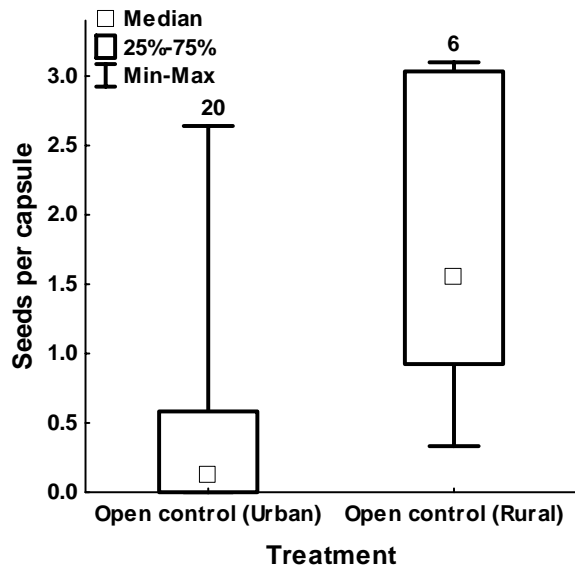


Figure 5.3. Comparison of mean number of seeds per capsule (2007) between unmanipulated *Brunsvigia littoralis* flowers in the urban and rural populations ($Z = 2.50$, $p = 0.01$, Mann-Whitney U -Test). Numbers above bars = number of plants.

Discussion

The results show that the long-tubed, scarlet flowers of *Brunsvigia litoralis* are specialized for pollination by the Malachite Sunbird, *Nectarinia famosa*. At one site, Malachite Sunbirds were frequent visitors to *B. litoralis* flowers. They followed a trap-lining method, visiting each individual inflorescence a few times daily, and fulfilled the role of pollination by making contact with the anthers and stigma (Fig. 5.1). In contrast, the shorter-billed Greater Double-collared Sunbird, *Cinnyris afra*, circumvented the reproductive organs and did not fulfil the ecological role of pollination, but obtained nectar through thieving or robbing. These findings are consistent with earlier work, which showed that a large guild of plants in the Cape Floral Region are adapted solely for pollination by Malachite Sunbirds (Geerts & Pauw 2009). Here I extend this earlier work by showing that Greater Double-collared Sunbirds, which have bills that are at least 6 mm shorter than those of Malachite Sunbirds, are not able to replace them as pollinators of a long-tubed plant species.

The high level of pollinator specificity in *B. litoralis* might be an important consideration in the conservation management of this endangered species (Bond 1994). Unfortunately, only one large population of *B. litoralis* remains and this makes it impossible to conduct a replicated statistical contrast of small urban populations and multiple large rural populations. Nevertheless, I observed strong differences in the pollination ecology of the small urban populations when compared with the single large rural population. Malachite Sunbirds were the only visitors in the large, rural population, while Greater Double-collared Sunbirds were the only visitors in the small, urban populations. This had two noticeable effects: firstly, evidence of flower robbing was observed in all examined flowers in the small, urban populations inside the town of Cape St. Francis, while no such damage was observed in the large rural population; secondly, seed set was significantly higher in the large, rural population than in the small, urban populations (Fig. 5.3). Pollen supplementation by hand in small, urban populations lead to a ten fold increase in seed set (Fig. 5.2), indicating that the relatively low seed set in small, urban populations is likely attributable to differences in pollen limitation rather than to nutrient or water limitation. Although direct evidence is lacking, the high level of seed set following supplemental hand pollination at urban sites suggests that seed set in the relatively large rural population is also pollen limited. Thus,

throughout its range, seed production in *B. littoralis* may be limited by its specialized pollinator (Lindberg & Olesen 2001).

The extent to which low levels of seed set are an immediate conservation concern depends on the importance of seeds in the demography of *B. littoralis* (Bond 1994). For example, if populations of *B. littoralis* are limited by density dependent factors such as increased seed predation in dense populations, an increase in seed set is unlikely to result in an increase in population growth rate (Steffan-Dewenter and Tschardt 1999; Johnson et al. 2004). Although I did not quantify herbivory in this study, a high level of seed predation in the large, rural population is suggestive of density dependent controls on population size (Antonovics & Levin 1980).

Adult longevity is another important factor in assessing the demographic importance of seeds for population persistence in *B. littoralis* (Silvertown et al. 1993). On the one hand, extreme longevity of genets is unlikely in this species because there is no evidence for belowground vegetative reproduction (Pauw and Hawkins 2010). On the other hand, the large bulbs indicate that adults are probably able to live for decades. Thus, the low levels of seed set observed in this study might be sufficient to maintain population growth, but this remains to be tested using demographic modelling.

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References

Agren, J. 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* 77, 1779-1790.

- Aguilar, R., L. Ashworth, L. Galetto, and M. A. Aizen. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* 9, 968-980.
- Aizen, M. A., and P. Feinsinger. 1994a. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology* 75, 330-351.
- Aizen, M. A., and P. Feinsinger. 1994b. Habitat fragmentation, native insect pollinators, and feral honey-bees in a Argentine Chaco Serrano. *Ecological Applications* 4, 378-392.
- Antonovics, J., and D. A. Levin. 1980. The ecological and genetic consequences of density-dependent regulation in plants. *Annual Review of Ecology and Systematics* 11, 411-452.
- Bond, W. J. 1994. Do mutualisms matter - assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 344, 83-90.
- Collins, B. G., C. Newland, and P. Briffa. 1984. Nectar utilization and pollination by Australian honeyeaters and insects visiting *Calothamnus-Quadrifidus* (Myrtaceae). *Australian Journal of Ecology* 9, 353-365.
- Cunningham, S. A. 2000. Depressed pollination in habitat fragments causes low fruit set. *Proceedings of the Royal Society of London Series B-Biological Sciences* 267, 1149-1152.
- Debinski, D. M., and R. D. Holt. 2000. A survey and overview of habitat fragmentation experiments. *Conservation Biology* 14, 342-355.
- Donaldson, J., I. Nanni, C. Zachariades, J. Kemper, and J. D. Thompson. 2002. Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. *Conserv. Biol.* 16, 1267-1276.
- Doutt, R. L. 1994. *Cape Bulbs*. Timber Press, Portland, Oregon.
- Faegri, K., and L. van der Pijl 1979. *The principles of pollination ecology*, 2nd ed. Pergamon, Oxford, UK.
- Fox, S. J. C., and P. A. R. Hockey. 2007. Impacts of a South African coastal golf estate on shrubland bird communities. *South African Journal of Science* 103, 27-34.

- Geerts, S., and A. Pauw. 2009. Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *South African Journal of Botany* 75, 699-706.
- Geerts, S., and A. Pauw. 2010. Easy technique for assessing pollination rates in the genus *Erica* reveals road impact on bird pollination in the Cape Fynbos, South Africa. *Austral Ecology*, doi:10.1111/j.1442-9993.2010.02201.x.
- Groom, M. J. 1998. Allee effects limit population viability of an annual plant. *American Naturalist* 151, 487-496.
- Hockey, P. A. R., W. R. J. Dean, and P. G. Ryan 2005. Roberts' birds of Southern Africa. Trustees of the John Voelcker Bird Book Fund, Cape Town.
- Inouye, D. W. 1980. The terminology of floral larceny. *Ecology* 61, 1251-1253.
- Jennersten, O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae) - effects of habitat fragmentation on visitation and seed set. *Conservation Biology* 2, 359-366.
- Johnson, S. D. 1992. Plant-animal relationships. In: Cowling, R.M. (Ed.), *The ecology of fynbos: nutrients, fire and diversity*. Oxford University Press, Cape Town, pp. 175-205.
- Johnson, S., and K. Steiner. 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution* 15, 140-143.
- Johnson, S. D., J. C. Manning, and A. Pauw. 2009. Advances in the pollination biology of South African plants. *South African Journal of Botany* 75, 625-629.
- Johnson, S. D., C. L. Collin, H. J. Wissman, E. Halvarsson, and J. Agren. 2004. Factors contributing to variation in seed production among remnant populations of the endangered daisy *Gerbera aurantiaca*. *Biotropica* 36, 148-155.
- Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: The conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* 29, 83-112.
- Lamont, B. B., P. G. L. Klinkhamer, and E. T. F. Witkowski. 1993. Population fragmentation may reduce fertility to zero in *Banksia goodii* - a demonstration of the Allee effect. *Oecologia* 94, 446-450.
- Lindberg, A., and J. M. Olesen. 2001. The fragility of extreme specialization, *Passiflora mixta* and its pollinating hummingbird *Ensifera ensifera*. *Journal of Tropical Ecology* 17, 323-329.

- Morgan, J. W. 1999. Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. *Conservation Biology* 13, 266-273.
- Murren, C. J. 2002. Effects of habitat fragmentation on pollination: pollinators, pollinia viability and reproductive success. *Journal of Ecology* 90, 100-107.
- Pauw, A. 2004. Variation in pollination across a fragmented landscape at the Cape of Africa. Ph.D Dissertation, University of Cape Town, Cape Town
- Pauw, A. 2007. Collapse of a pollination web in small conservation areas. *Ecology* 88, 1759-1769.
- Pauw, A., and J. A. Hawkins. 2010. Reconstruction of historical pollination rates reveals linked declines of pollinators and plants. *Oikos* doi: 10.1111/j.1600-0706.2010.19039.x.
- Proctor, M., P. Yeo, and A. Lack 1996. *The natural history of pollination*. Timber Press, Portland, Oregon, USA.
- Raimondo, D. C., L. Von Staden, W. Foden, J. E. Victor, N. A. Helme, R. C. Turner, D. A. Kamundi, and P. A. Manyama 2009. *Red List of South African plants*. *Strelitzia* 25. South African National Biodiversity Institute, Pretoria.
- Rebelo, A. G. 1987. Bird pollination in the Cape Flora. In Rebelo, A. G. (Ed.), *A preliminary synthesis of pollination biology in the Cape flora*. CSIR, Pretoria, pp. 83-108.
- Saunders, D. A., R. J. Hobbs, and C. R. Margules. 1991. Biological consequences of ecosystem fragmentation - a review. *Conservation Biology* 5, 18-32.
- Sih, A., and M. S. Baltus. 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. *Ecology* 68, 1679-1690.
- Silvertown, J., M. Franco, I. Pisanty, and A. Mendoza. 1993. Comparative plant demography - relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. *Journal of Ecology* 81, 465-476.
- Snijman, D. 2002. A guide to the *Brunsvigia* species of Namaqualand and the Cape. *Indigenous Bulb Association of South Africa* 51, 3-9.
- Steffan-Dewenter, I., and T. Tschardt. 1999. Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121, 432-440.
- Turner, I. M. 1996. Species loss in fragments of tropical rain forest: A review of the evidence. *Journal of Applied Ecology* 33, 200-209.

- Turner, M. G. 1989. Landscape ecology - the effect of pattern on process. *Annual Review of Ecology and Systematics* 20, 171-197.
- Ward, M., and S.D.Johnson. 2005. Pollen limitation and demographic structure in small fragmented populations of *Brunsvigia radulosa* (Amaryllidaceae). *Oikos* 108,253-262.
- Wilcock, C., and R. Neiland. 2002. Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science* 7, 270-277.

6 Habitat change and reduced pollination by nectar-feeding birds in response to fire in mature Cape fynbos, South Africa *

Nectar-feeding birds are important pollinators in fire-prone regions of the world, but the impact of fires on these bird communities has seldom been studied. Nectar-feeding bird communities were censused during peak flowering, before and after naturally occurring fires in the Cape Fynbos of South Africa. The abundance and species richness of nectar feeding birds decreased at all sites. In a controlled experiment, two common bird-pollinated plants, which flower profusely after a fire, were presented in floral arrays on both sides of the fire boundary. Birds visited flowers only in the unburnt areas. The results are surprising given the large number of bird-pollinated plants that have fire-stimulated flowering.

Introduction

Fire is an integral and essential component of the ecology of fynbos vegetation in the Cape Floristic Region (CFR) of South Africa (van Wilgen 1982; Cowling 1992). Research on the ecological role of fire has focused mainly on the effect of fire on plants, while considerably less attention has been paid to the effect of fire on animals and animal-plant interactions (Pyne 1997). In particular, the dearth of information on pollinator responses is a concern (Parr and Chown 2003).

About 4 % of plants in the CFR are dependent on birds for pollination, and a large number of these plants, particularly the geophytic monocotyledons, flower abundantly after a fire (Johnson 1992; Rebelo 2001). Ironically, the small number of studies that have considered the impact of fire on birds in the CFR have found that nectar-feeding birds, i.e. sunbirds (Nectarinidae) and sugarbirds (Promeropidae), suffer greater declines than other bird guilds after fires (Fraser 1989; Fraser and McMahon 1992). Taken together, these observations suggest that bird-pollinated plants flowering in a post-fire landscape might suffer severe pollinator limitation (Fraser 1989; De Swardt 1993; Pauw 2004).

* Malherbe, S.D.T. contributed the floral transplant data

Here I investigate fire impacts on species richness and abundance of nectar-feeding birds and the rate of bird-pollination in post-fire vegetation. I use two approaches: (1) a comparison of bird communities in marked plots before and after a fire; and (2) comparison of pollination rates in bird-pollinated plants that were transplanted between burnt and unburnt sites.

Materials and methods

Pre- and post-fire bird observations

I recorded nectar-feeding bird abundance and richness at six sites in the Cape Floristic Region of South Africa (Table 6.1). Only specialist nectar feeders (i.e. Nectarinidae and Promeropidae) were included in the census, while facultative nectar feeders were excluded. Pre-burn sites were dominated by dense stands of two-meter-high, bird-pollinated *Protea* shrubs, except for the Jonkershoek firebreak where *Protea* individuals were more sparsely distributed. The six sites were burned in two wildfires that occurred on Stellenbosch Mountain in February 2009 and on Paarl Mountain in March 2009. Post-fire vegetation consisted of the seedlings of *Protea* and other shrubs, as well as resprouting bushes. The vegetation was about 30 cm high with emergent skeletons of fire-killed *Protea* shrubs.

Point counts were conducted before and after the fire (May-July 2007 and June 2009) by standing on a ladder and recording all nectar feeding birds within a 25 meter radius (Bibby et al 2000). Observation periods lasted twenty minutes and were conducted early in the morning when avian nectarivores are most active (Fry 2000). Rainy and very windy days were avoided. Bird density estimates are a relative measure; with pre-fire bird observations part of a different study, no distance measurements were included (Johnson 2008).

Birds are more conspicuous in open vegetation and this will bias our results against the expected outcome of higher richness and abundance in unburnt vegetation. Differences in nectar-feeding bird richness and abundance before and after the fires were compared with a Wilcoxon matched-pairs test.

Inflorescence transplants between burnt and unburnt sites

Three areas that burnt during the February 2009 fire were used for a transplant experiment in July-September of the same year (Jonkershoek, 33°58'15"S, 18°55'47"E; Brandwacht, 33°57'17"S, 18°52'49"E; Dornier, 33°59'22"S, 18°53'12"E). Each site was approximately 4.5 km from next the nearest site. Two Iridaceae species, *Chasmanthe floribunda* (Salisb.) and *Chasmanthe aethiopica* (L.) were used in the experiment because they were in full bloom, flower most profusely in the years after a fire and are known to be visited or pollinated by sunbirds (Geerts and Pauw 2009b). *C. floribunda* is able of some selfing but sunbird visitation increases seed set significantly whilst *C. aethiopica* is autogamous (Geerts 2006). *C. floribunda* was used at all sites; *C. aethiopica* was used only at Brandwacht. Each of the three study sites were located at a border between burnt and unburnt vegetation. Four pairs of study plots were marked at each site. The members of each pair were 200 m apart with the border between burnt and unburnt vegetation halfway between them.

Between 10 and 15 inflorescences of either *C. floribunda* or *C. aethiopica*, each bearing 5 to 10 flowers, were collected and placed in water-filled test tubes. The test tubes were secured together in a punctured cardboard tray. This floral array was moved among plots and observed in each for a 30 min period. Burnt and unburnt plots in a pair were observed in succession following a randomly allocated order. For *C. floribunda*, sampling at all three sites was repeated on three separate days for a total of 9 sampling days, whilst for the one *C. aethiopica* site sampling was conducted for two days only. All observations were done in the morning.

All sites were lumped together and days were treated as independent replicates because there was a high level of temporal variability in bird visitation rate. Thus, the final numbers of pairs used in the tests were as follows: $N_{C. floribunda} = 4 \text{ plots} \times 3 \text{ sites} \times 3 \text{ days}$; $N_{C. aethiopica} = 4 \text{ plots} \times 1 \text{ site} \times 2 \text{ days}$. Total observation time equalled 44 hours (divided equally between burnt and unburnt plots). The data were analyzed with a Wilcoxon matched-pairs test due to the many zeros in the dataset. All analyses were conducted using STATISTICA 9.0 (StatSoft 2009, Tulsa, USA).

Results

Pre- and post-fire bird observations

I observed the following nectar-feeding birds: Cape Sugarbird (*Promerops cafer*) (Fig. 6.1a), Malachite Sunbird (*Nectarinia famosa*), Southern Double-collared Sunbird (*Cinnyris chalybea*) and the Orange-breasted Sunbird (*Anthobaphes violacea*). Species richness of nectar-feeding birds showed a near significant decline after the fire (Wilcoxon matched-pairs test, $Z = 1.89$, $N = 6$ pairs, $P = 0.06$; Fig. 6.2a). Nectar feeding bird abundance however was significantly higher before versus after the fire (Wilcoxon matched-pairs test, $Z = 2.2$, $N = 6$ pairs, $P = 0.028$; Fig. 6.2b).

Inflorescence transplants between burnt and unburnt sites

A total of 187 bird visits were recorded for the two flowering species combined. Orange-breasted Sunbirds were the most frequent visitors and responsible for 67% of the visits (Fig. 6.1b). Malachite Sunbirds (24%), Southern Double-collared Sunbirds (0.5%) and Cape White-eyes (*Zosterops virens*) (9%) were less frequent visitors. Cape White-eyes were excluded in our analysis as they consistently rob *Chasmanthe* flowers without pollinating (Geerts 2006). No sugarbirds were observed visiting the flowers. Sunbird visitation was significantly higher in the adjacent unburnt vegetation for both *C. floribunda* (Wilcoxon matched-pairs test, $Z = 2.37$, $N = 7$, $P = 0.018$; Fig. 6.3a) and *C. aethiopica* (Wilcoxon matched-pairs test, $Z = 2.02$, $N = 5$, $P = 0.043$; Fig. 6.3b).

Table 6.1. Study sites and dates of nectar-feeding bird recordings before and after the fires on Stellenbosch Mountain (February 2009) and Paarl Mountain (March 2009).

Site	Before	After	GPS Coordinates
Paarl mountain 1	2007/06/29	2009/06/12	S33 45.56.5 E18 56.75.2
Jonkershoek Swartboskloof	2007/05/15	2009/06/20	S33 59 23.6 E18 57 18.2
Jonkershoek Panorama trail	2007/05/15	2009/06/20	S33 59.54.2 E18 58 6.05
Jonkershoek dam	2007/07/02	2009/06/20	S33 58 40.6 E18 56 41.5
Paarl mountain 2	2007/06/29	2009/06/12	S33 44 17.9 E18 57 19.2
Jonkershoek	2007/05/15	2009/06/20	S33 59 25.6 E18 57 18.5

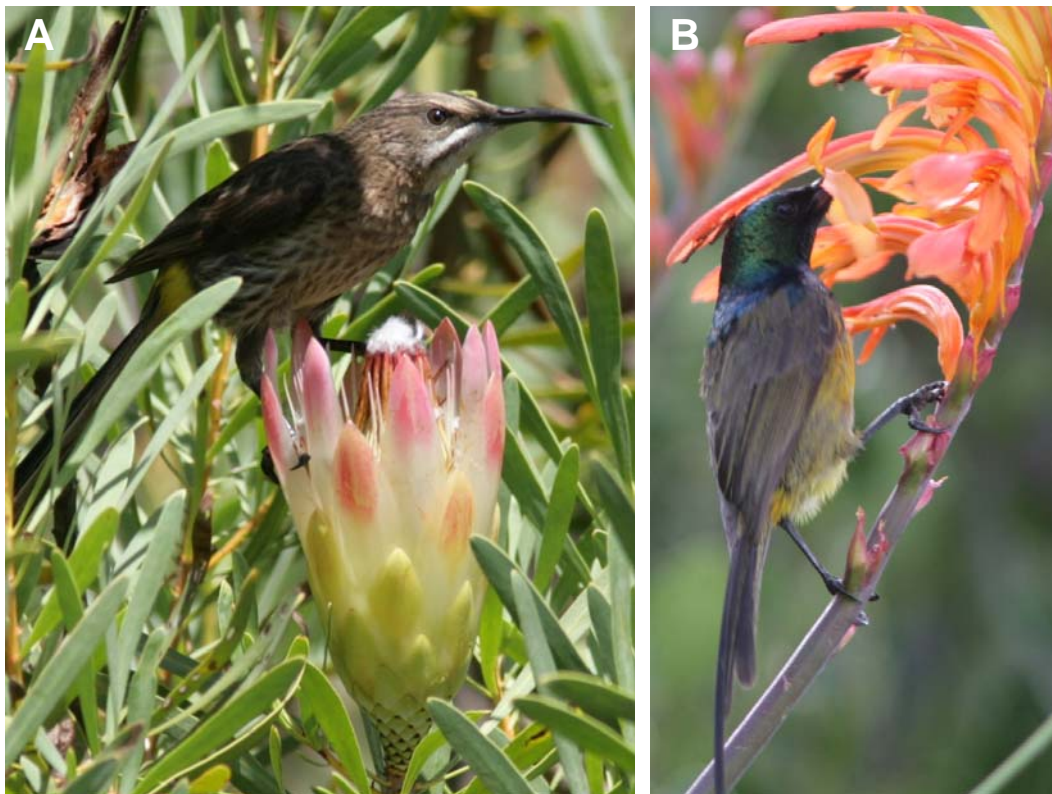


Figure 6.1 (a) Cape Sugarbird (*Promerops cafer*) female visiting a *Protea repens* inflorescence, during bird counts in mature fynbos vegetation. (b) Orange-breasted Sunbird (*Anthobaphes violacea*) male visiting a *Chasmanthe floribunda* flower.

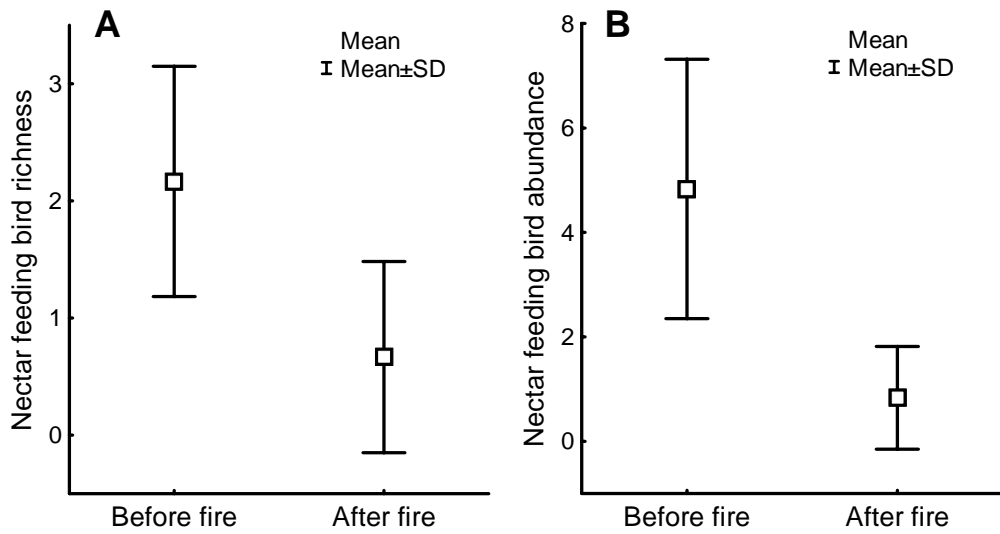


Figure 6.2. Comparison of specialist nectar-feeding bird species richness (a) and abundance (b) between pre and post-fire vegetation for the 25 meter radius, 20 min point counts ($N = 6$ before-after pairs).

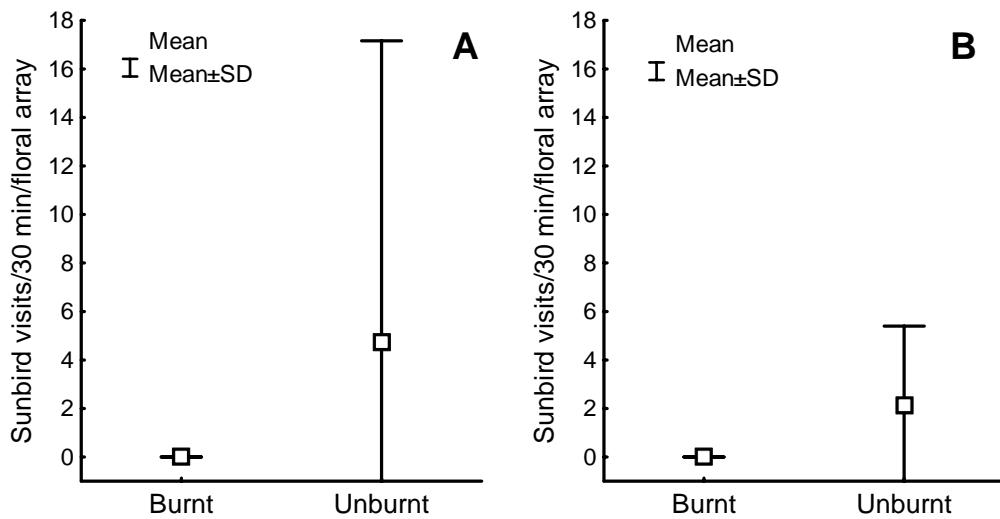


Figure 6.3. Nectar-feeding bird visitation to the floral arrays of (a) *Chasmanthe floribunda* ($N = 36$; 4 plots \times 3 sites \times 3 days) and (b) *Chasmanthe aethiopica* ($N = 8$; 4 plots \times 1 site \times 2 days) during 30 min observations in burnt and unburnt sites. Sunbirds visited floral arrays 7 out of 36 sampling periods for *C. floribunda* and 5 out of 8 sampling periods for *C. aethiopica*.

Discussion

Nectar-feeding bird abundance decreased significantly in post-fire vegetation whilst species richness showed a near significant decline. Floral arrays within unburnt vegetation were visited by nectar-feeding birds whilst arrays in burnt vegetation received no visits. These results suggest that nectar-feeding birds show a strong preference towards unburnt environments. Vegetation in an early successional stage probably produces insufficient nectar to attract and sustain nectar-feeding birds (Fraser 1989; Johnson 1992).

Surprisingly, a number of plant species dependant on birds for pollination, flower most profusely in the year directly after a fire – this includes members of the genera *Cyrtanthus*, *Haemanthus*, *Gladiolus* and *Watsonia* – with infrequent flowering in later years (Cowling 1992; Bond and Van Wilgen 1996). These species are not directly affected by fire because the bulbs or rhizomes are deeply buried. In contrast, the larger Proteaceae and Ericaceae shrubs are killed by fire (Rebelo 1987). These species flower mostly in late successional communities and are the most important nectar resources for nectar-feeding birds (Van Wilgen 1981; le Maitre and Midgley 1992).

Fire occurs as a natural disturbance in fynbos plant communities (Bond and Van Wilgen 1996). However, today almost all fires are started by humans and the escalation in frequency, and subsequent destruction of mature fynbos, could be unfavourable for nectar-feeding birds. High fire frequencies will maintain plant communities in an early successional stage whilst extensive fires decrease proximity to late successional vegetation. These factors might have a long-term negative effect on the nectar-feeding bird community since it appears that nectar-feeding birds require an extensive group of plant species to fulfill their nectar requirements. Nectar-feeding birds in this study were only observed in one of the six burnt sites. This site was closest to the urban edge. Other nectar sources, such as urban gardens or alien invasive plants, which can occur in great densities and are known to attract nectar-feeding birds (Geerts and Pauw 2009a; Le Roux et al. 2010), might partly substitute late successional vegetation by supplying abundant nectar.

The lack of nectar-feeding bird visitation to the floral arrays in post fire habitat, might partly be attributed to the temporal and spatial shortage of nectar in these floral arrays. In contrast, insects were observed to visit similar floral arrays of *Babiana* species (unpublished data). The pattern of low visitation in early successional communities might therefore differ between pollination guilds, with specific guilds favoured in early successional communities. So for example do orchids (*Pterygodium* and *Disperis*), which are dependant on oil-collecting bees for pollination, and which set most seed shortly after a fire, whilst seed set is lower in late successional vegetation (Pauw 2007). Potts et al. (2001) found a decrease in bee abundance and diversity in a burnt area, but no pollen limitation on their study plant, *Satureja thymbra*, at either the burnt or unburnt sites. These examples highlight that different pollination guilds might differ in their response to fire.

It is essential to improve the understanding of pollinator responses to fire. In particular nectar-feeding birds, with their extreme decrease in post fire environments, deserve more attention. Future studies should focus on nectar-feeding bird movement and changes in population numbers in burnt and adjacent unburnt vegetation. At what successional age are adequate resource levels achieved to attract and sustain the entire nectar-feeding bird community? Much needed are long-term studies to address these questions (Parr and Chown 2003; Jacquet and Prodon 2009). As short term alternative, chronosequence (a space-for-time substitution) can be used by sampling vegetation at different successional stages (Foster and Tilman 2000). In conclusion it is suggested that management decisions concerning biodiversity should aim to conserve the nectar-feeding bird and plant communities as a whole, rather than just focusing on individual species.

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References

- Bibby CJ, N. D. Burgess, D. A. Hill, S. H. Mustoe. (2000) Bird census techniques 2nd ed. Academic Press, London
- Bond WJ, Van Wilgen BW (1996) Fire and plants. Chapman & Hall, London
- Cowling RM (1992) The ecology of fynbos: nutrients, fire and diversity. Oxford University Press, Cape Town
- De Swardt DH (1993) Factors affecting the densities of nectarivores in Protea Roupelliae Woodland. Ostrich 64:172-177
- Foster BL, Tilman D (2000) Dynamic and static views of succession: Testing the descriptive power of the chronosequence approach. Plant Ecol 146:1-10
- Fraser M, McMahon L (1992) Habitat change by Cape Sugarbirds and Orange-breasted Sunbirds in an apparent response to fire in old mountain fynbos. Promerops 21:51-54
- Fraser MW (1989) Short-term responses of birds to fire in old mountain fynbos. Ostrich 60:172-182
- Fry CH (2000). The Birds of Africa. Vol. VI. Academic Press, London
- Geerts S (2006) Specialization within a floral syndrome: a Malachite Sunbird pollination guild within the Cape flora. Honours thesis. University of Stellenbosch, Stellenbosch
- Geerts S, Pauw A (2009a) African sunbirds hover to pollinate an invasive hummingbird-pollinated plant. Oikos 118:573-579
- Geerts S, Pauw A (2009b) Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. S Afr J Bot 75:699-706
- Jacquet K, Prodon R (2009) Measuring the postfire resilience of a bird-vegetation system: a 28-year study in a Mediterranean oak woodland. Oecologia 161:801-811
- Johnson DH (2008) In defense of indices: the case of bird surveys. J Wildl Manage 72:857-868
- Johnson SD (1992) Plant-animal relationships. In: Cowling RM (ed) The ecology of fynbos: nutrients, fire and diversity. Oxford University Press, Cape Town

- le Maitre DC, Midgley JJ (1992) Plant reproductive ecology. In: Cowling RM (ed) The ecology of fynbos: nutrients, fire and diversity. Oxford University Press, Cape Town
- Le Roux JJ, Geerts S, Ivey P, Krauss S, Richardson DM, Suda J, Wilson JRU (2010) Molecular systematics and ecology of invasive Kangaroo Paws in South Africa: management implications for a horticulturally important genus. *Biol Invasions* DOI: 10.1007/s10530-010-9818-4
- Parr CL, Chown SL (2003) Burning issues for conservation: A critique of faunal fire research in Southern Africa. *Austral Ecol* 28:384–395
- Pauw A (2004) Variation in pollination across a fragmented landscape at the Cape of Africa. Ph.D Dissertation, University of Cape Town, Cape Town
- Pauw A (2007) Collapse of a pollination web in small conservation areas. *Ecology* 88:1759-1769
- Potts SG, Dafni A, Ne'eman G (2001) Pollination of a core flowering shrub species in Mediterranean phrygana: variation in pollinator diversity, abundance and effectiveness in response to fire. *Oikos* 92:71-80
- Pyne SJ (1997) *World Fire: the culture of fire on earth*. University of Washington Press, Seattle
- Rebelo AG (1987) Bird pollination in the Cape Flora. In: Rebelo AG (ed) *A preliminary synthesis of pollination biology in the Cape flora*, 141 edn. CSIR, Pretoria, pp 83-108
- Rebelo AG (2001) *Proteas: A field guide to the Proteas of southern Africa*. Fernwood Press
- Van Wilgen BW (1981) Some effects of fire frequency on fynbos plant community composition and structure at Jonkershoek, Stellenbosch. *S Afr J For* 118:42-55
- van Wilgen BW (1982) Some effects of post-fire age on the above-ground plant biomass of fynbos (macchia) vegetation in South Africa. *J Ecol* 70:217-225

7 African sunbirds hover to pollinate an invasive hummingbird-pollinated plant*

Why do hummingbirds hover while Old World nectar-feeding birds perch? A unique opportunity to explore this question is presented by the invasion into Africa of a plant adapted for pollination by hovering hummingbirds. Like other hover-pollinated plants of the New World, the flowers of the tree tobacco *Nicotiana glauca* lack perches and are oriented towards open space. I find that Old World nectarivores, especially the Malachite Sunbird, *Nectarinia famosa*, hover 80% of the time when taking nectar from these flowers. They hover for up to 30 s, and are able to sustain this hovering lifestyle in an area where native nectar plants are absent. *Nicotiana glauca* greatly increases the local abundance of sunbirds compared with uninvaded areas. In turn, flowers visited by sunbirds formed significantly more capsules and set significantly more seed than sunbird-excluded flowers, possibly facilitating the invasion. The results suggest a prominent role for plant - rather than bird - traits in determining the occurrence of hover-pollination, begging the question of why plants adapted for hover pollination do not occur outside the New World.

Introduction

The invasive potential of alien flowering plants in a novel environment may depend on how well they integrate into existing pollination webs. If they integrate poorly — due, for instance, to a lack of evolutionary history with their new community — then seed production and invasion success may be pollinator limited (Richardson et al. 2000; Morales and Aizen 2006; Van Kleunen and Johnson 2007). Plants with flowers that are specialized for pollination by a small number of animal species might be particularly prone to pollinator limitation in their introduced range (Richardson et al. 2000), however, lack of knowledge about the role of specialized plant and pollinator traits in shaping interaction webs makes predictions difficult (Rezende et al. 2007), and apparently specialized organisms may interact with their new communities in surprising ways (Pemberton and Wheeler 2006).

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Conversely, the grand-scale transplant experiments provided by the introduction of alien species can teach us much about the role of plant and animal traits in mediating interactions. Such an opportunity occurs where the tree tobacco, *Nicotiana glauca*, a plant adapted for pollination by hovering hummingbirds, is introduced into the Old World where hummingbirds are absent. The observations made on this species in its introduced range in Africa highlight the question of why avian hover-pollination is limited to the New World.

Plants adapted for pollination by birds are widespread and are typified by having large volumes of dilute nectar, reddish colouration and unscented flowers (van der Pijl 1961). A subset of these species is adapted for hover-pollination by having their flowers oriented toward open space (Westerkamp 1990). This subset is essentially restricted to the New World, where they are pollinated by hovering hummingbirds. The coral trees of the genus *Erythrina* (Fabaceae) are a classical example: forty-two Old World and 15 New World species are adapted to perching birds; adaptations for hover-pollination occur in a paraphyletic assembly of 65 species restricted to the New World (Bruneau 1997).

The hummingbirds belong to the family Trochilidae, Order Apodiformes, and are restricted to the New World. The Trochilidae most often hover when feeding, and are superbly adapted to this mode of life, by having long, pointed wings, with long hands, light bodies, small feet, and long, straight bills (Collins and Paton 1989; Westerkamp 1990; Wells 1993; Altshuler and Dudley 2002; Warrick *et al.* 2005; Iwaniuk and Wylie 2007; Tobalske *et al.* 2007). In contrast, the nectarivorous birds of the Old World are in the order Passeriformes, and seldom hover and never do so consistently (Pyke 1981; Westerkamp 1990; Fleming and Muchhala 2008). Old World nectarivorous birds tend to have shorter wings, are larger on average, have bigger feet and curved bills. The main Old World groups are the Nectarinidae (sunbirds) of Africa and the Paleotropics, and the Meliphagidae (honeyeaters) of Australasia. Old World plants, such as the rattail, *Babiana ringens* (Iridaceae), sometimes go to great lengths to provide perches for these birds (Anderson *et al.* 2005). When Anderson *et al.* removed the perches, these individuals received fewer visits than unmanipulated plants.

The question of why the hover-pollination syndrome occurs only in the New World is not entirely resolved. The easy answer is that, by an accident of history, the Trochilidae clade is restricted to the New World, they are hover-feeders and most bird-pollinated plants in the

New World are adapted to them. That phylogenetic constraints are not the only factor, is suggested by anecdotal records of hovering in both the Old World sunbirds and honeyeaters, although admittedly, it is not clear that they are able to do so as a way of life (Burchell 1822; Paterson 1958; Skead 1967; Westerkamp 1990; Fry et al. 2000)(Burchell 1822; Paterson 1958; Skead 1967; Westerkamp 1990; Fry 2000; Fry and Keith 2000). In addition, a role for plant traits in determining the occurrence of hover-pollination is suggested by Miller's (1985) experiments and field observations, which show that hummingbirds prefer to feed from a perch whenever one is provided. Miller concluded that plants make hummingbirds hover.

Can plants also make Old World nectarivorous birds hover? The opportunity to answer this question does not exist in natural communities, because Old World plants have perches. Removal of perches from a few plants will also not be useful, because birds will have the choice of unmanipulated plants nearby and will not be forced to maintain a life-style of hovering. The ideal situation occurs when an arid Old World landscape, which in summer lacks nectar for birds, is invaded by a perch less hummingbird-pollinated plant such as *N. glauca*. Although anecdotal observations of sunbirds visiting *N. glauca* exists (Skead 1967; Williams et al. 1986; Tadmor-Melamed et al. 2004), the role of sunbirds in the pollination of *N. glauca* has not yet been determined, and the behaviour of the sunbirds at the plant has not been documented.

I attempt to answer the following questions: does *N. glauca* become integrated into a Old World pollination community, which lack Trochilidae? How does an Old World bird fauna respond behaviourally to a plant adapted for pollination by hovering birds? Can Old World birds enhance seed set and facilitate the invasion of a plant adapted for pollination by hovering birds? Can such plants, in turn, alter the distribution of Old World nectarivorous birds by supplying an abundant nectar source?

Materials and methods

Study species

Nicotiana glauca is a small fast-growing tree about 4 metres in height. It is native to northern Argentina where it occurs in riverbanks and along road sides (Goodspeed 1954; Hernandez 1981; Cronk and Fuller 1995; Nattero and Cocucci 2007). At least five species of hummingbird pollinate *N. glauca* in its native range (Nattero and Cocucci 2007). During the

last century *N. glauca* has become a cosmopolitan weed on four continents (Cronk and Fuller 1995; Thesis Appendix) including Africa, where it is declared a category 1 weed (Henderson 2001). In South Africa it is found mainly at disturbed sites, along roads, farmlands and in riverbeds, usually in small groups, but in the dry north western Cape populations can contain thousands of trees (Stirton 1978).

The plant can apparently flower throughout the year (Goodspeed 1954), as is the case in South Africa (Stirton 1978), but peaks in summer (Vahrmeijer 1981). The tubular, scentless flowers are a greenish-yellow when young, changing to a brighter yellow as they age. The stigma and anthers are situated within the mouth of the corolla. Anthesis occurs throughout the day, and flowers last for about 3 to 4 days (Schueller 2004; Nattero and Cocucci 2007). In its invasive range in California, *N. glauca* flowers are self-compatible and capable of self-pollination, but hummingbird pollination significantly enhances seed set (Schueller 2004). In invaded areas that lack nectar-feeding birds *N. glauca* apparently sets some seed through autogamy (Bogdanovic et al. 2006).

Study sites

I established an observation plot measuring 30 m² in a dense *N. glauca* population at Buffelsriver (29°44'82.9"S, 17°38'1.5"E) in the northern Cape. This population extended for approximately 10 km along a dry riverbed and contained thousands of plants (Fig. 7.1d). The site has been infested by *N. glauca* for at least 40 to 50 years (S. Whitlow pers. comm.). During the observation period, the landscape was, as far as I were able to ascertain, devoid of other nectar resources for birds. The effects of sunbird pollination on seed and fruit set were firstly investigated at the Buffelsriver site, but on my return four weeks later, most capsules had already opened. The experiment was therefore repeated at Leipoldtville (32°13'44.2"S, 18°29'3.82"E), a site with approximately 150 plants and several Malachite Sunbirds *Nectarinia famosa* (L.) in attendance.

Floral visitors

Pollinator observations were made with close focusing 8 x 40 binoculars on four days in November 2007 at Buffelsriver. The total time spent on formal observation of floral visitors was 8 hrs, but 8 days were spent in the population conducting experiments. Pollinator species, number of flowers visited and behaviour were recorded on a hand held dictaphone with a

timer. Behaviour was scored as perching or hovering and as legitimate visits (those making contact with the stigmas and anthers) and illegitimate visits (robbing the flower by piercing the corolla from the side). The duration of hovering flights could be calculated on transcription from the dictaphone and was accurate to the nearest second. Differences between the sunbird species in behaviour (hovering vs. perching) was tested with a χ^2 test. To test differences in the duration of foraging bouts between species a Mann-Whitney U-test was used.

Nectar properties

Nectar standing crop was measured on ten 2-3 day old flowers at two hour intervals from 6h30 to 16h30. Nectar standing crop is influenced by the rate of production, consumption and evaporation, and thus gives an indication of actual resource availability to birds. Nectar was extracted in the field using a 5 μ l capillary tube (Drummond Scientific Company, Broomall, PA, USA) and concentrations determined with a 0–50% field handheld refractometer (Bellingham and Stanley, Tunbridge Wells, UK). Nectar volume and concentration were converted to milligrams of sugar per flower. By estimating the number of flowers in the observation plot, nectar density per hectare could be calculated.

Effect of sunbirds on seed production

Two measures of plant fitness were determined namely, capsule set and number of seeds per capsule. Capsule set could be determined by counting filled capsules and the stem scars left by aborted flowers. Inflorescences (n-trees = 7; n-flowers = 82) were bagged in fine-mesh pollinator-exclusion bags whilst in bud phase, and kept bagged throughout the flowering period; a nearby inflorescence (n-trees = 7; n-flowers = 83) on each plant was marked and used as a control (open to all flower visitors). Number of flowers per treatment per plant (n=7) varied between 5 and 21 flowers (avg. 12 flowers per treatment per plant). Capsules were collected two weeks later.

Only a subset of capsules was used in determining seed set. To calculate the number of seeds per capsule, closed but mature capsules were collected and dried at 60°C for 48 h. The seeds were weighed with a balance (accuracy of 0.001 mg). To determine number of seeds per capsule, 100 seeds were weighted (n = 10 replicates) to obtain a calibration point. A non-

parametric Mann-Whitney U-test was used to test differences in seed set and a t-test to test for differences in capsule set.

Effect of N. glauca on sunbird distribution

To test the prediction that *N. glauca* increases nectar-feeding bird densities at a very local scale, 10 minute point counts (25m radius plots) were conducted at *N. glauca* present and adjoining *N. glauca* absent sites (between 1.5 and 4 km away). Point counts were conducted at Buffelsriver and surrounding areas (7 with *N. glauca* present and 9 where the plant was absent) on the 16-19 October 2007 and at Leipoldtville (1 present; 1 absent) on the 22 November. Several point counts per day were conducted between sunrise and 11:00. Thus, the midday lull in bird activity was avoided. The *Nicotiana glauca* absent sites were chosen to have similar vegetation structure as the *N. glauca* present sites. Observations were recorded with a dictaphone and later transcribed. Inclement weather was avoided. Differences in nectar feeding bird densities between *N. glauca* present and absent sites were tested with a Mann-Whitney U-test.

Results

Floral visitors

The most frequent visitor observed was the Malachite Sunbird (Table 7.1). Visitation rates by Southern Double-collared Sunbirds *Cinnyris chalybea* (L.) and Dusky Sunbirds *C. fuscus* (Vieillot) may be slightly underrepresented as they are less conspicuous in the dense *N. glauca* stands (Fig. 7.1d). All three observed sunbird species had the ability to hover when feeding on *N. glauca* flowers. The Malachite Sunbird (largest sunbird species) hover fed significantly more frequently than the smaller sunbird species ($\chi^2 = 68$, DF = 2, $p < 0.001$) (Fig. 7.1a, 7.2). When comparing hovering behaviour between species Malachite Sunbirds hover for significantly longer time periods than Dusky Sunbirds (Mann-Whitney *U*-test, $U = 1231$, $p < 0.001$, $n_{\text{dusky}} = 32$, $n_{\text{malachite}} = 151$), and visit more flowers sequentially, per foraging bout (Mann-Whitney *U*-test, $U = 1104$, $p < 0.001$, $n_{\text{dusky}} = 32$, $n_{\text{malachite}} = 130$) (Table 7.2). Only four hover feeding visits by Southern Double-collared Sunbirds were observed, therefore only malachite and Dusky Sunbird visits are compared. The longest hovering visit was by a malachite female visiting at least eight flowers and hovering continuously for over 30 s.

Pollen is mostly carried on the beak of the Malachite Sunbird (Fig. 7.1b), whereas there is contact between anthers and forehead feathers of the smaller sunbirds (Fig. 7.1c). Nearby branches are used if available (Fig. 7.1e). Malachite Sunbirds were never observed robbing flowers whilst Dusky Sunbirds robbed during 8 of 114 visits and Southern Double-collared Sunbirds robbed on 62 out of 102 visits. The flowers are flexible and give way when probed from the side therefore if the bird applies pressure towards the base of the flower it is sometimes able to pierce the corolla tube while perched, but is not able to do this while hovering. The only other flower visitors were Cape weavers, *Ploceus capensis* (L.), which destroyed flowers in an attempt to reach the nectar. Honey bees *Apis mellifera*, and other bee species, were visiting *Oncosiphon* species (Asteraceae) but were never observed visiting *N. glauca* flowers.

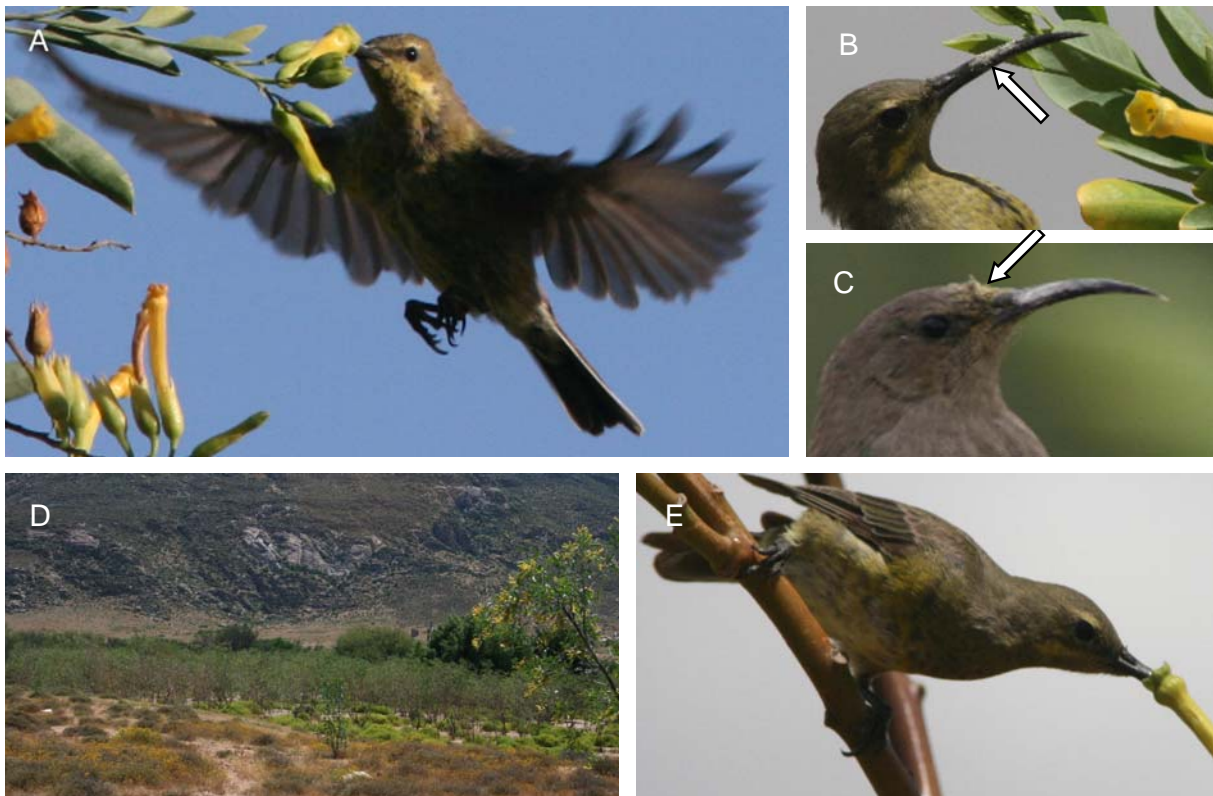


Figure 7.1 (a) *Nectarinia famosa* (Malachite Sunbird) hover feeding at and pollinating a *N. glauca* flower; (b) Pollen is mostly carried on the beak of the Malachite Sunbird; (c) There is contact between anthers and forehead feathers of the smaller sunbirds like the Southern Double-collared Sunbird (*C. chalybea*); (d) Dense stands of *N. glauca* at Buffelsriver in the Northern Cape Province; (e) Nearby branches of the same or different individuals are used when feeding on flowers while perched. (photographs by Sjirk Geerts)

Nectar properties

Field observations suggested that birds prefer the older yellow rather than the younger greener flowers, and therefore nectar was extracted from two to three day old flowers. Typical of bird pollinated plants, nectar volumes declined in the afternoon (one-way ANOVA, $F_{5, 54} = 3.15$, $p = 0.01$) (Fig. 7.3), but interestingly, sunbirds foraged throughout the day. Nectar density in the observation plot was 3208 g of sucrose per ha⁻¹.

Effect of sunbirds on seed production

Capsule set was significantly higher when flowers were left open to receive visits by sunbirds (t-test, $DF = 12$ $t = -2.5$ $p = 0.028$) (Fig. 7.4). Eighty two bagged flowers resulted in 46 capsules and 83 open flowers in 62 capsules. Capsules that resulted from open pollination also contained significantly more seeds than capsules that were formed inside pollinator exclusion bags (Mann-Whitney U-test, $U = 433.5$, $z = 4.60$, $n_{\text{bagged flowers}} = 34$, $n_{\text{open flowers}} = 49$, $p < 0.001$) (Fig. 7.5). Even when the sample size is reduce to the plant level, bagged flowers still have significantly lower seed set (Mann-Whitney U-test, $U = 6.0$, $z = 2.36$, $p = 0.018$, $n_{\text{group 1}} = 7$, $n_{\text{group 2}} = 7$). Thus from the flower to the seed dispersal stage the total difference in seeds set was 34881 seeds for 82 bagged flowers and 91641 seeds for 83 open flowers.

Effect of N. glauca on sunbird distribution

Only two sunbird species, the Southern Double-collared Sunbird and the Malachite Sunbird, were encountered during point counts. A significantly higher number of sunbirds (Southern Double-collared Sunbird and the Malachite Sunbird combined) were encountered during point counts at *N. glauca* present sites ($n = 29$ sunbirds) than at *N. glauca* absent sites ($n = 2$ sunbirds) (Mann-Whitney U- test, $U = 2.00$, $z = 3.38$, $p < 0.001$, $n_{\text{present}} = 8$, $n_{\text{absent}} = 10$) (Fig. 7.6).

Table 7.1. Visitation rate of *Nicotiana glauca* flower visitors at Buffelsriver.

Species	Visits per flower per hour
Malachite Sunbird	0.033
Dusky Sunbird	0.007
Southern Double-collared Sunbird	0.007
Total	0.047

Table 7.2. Comparisons of hover feeding behaviour in the Malachite and Dusky Sunbirds (mean and S.D.). Only four hovering visits by Southern Double-collared Sunbirds were observed and therefore excluded.

Sunbird species	Length of hovering bout in seconds	No. of consecutive flowers hovered	Hovering time per flower in seconds
Malachite Sunbird	4.0 (3.6)	3.1 (3.8)	1.5 (1.09)
Dusky Sunbird	1.8 (1.41)	1.5 (0.76)	1.2 (0.52)

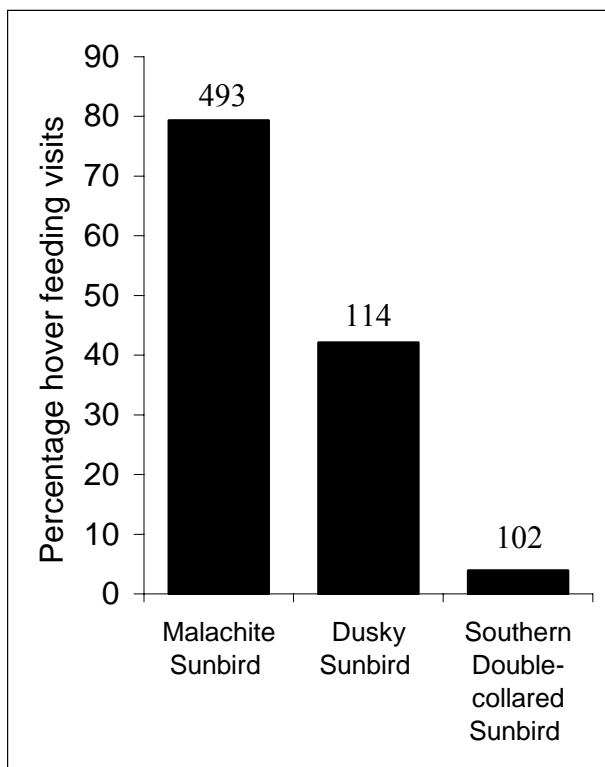


Figure 7.2. Percentage of hover feeding visits for the three observed sunbird species. Numbers above bars indicate number of visits observed.

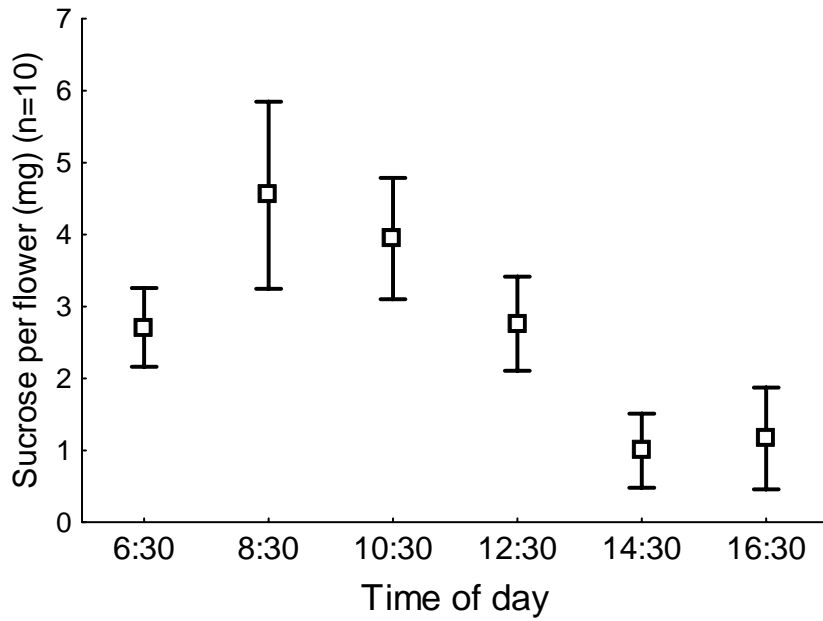


Figure 7.3. Sucrose availability to sunbirds throughout the day (n = 10 new flowers at each time interval)



Figure 7.4. Mean percentage of capsule set between pollinator excluded and flowers open to pollinators (numbers above bars indicate number of plants in each treatment; bars indicate S.D.).

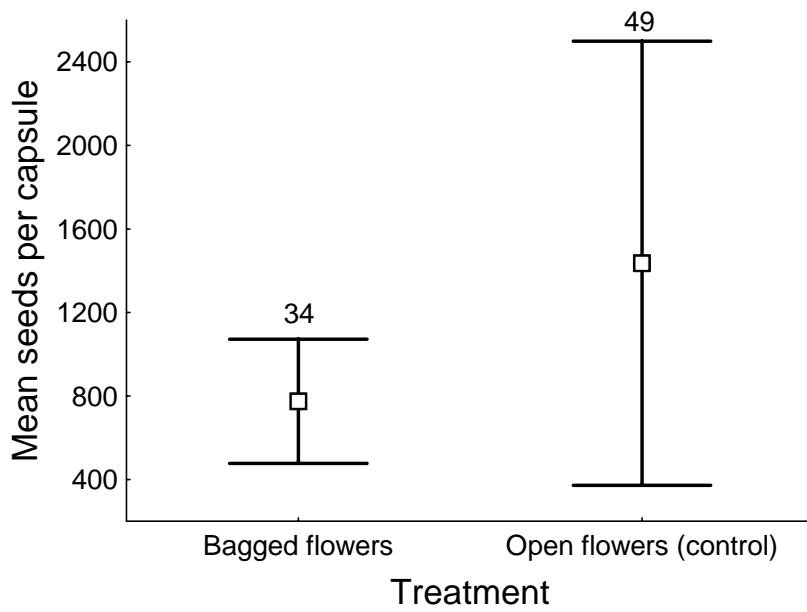


Figure 7.5. Seeds per capsule between pollinator excluded and pollinator visited flowers. Only flowers that set seed are included (numbers above bars indicate number of capsules; bars indicate S.D.).

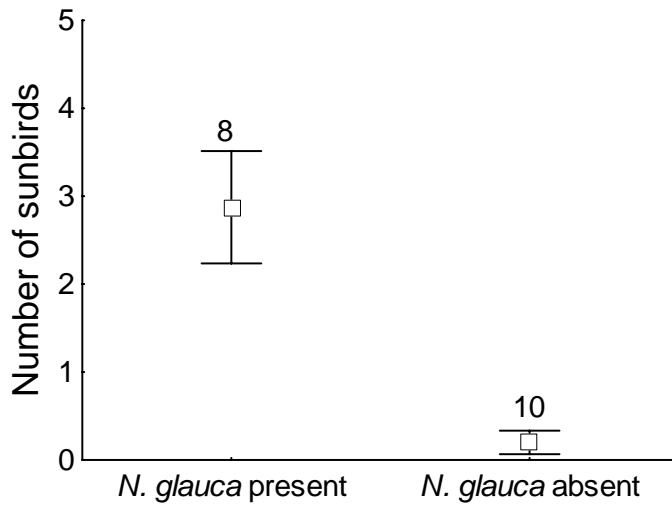


Figure 7.6. Average number of sunbirds observed at *N. glauca* absent and *N. glauca* present sites (numbers above bars indicate number of sites; bars indicate S.E.).

Discussion

Despite being specialized for pollination by hovering birds, the invasive tree tobacco (*N. glauca*) has become integrated into an Old World pollination community that lacks hummingbirds. The integration of this pollination specialist plant was made possible by unexpected behavioural plasticity of Old World nectarivorous birds. Although Old World nectarivorous birds have only very rarely been observed to hover feed on indigenous plants, three species of Old World sunbirds behaved like hummingbirds and hover-fed when they were presented with a New World plant adapted for hover-pollination. The largest of the species, the Malachite Sunbird, *Nectarinia famosa*, was the most inclined to hover and hovered for the longest period of time (Fig. 7.2). As is the case with hummingbirds (Miller 1985), the sunbirds used perches when these were available. Furthermore, sunbirds could apparently sustain a lifestyle in which they were obtaining up to 80% of their nectar intake by hovering at *N. glauca*. Alternative nectar sources for birds were absent from this semi-desert region during the time of the study (the dry season).

Nicotiana glauca in turn enhanced the local density of sunbirds: point count plots that contained *N. glauca* had more sunbirds than nearby uninvaded plots. The high local abundance of Malachite Sunbirds at the end of November was particularly surprising because this species normally starts migrating out of the northwest of South Africa during October, dropping to zero percentage reporting rate at the peak of the summer drought in December (Harrison et al. 1997). In exchange for nectar rewards, the birds successfully transferred pollen between the plants and enhanced seed set relative to pollinator-excluded flowers (Fig. 7.5). In addition, the birds are likely to enhance outcrossing, a benefit not quantified in this study. By enhancing seed set, and probably genetic diversity, the sunbirds might be facilitating the invasion of this alien species into Africa. This result supports the growing realization that the lack of the original pollinator assemblage seldom poses a limitation to plant invasion (Chittka and Schurkens 2001; Brown et al. 2002; Graves and Shapiro 2003; Moragues and Traveset 2005; Morales and Aizen 2006), although not as much evidence exists for successful integration of a pollination specialist into local pollination communities as many invasive plants are pollinator generalists that utilize a diversity of pollinators (Valentine 1977; Richardson et al. 2000).

The results highlight the question of the relative role of bird vs. plant traits as factors determining hover-pollination. The importance of bird traits has been stressed by

physiologists and ornithologists who have studied the remarkable adaptations of the Trochilidae for hover flight (reviewed in Collins and Paton 1989; Altshuler and Dudley 2002). Pyke (1981) for example has suggested that the unusually small size of the Trochilidae predisposes them to hovering, while the large size of Old World nectarivores precludes hovering. A possible, though disputed benefit of hover-feeding is decreased foraging time (Collins and Paton 1989). The obvious cost is greater energy expenditure. Pyke (1981) argues that in small bird species this cost is less and the benefit is more likely to outweigh the cost. It comes as a double surprise then that the heaviest of the three sunbird species observed in this study is the most inclined to hover (Hockey *et al.* 2005). Hovering behaviour was apparently not facilitated by unusually high nectar availability. Nectar density in the *N. glauca* stands (up to 3200 g sucrose per ha⁻¹) is similar to levels that I have observed in stands of native *Protea* species (750-15000 g sucrose per ha⁻¹) but much higher than in stands of the native *Erica coccinea* (33 g sucrose per ha⁻¹).

While phylogenetic constraints on hovering flight are almost certainly an important factor in determining the distribution of hover pollination, the results presented here, along with others (Miller 1985; Westerkamp 1990), suggest that the ability to hover may not be as constrained as previously thought. At least some Old World birds can also maintain a hovering lifestyle. If phylogenetic constraints are lifted, the question of why Old World plants have perches while New World plants often lack them, becomes a question for ecologists. Community context might be an important consideration. Is there stronger competition among Old World than among New World plants for attracting nectarivorous birds? Why could it be advantageous for plants to make birds hover? Are plants without perches less likely to be robbed? Does hover-pollination decrease pollen transfer among flowers on the same plant? Or, do plants make birds hover because hovering makes them hungry for nectar?

The apparent lack of a strong phylogenetic constraint on hover-pollination also has possible implications for invasion biology. Old World birds in other parts of the introduced range of *N. glauca*, for example in Australia and India, might also be enlisted as hovering accomplices of *Nicotiana glauca*. More generally, alien New World plants adapted for hover-pollination, are likely to find willing partners in the Old World Nectarinidae.

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References

- Altshuler DL, Dudley R (2002) The ecological and evolutionary interface of hummingbird flight physiology. *J Exp Biol* 205:2325-2336
- Anderson BW, Spencer WW, Barrett CH (2005) Specialized bird perch aids cross-pollination: A plant scores by providing an access point for visiting sunbirds to feed on its nectar. *Nature* 435:41
- Bogdanovic S, Mitic B, Ruscic M, Dolina K (2006) *Nicotiana glauca* Graham (Solanaceae), a new invasive plant in Croatia. *Acta Bot Croatia* 65:203-209
- Brown BJ, Mitchell RJ, Graham SA (2002) Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology* 83:2328-2336
- Bruneau A (1997) Evolution and homology of bird pollination syndromes in *Erythrina* (Leguminosae). *Am J Bot* 84:54-71
- Burchell WJ (1822) *Travels in the interior of southern Africa*, vol. 1. – Longman, Hurst, Rees, Orme and Brown, London
- Chittka L, Schurkens S (2001) Successful invasion of a floral market - An exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. *Nature* 411:653
- Collins BG, Paton DC (1989) Consequences of differences in body-mass, wing length and leg morphology for nectar-feeding birds. *Aust J Ecol* 14:269-289
- Cronk QCB, Fuller JL (1995) *Plant invaders: the threat to natural ecosystems*. Chapman and Hall, London
- Fleming TH, Muchhala N (2008) Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of vertebrate pollination systems. *J Biogeogr* 35:764-780
- Fry CH, Keith S, Urban EK (2000) *The birds of Africa*, vol VI. Academic, London
- Goodspeed TH (1954) The genus *Nicotiana*: origins, relationships, and evolution of its species in the light of their distribution, morphology, and cytogenetics. *Chronica Botanica Co.*
- Graves SD, Shapiro AM (2003) Exotics as host plants of the California butterfly fauna. *Biol Conserv* 110:413-433

- Harrison JA, Allan DG, Underhill LG, Herremans M, Tree AJ, Parker V, Brown CJ (eds) (1997) The atlas of southern African birds. BirdLife South Africa, Johannesburg
- Henderson L (2001) Alien weeds and invasive plants. Plant Protection Research Unit Handbook Agricultural Research Council. Paarl Printers, Cape Town
- Hernandez H (1981) Sobre la ecología reproductiva de *Nicotiana glauca* Grah.: una maleza de distribución cosmopolita. Bol de la Sociedad. Bot de Mex 41:47-73
- Hockey PAR, Dean WRJ, Ryan PG (2005) Roberts' birds of Southern Africa. Trustees of the John Voelcker Bird Book Fund, Cape Town
- Iwaniuk AN, Wylie DRW (2007) Neural specialization for hovering in hummingbirds: Hypertrophy of the pretectal nucleus lentiformis mesencephali. J Comp Neurol 500:211-221
- Miller RS (1985) Why Hummingbirds hover. The Auk 102:722-726
- Moragues E, Traveset A (2005) Effect of *Carpobrotus* spp. on the pollination success of native plant species of the Balearic Islands. Biol Conserv 122:611-619
- Morales CL, Aizen MA (2006) Invasive mutualisms and the structure of plant-pollinator interactions in the temperate forests of north-west Patagonia, Argentina. J Ecol 94:171-180
- Nattero J, Cocucci AA (2007) Geographical variation in floral traits of the tree tobacco in relation to its hummingbird pollinator fauna. Biol J Linn Soc 90:657-667
- Paterson J (1958) Hovering by *Nectarinia chalybea* and *Nectarinia venusta*. Ostrich 29:48
- Pemberton RW, Wheeler GS (2006) Orchid bees don't need orchids: Evidence from the naturalization of an orchid bee in Florida. Ecology 87:1995-2001
- Pyke GH (1981) Why hummingbirds hover and honeyeaters perch. Animal Behavior 29:861-867
- Rezende EL, Jordano P, Bascompte J (2007) Effects of phenotypic complementarity and phylogeny on the nested structure of mutualistic networks. Oikos 116:1919-1929
- Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmanek M (2000) Plant invasions - the role of mutualisms. Biol Rev 75:65-93
- Schueller SK (2004) Self-pollination in island and mainland populations of the introduced hummingbird-pollinated plant, *Nicotiana glauca* (Solanaceae). Am J Bot 91:672-681
- Skead CJ (1967) The sunbirds of southern Africa, also the sugarbirds, the white-eyes and the spotted creeper. Cape and Transvaal Printer

- Stirton CH (1978) Indringerplante: mooi, maar gevaarlik: 'n gids vir die uitkenning en die beheer van ses-en-twintig indringerplante van die Provinsie die Kaap die Goeie Hoop. Creda press, Cape Town
- Tadmor-Melamed H, Markman S, Arieli A, Distl M, Wink M, Izhaki I (2004) Limited ability of Palestine sunbirds *Nectarinia osea* to cope with pyridine alkaloids in nectar of tree tobacco *Nicotiana glauca*. *Funct Ecol* 18:844-850
- Tobalske BW, Warrick DR, Clark CJ, Powers DR, Hedrick TL, Hyder GA, Biewener AA (2007) Three-dimensional kinematics of hummingbird flight. *J Exp Biol* 210:2368-2382
- Vahrmeijer J (1981) Gifplante van Suider-Afrika wat veeverliese veroorsaak. Tafelberg Press, Cape Town
- Valentine DH (1977) The pollination of introduced species, with special reference to the British Isles and the genus *Impatiens*. - In: Richards, A. J. (ed), *The Pollination of Flowers by Insects*. Academic Press, New York, pp 117-123.
- van der Pijl L (1961) Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution* 15:44-59
- Van Kleunen M, Johnson SD (2007) Effects of self-compatibility on the distribution range of invasive European plants in North America. *Conserv Biol* 21:1537-1544
- Warrick DR, Tobalske BW, Powers DR (2005) Aerodynamics of the hovering hummingbird. *Nature* 435:1094-1097
- Wells DJ (1993) Ecological correlates of hovering flight of hummingbirds. *J Exp Biol* 178:59-70
- Westerkamp C (1990) Bird-flowers – hovering versus perching exploitation. *Bot Acta* 103:366-371
- Williams AJ, Braine SG, Bridgeford P (1986) The biology of the Dusky Sunbird in S.W.A./Namibia: a review. *Lanioturdus* 22:4-10

8 Molecular systematics and ecology of invasive Kangaroo Paws in South Africa: management implications for a horticulturally important genus*

Most legislation pertaining to non-native organisms is implicitly focussed at the individual species level. However, in some cases interspecific hybrids can be more invasive than any of the parent species. This is problematic for policy makers, and for horticulturists developing or trading in new ornamental cultivars. We explore these issues in the context of the need to manage naturalized populations of Kangaroo Paws (*Anigozanthos* species) in South Africa. Self-sustaining, dense populations of naturalized Kangaroo Paws occur at several localities and are highly attractive to local nectar-feeding birds. The populations show high levels of seed set with or without bird pollination. Given the known propensity of Kangaroo Paws to hybridise in their native range in Australia, and confusion about the species identity of naturalized populations in South Africa, it was essential to resolve some key taxonomic issues in the group. We constructed the first molecular phylogeny for *all* species of the Kangaroo Paw group (genera *Anigozanthos* and *Macropidia*; family Haemodoraceae). As previously determined by taxonomists working on herbarium specimens, naturalized populations were identified as *A. flavidus*. In addition, we also identified a second species, *A. rufus*. Relative genome size estimates for *Anigozanthos* species indicated that small inter-specific differences in genome sizes are positively correlated to hybrid fitness. *Anigozanthos flavidus* and *A. rufus* have relatively ‘compatible’ genomes and may produce fertile hybrids under field conditions. However, for species whose genome size differ more than ~30%, there is little inter-specific compatibility and consequently a very low risk of producing fertile hybrids. In conclusion, we recommend that trade in Kangaroo Paws in South Africa should be temporarily restricted and that particular cultivars should first be subjected to a careful risk assessment.

* Only the ecological part of this chapter is my own work, the molecular work was done by Le Roux, JJ and the flow cytometry work by Suda, J. JJ Le Roux, S Geerts, P Ivey, S Krauss, DM Richardson, J Suda, JRU Wilson. *Invasion Biology* in press, DOI 10.1007/s10530-010-9818-4

Introduction

Managing invasive populations by responding rapidly, even in the absence of insights from detailed natural history studies, is cost-effective (Simberloff 2003). This is because the cost and likelihood of success of an eradication programme decreases with an increase in infestation (Rejmánek and Pitcairn 2002). However, any action taken, or planned, must be justifiable. This is particularly important when the invasive species has commercial or other value. Increasingly, the justification for intervention needs to include objective verification that the putative invader has a high risk of spreading and/or causing damage. A key task in objective risk analysis for biological invasions is the accurate determination of the taxonomic identity of the subject and an assessment of its biogeographic status: native vs. alien (*sensu* Pyšek et al. 2004).

When the taxonomic identity of an organism is clear and the species is known to be invasive elsewhere in the world, initial risk assessment may require little additional information (Pheloung et al. 1999). However, if the taxonomy is uncertain (e.g. due to a lack of local expertise, cryptic invasions, or possible hybridisation), elucidation of the taxonomic status is crucial for legislation. Moreover, well-resolved taxonomy on its own is of little value if no additional information is available.

There have been several attempts to define which parameters and traits are pertinent to invasion risk for plants. For example, Richardson et al. (2000) suggested that spread of alien plants may be limited by a lack of suitable pollinators, and that pollinators in the introduced range may play an important role in maintaining reproductive fitness and in facilitating spread. Therefore, studies investigating the breeding system and/or pollination ecology of naturalized plants can both aid risk assessment, and help estimate the potential for hybridisation (Ellstrand and Schierenbeck 2000). Such basic natural-history studies should run concurrently with management programs. This dual approach, whereby evidence is gathered without compromising timely action to limit invasions, is particularly important if there is a conflict of interest or if co-operation needs to be encouraged or enforced to achieve eradication (Simberloff 2009).

South Africa is developing new legislation to regulate the use, sale, and control of non-native species: the National Environment Management: Biodiversity Act

(NEM:BA). NEM:BA follows on from the Conservation of Agricultural Resources Act (CARA) in listing regulated species (Nel et al. 2004). Much of the legislation relies on species being defined entities, but the legislation also attempts to deal with cultivars and hybrids. For example: “all seed producing species or hybrids of *Lantana* that are non-indigenous to Africa” are regulated. This specification, and prolonged engagement with horticulturists, resulted in an agreement to destroy all stock of the lantana cultivar ‘Sundancer’ when it was shown to produce fertile seeds, but to continue trading with the species *Lantana montevidensis*, which does not produce fertile seeds in South Africa (although Czarnecki & Deng [2009] showed some cultivars of *L. montevidensis* produce fertile pollen and seed in experimental trials in the USA). The regulations thus aim to be pro-active, but rely on focussed research to provide key biological information on which to base decisions. Continued support from horticulturists will depend on the understanding that trade restrictions will only apply to taxa with a high risk of invading, based on objective and transparent criteria.

In this paper we describe a case-study in South Africa of a group of Australian plants called Kangaroo Paws (the genera *Anigozanthos* and *Macropidia*). We assess the current extent of invasion of known naturalized populations, place this in a phylogenetic context, and, by exploring genome compatibility, assess the likelihood of hybridisation. Based on these findings we provide recommendations on how to accommodate this group of plants in developing regulations.

Materials and Methods

Study system

Kangaroo Paws are perennial herbs native to Western Australia from the genera *Anigozanthos* and *Macropidia* (Haemodoraceae). Adaptations to bird pollination, unusual flower morphology and colouration (Fig. 8.1) make them popular and important horticultural species in many parts of the world (Tsrör et al. 2005). Twelve species of Kangaroo Paws are currently recognized (Hopper 1987); 11 in the genus *Anigozanthos* and a single species in the genus *Macropidia* (*M. fuliginosa*). The taxonomy of Kangaroo Paws is problematic and has been the subject of numerous studies (Anderberg and Eldenäs 1991; Hopper 1980; Hopper and Campbell 1977; Hopper et al. 1999, 2009; Simpson 1990). In particular, the validity of *Macropidia* as

a monotypic genus has been discussed in detail, with some authors supporting its monotypic status (Hopper 1980; Hopper and Campbell 1977; Hopper et al. 1999, 2009) while others have argued in favour of it being lumped within *Anigozanthos* (Anderberg and Eldenäs 1991, Simpson 1990). Relationships between closely-related taxa within *Anigozanthos* are also unclear (Hopper 1980). Contributing to this taxonomic obscurity is the ease with which some species within *Anigozanthos* hybridise (Hopper 1977a, 1977b, 1978, 1980; Hopper and Burbidge 1978; Shchori et al. 1995).

Kangaroo Paws are cultivated commercially in several countries around the world and at least 26 cultivars are registered with the Australian Cultivar Registration Authority (<http://www.anbg.gov.au/acra/acra-list-2009.html#a>). In its native country, populations of *A. flavidus* have naturalized and are spreading in New South Wales and South Australia (Australian Native Plants Society 2009; Martin O’Leary, personal communication 2009) and are considered serious environmental weeds (Hoskins et al. 2007). Hopper (1993) noted that *A. flavidus* is extremely competitive and indeed the most invasive and robust species of Kangaroo Paw. Despite this, *A. manglesii* is the only taxon listed in Randall’s (2007) “The introduced flora of Australia and its weed status”.

In South Africa, Kangaroo Paws have been traded in the horticultural industry since the 1990s and currently about ten commercial hybrids are available (Jacques Malan, MalanSeuns Nursery, personal communication). Data from Compton Herbarium in Cape Town indicated that areas around Kleinmond, in South Africa’s Western Cape Province, are the only known locations where Kangaroo Paws occur (Fig. 8.2). These records identified naturalized populations as *A. flavidus* and, potentially, *A. manglesii*. These populations presumably are derived from plants introduced to a local flower farm (Honingklip) somewhere between 1960 and 1969. The record for this population in the South African Plant Invaders Atlas gives the taxon as *A. flavidus* (Henderson 2007), although Stephen Hopper (personal communication), the world authority on the group, has suggested that these populations represent “*A. flavidus* and hybrids”. The current invasion occurs within 10 km of one of South Africa’s most pristine and

important biodiversity-hotspot conservation areas, the Kogelberg Biosphere Reserve (Fig. 8.2).

Mapping of invasive populations and population size estimates

All known naturalized populations were systematically surveyed by walking parallel lines extending ~ 50 m beyond the most isolated plant found (see Zenni et al. 2009 for details). For smaller outlying populations the geographic position of each plant found was marked using a hand-held Global Positioning System (GPS Garmin® GPSmap 60CSx, maximum resolution of 3 m). For large populations (> 20 000 plants) the tracklogs from the tracking lines recorded in the GPS were used as the basis for drawing a polygon of the surveyed area in ArcView GIS v. 3.2. Three plots of 15 m × 15 m within these larger populations were then subdivided into quarters and the percentage plant coverage (rhizome mats) visually determined independently by two persons. We also used twelve 2 m × 2 m random plots within large populations to determine the number of sprouts/unit area covered by monotypic stands. We used sprouts because in most cases it was impossible to distinguish individual plants from sprouts (clusters of fans [leaves]) from the same rhizome in dense stands. Using the spatial analyst tool in ArcGIS we determined the total area of the polygon (population) for large populations. Using data for coverage and density estimates we were able to extrapolate estimates for population size/density and the percentage area covered.

Pollination biology

Flowers in the largest patch of Kangaroo Paws (Fig. 8.2) were observed for bird visitation for 1 h during peak activity (morning) before and after flower removal. Pollinator species, number of flowers visited and behaviour were also recorded. To determine accessibility for sunbirds and sugarbirds, flower depth was measured in 13 randomly selected flowers.

As an additional estimate of the attractiveness to pollinators, we estimated the standing crop of nectar. This is influenced by the rate of production, consumption, and evaporation of nectar and gives an indication of the actual resource available to birds at any time. Nectar standing crop was measured in the morning (when birds were still

very active) from 15 randomly selected young flowers. We determined nectar volume in the field with a 5 μ L capillary tube and nectar concentration with a handheld 0-50 % Bellingham and Stanley refractometer (Tunbridge Wells, Kent, UK).

Seed set

To determine the importance of pollinators on individual fitness, we compared capsule set and number of seeds set per capsule for bagged and unbagged flowers. Twenty clumps were selected from the largest population and inflorescences were bagged in fine-mesh pollinator exclusion bags while in bud phase, and kept bagged throughout the flowering period (a total of 748 flowers; 10 inflorescences). A nearby inflorescence in the same clump was marked and used as a control (flowers were open to all flower visitors, with a combined total of 912 flowers; 10 inflorescences). The proportion of capsules that set seed was determined by comparing the number of filled capsules with the number of stem scars left by aborted flowers after 12 weeks. Capsules were dissected and numbers of seeds counted. All statistical analyses were conducted in R (R Development Core Team, 2009). Differences in capsule set between open and bagged flowers were tested using generalised linear models with binomial errors. Differences in seed set were tested using both generalised linear models with a variety of error structures, to explore the impact of over-dispersion, and with generalised linear mixed-effect models, to account for between-plant variation.

Kangaroo Paw Taxonomy

Taxon sampling and DNA extraction

Leaf samples were collected from South Africa and Australia, dried and kept on silica gel (Appendix 1). Accessions of all *Anigozanthos* species and *M. fuliginosa* in Australia were obtained from collections held at Kings Park and Botanic Garden in Perth, Western Australia. Invasive taxa in South Africa were collected by JLR. Total genomic DNA was extracted using the CTAB extraction protocol described by Doyle and Doyle (1990).

PCR amplification and DNA sequencing

The spacer and intron regions of the plastid *trnL-F* region were amplified using the universal primers "c" and "f" (Taberlet et al. 1991). Each 50 µL PCR reaction contained approximately 50 ng of genomic DNA, 200 µM of each dNTP (AB gene, supplied by Southern Cross Biotechnologies, Cape Town, South Africa), 25 pmoles of each primer, 5 U *Taq* DNA polymerase (Super-Therm JMR-801, Southern Cross Biotechnologies, Cape Town, South Africa), 1 × PCR reaction buffer, 1.5 mM MgCl₂. PCR consisted of a thermocycle of initial denaturation of 95° C for 5 min; 35 cycles at denaturation at 94° C for 30 s, annealing at 58° C for 60 s, elongation at 72° C for 90 s; and final extension at 72° C for 10 min. All PCR amplifications were done in a Multigene gradient cycler (Labnet International, Inc., New Jersey, USA). Amplified DNA fragments were purified using the QIAquick PCR Purification Kit (Qiagen, Southern Cross Biotechnologies, Cape Town, South Africa) and sequenced in both directions using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit and an automated ABI PRISM 377XL DNA sequencer (PE Applied Biosystems, Foster City, California, USA).

DNA sequence alignment and analysis

Contiguous sequences were constructed, edited and aligned using BioEdit version 7.0.5.3 (Hall 1999). All edited sequences have been deposited in GenBank (<http://www.ncbi.nlm.nih.gov>, Appendix 1). We also included data for other Kangaroo Paw species that were available on GenBank. We used *Blancoa canescens*, a known sister species to Kangaroo Paws (Hopper et al. 1999), as outgroup taxon. The dataset was analyzed using maximum-likelihood search criteria with parameter estimates obtained from the program MODELTEST version 3.06 (Posada and Crandall 1998). We estimated base frequencies and the transition/transversion ratio from the data. Heuristic searches were carried out with TBR, MULTREES, and COLLAPSE options in effect and performed with PAUP* 4.0beta10 (Swofford 2002); 1000 bootstrap replicates (Felsenstein 1985) were used to assess branch support. Trees were visualised in TreeEdit version 1.0a1-19 (<http://evolve.zoo.ox.ac.uk/software/TreeEdit/main.html>).

Genome size estimates and hybrid reproductive output and fertility

Relative genome sizes of silica-dried specimens were determined by flow cytometry using a Partec PA II instrument (Partec GmbH., Münster, Germany) equipped with a mercury arc lamp for UV excitation. The methodology generally followed the two-step procedure (without centrifugation) described by Suda and Trávníček (2006). Otto I buffer (0.1 M citric acid, 0.5% Tween 20) was used for nuclei isolation and Otto II buffer (0.4 M Na₂HPO₄ × 12 H₂O), supplemented with AT-selective fluorochrome DAPI (at final concentration 4 µg/mL) and β-mercaptoethanol (2 µL/mL), was used to stain the nuclear suspension. *Bellis perennis* L. (2C = 3.38 pg) was selected as an appropriate internal reference standard and flow histograms were evaluated using the Partec FloMax software ver. 2.4d.

Flow cytometry analysis was conducted on all *Anigozanthos* species except *A. onycis* due to a lack of tissue material for this species (Table 8.1). A matrix of genome size ratios (differences) was constructed for all pairwise species combinations for which we had estimates. Similarly, we constructed matrices for numbers of seed set, seed germination success, and pollen fertility for the same pairwise species combinations (hybrids), using data from Hopper (1980). These datasets allowed us to explore how differences in parental genome sizes affect their resulting hybrids' fitness (seed set, germinability, pollen fertility). We used linear regression to examine the effect of the ratio of genome sizes (larger over smaller, log-transformed) on the number of seeds per capsule produced by hybrids (also log-transformed). We also tested a regression weighted by the number of capsules investigated, as this varied from 2 to >200, see Appendix 1 in Hopper (1980). The effect of the ratio of genome sizes on the probability of hybrid seed germination was tested using a generalised linear model with binomial and quasi-binomial errors. Finally, the effect of the ratio of genome sizes on the percentage pollen fertility of the F₁ hybrids (logit transformed and zeros excluded, as data on sample size was not available) was tested using a linear model. In this case, we also tested to see whether there was an effect of weighting by the number of hybrids the pollen was collected from. We assumed that different parental crosses are distinct entities (although obviously with the same ratio of genome sizes).

This may inflate the significance level of any results, but given the strength of the relationships obtained this should not affect the qualitative conclusions.

Results

Invasive Kangaroo Paw populations in South Africa

Population mapping and size estimates

We found one large population that covered around 6676 m² on Honingklip farm, the original point of introduction into the Western Cape (indicated by black arrow in Fig. 8.2). Within this stand, percentage cover by monotypic stands of plants in subplots ranged from 15% to 85% (average: 42%). These monotypic stands contained between 102 and 462 individual sprouts (average 265). Using these data we estimated that this area is currently infested by monotypic stands of Kangaroo Paws roughly corresponding to around 180 000 individual sprouts. This population was surrounded by scattered individuals up to 700 m away.

We also found two small populations further away. The first population had around 59 individuals in an urban area about 5.5 km from the main Honingklip population. It appears to have spread from a deliberate planting. The second population was about 7.0 km from the main Honingklip population in a mountainous area of natural vegetation and consisted of around 227 individuals. The origin of this population is unknown, but is probably the result of accidental, human-mediated, long-distance dispersal.

Floral visitors

The high density of Kangaroo Paws provided a nectar-rich environment that attracted high numbers of native nectarivorous birds, especially Cape Sugarbirds (*Promerops cafer*). We observed 425 sugarbird visits in our 1 h observation period (0.44 visits/flower/hour, with a maximum of ten observed at any one time). We also observed incidental visits of the Orange-breasted Sunbird (*Anthobaphes violacea*) (Fig. 8.1a) and Malachite Sunbirds (*Nectarinia famosa*) at smaller flowering patches. The longer billed Malachite Sunbirds and Cape Sugarbirds carry pollen on their beaks

whereas the shorter-billed Orange-breasted Sunbirds carry pollen on their head feathers (Fig. 8.1b). Sometimes pollen was also consumed by birds feeding on nectar (Fig. 8.1c). In comparison, only three Cape Sugarbirds and one Malachite Sunbird were observed during 1 h following the removal of all inflorescences.

Flower morphology and nectar properties

Kangaroo Paws have tubular flowers with tube lengths of 27.2 ± 1.8 mm (± 1 SD throughout) making nectar accessible to all sunbirds and sugarbirds (Geerts and Pauw 2009a). The protruding stamens ensure pollen placement on the head or upper part of the bill (Fig. 8.1). Standing nectar crop was relatively low at 8.4 ± 9.9 μ L but within the range previously reported for *Anigozanthos* species during dry, non-rainy, seasons (Hopper and Burbidge 1978). Sucrose concentrations were $16.8 \pm 2.7\%$, typical for bird pollinated taxa, and once again within the range previously reported for *Anigozanthos* species (Hopper and Burbidge 1978).

Capsule set and seed production

Bagging flowers did not affect the proportion of flowers that set capsules ($P = 0.25$ from a Chi-squared test comparing generalised linear models with and without bagging as a factor, a similar result is obtained if non-parametric tests are used). Both bagged and unbagged plants showed a high proportion of capsule set (88–100%, except for one bagged plant with a capsule set of 66%). Bagged flowers, however, tended to have fewer seeds per capsule (Fig. 8.3). When capsules were treated as replicates, capsules that resulted from bird pollination contained significantly more seeds than bagged plants (41%, 95% C.I. of 13–76%; $LR_{1,180} = 9.16$, $P(\text{Chi}) = 0.0025$, from the model with negative binomial errors, there was substantial over-dispersion if Poisson errors were used). This is similar to the findings of Hopper (1980) who showed a 40 % reduction in selfed vs. outcrossed populations of *A. flavidus*. However, if plant was included as a grouping factor in a mixed effects model, then there was no significant effect of bagging on the number of seeds per capsules ($\text{Chisq}_{2,4} = 0.88$, $P = 0.64$).

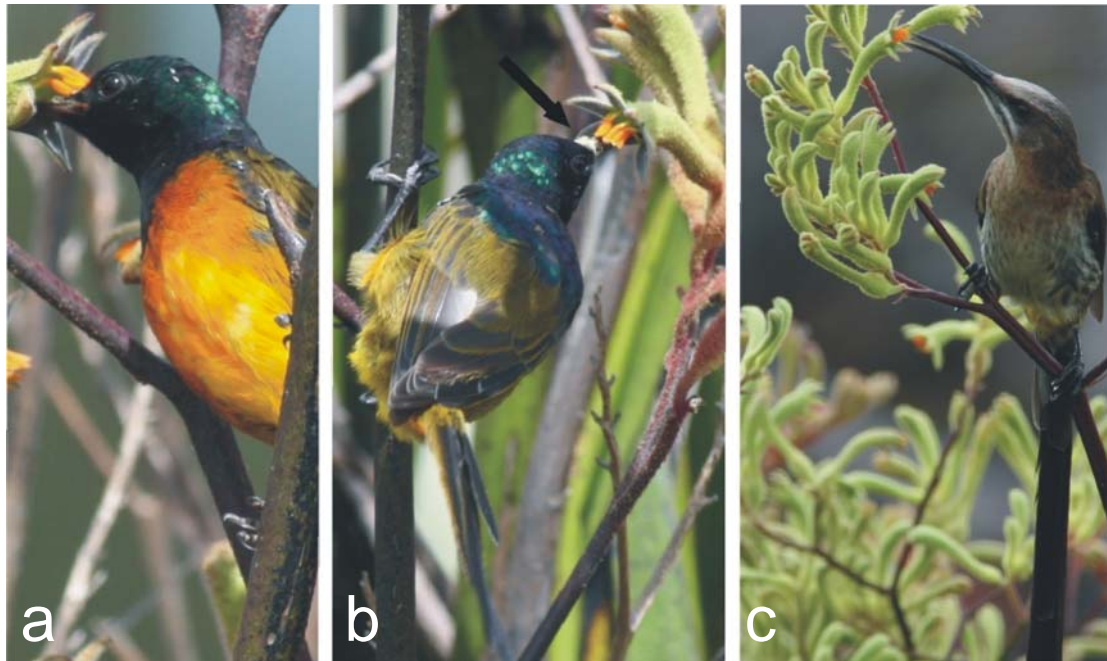


Figure 8.1 (a) Native nectarivorous birds such as the Orange-breasted Sunbird are attracted to kangaroo paw flowers. (b) Protruding stamens allow deposition of pollen on foreheads (black arrow) for cross-pollination, while (c) other birds (Cape Sugarbird) feed on pollen (photographs by Sjirk Geerts).

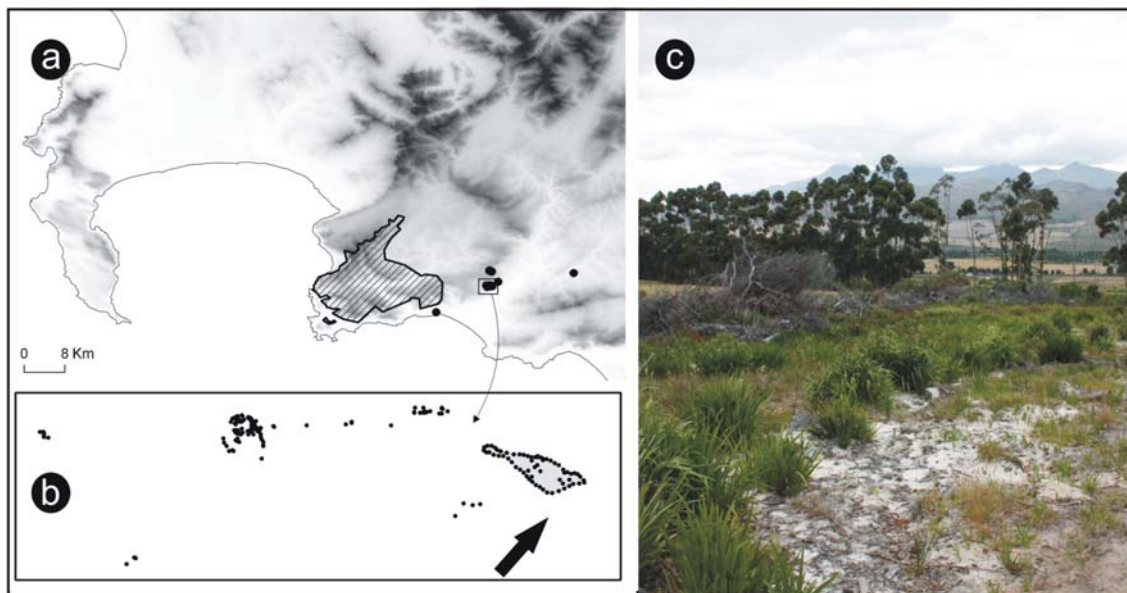


Figure 8.2 (a) Map illustrating the locations of *Anigozanthos* populations in South Africa (Western Cape). One large population (ca. 180 000 individual sprouts) was found on Honingklip farm (enlarged in b) and contained areas infested by monotypic stands of *Anigozanthos* (c) indicated by black arrow in (b). The polygon in (a) depicts the location of the Kogelberg Biosphere Reserve (photograph by Sjirk Geerts).

Kangaroo Paw Taxonomy

Sequence variation and phylogenetic analysis

The aligned *trnL-F* matrix contained 1076 characters. All DNA sequences have been deposited in GenBank (accession numbers GU223383-GU223407). The alignment matrix constructed using data generated in this study and additional sequence data obtained from GenBank required nine gaps (indels), ranging from 1 to 17 characters in size. The best-fit maximum likelihood model was a Hasegawa, Kishino, and Yano (1985) (HKY) model. This model with base frequencies determined from the data (A = 0.3296; C = 0.1326; G = 0.1530; T = 0.38480), ts/tv = 2.0887, Rates = equal. The score of the optimal tree was $-\ln \text{likelihood} = 1774.31$.

The ML tree revealed that Kangaroo Paw species from the genus *Anigozanthos* form a monophyletic clade joined by *Macropidia fuliginosa* as the sister lineage, with moderate support (70 % BS) (Fig. 8.4). Within *Anigozanthos* some species of section *Anigozanthos* (branched species) were more closely related to all species within section *Haplanthesis* (unbranched species) than to other species within section *Anigozanthos*. For example, *A. preissii* and *A. onycis* (section *Anigozanthos*) showed closer phylogenetic relationships to species within section *Haplanthesis* (e.g. *A. gabriellae*) than to other species within section *Anigozanthos* (Fig. 8.4). These findings are somewhat in agreement with Hopper (1980) who found *A. onycis* more capable of hybridising with species from section *Haplanthesis* than those from section *Anigozanthos*. Not surprisingly, a constrained maximum likelihood analysis enforcing monophyly for sections *Haplanthesis* and *Anigozanthos* resulted in a less likely tree topology ($-\ln \text{likelihood} = 1824.39$).

Our phylogenetic analysis identified invasive populations in South Africa to be a mixture of two species: *A. flavidus* and *A. rufus*. The commercial hybrids, Bush Pearl and Bush Gold, had plastid parental lineages that corresponded to *A. humilis*. This is in agreement with the known hybrid origins of these cultivars (*A. humilis* X *A. bicolor* X *A. flavidus* and *A. humilis* X *A. flavidus*, respectively).

Flow cytometry, hybridisation and fitness

We obtained estimates of relative nuclear DNA amounts for 19 samples (see Fig. 8.4, Table 8.1). Interspecific genome sizes varied 1.59-fold (min. value in *A. gabriellae* JLR 164, max. value in *A. preissii* JLR 165) while the intraspecific variation was usually low (< 4.5 %, except for *A. preissii* where there was 1.18-fold variation between the two accessions). Superimposing flow cytometry results on the phylogenetic tree (Fig. 8.4) showed a good agreement between genome size values of *A. flavidus* and one sample from Kleinmond (JLR 33) and two samples from Honingklip (JLR 34, JLR 35). In addition, a clade with a low nuclear DNA amount (comprising *A. bicolor*, *A. manglesii*, and *A. viridis*) was revealed. Genome size values in *A. flavidus* and *A. rufus*, the two species found to be invasive in South Africa, were similar and so we could not identify any hybrid individuals from the flow cytometry data. The ratio of genome sizes between parental species significantly affected the reproductive biology of hybrids. There was a marked decline in seed set of hybrids with an increase in genome size differences between the parents ($F_{1,63} = 27.7$, $P < 0.01$, Fig. 8.5a, weighting by number of capsules investigated did not significantly affect the parameter estimates). However, the resulting seeds did not show any difference in germination probability, with an average germination of around 14% (P [Chi_{1,60}] = 0.61, quasi-binomial errors provided a much better fit given the strong over-dispersion, Fig. 8.5b). The fertility of pollen in the F₁ declined sharply with an increase in genome size differences between the parental stock ($F_{1,34} = 28.6$, $P < 0.01$, Fig. 8.5c). It is important to note that some of the data on seed germination percentage and much of the data on F₁ pollen fertility are censored in the original experiment. If it was difficult to grow F₁ hybrids through to pollen fertility then the data are missing (and so not counted as zeros in the analysis).

Table 8.1. Relative genome size estimates (mean \pm SD) for selected *Anigozanthos* species. *Bellis perennis*, $2C = 3.38$ pg, was used as a unit value.

Species	Relative genome size (pg)	No. of samples
<i>A. bicolor</i>	0.615	1
<i>A. flavidus</i>	0.796 ± 0.010	3
<i>A. gabriellae</i>	0.609	1
<i>A. humilis</i>	0.7	1
<i>A. kalbarriensis</i>	0.712	1
<i>A. manglesii</i>	0.639 ± 0.010	3
<i>A. preissii</i>	0.897 ± 0.074	2
<i>A. pulcherrimus</i>	0.753	1
<i>A. rufus</i>	0.773 ± 0.017	2
<i>A. viridis</i>	0.643	1

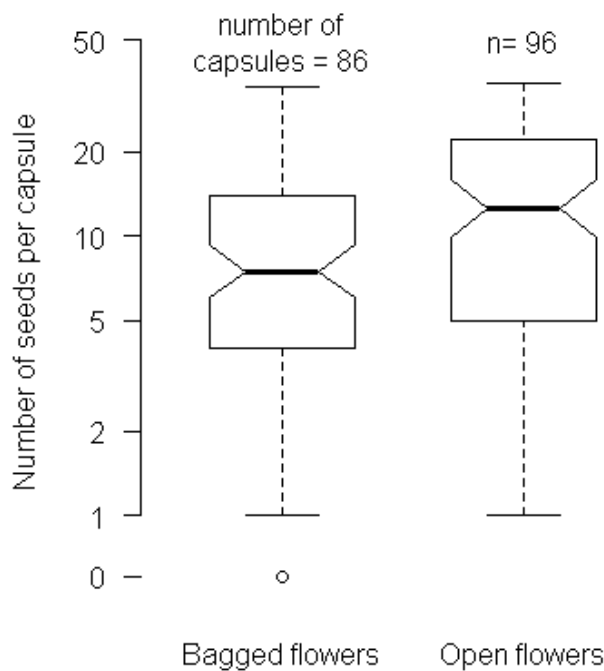


Figure 8.3. A boxplot of the effect of bagging flowers on the number of seeds per capsule. In one instance no seeds were found per capsule, as the y-axis is logarithmic this is plotted separately. The bold lines show the median, the boxes the quartile ranges, and the lines show either 1.5 times the interquartile range or the point furthest from the median, whichever is less. Outliers outside this range are plotted individually.

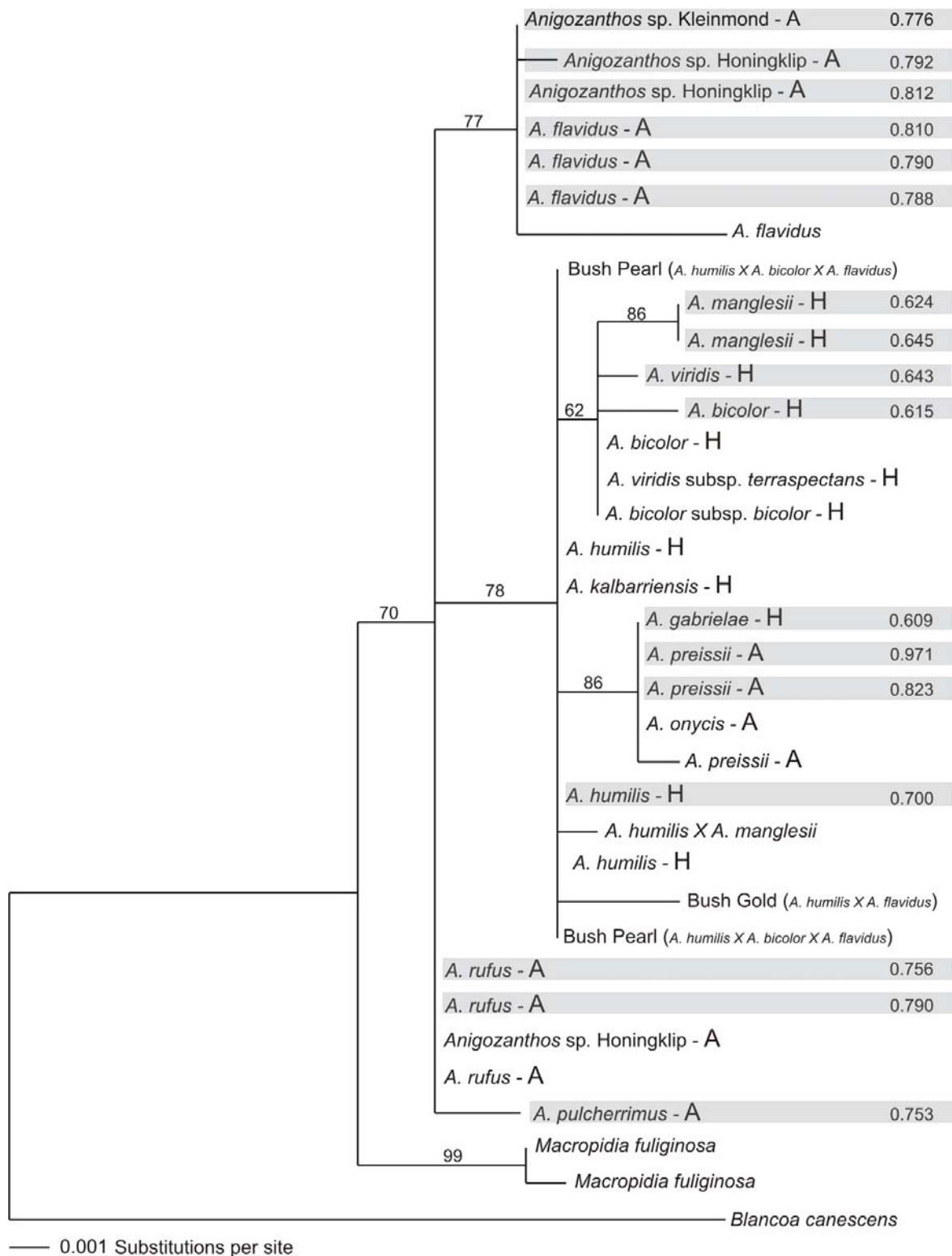


Figure 8.4. The maximum likelihood tree for Kangaroo Paws based on trnL-F DNA sequence data. Taxonomic subclassification into branched (section *Anigozanthos*) and unbranched (section *Haplantthesis*) is indicated as “A” and “H” respectively. Relative genome sizes are also shown for those taxa where estimates are available (shaded). Branch support is indicated as bootstrap values (1000 replicates).

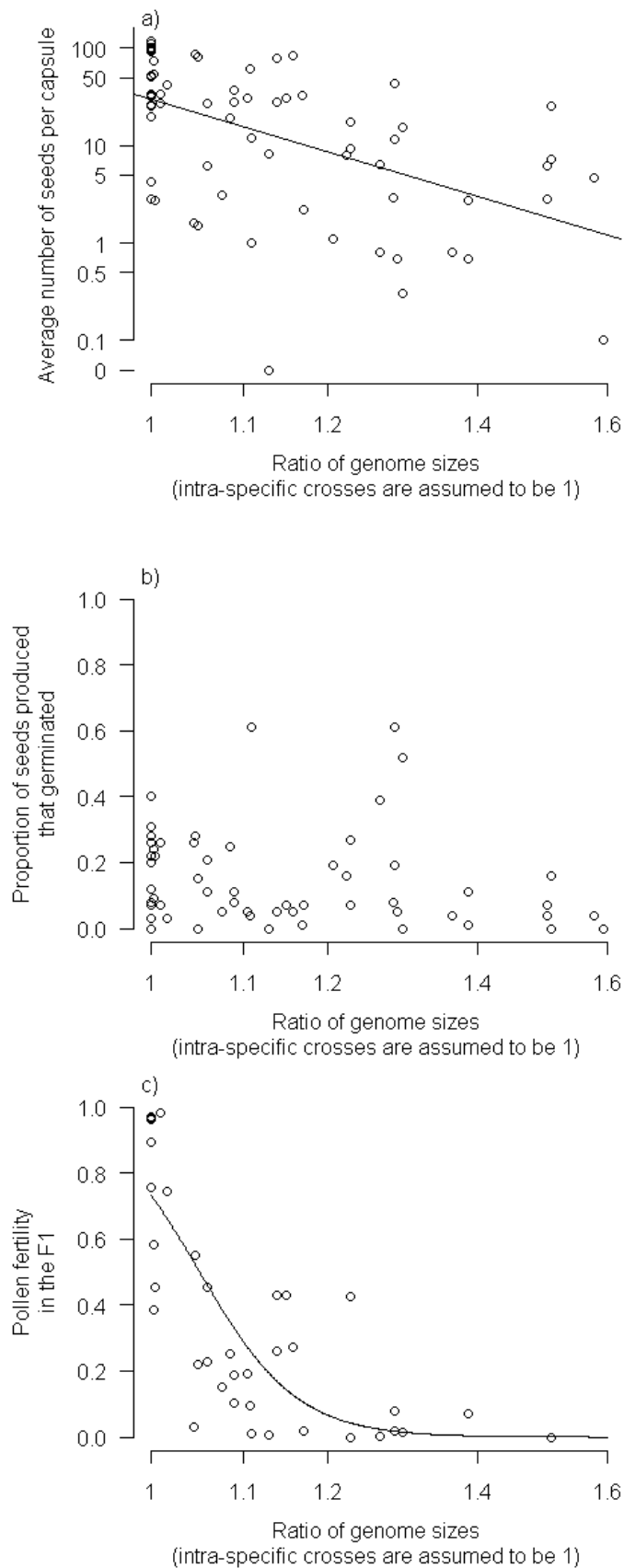


Figure 8.5. The effect of relative parental genome size on (a) the number of seeds per capsule, (b) the germination probability of resulting seeds, and (c) F_1 pollen fertility of various hybrids of Kangaroo Paw species. Seed set and pollen fertility data were obtained from Hopper (1980). Statistically significantly fitted relationships are shown a) $r^2=0.37$; c) $r^2=0.46$. In one instance no seeds were found in a capsule, and so this point was excluded from the log-linear regression and, as the y-axis is logarithmic, it is plotted separately on panel a.

Discussion

The potential for hybridisation in the introduced range

Hybridisation often results in increased invasiveness for plants (Ellstrand and Schierenbeck 2000; Prentis et al. 2008). Previous taxonomic work putatively identified *A. flavidus* and *A. manglesii* as naturalized in South Africa. In addition to confirming the identity of *A. flavidus*, our phylogenetic approach also identified the second species as *A. rufus*. Our single plastid-gene phylogeny precluded any assessment of whether hybrids are currently present within naturalized populations in South Africa. However, there are several reasons to suspect that there is a potential risk of hybridisation. Firstly, there should be pollen exchange, as the populations comprise mixes of two species with overlapping flowering times and a high frequency of visitation by pollinators. Secondly, most species of Kangaroo Paws are capable of inter-specific outcrossing (Hopper 1980; Shchori et al. 1995), and *A. flavidus* and *A. rufus* are known to produce hybrids in experimental crosses, although these have very low pollen fertility (Hopper 1980). With *A. flavidus* as the paternal lineage, hybrid seeds have a 29.0 % germination success, and mature hybrids show 2.9% pollen fertility (Hopper 1980). Unfortunately, reciprocal comparisons of parental crosses between *A. flavidus* and *A. rufus* are not available (the success of Kangaroo Paw hybrids varies with the identity of the paternal and maternal lineages; Hopper 1980). Thirdly, the small difference in genome size between *A. flavidus* and *A. rufus* (3.0%) suggests these species could produce some fertile hybrids. In Australia, populations of *A. manglesii* and *A. humilis* (with 8.5% difference in genome size) readily hybridise and back-cross in the wild (Hopper 1977a, 1977b). Moreover, it should also be noted that the variation in genome size between two accessions determined as *A. preissii* can most easily be explained by the incidence of interspecific hybridisation. Overall, the likelihood of fertile hybrids in Kangaroo Paws appears to be strongly affected by the genome size difference between parental strains, with no fertile hybrids produced above a cut-off of ~ 30% (Fig. 8.5). These data support the notion that differences in nuclear DNA amount can serve as a strong barrier for successful hybridisation (Magdalena Kubešová et al., unpublished data; Petr Bureš, personal communication 2009).

The need to control naturalized populations before they spread further

We also showed that *Anigozanthos flavidus* can set seed in South Africa in the absence of pollinators, and so might produce self-sustaining outlying foci after long-distance dispersal. Kangaroo Paws are a predominantly outcrossing group and selfing, on average, results in a 90 % decrease in seed set (Hopper 1980). Interestingly, compared to other conspecifics, both *A. flavidus* and *A. rufus* have relatively higher levels of self-compatibility and display relatively high levels of seed set when selfed (Hopper, 1980). We found only a 40% reduction in seed set in selfed vs. bird-pollinated individuals. This may explain the existence of small outlying populations surrounding the main infestation site on Honingklip farm, and clearly increases the potential for the plants to spread rapidly. Although the naturalized populations have not yet spread widely, plants can clearly spread and form mono-specific stands. The Early Detection and Rapid Response (EDRR) program in South Africa was established in part to deal with species before they become widespread. *Anigozanthos* populations are still containable at a relatively low cost, and it is prudent to act while control costs are small.

Anigozanthos species also show the potential to change pollination webs. The floral morphology of invasive *Anigozanthos* species present in South Africa (Armstrong 1979) closely matches that of many native plants in the Cape Floristic Region. Kangaroo Paws provide sunbirds and sugarbirds with a rich source of nectar at a time of nectar scarcity (late summer), in return being rewarded by increased reproductive output due to increased outcrossing. Kangaroo Paws could therefore ultimately increase sunbird and sugarbird abundance in South Africa (Geerts and Pauw 2009b).

Another important consideration is that invasive populations of Kangaroo Paws in South Africa occur in fynbos, an evergreen hard-leafed shrub land that occurs along the south-western coastal belt (100 – 200 km wide) of South Africa. Fire is a crucial ecological factor in the functioning of fynbos ecosystems. As an adaptive response to wild fires in Australia, flowering, branching, seed production, seed viability and seedling establishment of Kangaroo Paws are stimulated by smoke and heat (Lamont and Runciman 1993, Tieu et al. 2001). The frequent occurrence of fires in the currently invaded areas is likely to act as a stimulus for increased reproductive output,

spread and invasiveness. Fire is used as an integral part of management for well-established woody invasive species in fynbos, to kill seedlings and stimulate seed germination following mechanical clearing (van Wilgen et al. 1994). We cannot see any practical role of utilizing fire in an integrated management plan for Kangaroo Paws in fynbos. Even though these two species (or hybrids) currently occupy a relative small area, our studies indicate they have considerable invasive potential and should be immediately controlled. Although the naturalized populations have not yet spread widely, plants can clearly spread and form mono-specific stands. Current infestations are within 10 km of one of South Africa's most pristine and important biodiversity-hotspot conservation areas, the Kogelberg Biosphere Reserve, and so management is regarded as a priority.

Recommendations made from results of this study have already led to the eradication of one of the smaller outlying populations of Kangaroo Paws in the Western Cape (59 individuals 5.5 km away from the main infestation) which was flagged as the highest priority for intervention (since isolated populations of invading species are known to contribute disproportionately to population growth and invasion rates; Higgins & Richardson 1999). The results will also be used to formulate a longer-term strategy for dealing with the larger populations. The landowner has undertaken the initial "holding action" of removing, through mechanical brushcutting, the flower heads of as many mature plants as possible in the biggest populations to decrease pollinator abundance, reduce seed set, the potential for hybridisation, and the establishment of additional satellite foci.

Kangaroo Paw taxonomy

This is the first attempt to reconstruct a molecular phylogeny for *all* species of Kangaroo Paws (*Anigozanthos* and *Macropidia*). Given the taxonomic uncertainties within this group (Anderberg and Eldenäs 1991; Hopper 1978, 1980, 1999; Simpson 1990), the use of molecular systematics is particularly relevant here. While previous molecular phylogenies of the Haemodoraceae supported *Macropidia* as the monotypic sister group of *Anigozanthos* (Hopper et al. 1999, 2009), a molecular phylogeny that includes all Kangaroo Paw species would render indisputable support for the placement of *Macropidia*.

Our molecular phylogeny supports the view of Hopper and co-workers (1980, 1999, 2009) in suggesting that *Macropidia fuliginosa* is indeed the monotypic sister lineage of *Anigozanthos*. However, the subdivision of *Anigozanthos* into sections *Haplanthesis* (unbranched species) and *Anigozanthos* (branched species) (Hopper 1980) has little phylogenetic support and remains unresolved. Indeed, Hopper (1980) suggested that the division based on branched and unbranched stems is “to some extent artificial”. For example, here, the branched species *A. preissii* and *A. onycis* showed a closer phylogenetic relationship to the unbranched species *A. gabriellae*, than to other branched species (also see Hopper et al. 2009). Interestingly, Hopper (1980) reported that only slight crossing barriers exist between *A. onycis* and members of section *Haplanthesis*. The reconstruction of a molecular phylogeny using more variable gene regions is currently underway and should render better resolution of these relationships (Rhian Smith, personal communication 2009).

Implications for legislation

The South African nursery industry currently trades in various horticultural hybrids of *Anigozanthos* species. We included the ‘Bush Pearl’ and ‘Bush Gold’ varieties in our molecular analysis (*A. humilis* × *A. bicolor* × *A. flavidus* and *A. flavidus* × *A. humilis*, respectively). Inter-specific hybrids of *A. flavidus* are often produced in the horticultural industry for their increased vigour, longevity and floriferous properties (Hopper 1980). Hybridisation often results in sterility as afforded by chromosomal rearrangements and/or factors under direct genetic control (Rieseberg 2001). Previous work illustrated that in Kangaroo Paws, seed set, germination success and pollen fertility of back-crossed F₁ hybrids approximate or equals those of inter-specific hybrids (Hopper 1980). Our results indicate that ‘genome compatibility’ (genome size similarity) may be one of the underlying mechanisms that is correlated with this phenomenon. Several Kangaroo Paw species hybridise in disturbed habitats in their native range (Hopper 1977a, 1977b) with some hybrid combinations being fertile (Hopper 1980). Even though horticulturists claim that commercial hybrids are ‘mostly’ sterile (Angus Stewart, personal communication 2009) our results illustrate that these hybrids could have invasive potential. Consequently we propose that the trade in all species of Kangaroo Paws and their hybrids in South Africa should be

restricted until detailed studies can show complete and stable sterility in horticulturally important cultivars.

Conclusions

Naturalized populations of *Anigozanthos flavidus* and *A. rufus* in South Africa represent a threat to the biodiversity of the Cape Floristic Region, both by creating dense monocultures and potentially by altering pollination networks. The population appears to be spreading, and there is a high probability that the two species will hybridise if they have not already done so. This will have unknown and potentially undesirable consequences. One population has already been cleared, and the plans for treating the others areas as well as follow-up work are on-going.

Our study also highlights the importance of phylogenetic assessments in addressing plant invasions. By clarifying taxonomic issues of the Kangaroo Paw group, we have identified areas of potential concern. Parental species with similar-sized genomes (up to 30% difference) should not be grown together, and an assessment of fertility and risk of invasiveness needs to be done for different cultivars (hybrids). While some cultivars of Kangaroo Paw might be deemed ‘safe’, we would caution against allowing any taxa in this genus to be grown or sold until a thorough assessment, using both molecular and ecological data, has been conducted. These studies should investigate pollen fertility, outcrossing success and seed set ability of individual cultivars.

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References

- Anderberg AA, Eldenäs P (1991) A cladistic analysis of *Anigozanthos* and *Macropidia* (Haemodoraceae). *Aust Syst Bot* 4: 655-664.
- Armstrong JA (1979) Biotic pollination mechanisms in the Australian Flora - a review. *New Zeal J Bot* 17: 467-508.
- Czarnecki DM, Deng, ZN (2009) Pollen stainability and seed production of 32 *Lantana mantara* and 2 *Lantana montevidensis* cultivars. *HortScience* 44: 1054–1055.
- Doyle J, Doyle J (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13–15.
- Ellstrand NC, Schierenbeck KA (2000) Hybridisation as a stimulus for the evolution of invasiveness in plants? *Proc Natl Acad Sci USA* 97: 7041–7050.
- Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.
- Geerts S, Pauw A (2009a) Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *S Afr J Bot* 75: 699-706.
- Geerts S, Pauw, A (2009b) African sunbirds hover to pollinate an invasive hummingbird-pollinated plant. *Oikos* 118: 573-579.
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis. <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>.
- Hasegawa M, Kishino H, Yano T (1985) Dating the human-ape split by a molecular clock of mitochondrial DNA. *J Mol Evol* 22:160-174.

- Henderson L (2007) Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). *Bothalia* 37: 215–248.
- Higgins SI, Richardson DM (1999) Predicting plant migration rates in a changing world: the role of long-distance dispersal. *Am Nat* 153: 464-475.
- Hopper SD (1977a) The reproductive capacity of *Anigozanthos manglesii* D. Don, *A. humilis* Lindl. and their hybrids in a wild population. *Aust J Bot* 25: 423-428.
- Hopper SD (1977b) The structure and dynamics of a hybrid population of *Anigozanthos manglesii* D. Don and *A. humilis* Lindl. (Haemodoraceae). *Aust J Bot* 25: 413-422.
- Hopper SD (1978) An experimental study of competitive interference between *Anigozanthos manglesii* D. Don, *A. humilis* Lindl. and their F₁ hybrids (Haemodoraceae). *Aust J Bot* 26: 807-818.
- Hopper SD (1980) A biosystematic study of the Kangaroo Paws, *Anigozanthos* and *Macropidia* (Haemodoraceae). *Aust J Bot* 28: 659-680.
- Hopper SD (1987) *Blancoa*, *Anigozanthos*, *Macropidia*. *Flora of Australia* 45: 110-128.
- Hopper SD (1993). *Kangaroo Paws and Catspaws: a Natural History and Field Guide*. Pg. 26. Department of Conservation and Land Management, Perth.
- Hopper SD, Burbidge AH (1978) Assortative pollination by red wattlebirds in a hybrid population of *Anigozanthos* Labill. (Haemodoraceae). *Aust J Bot* 26:
- Hopper SD, Campbell NA (1977) A multivariate morphometric study of species relationship in Kangaroo Paws *Anigozanthos* and *Macropidia* Haemodoraceae. *Aust J Bot* 25: 523-544.
- Hopper SD, Fay MF, Rossetto M, Chase MW (1999) A molecular phylogenetic analysis of the bloodroot and kangaroo paw family, Haemodoraceae: taxonomic, biogeographic and conservation implications. *Bot J Linn Soc* 131: 285-299.
- Hopper SD, Smith RJ, Fay MF, Manning JC, Chase MW (2009) Molecular phylogenetics of Haemodoraceae in the Greater Cape and Southwest Australian Floristic Regions. *Mol Phylogenet Evol* 51: 19-30.
- Hoskins JR, Conn BJ, Lepschi BJ, Barker CH (2007) Plant species first recognised as naturalised for New South Wales in 2002 and 2003, with additional comments

- on species recognised as naturalised in 2000–2001. *Cunninghamia* 10: 139–166.
- Lamont BB, Runciman HV (1993) Fire may stimulate flowering, branching, seed production and seedling establishment in two kangaroo paws (*Haemodoraceae*). *J Appl Ecol* 30: 256-264.
- Nel JL, Richardson DM, Rouget M, Mgidi T, Mdzeke N, Le Maitre DC, van Wilgen BW, Schonegevel L, Henderson L, Naser S (2004) A proposed classification of invasive alien plant species in South Africa: towards prioritising species and areas for management action. *S Afr J Sci* 100: 53-64.
- Pheloung PC, Williams PA, Halloy SR (1999) A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. *J Environ Manage* 57: 239–251.
- Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ (2008) Adaptive evolution in invasive species. *Trends Plant Sci* 13: 288-294.
- Pyšek P, Richardson DM, Rejmánek M, Webster G, Williamson M, Kirschner J (2004). Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. *Taxon* 53: 131-143.
- R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org>
- Randall RP (2007) The introduced flora of Australia and its weed status. CRC for Australian Weed Management, Department of Agriculture and Food, Perth, Western Australia.
- Rejmánek M, Pitcairn MJ (2002) When is eradication of pest plants a realistic goal? In: Veitch C and Clout M (eds) *Turning the Tide: The Eradication of Invasive Species*, pp 249-253, IUCN SCC Invasive Species Specialist Group, Gland, Switzerland.
- Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmánek M (2000) Plant invasions - the role of mutualisms. *Biol Rev* 75: 65-93.
- Rieseberg LH (2001) Chromosomal rearrangements and speciation. *Trends Ecol Evol* 16: 351–358.

- Shchori Y, Kochba M, Afgin L (1995) Hybrid fertility in different crosses of *Anigozanthos manglesii*. *Acta Hort* 420: 147-149.
- Simberloff D (2003) How much information on population biology is needed to manage introduced species? *Conserv Biol* 17: 83-92.
- Simberloff D (2009) We can eliminate invasions or live with them. Successful management projects. *Biol Invasions* 11: 149–157.
- Simpson MG (1990) Phylogeny and classification of the Haemodoraceae. *Ann Mo Bot Gard* 77: 722-784.
- Suda J, Trávníček P (2006) Reliable DNA ploidy determination in dehydrated tissues of vascular plants by DAPI flow cytometry: new prospects for plant research. *Cytometry Part A*, 69A/4: 273-280.
- Swofford DL (2002) PAUP* 4 phylogenetic analysis using parsimony (and other methods), version 4. Sinauer Associates, Sunderland, Massachusetts.
- Taberlet P, Gielly L, Patou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl Mol Biol* 17: 1105–1109.
- Tieu A, Dixon KW, Menev KA, Sivasithamparam K, Barrett RL (2001) Spatial and developmental variation in seed dormancy characteristics in the fire-responsive species *Anigozanthos manglesii* (Haemodoraceae) from Western Australia. *Ann Bot* 88: 19-26.
- Tsrur L, Hazanovsky M, Lebiush-Mordechai S, Ben-David T (2005). Control of root rot and wilt caused by *Pythium myriotylum* in Kangaroo Paw (*Anigozanthos*). *J Phytopath* 153: 150-154.
- van Wilgen BW, Richardson DM, Seydack AHW (1994) Managing fynbos for biodiversity: constraints and options in a fire-prone environment. *S Afr J Sci* 90: 322-329.
- Zenni RD, Wilson JRU, Le Roux JJ, Richardson DM (2009) Evaluating the invasiveness of *Acacia paradoxa* in South Africa. *S Afr J Bot* 75: 485-496.

Appendix 1 Details of specimens of *Anigozanthos* and *Macropidia* species collected in this study.

Specimen ID	Species	Country	Lat/Long	GenBank Accession
JLR033	unknown	South Africa	-34°20'19.02", +19°2'16.56"	GU223383
JLR034	unknown	South Africa	-34°17'31.74", +19°8'10.08"	GU223384
JLR035	unknown	South Africa	-34°17'31.74", +19°8'10.08"	GU223385
JLR036	Bush Pearl (<i>A. humilis</i> X <i>A. bicolor</i> X <i>A. flavidus</i>)	Nursery stock		
JLR038	unknown	South Africa	NA	GU223392
JLR158	<i>A. manglesii</i>	Australia	-34°17'31.74", +19°8'10.08"	GU223387
JLR159	<i>A. manglesii</i>	Australia	-31°43'19.30", +115°51'43.70"	GU223388
JLR160	<i>A. humilis</i>	Australia	-31°50' 37.98", +116°19'32.34"	GU223389
JLR161	<i>A. kalbarriensis</i>	Australia	-32°0'47.28", +116°36'0.18"	GU223390
JLR162	<i>A. flavidus</i>	Australia	-31°57'59.13'', +115°50'17.56''	GU223391
JLR163	<i>A. viridis</i>	Australia	-34°57'30.30", +117°48'29.40"	GU223386
JLR164	<i>A. gabriellae</i>	Australia	-31°57'59.13'', +115°50'17.56''	GU223393
JLR165	<i>A. preissii</i>	Australia	-31°57'59.13'', +115°50'17.56''	GU223394
JLR166	<i>M. fuliginosa</i>	Australia	-34°51'15.40", +116°56'13.50"	GU223395
JLR167	<i>A. pulcherrimus</i>	Australia	-31°57'59.13'', +115°50'17.56''	GU223407
JLR168	<i>A. flavidus</i>	Australia	+115°50'17.56''	GU223404
JLR169	<i>A. flavidus</i>	Australia	-34°56'4.00", +117°56'38.30"	GU223396
JLR171	<i>A. humilis</i>	Australia	NA	GU223397
JLR172	<i>A. rufus</i>	Australia	-32°1'11.70", +115°58'55.62"	GU223398
JLR173	<i>A. rufus</i>	Australia	-34°19'41.50", +117°50'13.50"	GU223399
JLR174	<i>A. preissii</i>	Australia	NA	GU223400
JLR175	<i>A. bicolor</i>	Australia	-34°51'15.40", +116°56'13.50"	GU223401
JLR176	<i>A. bicolor</i>	Australia	-32°12'36.80", +116°18'41.80"	GU223402
JLR252	Bush Gold (<i>A. humilis</i> X <i>A. flavidus</i>)	Nursery stock	NA	GU223403
JLR254	Bush Pearl (<i>A. humilis</i> X <i>A. bicolor</i> X <i>A. flavidus</i>)	Nursery stock	NA	GU223405
		South Africa	NA	GU223406

9 Demography and life history of a shade intolerant geophytic plant in the Cape Floristic Region of South Africa

For a gap-colonizing species competition for light significantly determines the life history. This is well known for forest plants but less so for plants in Mediterranean systems. In these systems light competition is particularly important for geophytes due to their low stature. One way of studying the life history of these geophytes is with matrix population models. These models aid in understanding the life cycle and most important stages of a plant species. In this study, I report on a decade of data on permanently marked individual plants from the geophytic plant, *Brunsvigia orientalis*, in the Cape Floristic Region of South Africa. Lambda initially increased with time since disturbance, but in later successional vegetation plants are outshaded and lambda decreased again. The shaded part of the population had a significantly lower lambda. Sunshine hours during the peak growing season are positively correlated with lambda. Rainfall and temperature had no effect on growth rate, percentage of plants flowering or mortality. Lambda was most sensitive to alterations in the young adult reproductive stage class. The elasticity component, survival, was most important and higher than that for growth and reproduction. *B. orientalis* is in the middle of the demographic triangle space similar to iteroparous herbs of other studies. Reduced population growth rate in the shade and a large investment in a winged inflorescence suggest *B. orientalis* is a light demanding, well dispersed, gap colonizer.

Introduction

Competitive interactions between plants for sunlight have significant effects on population growth rates (Haag *et al.* 2004). In this competition for light, bigger plants in late successional communities typically have the advantage (Zobel 1992). This renders early successional, excellent dispersing species unable to compete with long-lived shrubs. However, vegetation of different ages are created in space and time through natural disturbances (Pickett & White 1985). This environmental heterogeneity allows good

dispersers and colonizers, capable of rapid growth, to utilize the newly available gaps before slower growing shrubs dominate the vegetation (Valverde & Silvertown 1997a). Indeed, for most gap-colonizing species competition for light, with gaps opening and closing, significantly determines population growth rates (see for example Pascarella & Horvitz 1998) with increased understory shade reducing growth and reproductive output (Luken et al. 1997). In forest habitats the life strategies of understorey species, in terms of tree fall and hurricane disturbances, are relatively well studied (Batista et al. 1998; Pascarella & Horvitz 1998; Horvitz et al. 2005; Pathikonda et al. 2009). Since the large majority of studies come from forest habitats, very little is known about overstorey and understorey interactions in Mediterranean shrub-lands (Pugnaire & Lazaro 2000).

In Mediterranean systems the main disturbances are caused by large mammals and fires. They fulfil an important role in creating gaps or temporarily removing the entire overstorey (Bond 2005; Vlok and Yeaton 2000). In some Mediterranean systems agricultural grazing appears to be a very effective tool in releasing less competitive herbaceous species, like geophytes, from light competition from taller plants (Perevolotsky 2006). Fire, however, is the main disturbance in most Mediterranean vegetation types and the openness of the vegetation is determined by time-since fire (Cowling 1992; Diadema *et al.* 2007). Reproductive success and survivorship typical decline with time-since-fire in “gap-specialists” such as the Florida shrub *Eryngium cuneifolium* (Menges & Quintana-Ascencio 2004).

Fires are important as a regeneration window for geophytes. Since geophytes produce new leaves annually from an underground bulb they have a low stature and are easily dominated by larger shrubs (Tilman 1994). Management recommendations on fire frequencies (Diadema *et al.* 2007) and grazing (Immanuel & Talya 2001) for geophytes in Mediterranean regions are scarce. This is particularly so in the Cape Floral Region of South Africa where recommendations on management of geophytes are sketchy at best. This is important, however, since the Cape Floral Region is a global centre for geophyte diversity with the approximately 1500 geophytic species. This is equal to 17% of the total flora and much higher than other Mediterranean shrublands (Manning *et al.* 2002).

Furthermore fire frequencies has both increased and decreased at the Cape. To explain, in small urban reserves, fire frequencies are lower; whilst an escalation in frequency fire in is the norm in most natural areas. Another important alteration is the invasion by large alien shrubs. Higher growth rates of many alien invasive plants result in a more rapid rate of canopy closure (Richardson & van Wilgen 2004). In light of this, life history of geophytic plants as weak competitors urgently needs to be assessed. This can be done by using a demographic matrix modelling approach.

Matrix population models are and have proven to be powerful tools to evaluate the demographic conditions of a population (Leslie 1945; Lefkovitch 1965). They have been widely used to project the population growth of species with complex lifecycles and under different ecological scenarios (Caswell 2001). These models have proved useful in conservation strategies (Kwit *et al.* 2004), exploring population viability of endangered species (Evans *et al.* 2008), simulating a variety of disturbance conditions (Valverde *et al.* 2004), recommending management (Ticktin *et al.* 2002; Hernandez-Apolinar *et al.* 2006) and assessing habitat fragmentation (Bruna & Kress 2002; Bruna & Oli 2005). One way of comparing life histories has been elasticity analyses (De Kroon *et al.* 1986; Caswell 2001). Elasticity analysis give insight into the life history of a plant species. Elasticity analyses have shown that changes in some vital rates could have a proportionately greater impact on population growth rates than others (Kalisz & McPeck 1992; Morris & Doak 2005). Elasticity analysis of a matrix indicates the relative effect on the population growth rate of small changes to matrix elements representing different transitions in the life cycle. Vital rates with high elasticities are important, since small changes in those particular vital rates could lead to proportionally large increases in lambda (Mills *et al.* 1999; de Kroon *et al.* 2000). Silvertown *et al.* (1993) and Franco and Silvertown (2004) used elasticity analysis to compare plants species differing in life histories. They found that perennial herb species have very high composite elasticity values for growth (G) and fecundity (F) whilst survival (S) was more important in woody plants (Silvertown *et al.* 1996; Franco & Silvertown 2004). Comparisons of these elasticity values between species and populations, or the same species over time, can be elegantly presented in the demographic triangle proposed by Silvertown *et al.* (1993).

The knowledge of plant survival, growth, reproduction and life expectancies is essential in understanding the population dynamics of a species (Harper 1977). The contribution of these components can be difficult to estimate since they can vary considerably between years. However, long-term studies provide a reliable way to uncover general patterns in plant demography. In general demographic data is scarce and often time-consuming and costly to collect (Heppell *et al.* 2000). In particular, few data on demographic traits are available for geophytic plant species. This might be due to the difficulties associated with the demography of geophytic plants (Harper 1967, 1977). Geophytic plants go dormant and might lose all aboveground parts for a particular season or year, which makes these species difficult to mark (Kery & Gregg 2004; Kery *et al.* 2005; Lesica & Crone 2007). A second potential difficulty is the long life spans involved which theoretically allow populations to survive short-term environmental variation (Higgins *et al.* 2000). If population numbers are reduced due to natural disturbance, recovery in long-lived species can be slow, therefore determining changes over the short-term is almost impossible (Drechsler *et al.* 1999). These difficulties together contribute to the relative lack of demographic studies of geophytic species (but see Kery & Gregg 2003; Shefferson *et al.* 2003; Kery & Gregg 2004; Lesica & Crone 2007; Hutchings 2010). Only a few matrix projection models have been produced for South African plants, and these deal with shrubs (Maze & Bond 1996; Pfab & Witkowski 2000; Raimondo & Donaldson 2003).

My aims in this study are to utilize a decade of demography data to explore the life history of a gap colonizing geophyte through a deterministic, stage based population model. I conducted elasticity analysis to determine the relative importance of survival, growth, and fecundity. In particular, I address the following: (1) What is the growth rate of a *B. orientalis* population in the years following a disturbance? (2) What are the relative contributions of survival, growth, and fecundity? (3) How does climate, shade and the cost of growth and reproduction influence growth rate? (4) How does the life history of *B. orientalis* compare to other plants?

Methods

Study species and sites

The geophytic *Brunsvigia orientalis*, family Amaryllidaceae, is one of the most spectacular and conspicuous flowering plants of the 17 *Brunsvigia* species (Arnold & De Wet 1993; Snijman & Archer 2003). *Brunsvigia orientalis* is a large bulbous plant with seasonal growth and reproduction. The broad prostrate leaves are produced in September and October, after the wet winter (Fig. 9.1a). In adult plants the leaves can measure ~80 cm or more across. Plants are leafless during the dry summer (October to April) when all above ground parts are shed and plants persist as dormant underground bulbs. The spherical umbellate inflorescences, ~35 cm in diameter, are produced at the end of summer (February to April) when the plants are leafless. Inflorescences bear about ~40 large, scarlet flowers, each of which produces a winged capsule. After flowering the inflorescence detaches from the bulb and is rolled large distances by the wind, dropping the large green seeds. Seed dispersal therefore coincides with the first winter rainfall. Seeds germinate immediately (recalcitrant), leaving no seed bank and producing either one- or two-leafed seedlings. Older plants produce more and bigger leaves of up to 12 leaves per plant (pers. obs). Plants can also stay dormant, not producing leaves or flowers in a particular year. Excavation of belowground parts has confirmed that *B. orientalis* is unable to reproduce vegetatively (Pauw 2004).

To calculate the importance of seeds for population persistence a long-term population monitoring study was initiated in 1999 at Rondevlei Nature reserve in the Cape Floristic Region of South Africa. Rondevlei is an urban conservation area of 290 ha. Most of the conservation area consists of wetlands but higher-lying areas support Cape Flats dune strandveld of which *B. orientalis* is a typical species (Mucina & Rutherford 2006). The area is characterized by hot dry summers with seasonal rains in the cold winters (annual rainfall of 518 mm).

After a fire in 1998 at Rondevlei Nature reserve three transects were established within the *B. orientalis* population on the south end of the reserve. Plants within these transects were marked by inserting a 35 cm long stake into the sand on the east side of the plant.

The stake was constructed of 40 mm wire with the end bent into a eye that held a metal tag with a number punched onto it. The distance between the plant and the tag varied depending on plant size (leaf number). The stakes could not project above the level of the sand because of possible interference with wildlife, especially from hippopotamus. Tags were therefore at ground level and were often buried by wind blown sand and mole-rat activity. To aid in the location of tagged plants each individual was mapped to the nearest 10 cm on graphing paper. On occasions, a metal detector was used to locate missing tags.

Typical of a healthy and expanding population, seedlings were over abundant compared to adult plants. Therefore not all seedlings are included and where possible I marked at least thirty plants per leaf number. To improve my sample size of very large plants, randomly distributed plants outside of transects were included. Field data thus represented cohorts that were monitored for growth and survival. Therefore the Rondevlei model is deterministic and I make the following assumptions: the chosen life history stages are valid and appropriate for describing population dynamics; the life history parameters used to calculate transition probabilities do not change over time and are independent of population density; all individuals within a stage share the same vital rates.

Flowering (April) and number of leaves (August) for 237 mapped individuals were recorded for the years 1999-2010 (some years were skipped; see under matrix modelling). Three large plants were added in subsequent years. Two inflorescences are very occasionally produced by large plants (less than 2 % of flowering plants) and seed set was then totalled across inflorescences. Seeds were either counted by hand, or sieved into size classes and weighed in order to calculate total seeds per plant.

Stage classification

Two approaches of defining stage classes have been used in the past, namely numerical (Vandermeer 1978; Moloney 1986) and biological (e.g. Lefkovitch 1965). In this study the latter approach is used by combining size (leaf number) with reproductive criteria, as reproduction was size and not age dependent. The population structure was divided into

four stages for two reasons (1) to be reproductively relevant and (2) for the sample size to remain large enough for satisfactory estimates of transition probabilities between stages (Ramula & Lehtila 2005). The potential fates and transitions of plants in the various stages are shown in the life cycle graph (Fig. 9.2). To justify leaf number as an accurate criteria for stage classification fifty plants across the size spectrum were excavated and the bulbs measured and correlated with number of leaves and leaf sizes (Fig. 9.1b). Excavations were conducted on the site of a planned development at Milnerton in 2001.

Matrix modelling

I developed a total of 8 yearly matrices for Rondevlei (1999-2000, 2000-2001, 2001-2002, 2005-2006, 2006-2007, 2007-2008, 2008-2009, 2009-2010); data for 2003 and 2004 was not available. Transitions between stages are annual and individuals could only move to a new stage class once a year. Plants gained leaves and moved to the next stage, lost leaves and moved to a previous stage, or stayed in the same stage class (Fig. 9.2). Retrogression from adult plants to seedlings never occurred and retrogression from young adults to the seedling stage rarely occurred (less than 1.5% of young adult transitions) and was added to young adult fecundity (Fig. 9.2). All other possible retrogressions were incorporated into the annual transition matrices.

Less than five percent of plants did not produce leaves i.e. stayed dormant (all plants in all years combined). Sample sizes for dormant plants were too small to warrant a separate dormancy category; dormant plants (zero values) were therefore substituted with the category of the year thereafter. Dormancy never lasted for more than one year (except for one two year dormancy period observed in a single plant). Plants not reappearing for two consecutive years were recorded as dead. Death was confirmed in a four instances by excavating the bulb, which was found to consist only of the dry, outer membranous tunic. Plants not appearing in the final year of the census had to be assigned to either dead or dormant. To do so dead and dormant plants for each year were summed. The percentage of plants being dormant or dead for each stage class could be calculated from this and this relationship used to estimate the percentage of dead and dormant plants for 2010.

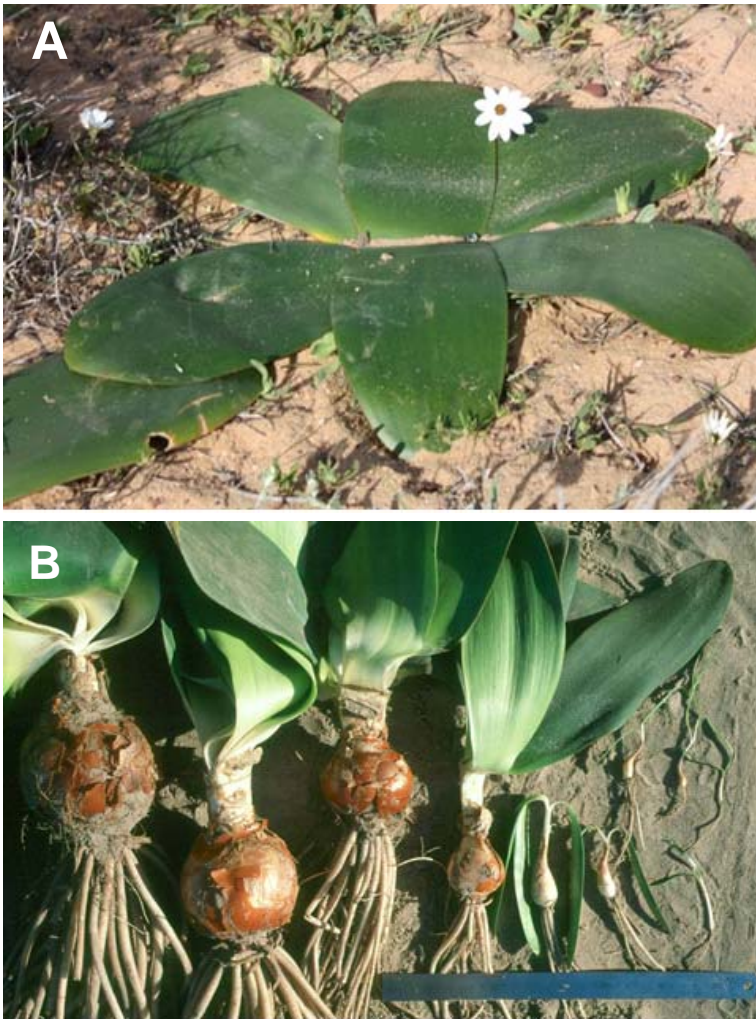


Figure 9.1 (a) Young adult *Brunsvigia orientalis* plant (leaf stage) in spring, surrounded by a few west coast daisies. (b) Excavated *Brunsvigia orientalis* bulbs, older plants (larger bulbs) are those consisting of more as well as bigger leaves (ruler is 30 cm)

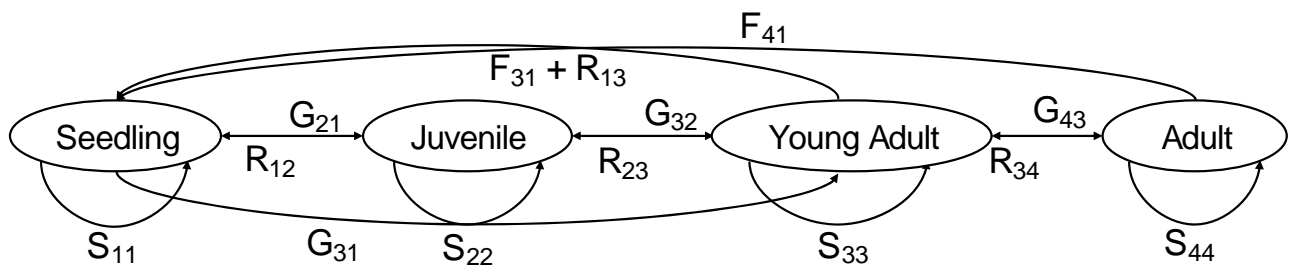


Figure 9.2. Life cycle graph of *B. orientalis*. Circles represent plant stages, arrows represent possible transitions and letters show connection between each transition corresponding to the matrix in Table 9.1.

The proportion of plants flowering (from the total plants alive) was available for 1999-2002, 2004-2006, 2009 and 2010. Flowering (April) was seen as following the leaf stage (September), thus flowering in year $t+1$ is grouped with leafing in year t . The annual decrease in proportion of plants flowering was calculated to estimate the proportion of plants flowering for 2007 and 2008 (not for 2003 as there was also no leaf data available for that year). Instead of using the average number of plants flowering, flowering in 2007 and 2008 was calculated more accurately by plotting a line graph of percentage plants flowering per year and estimating values for 2007 and 2008 by eye.

Seeds per capsules and number of capsules per plant were counted for all marked plants at Rondevlei for three years (1998, 2000 and 2001). The leaf stage of each of these plants was known and used to calculate the average number of seeds produced per life stage for each year. Because seed set per capsule was not significantly different between years (Kruskal-Wallis $H = 6.14$, $df = 2$, $P = 0.05$; seeds per capsule mean \pm SD: 1998, 4.78 ± 3.65 , $n = 30$ plants; 2000, 3.55 ± 3.16 , $n = 39$ plants; 2001, 5.38 ± 3.97 , $n = 49$ plants) it was averaged across years and extrapolated across all years; therefore annual variation in λ does not include variation in seed set. To test whether larger plants had a higher reproductive output due to more seeds per capsule or more flowers per plant a multiple regression analysis was used.

Brunsvigia orientalis does not have seed dormancy; hence there is no need for a seed stage in the matrix model. The number of seeds germinating and surviving for the first growing season was calculated from field experiments. Recruitment rates were estimated from observing emergence and survival of a set of planted seeds. Fifty viable seeds were laid out on the soil surface inside steel hoops (1m in diameter) near the Rondevlei population, but outside the seed dispersal shadow ($n = 22$ steel hoops). The hoops were 5 cm high and were submerged in the sand so that at least 2 cm protruded above the soil surface in order to contain the seeds. The hoops were placed randomly in open situations, or in more shaded patches under bushes. The number of seedlings was counted six months later. Some seeds would by definition not land in a patch where it could germinate whilst some seeds might also remain in the capsules, therefore our seedling survival must

be considered an over estimation. Annual variation in lambda does not include variation in seed to seedling transition.

By combining these three reproductive variables, fecundity (F) values for each stage could be calculated as follows: proportion of plants flowering in each year \times number of seeds per plant \times seedling survival after the first growing season.

I assumed Markovian fates for our projection model (Horvitz and Schemske 1995). In assembling the projection matrix I used fecundity and transition probabilities for each individual in a matrix model. The linear population projection matrix model used to analyze demography of species with a complex life cycle is: $n_{(t+1)} = A \cdot n_{(t)}$, With $n_{(t)}$ a vector of all individuals in the population at time t , classified by stage $n_{(t+1)}$ which is the vector for the next stage interval. A represents a non negative square matrix with the matrix elements a_{ij} representing transition rates amongst stages (Caswell 2001), i.e. representing the contributions from individuals in the j -th category to the i -th category after one time step and are given by survival, growth and fecundity. This equation is a model of exponential growth and does not include terms for density dependence. For the study population A is a 4 x 4 matrix, with S the survival within the same stage, F the fecundity, R the retrogression to a smaller stage and G the growth to the next stage (Table 9.1). These matrices were combined by taking the average for each transition for all 8 matrices. The stage-based models were constructed using a spreadsheet package (Microsoft Excel 2008) with the Pop-Tools plug-in (Hood 2008). The dominant right eigen value corresponds to the population growth Lambda (λ) were $\ln \lambda = r$, the intrinsic growth rate (Caswell 2001). The use of lambda in asymptotic growth rate, can predict future population size only if the environment stays the same, which it almost never does, however it can be used to look at demographic success or how small changes in the life history will affect population size in the long run.

Table 9.1. The projection matrix corresponding to the life-cycle in Figure 9.2. The elements of the matrix represent fecundity per reproductive individual (F), the probability of survival and growth from one stage to the next (G_{ij}), the probability regression to smaller stages (R_{ij}), or the probability of survival and remaining in the same stage from one time interval to the next (stasis S_i). Zero entries are transitions that are never observed.

Stage at time $t + 1$	Stage at time t			
	Seedling	Juvenile	Young Adult	Adult
Seedling	S_{11}	R_{12}	$F_{13} + R_{13}$	F_{14}
Juvenile	G_{21}	S_{22}	R_{23}	0
Young	G_{31}	G_{32}	S_{33}	R_{34}
Adult	0	0	G_{43}	S_{44}

Elasticity analysis

Elasticity analysis is a measure of proportional change in the population growth rate, lambda, for a proportional change in the individual matrix element. Since the elasticity of all matrix elements sum to one, elasticities can be compared among populations and species or among life history parameters. In addition, elasticity is also a measure of an element's contribution to fitness (De Kroon et al. 1986; Caswell 2001). Elasticity analysis was conducted to determine the proportional contribution of each matrix element to lambda. This was estimated as $e_{ij} = (a_{ij}/\lambda) \times (\partial\lambda/\partial a_{ij})$, where e_{ij} is the elasticity value for the element m_{ij} (i.e., the element in the i th row and j th column) of the inherent projection matrix (Caswell 1989). Elasticity values were calculated for every transition element in all matrices. Composite elasticity's were calculated by summing matrix elements for all stages representing survival (S), growth (G), and fecundity (F). To present the elasticity structure in a simple way, I summed elasticities for each column, excluding fecundity, generating total elasticities for the fate of seedlings, juveniles, young adults and adults. Elasticity of these four stages is also represented in relation to annual variation in lambda.

Shade impact

With increasing succession taller plant species are expected to outcompete species with a prostrate growth form. For all marked *B. orientalis* plants the percentage of its leaf area that was shaded at midday was recorded at the start of our monitoring period. Percentage

shade was calculated as that part of the plant in the shade at midday. Although the area burned approximately a year before monitoring started, shrubs were already resprouting and producing some shade. To estimate overall effect of shading on the population growth rate marked plants were divided in two groups: those with more and those with less than 50% shade. This cut-off was chosen in order to maximize sample size for both groups. For both groups an overall transition matrix for the period 1999-2010 was constructed and lambda calculated.

Fecundities were calculated separately for both groups as the percentage of plants flowering differed between shaded and unshaded plants. Yearly matrices could not be constructed as sample sizes for particular stage groups became very small in some years. To determine shade impact on seedling survival, 50 viable seeds were sown in steel rings ($n = 22$) in shade and in the open (see details under matrix modeling). Data was normally distributed and analyzed with a t-test.

Effect of reproduction and leaf gain on future leaf gain

There is a potential trade-off between current growth and reproduction and future growth and reproduction, because resources allocated in the first year are not available in the second year (Horvitz & Schemske 1988). Ideally, effect of current reproduction on future reproduction is assessed but I do not have enough sequential reproduction data. Therefore, I determine effect of reproduction on subsequent growth, with flowering in Autumn expected to reduce the energy available to produce new leaves in the subsequent Winter. Only reproductive plants (4 or more leaves) were included in the analysis. Leaf data from year t (September) and $t+1$ (September) was used with data from the relevant flowering period in-between (April of year $t+1$). Shaded plants might need more energy to produce leaves, masking reproduction costs, whilst plants in the sun have more energy for leaf and flower production. Therefore, the dataset was divided into shaded ($>50\%$ shade) and non shaded ($<50\%$ shade) plants.

For cost of growth I expect leaf gain in year t to reduce the energy available to produce leaves in year $t+1$. Change in leaf number was calculated for each plant from year t to $t+1$.

Dormancy was excluded in these analyses. Plants in year t were divided into 3 groups, those that have gained leaves, lost leaves and maintained the same amount of leaves from year $t-1$. Plants, according to the change in leaf number, were put into one of those three groups: leaf gain by plants that gained leaves in year t , leaf gain by plants that lost leaves in year t , leaf gain by plants with similar number of leaves in year t . A Kruskal-Wallis ANOVA was run to determine whether leaf gain differed significantly between the 3 groups; a post hoc multiple comparisons analysis identified where these differences were.

Leaf gain between flowering and non-flowering plants (in shaded and non shaded environment) were compared with a Mann-Whitney U Test. Analyses were done for individual years and for all years grouped together. The significance of the following predictor variables on changes in leaf number was assessed in a general linear model (GLM): (1) calendar year (categorical predictor, 2000-2001, 2004-2005, 2005-2006, 2008-2009, 2009-2010); (2) shade (continuous predictor, 0-100%); (3) flowering (categorical predictor); (4) Change in leaf number in the previous year (continuous predictor). 1999-2000 was excluded in the analysis since no previous leaf data existed as this was the first year of sampling. Sample sizes differ from other parts of this study, because plants that had less than 4 leaves in the previous year, were excluded in the GLM.

Climate

Rainfall, temperature and hours of sunshine is known to influence growth rate, mortality and flowering. Therefore lambda, mortality and the number of plants flowering at Rondevlei were correlated with rainfall in year $t-1$, year t and $t+1$ using annual, monthly and winter (May-August) rainfall. Temperature (daily maximum, daily minimum and monthly mean) and sunshine hours were also correlated with lambda, mortality and the number of plants flowering. Rainfall data is from Rondevlei itself, with temperature data from the Cape Town weather station. Weather data were available up till July 2010. Pearson's correlations with a Bonferroni correction were used. All analysis was performed in STATISTICA 9.0 (StatSoft, Inc. 2009, Tulsa, USA).

Results

Bulb size is positively correlated with leaf width (Pearson correlation $r^2 = 0.94$, $p < 0.001$; Fig. 9.1b, 9.3a), leaf length (Pearson correlation $r^2 = 0.89$, $p < 0.001$; Fig. 9.3b) and with number of leaves per plant (Pearson correlation $r^2 = 0.90$, $p < 0.001$; Fig. 9.3c). Although bulb size correlated better with leaf size, leaf number was preferred since it could be easily recorded and was repeatable across observers and populations. As a justification for leaf category classification the number of leaves is plotted against the proportion of plants flowering, with the selected stage categories demarked with dotted lines (Fig. 9.4).

Matrix computations

Predicted mean age of parents averaged forty-eight years (± 31.0 SD) but dropped below thirty years for the last two years. Generation time fluctuated around nine years (± 3.3 SD). Transitions between different life stages for the annual matrices and reproductive values for each stage are shown in Table 9.2. For all stages, except juveniles in some years, staying in the same stage was the most likely (Table 9.2). Lambda increased with time, since the fire in 1998, to a maximum of 2.2 in year seven where after it decreased over the last few years of monitoring (Table 9.2). Annual mortality for the smaller stage classes was higher than that for the older stage classes (Fig. 9.5). Seedling survival however was still very high with more than 80% surviving on an annual basis. The apparent immortality of adults is deceptive, because adults diminish in size before dying in a smaller stage class. To illustrate this, the growth trajectory of a shrinking adult plant is compared to a growing juvenile plant (Fig. 9.6).

A higher proportion of adult versus young adult plants flowered; 60 % vs. 21% (average over all years). Adult plants also produced more seeds than young adults (Table 9.3). Reproductive output for the adult stage was higher compared to the young adult stage, due to more flowers per plant rather than more seeds per capsule (Multiple Regression $F_{(3,86)} = 24.95$; $t = 4.38$; $p < 0000$; Fig. 9.7). No distinction was made between seeds from adults and young adults in the germination trail as no obvious difference in seed size was observed. Germination trails in the sun and shade did not differ significantly (see shade analysis) and were subsequently pooled. An average of 41.5 % seeds surviving as seedlings after the first growing season ($n = 1100$ seeds sown in 22 hoops resulting in 456 seedlings).

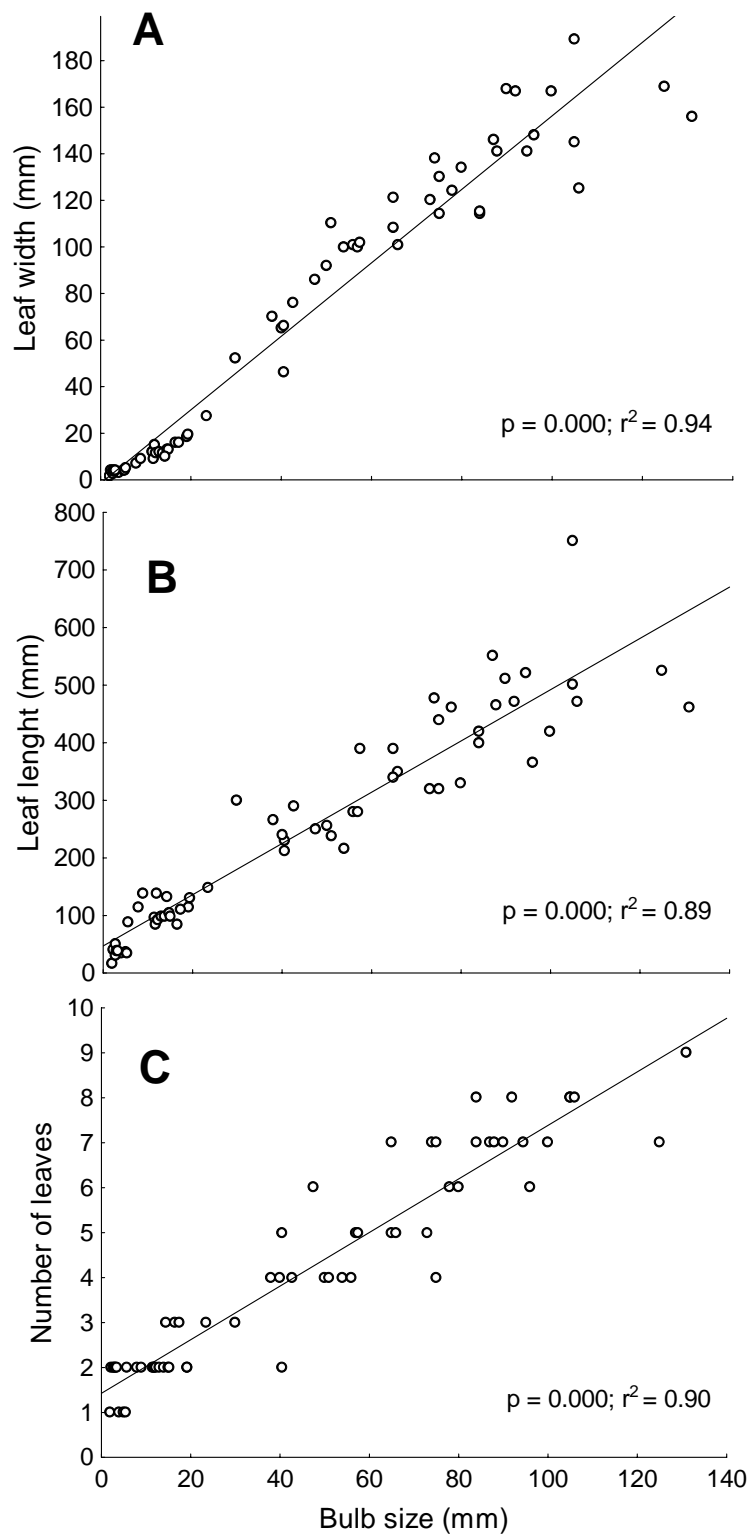


Figure 9.3. Relationship between bulb diameter and (a) leaf width, (b) leaf length and (c) the number of leaves per plant. All correlations are highly significant ($p < 0.001$).

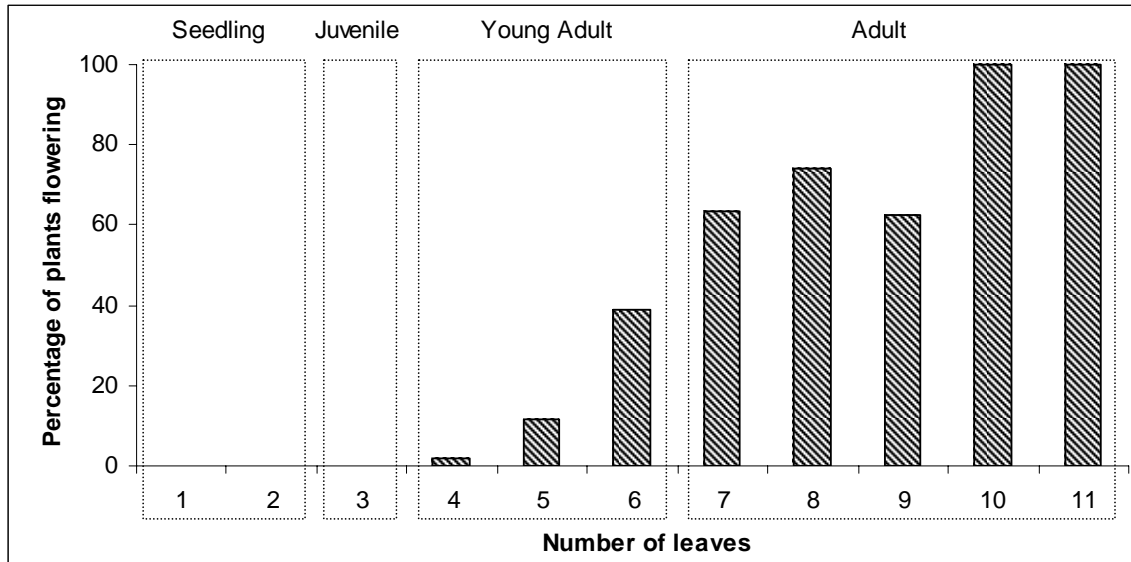


Figure 9.4. As a justification for the categories number of leaves are plotted against proportion of plant flowering with selected categories demarked in the dotted line blocks (All plants over all years)

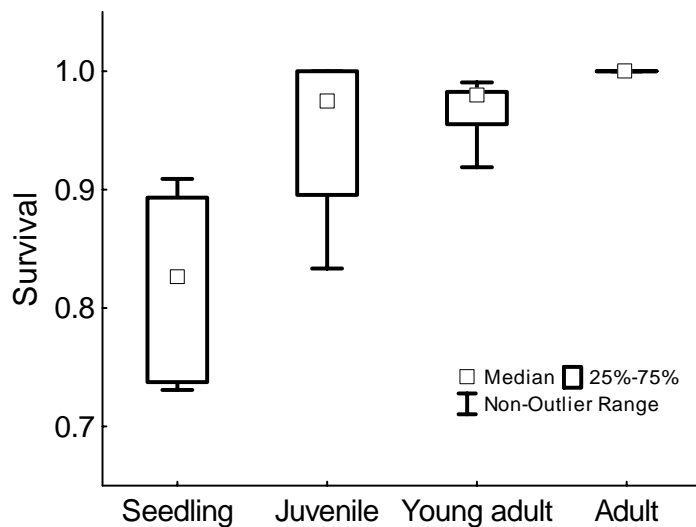


Figure 9.5. Survival in each stage class averaged over all transition matrices.

Table 9.2. Transition probabilities and annual growth rate (λ) for annual transition matrices with columns $t+1$. For the young adult and adult stage classes the top row represents the average number of seedling recruits produced by that size class (due to mortality columns do not always sum to one; note that no data was available for 2002 or 2003).

Stage at time $t + 1$	Stage at time t				
1999-2000 $\lambda = 1.69$	Seedling	0.80	0.14	17.76	81.08
	Juvenile	0.07	0.43	0.02	-
	Young Adult	0.00	0.43	0.80	0.20
	Adult	-	-	0.16	0.80
2000-2001 $\lambda = 1.60$	Seedling	0.84	0.12	20.87	78.67
	Juvenile	0.06	0.47	0.04	-
	Young Adult	0.00	0.35	0.86	0.20
	Adult	-	-	0.08	0.80
2004-2005 $\lambda = 2.10$	Seedling	0.51	0.19	29.20	70.63
	Juvenile	0.23	0.44	0.03	-
	Young Adult	0.00	0.38	0.80	0.19
	Adult	-	-	0.15	0.81
2005-2006 $\lambda = 1.85$	Seedling	0.85	0.40	20.33	69.67
	Juvenile	0.00	0.20	0.05	-
	Young Adult	0.04	0.25	0.86	0.37
	Adult	-	-	0.09	0.63
2006-2007 $\lambda = 2.24$	Seedling	0.73	0.00	16.49	61.16
	Juvenile	0.12	0.43	0.03	-
	Young Adult	0.06	0.57	0.86	0.19
	Adult	-	-	0.10	0.81
2007-2008 $\lambda = 1.27$	Seedling	0.69	0.44	12.64	52.65
	Juvenile	0.04	0.22	0.04	-
	Young Adult	0.00	0.33	0.86	0.43
	Adult	-	-	0.05	0.58
2008-2009 $\lambda = 1.40$	Seedling	0.41	0.14	7.26	31.53
	Juvenile	0.14	0.43	0.07	-
	Young Adult	0.00	0.43	0.88	0.54
	Adult	-	-	0.02	0.46
2009-2010 $\lambda = 1.39$	Seedling	0.45	0.21	10.31	59.35
	Juvenile	0.09	0.29	0.04	-
	Young Adult	0.00	0.43	0.89	0.36
	Adult	-	-	0.03	0.64

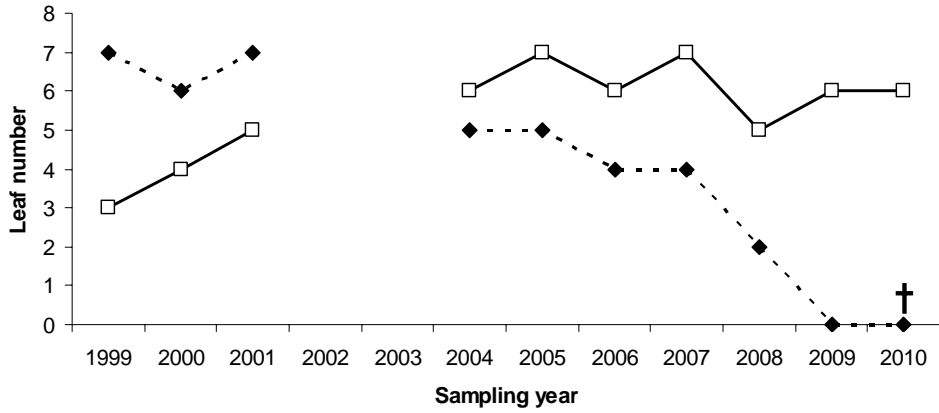


Figure 9.6. Comparative fates of a sun versus a shade plant. Consistent with carbon limitation of growth and survival, shade plants decline to small stage classes before dying. Dotted line and solid markers follow the fate of in plant growing in 70% shade, solid line with open markers follow a plant growing in full sun. No data available for 2002 or 2003.

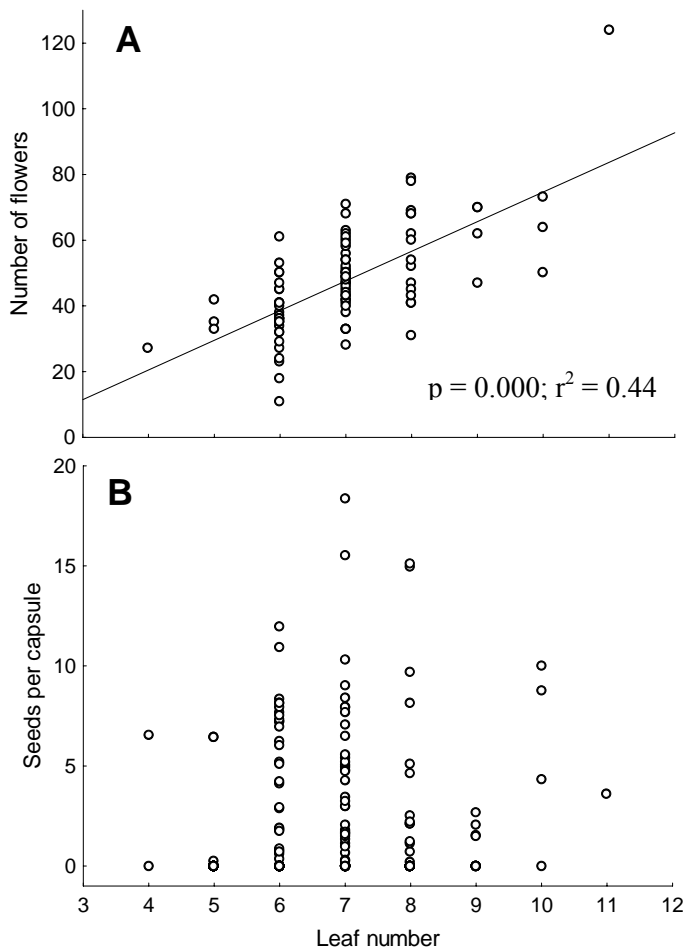


Figure 9.7. Plants with more leaves have a higher reproductive output because (a) they have more flowers per inflorescence, rather than (b) more seeds per capsule.

Elasticity analysis

Certain transitions are clearly of more importance than others in the life cycle of *B. orientalis*. Of high importance are terms related to the growth of smaller plants into larger size categories, and the reproductive contribution of larger plants (Table 9.4). The elasticity matrix for this population showed that the reproductive component (F) contributed a large part, 18.6%, to the population elasticity. The increment G, growth, contributed 37.4% and survival, S, contributed the most with 44%. Survival consists of the contribution of stasis (43%) and an insignificant contribution of retrogression (1%). The two elements of the elasticity matrix that contribute most to the dominant eigenvalue are those representing the survival and fecundity of young adults (Fig. 9.8; Table 9.4). Three other important transitions are seedling-seedling, seedling-juvenile and juvenile to young adult. These five transitions make up 76% of the contribution to lambda. Despite being a potentially long-lived plant, the seedling stage is an important stage class; the contribution of seedlings to the entire life cycle is second only to the contribution of young adults (Fig. 9.8; Table 9.4) From a conservation perspective, elasticities did suggest particularly sensitive life history stages for this plant namely fecundity and survival of young adults. Interestingly, adult plants have the lowest elasticity and are even less important than seedlings.

The higher the lambda of a particular matrix the greater the contribution by the seedling stage, whilst for lower lambdas the young adult stage becomes more important (Fig. 9.9a). If relative contributions towards lambda are compared over the entire study period, seedlings contribute significantly over the first number of years, where after their contribution decreases (Fig. 9.9b) and the contribution of young adult plants increased. With a decrease in population growth rates, seedlings became less important and young adult plants – with a potentially long reproductive life span and very little chance of dying – became more important. The contributions from adult plants remained similar throughout. All annual matrices group closely together with forest herbs or shrubs in the demographic triangle proposed by Silvertown et al (1996) (Fig. 9.10).

Table 9.3. Fecundity calculations for each of the four stage classes. Fecundity is calculated as the number of seedlings produced by a reproductive plant and is calculated as follows: the proportion of plants flowering \times number of seeds produced per inflorescence annually \times survival of seeds to seedlings. Values displayed are averages over all years of the study.

Stage class	Proportion of plants flowering	Seeds per inflorescence (mean \pm SD)	Seed to seedling survival	Fecundity
1	0	-	-	0
2	0	-	-	0
3	0.207	186 \pm 128.5	0.415	16.0
4	0.604	243 \pm 197.3	0.415	61.0

Table 9.4. The average elasticity values (and range) for each transition probability and the total elasticity for each life stage.

	Seedling	Juvenile	Young adult	Adult
Seedling	0.128 (0.053-0.205)	0.002 (0-0.004)	0.144 (0.089-0.202)	0.041 (0.013-0.072)
Juvenile	0.138 (0-0.217)	0.045 (0-0.079)	0.004 (0-0.011)	0.000 (0-0)
Young adult	0.050 (0-0.243)	0.140 (0-0.214)	0.221 (0.135-0.311)	0.006 (0.002-0.012)
Adult	0.00 (0-0)	0.00 (0-0)	0.047 (0.016-0.079)	0.035 (0.008-0.071)
Total elasticity	0.316	0.186	0.416	0.082

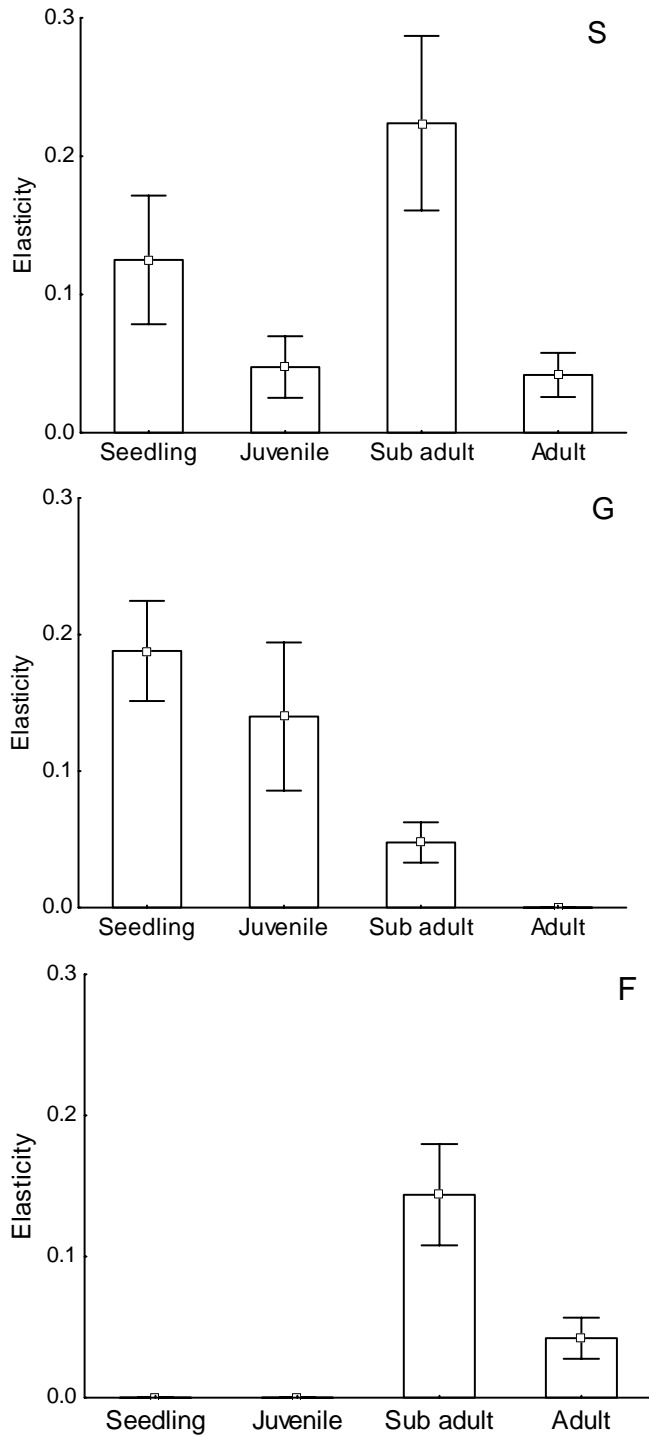


Figure 9.8. Elasticities of the transition matrices over all sampling years, with respect to: survival probabilities of plants in the same stage class (S), probability of growth to the next stage class (G), and fecundity per plant in each stage class (F). Bars denote the 0.95 confidence intervals.

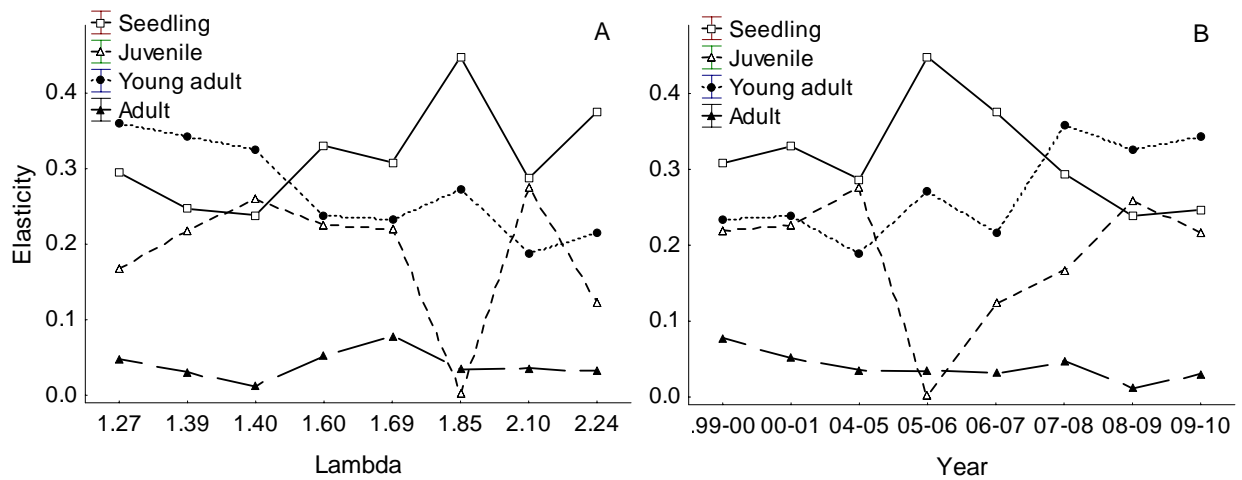


Figure 9.9. Elasticity values for each stage class was calculated by summing each column in the annual elasticity matrices (excluding fecundity) to compare relative contributions to lambda (a), and (b) over the entire study period.

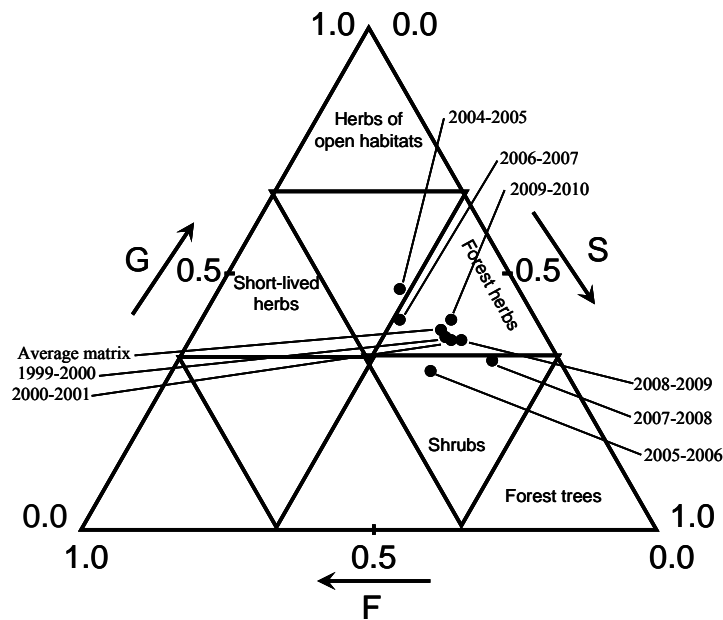


Figure 9.10. The position in the “demographic triangle” of the growth, survival, and fecundity components of the elasticities of *B. orientalis* as calculated from the 8 annual matrices and the average matrix. (Figure and legends from Silvertown et al 1996).

Shade impact

Seedling survival in shaded hoops – after the first growing season – was higher but not significantly so than seedling survival in the sun (t-test: $t = 1.49$, $df = 20$ $p = 0.15$; $N_{\text{sun}} = 13$ hoops, 24.08 seeds out of 50 survived; $N_{\text{shade}} = 9$ hoops, 15.89 seeds out of 50 survived). Although sample size was low after two growing seasons there was no difference between groups (t-test: $t = -1.18$, $df = 6$ $p = 0.86$; $N_{\text{sun}} = 5$, $N_{\text{shade}} = 3$). When comparing growth rates between marked plants in the shade (more than 50% shade) versus those in the sun (shade less than 50%), lambda of shaded plots was lower (1.60 versus 2.26). Particular transitions could not be identified to have changed drastically, but rather a change in most transitions, with retrogression more likely, growth less likely and fecundity lower (71.4% versus 75.3%) due to a reduced probability of flowering in the shaded versus the unshaded matrix (Table 9.5). In the shade matrix, stasis become more important (46% vs. 29%), whilst fecundity (21% vs. 29%) and growth (33% vs. 41%) become less important.

Effect of reproduction and leaf gain on future leaf gain

Changes in leaf number of flowering plants did not differ significant from non-flowering plants for (1) individual years (Mann Whitney U test $p > 0.05$ in all cases) and (2) for all years combined (Mann Whitney U test $U = 81610$ $Z = -0.822$ $P = 0.41$ $N_{\text{non-flowering}} = 567$ $N_{\text{flowering}} = 298$; Fig. 9.11). When plants were divided into shade ($> 50\%$ shade) or non shade ($< 50\%$ shade), there was no significant difference in changes in leaf number between flowering and non flowering plants for individual years (Mann Whitney U test $p > 0.05$ in all cases) or for all years grouped together (Shade: Mann Whitney U test $U = 8618$ $Z = -0.592$ $P = 0.44$ $N_{\text{non flowering}} = 206$ $N_{\text{flowering}} = 83$; Non shade: Mann Whitney U test $U = 37996$ $Z = -0.46$ $P = 0.62$ $N_{\text{non flowering}} = 360$ $N_{\text{flowering}} = 216$).

Leaf gain in year t significantly reduced leaf gain in year $t+1$ (Kruskal-Wallis test: $H_2 = 170$, $p < 0.001$, $N = 4164$). In the general linear model, calendar year and leaf change in previous growing season was important in predicting changes in leaf number, with flowering and the percentage shade less so (Table 9.6). Plants with leaf gain or no change

in leaf number in year t , had significantly fewer leaves in year $t+1$, whilst plants with a reduced leaf number in year t gained leaves in year $t+1$ (Fig. 9.12).

Climate

Neither rainfall (annual, monthly and winter; May- August), nor temperature (average daily maximum, average daily minimum, mean monthly) for year $t-1$, year t , or year $t+1$ had a significant impact on population growth rate after Bonferroni corrections (Table 9.7). Climate parameters had no effect on flowering ($p>0.05$ in all cases) or mortality ($p>0.05$ in all cases), with mortality declining constantly over the study period ($r^2 = 0.996$; $p<0.0001$). However, sunshine hours in September of year $t+1$ was significantly correlated with growth rate from year t to $t+1$ ($r^2 = 0.88$, $t = 6.06$, $P = 0.002$, $N = 7$; Fig. 9.13).

Table 9.5. Matrix comparison for plants in the shade and in the sun for all years combined ($N_{\text{shade}} = 743$ plant-years; $N_{\text{sun}} = 1031$ plant-years).

Stage at time $t + 1$	Plants in the shade (>50% shade)				Plants in the sun (<50% shade)			
	Stage at time t				Stage at time t			
	Seedling	Juvenile	Young Adult	Adult	Seedling	Juvenile	Young Adult	Adult
Seedling	0.75	0.21	13.20	71.09	0.62	0.22	20.10	75.38
Juvenile	0.04	0.44	0.06	0.00	0.15	0.34	0.03	0.00
Young	0.03	0.29	0.82	0.35	0.03	0.38	0.85	0.30
Adult	0.00	0.00	0.06	0.65	0.01	0.00	0.08	0.70

Table 9.6. The ability of a range of predictor variables to explain leaf change in *B. orientalis* in a general linear model with (1) calendar year (categorical predictor, 2000-2001, 2004-2005, 2005-2006, 2008-2009, 2009-2010); (2) shade (continuous predictor, 0-100%); (3) flowering (categorical predictor); (3) change in leaf number in the previous year (continuous predictor) included as predictor variables. Adjusted $R^2 = 0.11$ $p < 0.001$, type III sum of squares.

	SS	df	MS	F	p
Intercept	1.97	1	1.97	2.10	0.1481
Percentage shade	2.00	1	2.00	2.12	0.1455
Leaf change in previous growing season	36.89	1	36.89	39.25	0.0000
Flowering	0.41	1	0.41	0.43	0.5117
Calendar year	23.71	4	5.93	6.31	0.0001
Flowering \times calendar year	5.76	4	1.44	1.53	0.1908
Error	629.61	670	0.94		

Table 9.7. Correlations between lambda and various rainfall parameters. Leaf data is collected from spring (August to October) from year t to year $t+1$. The matrix will for example be named 2004-2005 with $t-1$ rainfall for 2003, rainfall for 2004 and $t+1$ rainfall for 2005. N equals eight in all cases, which equals the number of matrices since 2010 data was not available yet. After Bonferroni correction there was no significance in any parameter. NS = non significant.

Variables correlated with Lambda	Mean	Std.Dv.	r^2	t	p
t-2 Total rainfall for entire year	627.17	205.19	0.13	-0.88	NS
t-2 Total May-August	384.59	161.55	0.21	-1.15	NS
t -1 Total rainfall for entire year	681.34	261.10	0.22	-1.18	NS
t -1 Total May-August	427.93	191.25	0.24	-1.24	NS
t Total rainfall for entire year	696.97	302.56	0.15	0.96	NS
t Total May-August	474.27	203.87	0.11	0.80	NS
Individual months					NS in all cases

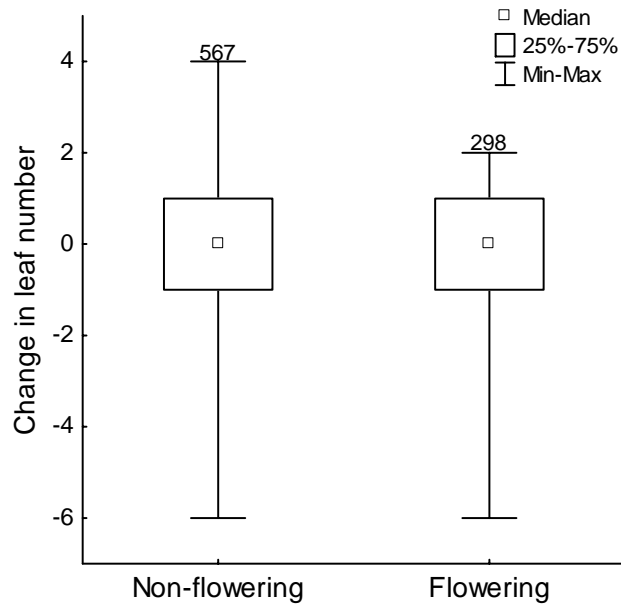


Figure 9.11. Change in leaf number for flowering and non-flowering plants (only young adult and adult stage classes used) for all years grouped together (numbers above bars indicate number of plant years).

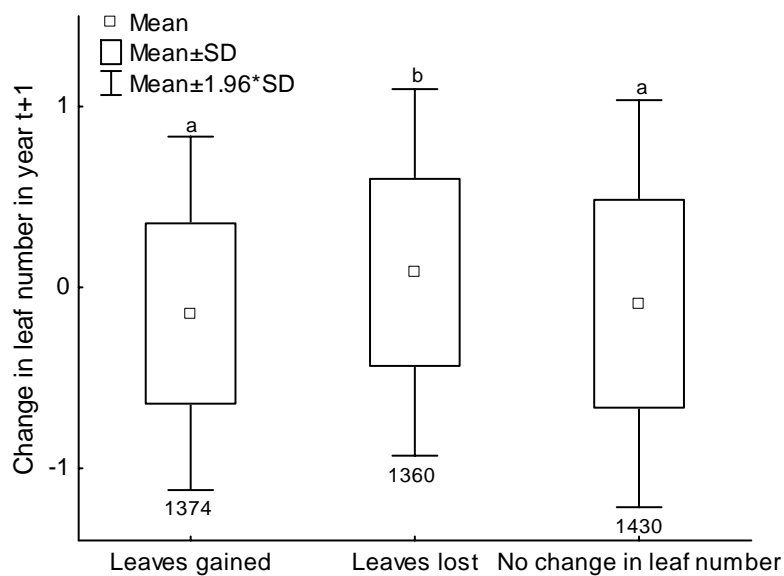


Figure 9.12. Plants in year t were divided into 3 groups, those that have gained leaves, lost leaves and maintained the same amount of leaves from year $t-1$ to year t . Plants, according to the change in leaf number, were put into one of those three groups: leaf gain by plants that gained leaves in year t , leaf gain by plants that lost leaves in year t , leaf gain by plants with similar number of leaves in year t .

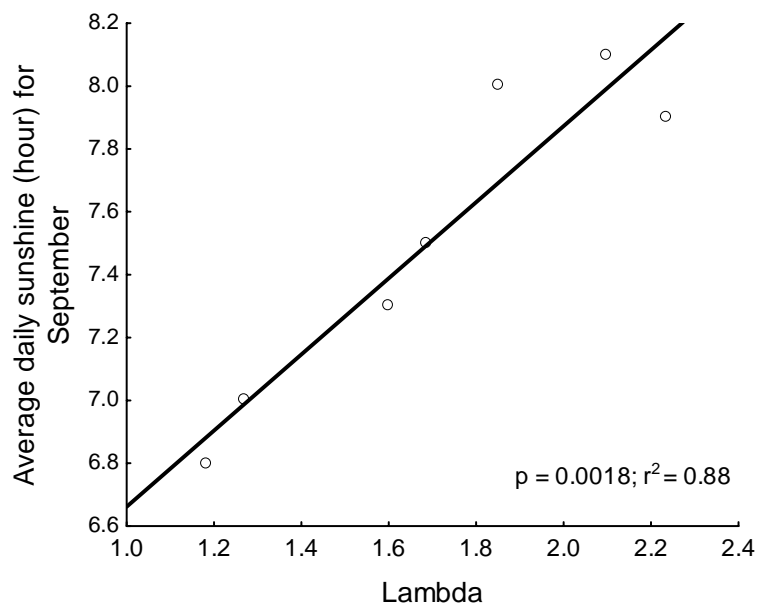


Figure 9.13. The only significant climate variable after bonferonni corrections was sunshine hours per day during the leaf stage of year t+1 on lambda from year t to t+1 ($r^2 = 0.88$, $t = 6.06$, $p = 0.002$, $N = 7$ (Note: climate data for 2010 not included as this data was not available in time)).

Discussion

The high growth rate observed in this study, a lambda of up to 2.2, compare well to spreading or growing populations with average growth rates of 2.60 for herbs, 2.21 for trees and 1.93 for a shrub (Werner & Caswell 1977; Silvertown et al. 1993; Shea & Kelly 1998; Parker 2000). When dividing plants into those in the sun and shade, the estimated lambda decreased from 2.26 in the sun to 1.60 in the shade. No particular transition were impacted, but shade decreased growth rate and reproduction (lower number of plants flowering) and increased retrogression for most life stages. Therefore, typical of a gap colonists and early successional species, *B. orientalis* population growth rate is reduced in the shade (Batista et al. 1998; Pascarella & Horvitz 1998; Valverde & Silvertown 1998).

One of the significant features of *B. orientalis* as a gap-colonising geophyte, is that adult plants are long-lived, up to a few decades. *B. orientalis* has a survival pattern similar to tree species, as plant size increases, survival probabilities increase to almost one (Condit et al. 1995; Abe et al. 1998; Schwartz et al. 2000). Resources stored in large, belowground bulbs seem to buffer individuals against mortality. A low mortality for young adult and adult plants was coupled with the ability to adjust in response to environmental changes; as plants tended to shrink rather than die. Of all the life-cycle stages, seed and seedlings have the lowest survival probabilities.

The probability of remaining in the same stage (S) was the most important contributor to lambda, similar to other long-lived plants (see for example Raimondo & Donaldson 2003; Alfonso-Corrado et al. 2007). In this study the young adult stage contributed the most to population growth, since plants in this stage can potentially live and reproduce for many years (Garcia & Ehrlén 2002; Koop & Horvitz 2005). For the non-reproductive stages growth was the most significant contributor to lambda. However, elasticity structure and population growth rates may differ between habitats, depending on the level of disturbance (Koop & Horvitz 2005). In disturbed sites, the contribution of the seedlings component might be relatively high initially, but with time since disturbance, contribution of plant persistence will dominate (Horvitz & Schemske 1995; Valverde & Silvertown

1998; Valverde et al. 2004; Octavio-Aguilar et al. 2008). This might explain why reproduction and seedlings were unexpectedly important for population growth in this study at 18.6% relative to other long-lived herbaceous plants (Silvertown et al 1993).. This pattern is similar to other gap-colonizing species and explains their behaviour as iteroparous forest herbs in the context of the Elasticity Triangle (Fig. 9.10) (Silvertown *et al.* 1993).

Ten years of demographic data was collected in this study. Although this is rather long compared to most demographic plant studies, it is still short when it comes to establishing the relationships between environmental variables and vital rates. *B. orientalis* is confined to the winter rainfall region of South Africa. Summers are hot and dry, so soil moisture early in the growing season is expected to be important. Surprisingly, this is not the case, rainfall had no effect on growth, mortality or proportion of plants flowering. Similarly, and in contrast to Hutchings (2010), there was no effect of temperature on growth rate, proportion of plants flowering or mortality. Mortality risk is largely independent of age (after the first year of survival) with no sign of good or bad years due to climate. Mortality was not caused by herbivory either, which might be attributable to high levels of toxicity (Van Wyk et al. 2005).

A potential mortality risk for *B. orientalis* is bulb excavation by collectors. The spectacular inflorescences appeal to anyone who has witnessed large stands of these plants flowering. *B. orientalis* is still a widespread and common plant, but in the closely related *B. josephinae* and endangered *B. litoralis*, illegal collecting is a problem (pers. obs.). Bulb collectors typically prefer larger more profusely flowering plants, but for long lived plant species the survival of larger stages is typically the most important (Fig. 9.8) (Franco & Silvertown 2004). Consequently, the best way to enhance the likelihood of persistence is to protect the larger reproductive individuals (Portela *et al.* 2010).

In accordance with Horvitz and Schemske (1988) and Shefferson *et al.* (2003) there was no detectable cost of reproduction (Fig. 9.11). Although I did not have enough sets of consecutive years of flowering data for a cost of reproduction analysis, flowering

individuals exhibited a strong tendency to remain flowering, with 60% of plants flowering in year t also flowering in year $t+1$. In contrast, Dutch populations of *Spiranthes spirales* tended to enter a vegetative state following a flowering event (Willems & Dorland 2000). Perhaps individuals may not risk flowering until they have a relatively large resource base. Cost of reproduction might not be measurable in leaf number, as leaves are important for maximizing the amount of sunlight a plant could obtain but might be compensated by bulb size, mortality or dormancy. Although dormancy was too low for proper analysis, 63% of plants that went dormant somewhere during the monitoring period died before the end of the study. Dormancy in *B. orientalis* thus occurs at a cost to survival, similar to orchids in Hutchings (2010) or Shefferson *et al.* (2003) but in contrast to *Silene spaldingii* for which dormancy is beneficial (Lesica & Crone 2007).

The data presented here show that *B. orientalis* is a light-demanding gap colonizer. The prostrate leaves, reduced population growth rate in the shade, and large investment in a winged inflorescence, which acts as a long-distance dispersal unit, are consistent with this interpretation. Therefore, a broader analysis of the importance of seeds, and hence pollinators, should take a metapopulation perspective that models the effect of changes in seed set on the rate of gap colonization (Valverde & Silvertown 1997b) which becomes even more important when other aspects, like climate change, are considered (Opdam & Wascher 2004).

This study has three important limitations. Firstly, I assume that annual variation in λ does not include variation in seed to seedling transition, nor variation in seed set. Secondly, when I model future population size, I assume that population growth rate remains constant and is not depressed by increased mortality at high densities. Thus, the results obtained in this study must be interpreted as reflecting demography under current density conditions (Caswell 2001). Thirdly, my results are based on the analysis of just a single population of *B. orientalis*. To completely understand population dynamics more populations under variable environmental conditions are needed. In the next chapter I will address the question of pollination variance across the landscape and impacts thereof on

population growth rates, since the pollinators of *B. orientalis* are known to be adversely impacted by habitat fragmentation (Pauw 2004).

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References

- Abe, S., T. Nakashizuka, and H. Tanaka. 1998. Effects of canopy gaps on the demography of the subcanopy tree *Styrax obassia*. *Vegetation Science* 9:787-796.
- Alfonso-Corrado, C., R. Clark-Tapia, and A. Mendoza. 2007. Demography and management of two clonal oaks: *Quercus eduardii* and *Q-potosina* (Fagaceae) in central Mexico. *Forest Ecology and Management* 251:129-141.
- Arnold, T. H., and B. C. De Wet 1993. *Plants of Southern Africa: Names and distribution*. Memoirs of the Botanical Survey of South Africa No. 62, NBI, Pretoria.
- Batista, W. B., W. J. Platt, and R. E. Macchiavelli. 1998. Demography of a shade-tolerant tree (*Fagus grandifolia*) in a hurricane-disturbed forest. *Ecology* 79:38-53.
- Bond, W. J. 2005. Large parts of the world are brown or black: A different view on the 'Green World' hypothesis. *Journal of Vegetation Science* 16:261-266.
- Bruna, E. M., and W. J. Kress. 2002. Habitat fragmentation and the demographic structure of an Amazonian understory herb (*Heliconia acuminata*). *Conservation Biology* 16:1256-1266.
- Bruna, E. M., and M. K. Oli. 2005. Demographic effects of habitat fragmentation on a tropical herb: Life-table response experiments. *Ecology* 86:1816-1824.
- Caswell, H. 1989. *Matrix population models: Construction, analysis, and interpretation*. Sinauer, Sunderland, MA, USA
- Caswell, H. 2001. *Matrix population models: construction, analysis, and interpretation*, 2nd edn. Sinauer Associates, Sunderland, MA.
- Condit, R., S. P. Hubbell, and R. B. Foster. 1995. Mortality rates of 205 Neotropical tree and shrub species and the impact of a severe drought. *Ecological Monographs* 65:419-439.

- Cowling, R. M. 1992. The ecology of fynbos: nutrients, fire and diversity. Oxford University Press, Cape Town.
- De Kroon, H., A. Plaisier, J. van Groenendael, and H. Caswell. 1986. Elasticity: the relative contribution of demographic parameters to population growth rate. *Ecology* 67:1427-1431.
- de Kroon, H., J. van Groenendael, and J. Ehrlén. 2000. Elasticities: A review of methods and model limitations. *Ecology* 81:607-618.
- Dekroon, H., A. Plaisier, J. Vangroenendael, and H. Caswell. 1986. Elasticity - the relative contribution of demographic parameters to population-growth rate. *Ecology* 67:1427-1431.
- Diadema, K., F. Medail, and F. Bretagnolle. 2007. Fire as a control agent of demographic structure and plant performance of a rare Mediterranean endemic geophyte. *Comptes rendus biologies* 330:691-700.
- Drechsler, M., B. B. Lamont, M. A. Burgman, H. R. Akcakaya, E. T. F. Witkowski, and Supriyadi. 1999. Modelling the persistence of an apparently immortal *Banksia* species after fire and land clearing. *Biological Conservation* 88:249-259.
- Evans, M. E. K., K. E. Holsinger, and E. S. Menges. 2008. Modeling the effect of fire on the demography of *Dicerandra frutescens* ssp *frutescens* (Lamiaceae), an endangered plant endemic to Florida scrub. *Population Ecology* 50:53-62.
- Franco, M., and J. Silvertown. 2004. Comparative demography of plants based upon elasticities of vital rates *Ecology* 85:531-538.
- Garcia, M. B., and J. Ehrlén. 2002. Reproductive effort and herbivory timing in a perennial herb: fitness components at the individual and population levels. *American Journal of Botany* 89:1295-1302.
- Haag, J., M. Coupe, and J. Cahill. 2004. Antagonistic interactions between competition and insect herbivory on plant growth. *Journal of Ecology* 92:156-167.
- Harper, J. L. 1967. A Darwinian approach to plant ecology. *Journal of Ecology* 55:247-270.
- Harper, J. L. 1977. Population biology of plants. Academic Press, London.

- Heppell, S. S., H. Caswell, and L. B. Crowder. 2000. Life histories and elasticity patterns: perturbation analysis for species with minimal demographic data. *Ecology* 81:654-665.
- Hernandez-Apolinar, M., T. Valverde, and S. Purata. 2006. Demography of *Bursera glabrifolia*, a tropical tree used for folk woodcrafting in Southern Mexico: An evaluation of its management plan. *Forest Ecology and Management* 223:139-151.
- Higgins, S. I., S. T. A. Pickett, and W. J. Bond. 2000. Predicting extinction risks for plants: environmental stochasticity can save declining populations. *Trends in Ecology & Evolution* 15:516-520.
- Hood, G. M. 2008. PopTools version 3.0.6. Available on the internet URL <http://www.cse.csiro.au/poptools>.
- Horvitz, C. C., and D. W. Schemske. 1988. Demographic cost of reproduction in a neotropical herb - an experimental field-study. *Ecology* 69:1741-1745.
- Horvitz, C. C., and D. W. Schemske. 1995. Spatiotemporal variation in demographic transitions of a tropical understory herb - projection matrix analysis. *Ecology* 65:155-192.
- Horvitz, C. C., S. Tuljapurkar, and J. B. Pascarella. 2005. Plant-animal interactions in random environments: Habitat-stage elasticity, seed predators, and hurricanes. *Ecology* 86:3312-3322.
- Hutchings, M. J. 2010. The population biology of the early spider orchid *Ophrys sphegodes* Mill. III. Demography over three decades. *Journal of Ecology* 98:867-878.
- Immanuel, N., and O. Talya. 2001. Effects of grazing on geophytes in Mediterranean vegetation. *Journal of Vegetation Science* 12:749-760.
- Kalish, S., and M. A. McPeck. 1992. Demography of an age-structured annual: resampled projection matrices, elasticity analyses, and seed bank effects. *Ecology* 73:1082-1093.
- Kery, M., and K. B. Gregg. 2003. Effects of life-state on detectability in a demographic study of the terrestrial orchid *Cleistes bifaria*. *Journal of Ecology* 91:265-273.
- Kery, M., and K. B. Gregg. 2004. Demographic analysis of dormancy and survival in the terrestrial orchid *Cypripedium reginae*. *Journal of Ecology* 92:686-695.

- Kery, M., K. B. Gregg, and M. Schaub. 2005. Demographic estimation methods for plants with unobservable life-states. *Oikos* 108:307-320.
- Koop, A. L., and C. C. Horvitz. 2005. Projection matrix analysis of the demography of an invasive, nonnative shrub (*Ardisia elliptica*). *Ecology* 86:2661-2672.
- Kwit, C., C. C. Horvitz, and W. J. Platt. 2004. Conserving slow-growing, long-lived tree species: Input from the demography of a rare understory conifer, *Taxus floridana*. *Conservation Biology* 18:432-443.
- Lefkovich, L. P. 1965. The study of population growth in organisms grouped by stages. *Biometrics* 21:1-18.
- Lesica, P., and E. E. Crone. 2007. Causes and consequences of prolonged dormancy for an iteroparous geophyte, *Silene spaldingii*. *Journal of Ecology* 95:1360-1369.
- Leslie, P. H. 1945. On the use of matrices in certain population mathematics. *Biometrika* 33:183-212.
- Luken, J. O., L. M. Kuddes, and T. C. Tholemeier. 1997. Response of understory species to gap formation and soil disturbance in *Lonicera maackii* thickets. *Restoration Ecology* 5:229-235.
- Manning, J. C., P. Goldblatt, and D. Snijman. 2002. *The Color Encyclopedia of Cape Bulbs*. Timber Press.
- Maze, K. E., and W. J. Bond. 1996. Are Protea populations seed limited? Implications for wildflower harvesting in Cape fynbos. *Australian Journal of Ecology* 21:96-105.
- Menges, E. S., and P. F. Quintana-Ascencio. 2004. Population viability with fire in *Eryngium cuneifolium*: Deciphering a decade of demographic data. *Ecological Monographs* 74:79-99.
- Mills, L. S., D. F. Doak, and M. J. Wisdom. 1999. Reliability of conservation actions based on elasticity analysis of matrix models *Conservation Biology* 13:815-829
- Moloney, K. A. 1986. A generalized algorithm for determining category size. *Oecologia* 69:176-180.
- Morris, W. F., and D. F. Doak. 2005. How general are the determinants of the stochastic population growth rate across nearby sites? *Ecological Monographs* 75:119-137.
- Mucina, L., and M. C. Rutherford. 2006. *The vegetation of South Africa, Lesotho and Swaziland*. South African National Biodiversity Institute, Pretoria South Africa.

- Octavio-Aguilar, P., J. Gonzalez-Astorga, and A. P. Vovides. 2008. Population dynamics of the Mexican cycad *Dioon edule* Lindl. (Zamiaceae): life history stages and management impact. *Botanical Journal of the Linnean Society* 157:381-391.
- Opdam, P., and D. Wascher. 2004. Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. 117:285-297.
- Parker, I. M. 2000. Invasion dynamics of *Cytisus scoparius*: A matrix model approach. *Ecological Applications* 10:726-743.
- Pascarella, J. B., and C. C. Horvitz. 1998. Hurricane disturbance and the population dynamics of a tropical understory shrub: Megamatrix elasticity analysis. *Ecology* 79:547-563.
- Pathikonda, S., A. S. Ackleh, K. H. Hasenstein, and S. Mopper. 2009. Invasion, disturbance, and competition: modeling the fate of coastal plant populations. *Conservation Biology* 23:164-173.
- Pauw, A. 2004. Variation in pollination across a fragmented landscape at the Cape of Africa. Ph.D Dissertation, University of Cape Town, Cape Town
- Perevolotsky, A. 2006. Livestock grazing and biodiversity conservation in Mediterranean environments: The Israeli experience. *Options Mediterraneennes*.
- Pfab, M. F., and E. T. F. Witkowski. 2000. A simple population viability analysis of the Critically Endangered *Euphorbia clivicola* RA Dyer under four management scenarios. *Biological Conservation* 96:263-270.
- Pickett, S. T. A., and P. S. White 1985. The ecology of natural disturbance and patch dynamics. Academic Press, Orlando, Florida, USA.
- Portela, R. d. C. Q., E. M. Bruna, and F. A. M. d. Santos. 2010. Demography of palm species in Brazil's Atlantic forest: a comparison of harvested and unharvested species using matrix models. *Biodiversity and Conservation* 19:2389-2403.
- Pugnaire, F. I., and R. Lazaro. 2000. Seed bank and understory species composition in a semi-arid environment: The effect of shrub age and rainfall. *Annals of Botany* 86:807-813.
- Raimondo, D. C., and J. S. Donaldson. 2003. Responses of cycads with different life histories to the impact of plant collecting: simulation models to determine

- important life history stages and population recovery times. *Biological Conservation* 111:345-358.
- Ramula, S., and K. Lehtila. 2005. Matrix dimensionality in demographic analyses of plants: when to use smaller matrices? *Oikos* 111:563-573.
- Richardson, D. M., and B. W. van Wilgen. 2004. Invasive alien plants in South Africa: how well do we understand the ecological impacts? *100*:45-52.
- Schwartz, M. W., S. M. Hermann, and P. J. Van Mantgem. 2000. Population persistence in *Florida torreyi*: comparing modeled projections of a declining coniferous tree. *Conservation Biology* 14:1023-1033.
- Shea, K., and D. Kelly. 1998. Estimating biocontrol agent impact with matrix models: *Carduus nutans* in New Zealand. *Ecological Applications* 8:824-832.
- Shefferson, R. P., J. Proper, S. R. Beissinger, and E. L. Simms. 2003. Life history trade-offs in a rare orchid: The costs of flowering, dormancy, and sprouting. *Ecology* 84:1199-1206.
- Silvertown, J., M. Franco, and E. Menges. 1996. Interpretation of elasticity matrices as an aid to the management of plant populations for conservation. *10*:591-597.
- Silvertown, J., M. Franco, I. Pisanty, and A. Mendoza. 1993. Comparative plant demography - relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. *Journal of Ecology* 81:465-476.
- Snijman, D. A., and R. H. Archer. 2003. Amaryllidaceae. In G. Germishuizen & N.L. Meyer (eds.). *Plants of southern Africa: an annotated checklist*. National Botanical Institute, Pretoria, RSA. *Strelitzia* 14: 957-967.
- Ticktin, T., P. Nantel, F. Ramirez, and J. T. 2002. Effects of variation on harvest limits for nontimber forest species in Mexico. *Conservation Biology* 16:691-705.
- Tilman, D. 1994. Competition and biodiversity in spatially structured habitat. *Ecology* 75:2-16.
- Valverde, T., S. Quijas, M. Lopez-Villavicencio, and S. Castillo. 2004. Population dynamics of *Mammillaria magnimamma* Haworth. (Cactaceae) in a lava-field in central Mexico. *Plant Ecology* 170:167-184.
- Valverde, T., and J. Silvertown. 1997a. Canopy closure rate and forest structure. *Ecology* 78:1555-1562.

- Valverde, T., and J. Silvertown. 1997b. A metapopulation model for *Primula vulgaris*, a temperate forest understorey herb. 85:193-210.
- Valverde, T., and J. Silvertown. 1998. Variation in the demography of a woodland understorey herb (*Primula vulgaris*) along the forest regeneration cycle: projection matrix analysis. *Journal of Ecology* 86:545-562.
- Van Wyk, B.-E., F. R. Van Heerden, and B. Van Oudtshoorn 2005. Poisonous plants of South Africa. Briza Publications, Pretoria.
- Vandermeer, J. 1978. Choosing category size in a stage projection matrix. *Oecologia* 32:79-84.
- Vlok, J.H.J., Yeaton, R.I. 2000. Competitive interactions between overstorey proteas and sprouting understorey species in South African mountain fynbos. *Diversity and Distributions* 6:273–281.
- Werner, P. A., and H. Caswell. 1977. Population-growth rates and age versus stage-distribution models for teasel (*Dipsacus-Sylvestris-Huds*). *Ecology* 58:1103-1111.
- Willems, J. H., and E. Dorland. 2000. Flowering frequency and plant performance and their relation to age in the perennial orchid *Spiranthes spirales* (L.) Chevall. *Plant Biology* 2:344-349.
- Zobel, M. 1992. Plant species coexistence – the role of historical, evolutionary and ecological factors. *Oikos* 65:314-320

10 The effect of pollen limitation on population growth rates in a long-lived geophyte

An important question in plant ecology is whether elevated pollination levels translate into population growth rate and vice versa, whether depressed pollination levels lead to population declines. By combining plant demography and plant reproduction the population growth rate under different pollination scenarios can be predicted. This is particularly important for small and fragmented plant populations. In this study I focus on *Brunsvigia orientalis*, a geophytic, sunbird pollinated plant from the highly transformed Cape lowlands of South Africa. Pollinator intensity determines seed set in *B. orientalis*, but whether this is important for population growth remains untested. Therefore, for 34 *Brunsvigia orientalis* populations I determined seed set, percentage of plants flowering, germination rate, population size and population structure. To calculate population growth rates, matrix models were constructed with the transition rates of Rondevlei (Chapter 9). Seed set varied hugely between populations, from 0.08 to 20.5 seeds per capsule. Seed set is positively correlated to population structure (seedling to adult ratio) and population growth rate, λ . By using population projection analyses, population fates under different pollination intensities are assessed. I show that the previously found link between pollination intensity and seed set can be extended to population growth rate, so that pollination intensity predicts population growth rate with a decline in the most pollen limited populations.

Introduction

Demographic modelling is a powerful tool for evaluating anthropogenic and natural impacts on plant populations (Caswell 1989; Caswell 2001). Typically plant demographic studies consider different harvesting scenarios (Raimondo and Donaldson 2003; Alfonso-Corrado *et al.* 2007; Portela *et al.* 2010), fragmentation impacts (Bruna and Kress 2002; Tomimatsu and Ohara 2010), restoration success (Colas *et al.* 2008), conservation questions (Griffith and Forseth 2005), competition for light (Silvertown *et al.* 1996;

Batista *et al.* 1998) and fire impacts (Silva *et al.* 1991; Evans *et al.* 2008). Currently few studies model plant animal-interactions, and where they do, the focus is generally on herbivory (Maron and Crone 2006; Miller *et al.* 2009) or seed dispersal (Melo and Tabarelli 2003) with very little work on pollination (Ashman *et al.* 2004).

With the large number of pollination studies on the one hand and an increasing number of demographic studies on the other, one might suppose that the population-level consequence of variable pollination is well studied. Surprisingly, this is not the case (Ashman *et al.* 2004); studies relating variation in pollination success to demography remain uncommon (Parker 1997; Ehrlen 2002; Lennartsson 2002; Hegland and Totland 2007; Price *et al.* 2008). Traditionally pollination ecologists measure fruit and seed set as an indicator of natural pollination success (Chapters 5, 7, 8). Several studies improve on this by adding a germination trial (Morgan 1999; Lienert and Fischer 2004; Hegland and Totland 2007), but very few consider the entire plant life-cycle. Possible reasons are the time span involved and the assumption that seeds are generally of low importance in the demography of perennial plants (Silvertown *et al.* 1996).

Currently one of the major pressures increasing spatial variability in plant reproduction is habitat fragmentation (Wilcove *et al.* 1986; Saunders *et al.* 1991; Tomimatsu and Ohara 2010). Habitat fragmentation is considered a major threat for biodiversity as it affects plant fitness and population viability (Lienert and Fischer 2004; Fischer and Lindenmayer 2006). In plants, smaller, more fragmented populations often exhibit lower reproductive success since fragments become too small to sustain pollinator communities (Steffan-Dewenter and Tschardtke 1999; Steffan-Dewenter *et al.* 2002; Honnay *et al.* 2005; Aguilar *et al.* 2006; Valdivia *et al.* 2006). Most studies only consider consequences of fragmentation for plant reproduction, so that the extent to which variation in recruitment translates into subsequent population dynamics is still relatively unknown (but see Lennartsson 2002; Bruna 2003; Hobbs and Yates 2003; Bruna *et al.* 2009; Tomimatsu and Ohara 2010).

In the Cape lowlands of South Africa over 90% of the area is transformed by agriculture and urbanization (Heijnis *et al.* 1999; Kemper *et al.* 2000). Despite the fact that the Cape Floristic Region is characterized by exceptional botanical diversity and highly specialized pollination systems (Goldblatt and Manning 2002) the effect of habitat fragmentation on pollination has rarely been studied (but see Donaldson *et al.* 2002; Pauw 2007). Birds, and hence bird-pollinated plants, are likely to be among the most sensitive to anthropogenic habitat fragmentation (Bond 1994; Allen-Wardell *et al.* 1998; Kearns *et al.* 1998; Lindberg and Olesen 2001). Therefore, they present an ideal study system to assess pollen limitation and subsequent population growth rates in fragmented habitats

Here I focus on *Brunsvigia orientalis*, a geophytic species from the Cape Floristic Region. *B. orientalis* is pollinated by two sunbird species, the Lesser Double-collared Sunbird and Malachite Sunbird, both of which decrease in abundance in smaller and fragmented areas (Chapter 5, Cameron 1999; Hockey 2003; Pauw 2004; Fox and Hockey 2007; Randrianasolo 2003). Indeed, *B. orientalis* set fewer seed set in small conservation areas, early successional vegetation and in smaller populations (Pauw 2004). These plants are not resource limited, since hand pollination has shown a significant increase in seed set. Seed set is thus strongly dependent on pollination intensity (Pauw 2004). Although relative long lived, elasticity analysis has indicated that seeds play an important role in the life history of *B. orientalis* (Chapter 9). In light of this, a strong link between seeds and lambda is expected, but untested. Therefore, in this study I take the established link between pollination intensity and seed set one step further and determine the effect of seed set on population growth rate. In particular, I address the following: (1) Does variation in seed set translate into variation in population structure? (2) Is seed set an accurate predictor of population growth rate? (3) Are pollinator limited populations heading for extinction?

Methods

Study species and sites

Brunsvigia orientalis is a bulbous, long living plant with seasonal growth and reproduction. Inflorescences are produced at the end of summer (February to April),

when nectar resources for sunbirds are most limited. *B. orientalis* is self-incompatible and incapable of vegetative reproduction. Once the basic life history was known (Chapter 9), 34 *Brunsvigia orientalis* populations, across their range in the Western Cape Lowlands of South Africa, were sampled for seed set (autumn) and a snapshot in time for population structure (spring) (Fig. 10.1). For each population, seed set per capsule, number of capsules per plant and the population size in leaf and flowering stage was determined (Table 10.1).

Fecundity variables for matrix modelling

For each population a dense part in the centre of the population was selected and the number of leaves noted for each individual plant in a 50 by 2 meter transect (100 m²). Leaf data was collected in one growing season only (Table 10.1). Total population size during the leaf stage was either counted (<100 plants) or estimated by multiplying plant density per square meter with the total population area. During flowering, the conspicuous inflorescences were counted. The percentage of plants flowering differs between adults and young adults (Chapter 9). Since no plants were marked in this study the percentage of adult and young adult plants flowering could be calculated by assuming similar ratios of adult to young adult plants as was typically found in Chapter 9.

Seed data was collected over a range of sites, mostly in 2009 and 2010, but for nine sites seed data from 1998-2000 was included (Pauw 2004). For three additional sites seed data was available for both 2000 and 2009. For two of those there was no significant difference between years and data was pooled (Kleinmond, Mann-Whitney *U*-test, $U = 41$ $P = 0.97$; Milnerton, Mann-Whitney *U*-test, $U = 331$ $P = 0.08$). For the third site, seed set increased significantly and only 2009 data was used (Vermont, t-test, $t = 2.36$, $p = 0.02$). Seeds were either counted by hand, or sieved into size classes and weighed in order to calculate total seeds per plant. Average number of seeds per capsule for each population was square root transformed to obtain normality.

Seed to seedling transition rates were estimated in the field from observing emergence and survival of a set of planted seeds. Germination trails were conducted in populations

which were representative of a range of geographic regions: two sites in the Overberg (Vogelgezung, 34°40'37.0"S 20°06'37.6"E; Windheuwel, 34°08'11.3"S 20°17'13.1"E), two sites in the West coast area (Velddrif, 32°45'04.3"S 18°10'15.5"E; Langebaan, 32°57'10.0"S 18°10'08.0"E) and a site representative of the Cape flats (Milnerton, 33°51'28.4"S 18°29'21.8"E). Rondevlei was also representative of the Cape flats and data from Chapter 9 was included in this analysis. Fifty viable seeds were laid out in hoops (0.70 m in diameter) made by thick plastic strips at 5 populations (n = 5-14 hoops) (Vogelgezung, Windheuwel, Velddrif, Langebaan, Milnerton). The enclosures were protruding 5 cm above the soil surface in order to contain the seeds. Seedlings were counted six months later.

By combining these three reproductive variables, fecundity values for each population could be calculated as follows: proportion of plants flowering \times number of seeds per plant \times seedlings survival after six months. For each population these fecundity values were included into a transition matrix, with stage class division and average transition probabilities obtained from Chapter 9. I therefore assume that transition probabilities are similar to the Rondevlei matrix, but partly test this assumption by checking whether seed to seedling transitions are homogenous across sites. Our models assume density independence (Koop and Horvitz 2005). The stage-based models were constructed using a spreadsheet package (Microsoft Excel 2008) with the Pop-Tools plug-in (Hood 2008).

Population projections

Extreme pollen limitation in out-crossing plants is commonly assumed to be problematic for population persistence, but this is rarely tested. Therefore, I run population projections for a hundred years with an input of 25 plants per stage for a total of 100 plants. Three populations are presented graphically, small urban (Mosselbay), small natural (Elim road 2) and large natural (Vogelgezung). A projection from Rondevlei is included for comparison. All projections were done using a spreadsheet package (Microsoft Excel 2008) with the Pop-Tools plug-in (Hood 2008). All other analysis was performed in STATISTICA 9.0 (StatSoft, Inc. 2009, Tulsa, USA).

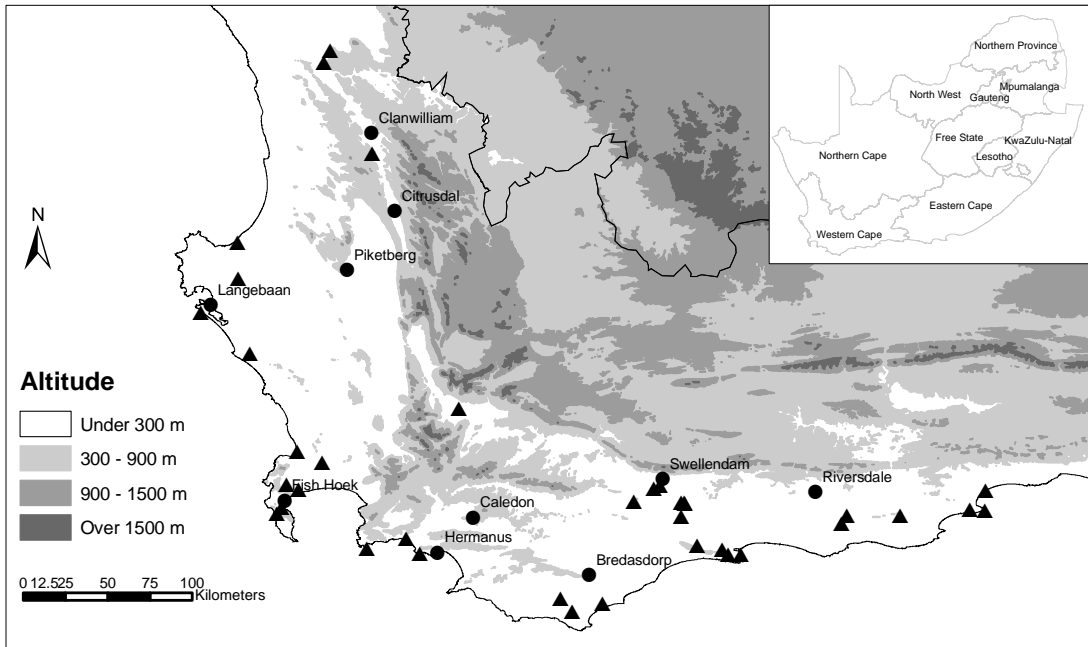


Figure 10.1. Distribution of the sampled *Brunsvigia orientalis* populations in the Cape floral region of South Africa.

Table 10.1. *Brunsvigia orientalis* study sites, year of seed collection, population size, seeds per capsule, the number of flowering plants and lambda for each population.

Site	Year of seed counts	Population size (leaf stage)	Seeds per capsule	Number of flowering plants	Lambda
Cape flats	2000	25	0.95	11	1.16
Danabaai	2009	2000	4.85	70	1.26
Hamerkop	2009	63	3.18	4	1.73
Cape Infanta	2009	23830	18.31	159	1.96
Klawer	1998	4134	7.28	300	1.89
Kleinmond	2000, 2009	172	4.34	24	1.61
Langebaan	2000	287583	3.71	2500	1.12
Elim road 2	2009	100	1.19	24	1.14
Elim road 1	Used Elim road 2	250	1.19	13	1.34
Millers point	2000	745	4.81	85	2.18
Milnerton	2000, 2009	1090	0.31	86	1.03
Mosselbay cemetery	2010	800	0.08	50	0.99
Nooitgedacht, Citrusdal	1998	1160	8.96	150	1.50
Potberg 1	2009	125	4.85	13	1.39
Potberg 2	2009	212	5.18	24	1.43
Rawsonville	2010	1000	2.88	300	1.37
Windheuwel	2009	16080	3.75	400	1.08
Rietvlei	2000	173	1.56	60	1.63
Riversdal, Stilbaai Road	2010	300	1.25	25	1.58
Road to sandkraal 1	2009	0	1.60	9	
Road to sandkraal 2	2009	8	1.42	6	1.59
Rooibrug	2009	400	9.60	111	1.95
Sandkraal	2009	164000	5.63	2890	1.21
Scaborough	2000	588	0.08	150	1.00
Seafarm	2000	109089	6.14	2000	1.33
Swellendam	2010	15	13.10	11	2.77
Tokai	2000	70	0.48	9	1.10
Velddrif	2008	4018	6.08	70	1.33
Vermont	2009	6250	9.41	60	1.67
Vogelgezang	2010	5570	20.53	100	2.18
West coast national park	2010	8	3.60	3	2.27
West coast national park, Postberg	2010	20	7.88	6	1.75
Wiedouw	1998	68800	8.83	400	1.14
Xama camp	2009	6264	8.88	56	1.35

Results

Fecundity variables and matrix modelling

Seed set varied hugely between sites, from 0.08 seeds per capsule in Mosselbay to 20.5 seeds per capsule at Vogelgezung. Seed germination varied somewhat across the landscape, with the lowest percentage of seeds germinating at Velddrif (mean and SD; 16.2 ± 15.6 %) and the highest percentage of seeds germination at Vogelgezung (mean and SD; 48.8 ± 22.6 %). Although there was a trend towards higher germination in wetter sites, correlations with average annual rainfall were not significant (Pearson's correlation $t = 1.523$, $p = 0.188$, $r^2 = 0.32$, $N = 7$ sites) and seed germination did not differ significantly between sites (Kruskal-Wallis $H = 12.36$, $df = 55$, $P = 0.06$). Germination rate was subsequently averaged across all sites, except for individual sites for which germination rates were available.

Population sizes varied from as little as 8 plants to extensive populations with thousands of plants (Table 10.1). Similarly, the percentage of plants flowering per population varied hugely. At a population level the number of seeds produced translates well into the number of seedlings, since the seedling to adult ratio is positively correlated to seeds per capsule (Pearson's correlation $t = 2.74$ $r^2 = 0.19$, $p = 0.010$, $N = 34$ sites; Fig. 10.2).

Lambda between populations varied from far above two, to just below one (Fig. 10.3). The population with the lowest seed set also had the lowest lambda. Seed set is an accurate predictor of lambda with lambda positively correlated with the number of seeds per capsule (Pearson's correlation, $t = 4.5$, $r^2 = 0.40$ $p < 0.001$, $N = 34$ sites; Fig. 10.3).

Population projections

Most populations have a high projected population growth rate under a density independent scenario. Only once the number of seeds per capsule decreased to 0.08 is population extinction predicted. Extinction is only predicted for one population, a small urban population (Fig. 10.4).

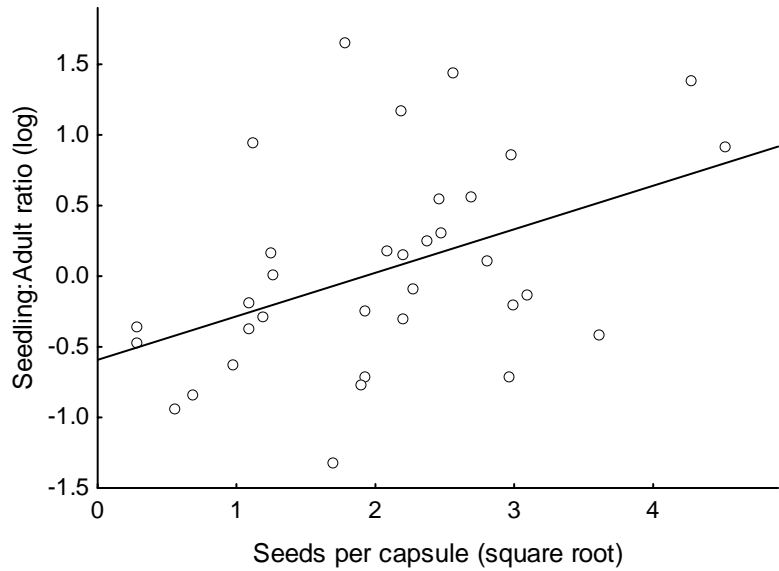


Figure 10.2. Correlation between the number of seeds per capsule and population structure (Pearson's correlation $t = 2.74$ $r^2 = 0.19$, $p = 0.010$, $N = 34$ sites). A negative ratio of juveniles to adults (logged) indicates there are more adults than juveniles in the population.

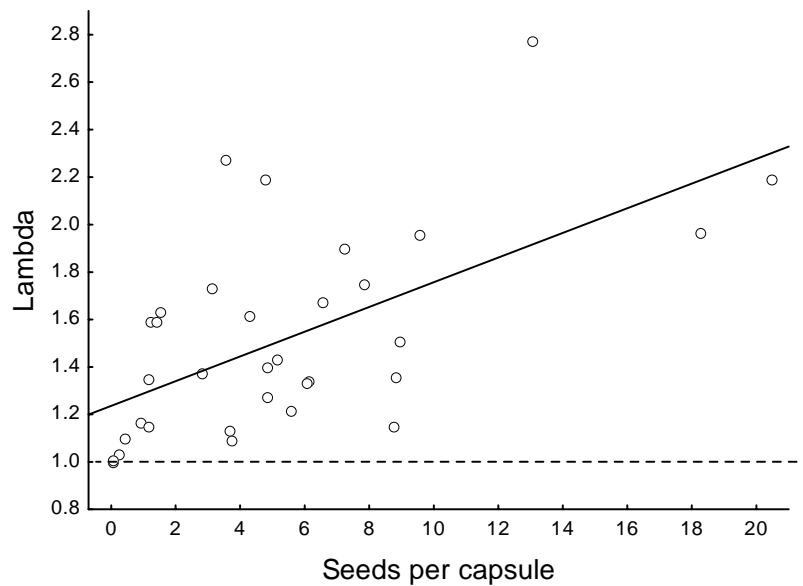


Figure 10.3. Lambda for all the study populations is correlated to the number of seeds per capsule. The dotted line indicates the point where populations will be at equilibrium. Populations below the line are decreasing, whilst populations above the line have the potential to expand in a density independent scenario (Pearson's correlation, $t = 4.5$, $r^2 = 0.40$ $p < 0.001$, $N = 34$ sites).

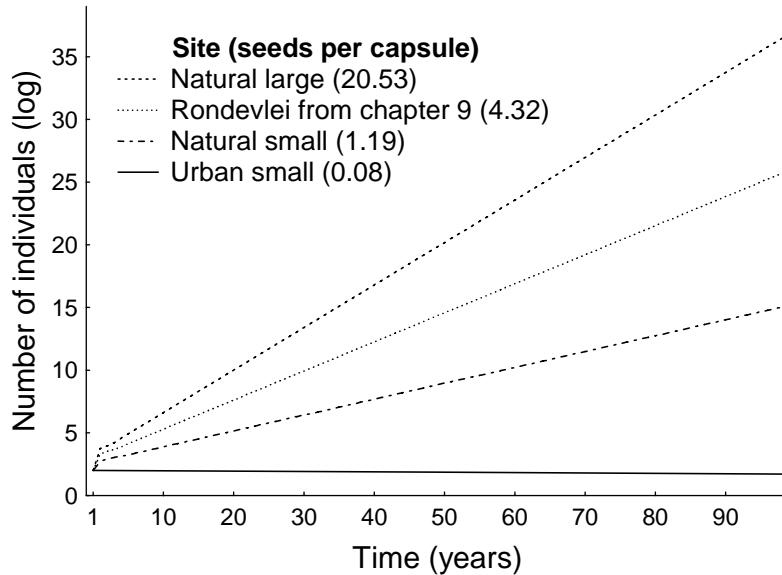


Figure 10. 4. Population projections for a population within a natural large environment (Vogelgezang), natural small environment (Elim road 2) and a small urban site (Mosselbay). A Rondevlei projection under conditions described in Chapter 9 is included for comparison. Note that population sizes are logged in order to be able to compare all populations on one graph.

Discussion

In a previous study the pollination intensity of *Brunsvigia orientalis* was found to be directly related to seed set (Pauw 2004). Here I take this a step further and show that seed set is directly related to population structure and growth rate (Fig.10. 2, 10.3). Lambda is found to be sensitive to changes in seed production (Chapter 9)(Ashman *et al.* 2004). In contrast, Horvitz *et al.* (2010) found that the increase in population growth rate due to supplemental pollination, in the understory herb *Lathyrus vernus*, was insignificant with a huge increase in seed set. In a review, Ashman *et al.* (2004) performed elasticity analyses for six populations from the literature where pollen supplementation improved seed production. In only three populations was there a strong positive effect of pollen supplementation on population growth rate (Bierzychudek 1982 and two populations from Parker 1997).

Elasticity analysis gives insight into the life history of a plant species where vital rates with high elasticities are important, since small changes in those particular vital rates could lead to proportionally large increases in lambda (Mills *et al.* 1999; de Kroon *et al.*

2000). When seed set is low, the importance of seeds decreases disproportionately and survival becomes more important. For example, at the pollen limited urban site (Mosselbay), the contribution of stasis is 84.0%, with growth contributing 13.5% and fecundity contributing a minor 2.6%. In comparison at a large natural site (Vogelgevang) where pollination is high, stasis contributed 31.5%, growth 44%, and fecundity 24.5%.

Most *Brunsvigia orientalis* populations produced many seeds per capsule and subsequently populations had very high projected growth rates. Some of these high growth rates compare well to the growth rates of spreading and rapidly expanding populations (Werner and Caswell 1977; Shea and Kelly 1998; Parker 2000; Koop and Horvitz 2005). Because of these high levels of seed set and growth rate a reduction in pollination and hence seed set is insignificant for population persistence (reviewed in Turnbull *et al.* 2000). However, this apparent overabundance of seeds is an important part of the survival strategy of a bet hedging, gap-colonizing life history (Chapter 9).

B. orientalis is a gap colonist, quickly colonising new areas and potentially moving huge distance with their winged, wind dispersed inflorescences (Chapter 9). Under low pollination scenarios a population might not decline, but the low seed set will be insufficient to colonise new areas. In late successional vegetation a few *B. orientalis* plants typically survive in small vegetation gaps until the next disturbance, where after they can recolonise the newly created opening (Horvitz and Schemske 1995; Bruna and Kress 2002; Kwit *et al.* 2004). If, however, disturbances are too few and too far apart, population persistence depends on colonisation from adjacent populations. If not enough seeds are produced, this is unlikely. Furthermore, in small urban fragments this is impossible and once a population has disappeared there are no adjacent populations to be colonized from. Therefore maintaining connectedness in small fragments is important for seed dispersal (Tewksbury *et al.* 2002; Schmucki and de Blois 2009). Connectedness between natural habitat will also benefit the movement of sunbirds as important pollinators of *B. orientalis* (Cameron 1999; Riandrianasolo 2003).

In our projection analysis population extirpation is predicted for one population only (Fig. 10.4). This is a small urban site where sunbird abundance is very low or zero (Pauw 2004). Counterbalancing low pollinator abundance is the fact that plants are long lived, and that one or two seeds per inflorescence can be produced through selfing (Pauw 2004). In addition, herbivory is low under these conditions and a larger percentage of seeds will survive to the seedling stage (Chapter 5). Furthermore, individual *B. orientalis* plants in urban populations are potentially longer lived since shrubs are cleared for esthetical reasons and plants are not overshadowed (Chapter 9). These factors might partly explain the observed anomaly of low seed set and unexpected large numbers of seedlings at some sites (see population structures in Appendix 1).

An important shortcoming of this study is the assumption that transition rates in all populations are similar to that of the model population at Rondevlei. This is a valid assumption for populations in close proximity, but less so for populations encountering different environmental conditions. Furthermore, population projections in this study are based on a density independence scenario, but populations will never be able to sustain such growth due to density related factors and ever increasing shade with vegetation succession.

This study focuses on the role of pollinators in determining population growth rates, but the demographic models provided here and in Chapter 9 could aid in the understanding of responses to other impacts as well. Firstly, studies modelling climate change and plant shifts will greatly benefit by simulating and including biotic interactions and pollinator range shifts within their models (Midgley *et al.* 2003; Simmons *et al.* 2004; Bomhard *et al.* 2005; Best 2007). Secondly, the extent to which pollen limited plants should be hand pollinated to ensure population persistence can be calculated (Chapter 5). In conclusion, even in the absence of comprehensive data sets for all populations, population projection matrices can inform management decisions and contribute to the conservation of species (Doak *et al.* 1994).

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References

- Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* 9:968-980
- Alfonso-Corrado C, Clark-Tapia R, Mendoza A (2007) Demography and management of two clonal oaks: *Quercus eduardii* and *Q-potosina* (Fagaceae) in central Mexico. *For. Ecol. Manage.* 251:129-141
- Allen-Wardell G et al. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conserv. Biol.* 12:8-17
- Ashman TL et al. (2004) Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology* 85:2408-2421
- Batista WB, Platt WJ, Macchiavelli RE (1998) Demography of a shade-tolerant tree (*Fagus grandifolia*) in a hurricane-disturbed forest. *Ecology* 79:38-53
- Best AS (2007) Which species will successfully track climate change? The influence of intraspecific competition and density dependent dispersal on range shifting dynamics. *Oikos* 116:1531-1539
- Bierzychudek P (1982) The demography of jack-in-the-pulpit, a forest perennial that changes sex. *Ecological Monographs* 52:335-351
- Bomhard B et al. (2005) Potential impacts of future land use and climate change on the Red List status of the Proteaceae in the Cape Floristic Region, South Africa. *Glob. Change Biol.* 11:1452-1468
- Bond WJ (1994) Do mutualisms matter - assessing the impact of pollinator and disperser disruption on plant extinction. *Philos. Trans. R. Soc. Lond. Ser. B-Biol. Sci.* 344:83-90

- Bruna EM (2003) Are plant populations in fragmented habitats recruitment limited? Tests with an Amazonian herb. *Ecology* 84:932-947
- Bruna EM, Fiske IJ, Trager MD (2009) Habitat fragmentation and plant populations: is what we know demographically irrelevant? *J. Veg. Sci.* 20
- Bruna EM, Kress WJ (2002) Habitat fragmentation and the demographic structure of an Amazonian understory herb (*Heliconia acuminata*). *Conserv. Biol.* 16:1256-1266
- Cameron A (1999) The effects of fragmentation of renosterveld vegetation on bird community composition. MSc mini-thesis, University of Cape Town, Cape Town.
- Caswell H (1989) Matrix population models: Construction, analysis, and interpretation. Sinauer, Sunderland, MA, USA
- Caswell H (2001) Matrix population models: construction, analysis, and interpretation, 2nd edn. Sinauer Associates, Sunderland, MA.
- Colas B et al. (2008) Restoration demography: a 10-year demographic comparison between introduced and natural populations of endemic *Centaurea corymbosa* (Asteraceae). *J. Appl. Ecol.* 45:1468-1476
- de Kroon H, van Groenendael J, Ehrlén J (2000) Elasticities: A review of methods and model limitations. *Ecology* 81:607-618
- Doak D, Kareiva P, Klepetka B (1994) Modeling population viability for the desert tortoise in the western Mojave Desert. *Ecological Applications* 4:446-460
- Donaldson J, Nanni I, Zachariades C, Kemper J, Thompson JD (2002) Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. *Conserv. Biol.* 16:1267-1276
- Ehrlén J (2002) Assessing the lifetime consequences of plant-animal interactions for the perennial herb *Lathyrus vernus* (Fabaceae). *Perspectives in plant ecology evolution and systematics* 5: 145-163
- Evans MEK, Holsinger KE, Menges ES (2008) Modeling the effect of fire on the demography of *Dicerandra frutescens* ssp *frutescens* (Lamiaceae), an endangered plant endemic to Florida scrub. *Population Ecology* 50:53-62
- Fischer J, Lindenmayer DB (2006) Beyond fragmentation: the continuum model for fauna research and conservation in human-modified landscapes. *Oikos* 112:473-480

- Fox SJC, Hockey PAR (2007) Impacts of a South African coastal golf estate on shrubland bird communities. *South African Journal of Science* 103:27-34
- Goldblatt P, Manning JC (2002) Plant diversity of the Cape Region of southern Africa. *Ann. Mo. Bot. Gard.* 89:281-302
- Griffith AB, Forseth IN (2005) Population matrix models of *Aeschynomene virginica*, a rare annual plant: Implications for conservation. *Ecological Applications* 15:222-233
- Hegland SJ, Totland O (2007) Pollen limitation affects progeny vigour and subsequent recruitment in the insect-pollinated herb *Ranunculus acris*. *Oikos* 116:1204-1210
- Heijnis CE, Lombard AT, Cowling RM, Desmet PG (1999) Picking up the pieces: a biosphere reserve framework for a fragmented landscape - the coastal lowlands of the Western Cape, South Africa. *Biodiversity and Conservation* 8:471-496
- Hobbs RJ, Yates CJ (2003) Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. *Australian Journal of Botany* 51:471-488
- Hockey P (2003) Conserving renosterveld - do birds matter? *African Birds and Birding* April/May:13
- Honnay O, Jacquemyn H, Bossuyt B, Hermy M (2005) Forest fragmentation effects on patch occupancy and population viability of herbaceous plant species. *New Phytologist* 166:723-736
- Hood GM (2008) PopTools version 3.0.6. In. Available on the internet URL <http://www.cse.csiro.au/poptools>
- Horvitz CC, Ehrlen J, Matlaga D (2010) Context-dependent pollinator limitation in stochastic environments: can increased seed set overpower the cost of reproduction in an understory herb? *Journal of Ecology* 98:268-278
- Horvitz CC, Schemske DW (1995) Spatiotemporal variation in demographic transitions of a tropical understory herb - Projection Matrix Analysis. *65:155-192*
- Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: The conservation of plant-pollinator interactions. *Annu. Rev. Ecol. Syst.* 29:83-112
- Kemper J, Cowling RM, Richardson DM, Forsyth GG, McKelly DH (2000) Landscape fragmentation in South Coast Renosterveld, South Africa, in relation to rainfall and topography. *Austral Ecol.* 25:179-186

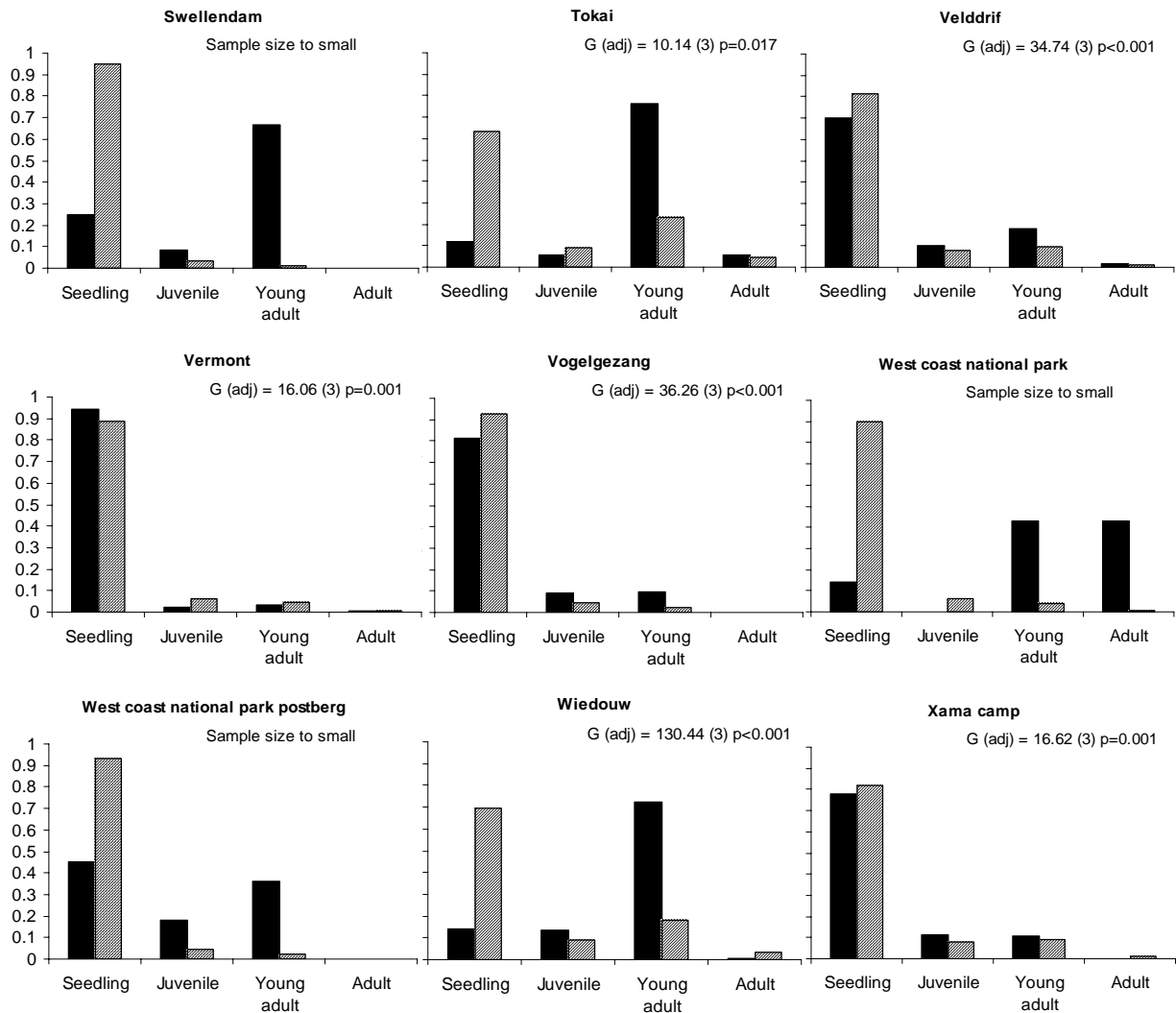
- Koop AL, Horvitz CC (2005) Projection matrix analysis of the demography of an invasive, nonnative shrub (*Ardisia elliptica*). *Ecology* 86:2661-2672
- Kwit C, Horvitz CC, Platt WJ (2004) Conserving slow-growing, long-lived tree species: Input from the demography of a rare understory conifer, *Taxus floridana*. *Conserv. Biol.* 18:432-443
- Lennartsson T (2002) Extinction thresholds and disrupted plant-pollinator interactions in fragmented plant populations. *Ecology* 83:3060-3072
- Lienert J, Fischer M (2004) Experimental inbreeding reduces seed production and germination independent of fragmentation of populations of *Swertia perennis*. *Basic Appl. Ecol.* 5:43-52
- Lindberg A, Olesen JM (2001) The fragility of extreme specialization: *Passiflora mixta* and its pollinating hummingbird *Ensifera ensifera*. *Journal of Tropical Ecology* 17:323-329
- Maron JL, Crone E (2006) Herbivory: effects on plant abundance, distribution and population growth. *Proc. R. Soc. Lond. B* 273:2575-2584
- Melo FPL, Tabarelli M (2003) Seed dispersal and demography of pioneer trees: The case of *Hortia arborea*. *Plant biology* 5:359-365
- Midgley GF, Hannah L, Millar D, Thuiller W, Booth A (2003) Developing regional and species-level assessments of climate change impacts on biodiversity in the Cape Floristic Region. *Biol. Conserv.* 112:87-97
- Miller TEX, Louda SM, Rose KA, Eckberg JO (2009) Impacts of insect herbivory on cactus population dynamics: experimental demography across an environmental gradient. *Ecological Monographs* 79:155-172
- Mills LS, Doak DF, Wisdom MJ (1999) Reliability of conservation actions based on elasticity analysis of matrix models. *Conserv. Biol.* 13:815-829
- Morgan JW (1999) Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. *Conserv. Biol.* 13:266-273
- Parker IM (1997) Pollinator limitation of *Cytisus scoparius* (Scotch broom), an invasive exotic shrub. *Ecology* 78:1457-1470
- Parker IM (2000) Invasion dynamics of *Cytisus scoparius*: A matrix model approach. *Ecological Applications* 10:726-743

- Pauw A (2004) Variation in pollination across a fragmented landscape at the Cape of Africa. Ph.D Dissertation, University of Cape Town, Cape Town
- Pauw A (2007) Collapse of a pollination web in small conservation areas. *Ecology* 88:1759-1769
- Portela RdCQ, Bruna EM, Santos FAMd (2010) Demography of palm species in Brazil's Atlantic forest: a comparison of harvested and unharvested species using matrix models. *Biodiversity and Conservation* 19:2389-2403
- Price MV, Campbell DR, Waser NM, Brody AK (2008) Bridging the generation gap in plants: pollination, parental fecundity, and offspring demography. *Ecology* 89:1596-1604
- Raimondo DC, Donaldson JS (2003) Responses of cycads with different life histories to the impact of plant collecting: simulation models to determine important life history stages and population recovery times. *Biol. Conserv.* 111:345-358
- Randrianasolo H (2003) Birds in West Coast Renosterveld fragments: implications for a threatened habitat. MSc mini-thesis, University of Cape Town, Cape Town
- Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation - a review. *Conserv. Biol.* 5:18-32
- Schmucki R, de Blois S (2009) Pollination and reproduction of a self-incompatible forest herb in hedgerow corridors and forest patches. *Oecologia* 160:721-733
- Shea K, Kelly D (1998) Estimating biocontrol agent impact with matrix models: *Carduus nutans* in New Zealand. *Ecological Applications* 8:824-832
- Silva JF, Raventos J, Caswell H, Trevisan MC (1991) Population responses to fire in a tropical savanna grass, *Andropogon-semiberbis* - a matrix model approach. *Journal of Ecology* 79:345-356
- Silvertown J, Franco M, Menges E (1996) Interpretation of elasticity matrices as an aid to the management of plant populations for conservation. *Conservation Biology* 10:591-597
- Simmons RE, Barnard P, Dean WRJ, Midgley GF, Thuiller W, Hughes G (2004) Climate change and birds: perspectives and prospects from southern Africa. *Ostrich* 75:295-308

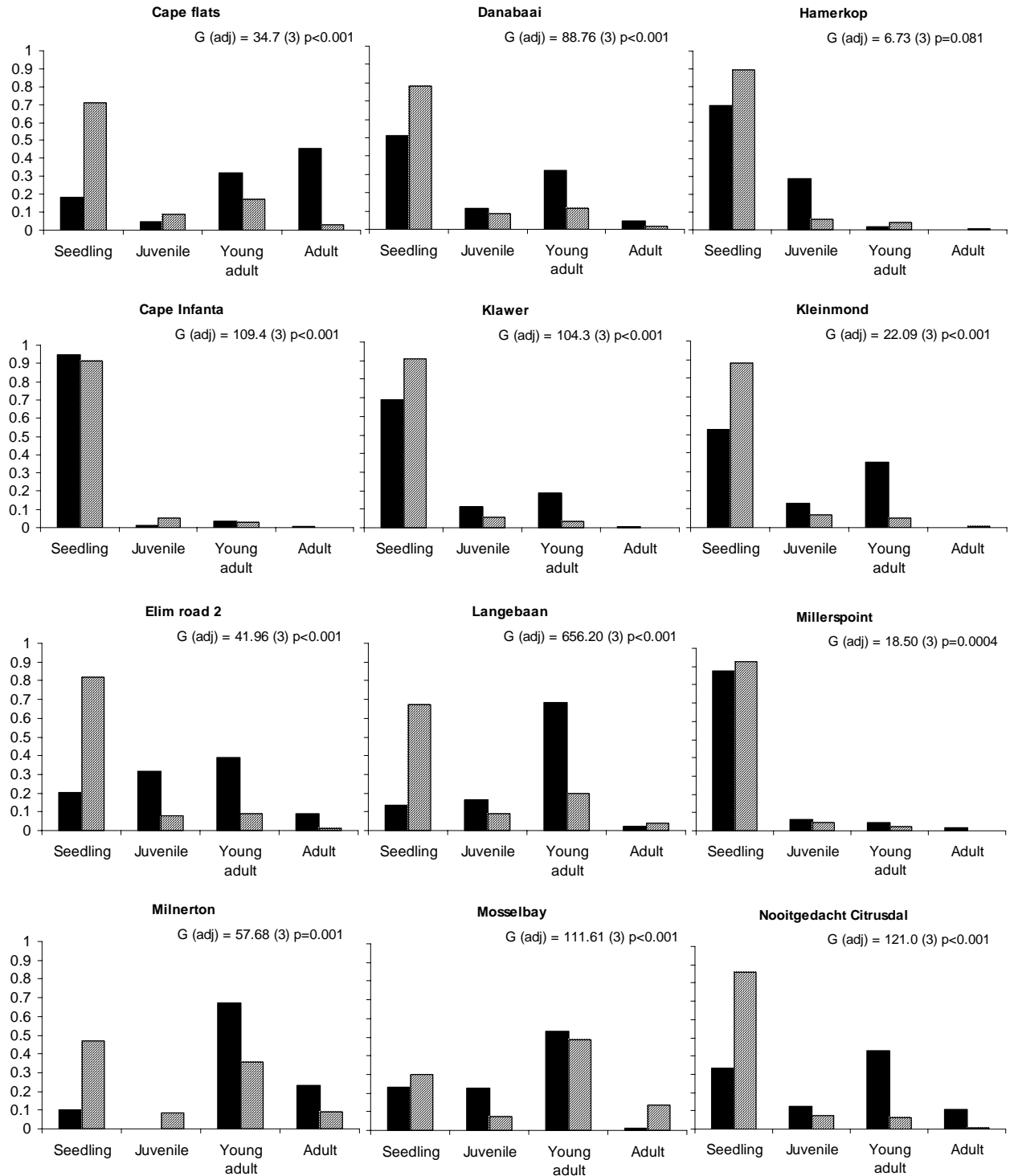
- Steffan-Dewenter I, Tschardt T (1999) Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121:432-440
- Steffan-Dewenter I, Munzenberg U, Burger C, Thies C, Tschardt T (2002) Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* 83:1421-1432
- Tewksbury JJ et al. (2002) Corridors affect plants, animals, and their interactions in fragmented landscapes. *PNAS* 99:12923-12926
- Tomimatsu H, Ohara M (2010) Demographic response of plant populations to habitat fragmentation and temporal environmental variability. *Oecologia* 162:903-911
- Turnbull LA, Crawley MJ, Rees M (2000) Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* 88:225-238
- Valdivia CE, Simonetti JA, Henriquez CA (2006) Depressed pollination of *Lapageria rosea* Ruiz et Pav. (Philesiaceae) in the fragmented temperate rainforest of Southern South America. *Biodiversity and Conservation* 15:1845-1856
- Werner PA, Caswell H (1977) Population-growth rates and age versus stage-distribution models for teasel (*Dipsacus sylvestris* Huds). *Ecology* 58:1103-1111
- Wilcove DS, McLellan CH, Dobson AP (1986) Habitat fragmentation in the temperate zone. In: Soule ME (ed) *Conservation biology: the science of scarcity and diversity*. Sinauer, Sunderland, pp 237-256

Appendix 1

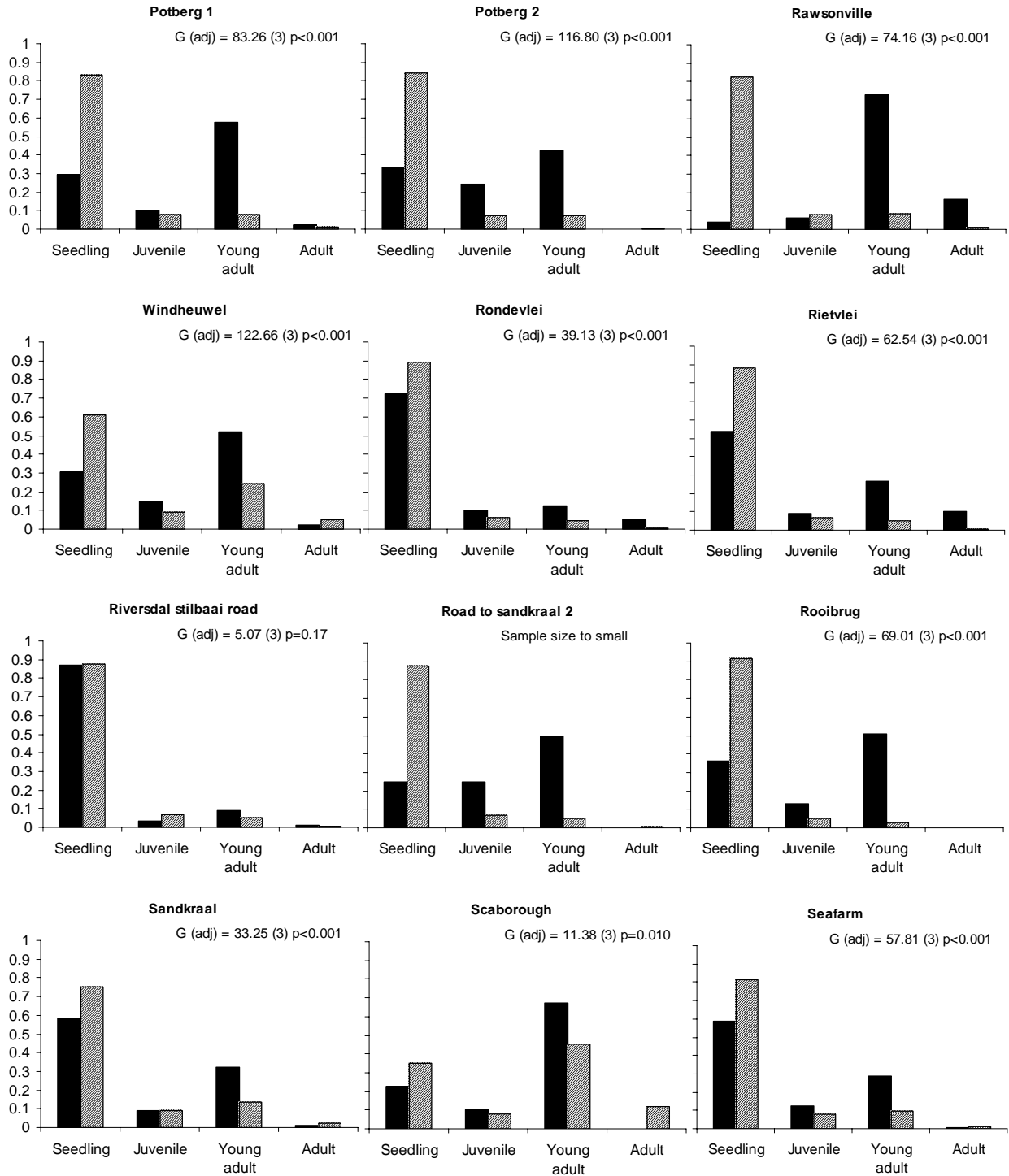
Population structures for all sampled populations are shown. The residual variation in Figure 10.2 can be partly explored by considering the disturbance history and the age of the population, which in turn determines seed production and population structure. This can be done by comparing the observed stage distribution (OSD) (solid bars) and stable stage distribution (SSD) (hatched bars) for each population. Y-axis presents the proportion of individuals and the x-axis the four stage classes. Values of the log-likelihood ratio tests (G) and their associated level of significance (P) are shown for each site. The G -statistic indicates whether there is a difference between OSD and SSD.



Appendix 1 cont.



Appendix 1 cont.



11 General conclusion

In this thesis I set out to describe the assembly and disassembly of the bird pollination community in the Cape Floristic Region of South Africa. This was done to address the knowledge gap on pollinator declines in areas outside Europe and North America, and on groups other than insects. Furthermore, pollinator declines are even more important in hugely diverse plant areas such the Cape.

I started this thesis by exploring the assembly of the nectar-feeding bird community and their dependent plant communities and showed that bird-plant pollination mutualisms are an important ecological factor structuring ornithophilous Proteaceae and nectar-feeding bird communities (Chapter 2). The close association between plant and bird communities found in this study, therefore suggests an important role for community-wide pollination mutualisms. I found that species-rich communities are more likely to contain vulnerable species, suggesting that persistence of these species is dependent on the nectar-feeding bird community. These findings will hopefully change the way we think about plant and animal communities; apart from factors such as soils, climate and fire, biotic interaction also shape these communities on a landscape scale. Furthermore, my work revealed major gaps in our knowledge of plant-pollinator interaction in South Africa. Proteaceae and Ericaceae are some of the biggest and most spectacular flowering families in the Cape, but apart from a few studies in the 1980s, detailed pollination studies of these groups are almost non-existent.

In this thesis I set out to consider a number of wide-ranging anthropogenic influences on the bird pollination community and their dependant plants. The first anthropogenic impact addressed was the artificial increase of bee numbers through bee keeping (Chapter 3). I found that as long as stocking densities are modest, honeybee farming is not impacting on the bird pollination community and can be allowed into nature reserves. However, effects on other pollinator groups and plant species need to be assessed before recommendations can be made (Brand 2009).

As set out in Chapter 4, roads negatively affected the bird pollination community. My results suggest that even roads with relatively low traffic volumes have a negative impact on bird pollination in close proximity to roads. To my knowledge this is the first study considering pollination in relation to roads. Therefore, the two-fold decline in pollination along road sides should have important implications for the way we view and manage road verges as refugia for species and ecological processes. The effectiveness of road side verges to conserve plant-pollinator mutualisms over the long term deserves more attention within the scientific and conservation communities.

Together with roads comes the extensively studied topic of habitat fragmentation. In Chapter 5 I found that the bird pollination community breaks down in small urban fragments. When the sole pollinator of *B. littoralis* is absent from these small fragments, other members of the nectar-feeding bird community cannot act as substitute pollen vectors, since the system is too specialized (Geerts and Pauw 2009), and seed set is extremely low. On the up side, with anthropogenic fragmentation plant populations become easily accessible for monitoring and increasing seed set through hand-pollination. The extent to which low levels of seed set are an immediate conservation concern depends on the importance of seeds in the demography of the plant. The low levels of seed set observed in this study might be sufficient to maintain population growth. This can potentially be tested by utilising parts of the demographic models developed in Chapter 9 for the closely related *B. orientalis*.

As set out in Chapter 6, nectar-feeding birds occur in very low abundance in post-fire vegetation and plants that flower in burnt landscapes are rarely visited. Surprisingly, many bird-pollinated plants flower in post fire habitat. These plants thus either flower en masse to attract nectar-feeding birds, or the low seed-set is offset by reduced seed predation and competition. Further research on the cost and benefits of fire-stimulated flowering is clearly needed.

In this thesis I have considered a number of wide-ranging anthropogenic influences on bird pollination communities with sometimes surprising outcomes. Despite being

specialized for pollination by hovering birds of the New World, the invasive tree tobacco has become integrated into pollination community that lacks hummingbirds (Chapter 7). Although Old World nectarivorous birds have only very rarely been observed to hover feed on indigenous plants, sunbirds hover feed at flowers of the tree tobacco. The tree tobacco supplies a rich nectar source in an otherwise dry landscape, and significantly increases sunbird numbers. In turn, flowers visited by sunbirds formed significantly more capsules and set significantly more seed than sunbird-excluded flowers, possibly facilitating the invasion. Similarly sunbirds visited, this time by conventional perching, the potentially invasive Australian kangaroo paws (Chapter 8). Kangaroo paws provide sunbirds and sugarbirds with a rich source of nectar at a time of nectar scarcity (late summer), in return, being rewarded by increased reproductive output. When inflorescences were removed, nectar-feeding bird abundance decreased significantly. Therefore, invasive alien plants could be beneficial for nectar-feeding birds by supplying additional nectar, particularly since mature, nectar rich vegetation is decreasing with increasing fire frequencies. Chapter 8 is a great example of research and management integration. As soon as it became clear that plants produced many seeds, all inflorescences were removed a few times during flowering until the best mechanism for total eradication had been found.

To conclude this thesis I determine whether the breakdown of the bird pollination community really matters. Therefore in Chapter 9 a demographic model for the bird-pollinated *Brunsvigia orientalis* was built, which showed that seeds are important in the life history of this shade intolerant, gap colonising species. In Chapter 10, this model is used to make the link between seed set and population growth rate. From this, effects of variable pollination levels could be predicted. Populations in human altered habitats had lower seed set and population extirpation was predicted for the most urban population. Only when *Brunsvigia orientalis* populations experience very low pollination rates are they at an extinction risk.

As demonstrated by *Brunsvigia orientalis*, most plant species have some sort of backup mechanism (Bond 1994). These might include asexual reproduction, selfing or being

long-lived. When the environment improves and plant-pollinator communities are restored these populations can bounce back. Some plants, like the pollinator absent *B. litoralis* of Chapter 5 and the pollen limited *B. orientalis* populations of chapter 10 are long lived and give the slow research to conservation circle opportunity to complete and give us, humans, time to react before it is too late. In contrast, alien invasive plants do not wait for the research to application circle to complete and here research and immediate application should go hand in hand.

References

- Bond WJ (1994) Do mutualisms matter - assessing the impact of pollinator and disperser disruption on plant extinction. *Philos. Trans. R. Soc. Lond. Ser. B-Biol. Sci.* 344:83-90
- Brand MR (2009) The short term impact of a collection of commercial Cape honeybee (*Apis mellifera capensis* Esch.) colonies on invertebrate flower visitors within a near pristine Fynbos habitat in the Cape Floristic Region. MSc Dissertation, Stellenbosch University, Stellenbosch.
- Geerts S, Pauw A (2009) Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *S. Afr. J. Bot.* 75:699-706

Thesis Appendix

Pollination ecology of the invasive tree tobacco *Nicotiana glauca*: comparisons across native and non-native ranges

Jeff Ollerton^{1*}, Stella Watts^{1,6}, Shawn Connerty¹, Julia Lock¹, Leah Parker¹, Ian Wilson¹, Sheila Schueller², Julieta Nattero³, Andrea A. Cocucci³, Ido Izhaki⁴ Sjikr Geerts⁵ and Anton Pauw⁵

¹Natural Environment Research Group, School of Science and Technology, University of Northampton, Avenue Campus, Northampton, NN2 6JE, UK. ²School of Natural Resources and Environment, University of Michigan 440 Church Street Ann Arbor, MI 48109-1115, USA. ³Instituto Multidisciplinario de Biología Vegetal (IMBIV). Conicet-Universidad Nacional de Córdoba. Casilla de Correo 495. 5000, Córdoba. Argentina. ⁴Department of Evolutionary and Environmental Biology, Faculty of Science and Science Education, University of Haifa, 31905 Haifa, Israel.

⁵Dept of Botany and Zoology, Stellenbosch Univ., Private Bag X1, Matieland, 7602, South Africa.

⁶Current address: Laboratory of Pollination Ecology, Institute of Evolution, University of Haifa, Haifa 31905, Israel.

Abstract

Interactions with pollinators are thought to play a significant role in determining whether plant species become invasive, and ecologically generalised species are predicted to be more likely to invade than more specialised species. Using published and unpublished data we assessed the floral biology and pollination ecology of the South American native *Nicotiana glauca* (Solanaceae) which has become a significant invasive of semi-arid parts of the world. In regions where specialised bird pollinators are available, for example hummingbirds in California and sunbirds in South Africa and Israel, *N. glauca* is integrated into local pollination webs and sets seed by both out-crossing and selfing. In areas where there are no such birds, such as the Canary Islands and Greece, abundant viable seed is set by selfing, facilitated by the shorter stigma-anther distance compared to plants in native populations. Surprisingly, in these areas without pollinating birds, the considerable nectar resources are not exploited by other flower visitors such as bees or butterflies, either legitimately or by nectar robbing. We conclude that *Nicotiana glauca* is a successful invasive species outside of its native range, despite its functionally specialised hummingbird

pollination system, and therefore its invasion success is not predictable from its pollination biology.

Introduction

Plant-flower visitor relationships evolve and are maintained within a fluctuating ecological context in which populations of pollinating, pollen collecting and nectar robbing animals can change significantly from year to year (e.g. Herrera 1988, Fishbein and Venable 1996, Ollerton 1996, Lamborn and Ollerton 2000, Alarcón et al. 2008). This is particularly relevant to introduced invasive plant species which lack ecological or functional pollinator specificity and are therefore ecological generalists (sensu Waser et al. 1996, Fenster et al. 2004, Ollerton et al. 2007). Such plants can form relationships with pollinators and maintain viable populations following human dispersal beyond their native range, negatively affecting local habitats by monopolising space and soil resources, and in the process out-competing native species (Theoharides and Dukes 2007). Invasive plants have also been shown to have more subtle, but still potentially important, detrimental effects on the native flora by becoming integrated into local pollination interaction webs (sensu Memmott and Waser 2002, Vilá et al. 2009, Padrón et al. 2009) and influencing patterns of flower visitation and pollen flow, resulting in lower seed set and quality, and reduced pollinator abundance (Chittka and Schurkens 2001, Schurkens and Chittka 2001, Moragues and Traveset 2005, Traveset and Richardson 2006, Bjerknes et al. 2007, Aizen et al. 2008, Morales and Traveset 2009, Stout and Morales 2009). Other studies, however, have found no negative effects (e.g. Aigner 2004) indicating that the outcomes of such indirect interactions are likely to be species and/or community specific (Moragues and Traveset 2005) and also to depend on spatial scale (Cariveau and Norton, 2009). It is therefore important for us to understand why some plant species are more likely than others to become a threat to local plants, and particularly whether such plants can be predicted from their floral traits (Rodger et al. 2010). For example, Chittka and Schurkens' (2001) study suggests that introduced plants with very high rates of nectar production may draw pollinators away from native plants, reducing their reproductive success. High rates of nectar production may therefore be a predictive trait for such negative indirect effects (though see Nienhuis et al. 2009).

Other than self-pollinating species, plants with ecologically generalized pollination systems, which can attract, reward and therefore utilise a wide range of

pollinators, have been considered the most probable invasive species (Baker 1974; Richardson et al. 2000; Olesen et al. 2002; Vilá et al. 2009). Such plants are theoretically more likely to co-opt native or introduced flower visitors as pollinators, ensuring their reproductive success and subsequent invasiveness, and there is growing evidence that this is the case (e.g. Forster 1994; Bartomeus et al. 2008; Vilá et al. 2009). Nevertheless more studies of the integration of invasive plants into local interaction webs are required to test the generality of this idea. In particular we should compare the pollination ecologies of invasive plants within their normal distributional range and within the areas of invasion. The only such published study that we know to exist is of *Rhododendron ponticum* (Stout et al. 2006, though see Rodger et al. 2010) and this is a gap in the knowledge of the ecology of invasive species generally (Tillberg et al. 2007).

This present research focuses on the invasive tree tobacco *Nicotiana glauca*. Graham (Solanaceae), a native of central and north west Argentina and Bolivia (Goodspeed 1954) which has been widely introduced to the subtropics as a garden ornamental, only to escape and densely colonise native habitats across the globe, including other parts of South America (Cocucci, Watts, pers obs.); Australia (Florentine and Westbrook 2005; Florentine et al 2006); California (Schueller 2004); Hawaii (Izhaki, pers. obs.); the north and east Mediterranean region (Tadmor-Melamed et al. 2004, Bogdanović et al. 2006) including Israel where *N. glauca* was first observed in 1890 (Bornmuller 1898); Mexico (Hernández, 1981); North Africa (Ollerton., pers. obs.); Southern Africa (Geerts and Pauw 2009, Henderson 1991, R. Raguso, pers comm.); and the Canary Islands (Kunkel 1976, Ollerton pers. obsv.). The species is listed in the Global Invasive Species Database (<http://www.issg.org/database/welcome/>), and a number of regional organisations consider it invasive, for example in Hawai'i (http://www.hear.org/pier/species/nicotiana_glauca.htm), Europe (<http://www.europe-aliens.org/index.jsp>) and South Africa (<http://www.agis.agric.za>).

In its native range, *N. glauca* is strictly hummingbird pollinated (Nattero and Cocucci 2007) although bees and other insects may pierce the base of the corolla tube to rob nectar. Our study therefore addresses the following two questions:

“(1) Is *N. glauca*, with its apparently functionally specialised pollination system and abundant nectar resources, pollinated by functionally equivalent pollinators (i.e. flower-feeding birds) throughout its native and non-native range?

(2) Is integration into the local pollination web a prerequisite for reproductive success in this highly invasive species?

Data collection and synthesis

We have synthesised published and unpublished data from studies of the species in north western Argentina and Bolivia (Nattero and Cocucci 2007, Nattero et al. 2010, and unpublished data), where the species is native, with research from areas where the species is introduced, including South America (Peru - Watts unpublished data); other populations of Argentina outside the native range - Nattero and Cocucci 2007 and unpublished data); North America (México – Hernández 1981, California – Schueller 2004 and 2007 and unpublished data); and the Old World, including Tenerife (Ollerton et al. unpublished data), Greece (Schueller 2002 and unpublished data) and Crete (Ollerton unpublished data); Israel (Tadmor-Melamed 2004, Tadmor-Melamed et al. 2004, Izhaki unpublished data); and South Africa (Skead 1967, Knuth 1898-1905, Marloth 1901, Geerts and Pauw 2009, and unpublished data). The methods for the published data collection can be found in the relevant papers; methods for the unpublished data are summarised only briefly and more details can be obtained via the corresponding author. Measurements of floral traits, including nectar production, followed standard protocols (Kearns and Inouye 1993, Dafni et al. 2005). Unless otherwise stated, the authors cited above were responsible for the data collected in specific geographical regions. Data were analysed using SPSS 17.0: all data fulfilled assumptions of normality (one-sample Kolmogorov-Smirnov Test) and mean values are presented as \pm SD.

The local distribution and abundance of *N. glauca*

Within its native range *N. glauca* is an occasional plant of dry, naturally and anthropogenically disturbed areas such as river banks, track sides and abandoned quarries. It is found mainly in semi-arid environments from low to high altitudes (0-3500 m), but never at wet localities. The plant is rarely abundant and is mainly found as scattered, usually multi-stemmed individuals, though stem densities on anthropogenically disturbed sites can range from 3.0 to 12.5 plants m^{-2} (Nattero and Cocucci 2007). Mean population fruit set per plant ranges from about 28.0% to 66.7% (grand mean = $42.4 \pm 13.1\%$ - Table 1).

In its non-native range *Nicotiana glauca* is a conspicuous, profusely blooming invasive species growing predominantly along roadsides and on disturbed land in semi-arid regions. It can be extremely abundant; for example, in Tenerife we have recorded densities of flowering stems in 3m x 3m quadrats of 6.7 ± 2.3 to 20.0 ± 4.4 , covering hundreds of square metres, and in an extensive population in South Africa (Buffelsrivier) we recorded stem densities of 48.8 ± 9.4 in 5 x 5m quadrats. Similarly, in Israel it forms relatively dense scrub in both mesic and semi-arid regions (Izhaki pers. obs.). These high densities are achieved mainly from seed production; there is no clonal growth, though broken stems can re-sprout and there may be some rooting from horizontal branches in contact with the soil (pers. obs.). Fruit set in populations can be high (Table 1). Each fruit contains hundreds of tiny, dry seeds (mean seeds per fruit of Israeli plants = 1122.7 ± 655.8 , n = 12 plants; mean seeds per fruit of South African plants = 1435.8 ± 1063.6 , n = 7 plants; mean seeds per fruit of California plants = 655 ± 247 (n = 16 plants). Seedlings are common in non-native populations and seeds from Tenerife showed high viability: mean percentage seed germination was $85.5 \pm 6.4\%$ following sowing on damp filter paper (n = 20 seeds in each of 10 Petri dishes). Plants in Israel also produce seeds with high viability (mean rate of germination = $92.7 \pm 5.2\%$, n = 25 seeds in each of 10 Petri dishes), and likewise, in South Africa (mean rate of germination = $87.5\% \pm 10.6\%$ n = 20 seeds in each of 10 Petri dishes)

The floral biology of *N. glauca*

Within its native range, the flowers of *N. glauca* are typically yellow and tubular, ranging from 30 to 57 mm in length (n = 38 populations). The mouth of the corolla is green when the flower first opens, but changes to yellow over several days, until the flower is a single hue. In scattered populations of northwest Argentina, a flower colour polymorphism is present which includes dark red, reddish yellow and yellow morphs.

All non-native populations studied to date possess only the typical yellow flower colour variant which may reflect the introduction of limited genotypes into the alien range. Corolla length also tends to be shorter in non-native populations; for example, flowers on Tenerife are on average 37.6 ± 1.7 mm in length (n = 21 flowers from 5 plants); South Africa (Buffelsrivier) = 33.7 ± 0.5 mm (n = 10 flowers on each

of 16 plants); California = 35.5 ± 1.8 mm (n = 10 flowers per plant on 85 plants across 4 sites) though island populations (more recently colonized and containing shorter billed hummingbird visitors) have slightly shorter corollas than the mainland plants (Schueller 2007); northern Israel = 34.8 ± 2.0 mm (n = 10 flowers on each of 10 plants); Peru = 33.3 ± 1.5 mm (n = 10 flowers on each of 4 plants); finally, Greek populations have the shortest recorded corolla lengths with an average of 31.8 ± 2.5 mm (n = 95 flowers on 9 plants).

Nectar is abundantly produced and of moderate sugar concentration, with a mean volume of 20.0 ± 8.1 μ l per flower and concentration of 25.2 ± 3.7 % sucrose equivalent in pollinator-excluded flowers from a native population (Galetto and Bernardello, 1993a) with a sugar composition of 48.6%: 38.9%: 13.2% (sucrose: fructose: glucose) (Galetto and Bernardello 1993b). Data from non-native populations were obtained using a variety of protocols, e.g. bagged for various periods versus standing crop from open flowers, at various times of the day. But they largely agree with the results from the native populations in that they show that *N. glauca* flowers produce substantial quantities of moderately concentrated nectar. For example, in North America, bagged flowers in California had a mean volume of 25.4 ± 16.8 μ l and concentration of 25.1 ± 6.0 % (n = 145) whilst in Mexico, it was 2.2 ± 5.78 μ l and 36.0 ± 1.7 % (n = 20 flowers). In Peru the values were 12.7 ± 12.1 μ l and 20.2 ± 5.8 % (n = 49). On Tenerife it was 5.7 ± 4.7 μ l and 26.8 ± 7.4 % (n = 30), in Greece 23.5 ± 8.6 μ l (no concentration data available – n = 53). In Israel a daily rhythm of nectar volume was detected with the lowest volumes at 0800 in the morning (5.7 ± 3.4 μ l), rising in the afternoon (1400) to 9.8 ± 3.8 μ l; the concentration was unchanged 20.4 ± 1.0 % at 0800, 19.9 ± 3.7 % at 1400 (n = 66). Perhaps more expected for a bird pollinated plant is the observation of peak nectar volumes in the early morning in a population in South Africa, dropping from 15.5 ± 14.4 μ l at 0830 to 2.8 ± 4.7 μ l at 1430; once again, concentration was largely unchanged (26.9 ± 4.0 % at 0830, 31.8 ± 6.4 % at 1430 – n = 10 flowers per time period).

Although, as we mentioned, the nectar data have been collected using a range of protocols and are therefore not directly comparable, nonetheless these results emphasise our main point that *N. glauca* produces abundant nectar in all populations, even those that are predominantly selfing (see below).

The population mean minimum stigma-anther (S-A) distance is a measure of the average ability of flowers to autogamously self pollinate. In *N. glauca* in California, island populations have shorter S-A distances than mainland populations. This is probably a result of the initial colonising plants being predominantly selfing rather than a result of natural selection favouring self pollinating genotypes, as the island populations (contrary to expectation) did not experience lower pollinator visitation rates compared to mainland populations (Schueller 2004). However, S-A distances vary greatly between populations (Table 1) and there is a trend of smaller S-A distances when one compares native populations, with non-native populations where specialised bird pollinators are present and populations with no pollinators (Figure 1). The difference between mean SA of plants in their native range (2.4 ± 0.9 mm, $n = 7$ populations) versus those from introduced populations where there are no pollinators (1.5 ± 0.3 mm, $n = 6$ populations) is small in absolute terms (only 0.9 mm on average). But in proportional terms this represents a decrease in stigma-anther distance of over one third from plants in the native ancestral range to the introduced invasive populations.

The small S-A distances of invasive compared to native populations may play a role in the ability of invasive populations to produce greater proportional fruit set (Table 1) as on average introduced populations have marginally statistically significantly greater reproductive output than native populations [mean fruit set: Native = $42.4 \pm 13.1\%$ ($n = 7$ populations); Introduced = $57.3 \pm 16.9\%$ ($n = 12$ populations); independent samples t-test: $t = -2.0$, $df=17$, $p=0.06$]. However, there is some geographical variation to this pattern; for example, self pollination in Israel is rare and occurs in only 6% of bagged *Nicotiana glauca* flowers (Tadmor-Melamed 2004), bagged flowers in a Mexican population studied by Hernández (1981) did not set fruit, whilst within California populations, mean fruit set of bagged flowers varied from 6 to 29% (Schueller 2004).

Flower visitors to *N. glauca*

In its native range in South America, *N. glauca* is pollinated by several species of hummingbirds and nectar robbed by *Xylocopa* carpenter bees (Table 2). In addition, the hummingbird *Chlorostilbon aureoventris* behaved as a secondary nectar robber in a population from northern Argentina and as a legitimate pollinator in others

(Table 2). None of these hummingbirds, nor the *Xylocopa*, are *Nicotiana* specialists: all visit the flowers of other plants for nectar.

Outside of its native range, two distinct patterns emerge, depending upon whether or not the populations fall within the range of specialist flower visiting birds. In Argentina, Peru, the south western USA and Mexico, hummingbirds once again act as pollinators, with bees and flies also making occasional legitimate visits to flowers (Table 2). In addition, the flowers are nectar robbed by native bees, honeybees, hoverflies (Syrphidae) and flower piercers of the genus *Diglossa*. In Israel, sunbirds (*Nectarinia osea*) are likely to be the main pollinators: 60% of their visits were legitimate, with nectar being accessed from the front of the flower; in the other 40% of visits the birds pierced the corolla base, and thus acted as nectar robbers. The Hummingbird Hawkmoth (*Macroglossum stellatarum*) was also observed as an occasional legitimate visitor in Israel, with *Xylocopa* and also several species of ants acting as nectar robbers (Cohen et al., pers. obs.; see Table 2). In South Africa, three species of sunbirds, the Malachite sunbird (*Nectarinia famosa*), the Dusky sunbird (*Cinnyris fuscus*) and the Southern double-collared sunbird (*C. chalybea*) have been confirmed as pollinators (Geerts and Pauw 2009). The former species is the most effective and frequent pollinator, while the latter two species also rob during 7% and 61% of visits respectively. There are also records of flower visitation of honeyeaters for Australia but their role in pollination is not clear (Table 2).

In the northern Mediterranean and the Canary Islands, however, where there are no specialist flower visiting birds, flower visitors have never been observed in any populations, despite extensive observations (Table 2). For example, five contrasting populations in the arid south west of Tenerife were surveyed during peak *N. glauca* flowering in April 2006. These populations had different abundances and densities of plants, and ranged from suburban post-demolition sites to rural, goat-grazed semi-natural habitats. Despite the presence in all of these habitats of potential flower visiting insects (including bees and butterflies) and birds (principally the Canary chiffchaff *Phylloscopus canariensis* a generalist bird that opportunistically visits flowers for nectar – see below) visits to flowers were never observed. In addition we checked over 1600 flowers (on average 330 per population) and found no evidence of nectar robbing. Finally, one population was surveyed for nocturnal visitors, particularly large night-flying moths, on three evenings. This population was chosen because of the presence of larvae of the Barbary Spurge Hawkmoth (*Hyles tithymali*

tithymali) feeding on *Euphorbia broussonetii*, indicating that these potential pollinators were present in that community. As well as checking flowers with flashlights, we added fluorescent dye powder (see Kearns and Inouye 1993, Dafni et al. 2005) to 10 flowers on each of 4 trees on one evening. On the two subsequent evenings we checked for dye transfer to nearby flowers but none was observed. These results confirmed previous observations by Ollerton et al. in 2003, 2004 and 2005 that *N. glauca* flowers on Tenerife are rarely, if ever, visited by nectar-feeding animals. These results strongly suggest that Canary Island populations are wholly selfing.

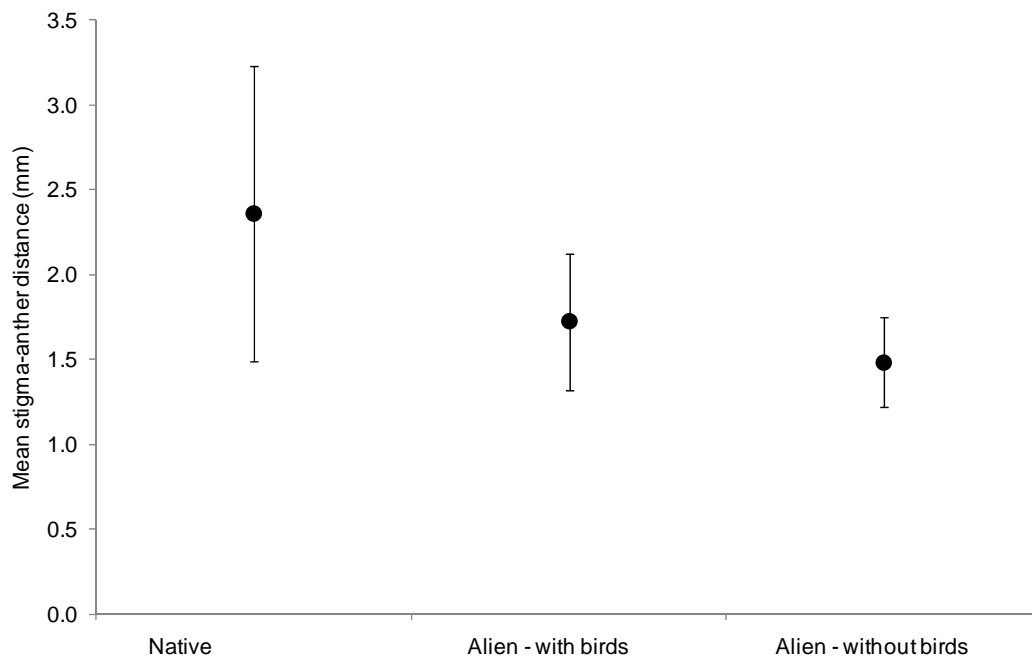


Figure 1: Mean ($\pm 95\%$ confidence interval) stigma-anther distance for native populations of *Nicotiana glauca* (“Native” – $n=7$ populations) compared to non-native populations within (“Alien-with birds” – $n=11$ populations) and outside (“Alien-without birds” – $n=6$ populations) of the range of specialised flower visiting birds (hummingbirds and sunbirds). One-way ANOVA: $F_{2,20} = 4.4$, $p=0.027$. Only the contrasts between Native vs. Alien-with birds (LSD post-hoc test: $p=0.03$) and Native vs. Alien-without birds (LSD post-hoc test: $p=0.01$) are significantly different, but there is an apparent trend and the latter has only a small sample size.

Discussion

In its native range, *Nicotiana glauca* forms dense stands mainly in disturbed sites with recent soil exposure, for example dry river beds and road sides (Nattero and Cocucci 2007). Outside of its native range *N. glauca* is clearly a successful invasive weed of disturbed areas where it forms dense, monodominant colonies because of the high rate of fruit and seed set, the viability of seeds and the frequent recruitment of seedlings into the population.

The pollination system of *Nicotiana glauca* can be best described using the terminology of Fenster et al. (2004) and Ollerton et al. (2007) as functionally specialized for hummingbird pollination, but ecologically generalized in that a range of hummingbird species can act as pollinators (Nattero et al. 2010). In this respect it seems to be an unlikely candidate as an ecologically invasive species, in relation to its ability to co-opt other pollinators (Richardson et al. 2000; Olesen et al. 2002). In parts of Argentina, Peru, Mexico, California, South Africa and Israel, however, where *N. glauca* has been introduced for at least 100 years, the species is clearly well integrated into the local pollination web via its interactions with specialist flower feeding birds such as hummingbirds (Hernández 1981; Schueller 2004, Schueller 2007) and sunbirds (Tadmor-Melamed et al. 2004, Geerts and Pauw 2009).

The successful integration of *N. glauca* into Old World pollination webs, which lack hummingbirds, comes as a surprise. Like many other hummingbird pollinated New World flowers, the flowers of *N. glauca* are oriented towards open space, an adaptation for pollination by birds that hover while feeding. According to conventional wisdom, Old World birds perch while feeding, so Old World flowers need to be oriented towards a perch in order to receive pollination (Westerkamp 1990). Unexpectedly, Old World sunbirds were found to adapt their behaviour and hover for extended periods of time while feeding from the hummingbird adapted flowers of *N. glauca* (Geerts and Pauw 2009). It remains to be determined whether Australian honeyeaters are also able to adopt this novel behaviour and act as pollinators of *N. glauca*.

In the northern Mediterranean and the Canary Islands, in contrast, *N. glauca* has not become integrated into the local flower visitation web, either via pollinators or nectar robbers. There are a restricted number of native Canarian and European taxa which could potentially pollinate *N. glauca*, for example long tongued bees such as *Xylocopa* and *Bombus*, and the larger Lepidoptera, including various hawkmoths

(Sphingidae). These bees, and their smaller relatives, frequently act as nectar robbers to long-tubed non-native plants such as *Fuchsia* spp. (Ollerton pers. obs.). Non-flower specialist passerine birds, particularly chiffchaffs (*Phylloscopus canariensis* and *P. collybita*), are known to pollinate a number of native Canarian plants (Vogel et al. 1984; Valido et al. 2004, Ollerton et al. 2008), at least one European species (Ortega-Olivencia et al. 2005) and are opportunistic feeders at the flowers of other non-native plants (Clement 1995; Ollerton pers. obs.). Their beak and tongue lengths are too short for them to legitimately access the nectar of *N. glauca*; however they are known to nectar rob other plants in the Canary Islands, for example *Aloe* spp. (Bramwell 1982). Extensive observation of populations of *N. glauca* on Tenerife and in Greece revealed no instances of nectar robbery, however. This is despite the presence of chiffchaffs in all populations on Tenerife, some of which were observed to perch in the larger *N. glauca* trees.

The nectar available in flowers of *N. glauca* is a significant energy and water resource for animals in semi-arid habitats. Multiplying the nectar values in obtained in Tenerife (see above) by the mean number of open flowers per inflorescence and the mean number of inflorescences per stem, suggests that on Tenerife, each stem on average maintains a standing crop of $374.8 \pm 820.7 \mu\text{l}$ of relatively sugar-rich nectar. Using the data for flowering stem densities (above), the nectar resources available to animals that can exploit these flowers would be of the order of $277.6 \mu\text{l m}^2$ in low density areas to $832.9 \mu\text{l m}^2$ in high density areas. We do not know the rate of replenishment of nectar in these flowers in Tenerife (though for an Argentinean population it was $0.2 \pm 0.2 \mu\text{l/h}$ - Galetto and Bernardello 1993a); nonetheless this standing crop represents a large potential resource of energy and water to any flower visiting animals within the semi-arid zone of Tenerife. It far exceeds the standing crops of most native species, with the exception of some of the specialised passerine-pollinated endemics (Ollerton et al. 2008) which are mainly restricted to the wetter laurel forest communities of the island. Why this resource is not utilised, resulting in the subsequent integration of the species into the local flower visitation web, is unclear. It is possible that the alkaloid content of the nectar of *N. glauca* deters animals that might otherwise exploit the nectar (Tadmor-Melamed et al. 2004) which would suggest that pollinators and nectar robbers within the native range of the plant, as well as in California, Israel and South Africa, have digestive strategies adapted to

cope with these compounds. The relatively high fraction of sucrose, which can only be digested by specialized nectarivores, i.e. hummingbirds and sunbirds, might additionally deter generalist passerines such as chiffchaffs. These areas deserve further research.

Despite the absence of pollinators in some parts of its modern range, *N. glauca* is a plant which is reproductively successful to the point of being a problematical invasive. High fruit and seed set, and relatively small S-A distances, suggest that these populations are largely selfing; apomixis is unlikely as emasculated and bagged flowers of plants in California never resulted in fruit or seed set (Schueller 2002). The difference in average S-A distances in native versus non-native habitats implies that populations in the native range are less frequently selfing. In native populations in Bolivia, where S-A distance is greatest, and presumably with a long history of interaction with the giant hummingbird (*Patagona gigas*), fruit set is relatively low (Nattero et al. 2010, Loayza et al. 1999). The populations with a small S-A distance therefore have pre-adapted the species to be a successful invader and fits with the ecology of the plant as a weedy colonising small tree of disturbed soil in South America. Nevertheless, despite small S-A distances and high levels of selfing, seed set of plants in South Africa that receive visits from sunbirds set significantly more fruit and seeds than pollinator excluded controls (Geerts and Pauw 2009). The trend of decreasing S-A distances from native populations, to invasive populations that are within the range of specialised flower visiting birds with those where no birds are present (Figure 1) is precisely what we would expect if initial founder events by largely self pollinating, isolated individuals are important prior to the establishment of larger populations that then subsequently attract significant numbers of native bird pollinators (if available) or remain as selfing populations if no suitable pollinators exist in the locality.

Invasive plants with a high rate of nectar production almost invariably have a high rate of pollinator visits to flowers, for example *Buddleia davidii* and *Impatiens glandulifera* in Europe and *Lantana camara* and *Melaleuca quinquenervia* in subtropical North America (Chittka and Schurkens 2001, Koptur 2006). The assumption is that many of these species are likely to be ecological and/or functional generalists in their native habitats (reviewed by Corbet 2006 and Traveset and Richardson 2006; see also Rodger et al. 2010). For instance, in Europe *Impatiens glandulifera* is a functional specialist but an ecological generalist (it is pollinated by a

range of bumblebees *Bombus* spp. – Chittka and Schurkens 2001, Lopezaraiza-Mikel et al. 2007, Nienhuis et al. 2009, Nienhuis and Stout 2009). In its native Himalayan habitats it is also *Bombus* pollinated (Saini and Ghattor 2007) which suggests that this plant was pre-adapted to be a successful invader in terms of its ability to integrate into existing pollination webs containing *Bombus* spp. or functionally equivalent medium to large sized bees. Invasive species may therefore re-establish their pollination niche only if they can appropriately interact with suitable local pollinators. Intuitively we might consider this to be less likely if the species is an ecological, functional or phenotypic specialist, but the data here are contradictory: functionally and phenotypically specialised (“euphilous”) introduced plants were shown by Corbet (2006) to be the most successful group of plants within the British and Irish flora, in terms of range increase. This may be due to the tendency of gardeners to introduce plants with large, showy flowers. *Nicotiana glauca* was introduced to Tenerife as a garden ornamental in the early 19th Century (Kunkel 1976) and so there would seem to have been ample time for the species to establish itself as part of the local flower interaction web, if suitable pollinators or nectar robbers were available. However in that time *N. glauca* has largely forsaken outcrossing for a successful selfing reproductive strategy. Island ecosystems are especially vulnerable to plant invasions (Simberloff 1995; Olesen et al. 2002) and *N. glauca* is only one of a large number of introduced plant species which may be negatively impacting on the flora and fauna of the Canary Islands and two of the California Channel Islands studied by Schueller (2002). *N. glauca* seems to have some degree of frost tolerance (Ollerton, pers. obsv.) and the increasingly arid environments predicted by future climate change may result in the species spreading even further north in Europe and North America.

In order to test our hypothesis that shorter SA distances have evolved in invasive populations of *N. glauca* that lack pollinators, further research is required. In particular we would like to know why it is that seeds produced from self pollination show such high viability. In addition, the role of other mechanisms that could prevent or facilitate selfing (such as reduced incompatibility of self-pollen, and synchronization of pollen viability and stigma receptivity) in native and invasive populations with and without pollinators.

In conclusion, we can state that *Nicotiana glauca* is a successful invasive species outside of its native range, despite its functionally specialised pollination system. In areas where suitable bird pollinators are available, for example

hummingbirds in California and sunbirds in South Africa and Israel, *N. glauca* becomes integrated into the local pollination web and sets seed by both out-crossing and selfing. In regions where there are no specialised flower visiting birds, such as the Canary Islands and Greece, abundant seed set is maintained by selfing, and the considerable nectar resources are not utilised by native flower visitors, either legitimately or by nectar robbing. In the case of *N. glauca*, invasion success is therefore not predictable from its pollination biology.

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References

- Aigner PA (2004) Ecological and genetic effects on demographic processes: pollination, clonality and seed production in *Dithyrea maritima*. *Biological Conservation* 116: 27-34
- Aizen MA, Morales CL and Morales JM (2008) Invasive mutualists erode native pollination webs. *PLOS Biology* 6: 396-403
- Alarcón R, Waser NM and Ollerton J (2008) Year-to-year variation in the topology of a plant-pollinator interaction network. *Oikos* 117: 1796-1807
- Baker, HG (1974) The evolution of weeds. *Annual Review of Ecology and Systematics* 5: 1-24
- Bartomeus I, Vila M and Santamaria L (2008) Contrasting effects of invasive plants in plant-pollinator networks. *Oecologia* 155: 761-770
- Bjerknes, A-L, Totland Ø, Hegland SJ and Nielsen A. (2007) Do alien plant invasions really affect pollination success in native plant species? *Biological Conservation* 138: 1-12
- Bogdanović S, Mitić B, Ruščić M and Dolina K (2006) *Nicotiana glauca* Graham (Solanaceae), a new invasive plant in Croatia. *Acta Botanica Croatica* 65:203-209

- Bornmuller J (1898) Ein beitrage zur kenntnis der flora von Syrien und Palastina. Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien 48: 544–653
- Bramwell D (1982) Conserving biodiversity in the Canary Islands. *Annals of the Missouri Botanical Gardens* 77: 28-37
- Cariveau DP and Norton AP (2009) Spatially contingent interactions between an exotic and native plant mediated through flower visitors. *Oikos* 118:107-114
- Chittka L and Schurkens S (2001) Successful invasion of a floral market. *Nature* 411:653-653
- Clement P (1995) *The chiffchaff*. Hamlyn, London
- Cohen N (2007) The effects of alkaloids in the floral nectar on the relationships between *Nicotiana glauca* and ants. M.Sc. Thesis. University of Haifa, Israel
- Dafni A, Kevan PG and Husband BC (2005) *Practical pollination biology*. Enviroquest, Cambridge Ontario
- Fenster CB, Armbruster WS, Wilson P, Dudash MR and Thomson JT (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics* 35:375-403
- Fishbein M and Venable DL (1996) Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology* 77: 1061-1073
- Florentine SK and Westbrooke ME (2005) Invasion of the noxious weed *Nicotiana glauca* R. Graham after an episodic flooding event in the arid zone of Australia. *Journal of Arid Environments* 60: 531-545
- Florentine SK, Westbrooke ME, Gosney K, Ambrose G and O'Keefe M (2006) The arid land invasive weed *Nicotiana glauca* R. Graham (Solanaceae): Population and soil seed bank dynamics, seed germination patterns and seedling response to flood and drought. *Journal of Arid Environments* 66: 218-230
- Forster PI (1994) Diurnal insects associated with the flowers of *Gomphocarpus physocarpus* E Mey (Asclepiadaceae), an introduced weed in Australia. *Biotropica* 26: 214-217
- Galetto L and Bernardello L (1993a) Nectar sugar composition in angiosperms from Chaco and Patagonia (Argentina): an animal visitor's matter? *Plant Systematics and Evolution* 238: 69–86
- Galetto L and Bernardello L (1993b) Nectar secretion pattern and removal effects in 3 species of Solanaceae *Canadian Journal of Botany* 71: 1394-1398
- Geerts S and Pauw A (2009) African sunbirds hover to pollinate an invasive

- hummingbird-pollinated plant. *Oikos* 118: 573-579
- Henderson L (1991) Invasive alien woody plants of the Northern Cape. *Bothalia* 21: 177-189
- Hernández HM (1981) Sobre la biología reproductiva de *Nicotiana glauca* Graham: una maleza de distribución cosmopolita. *Boletín de la Sociedad de Botánica de México* 41: 47-73
- Herrera CM (1988) Variation in mutualisms - the spatio-temporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society* 35:95-125
- Hobbs J N (1961) The birds of Southwest New South Wales. *Emu* 61: 21-55
- Kearns CA and Inouye DW (1993) *Techniques for pollination biologists*. University Press of Colorado, Niwot
- Knuth P (1898-1905) *Handbuch der Blütenbiologie*. Engelmann, Leipzig
- Kunkel G (1976) Notes on the introduced elements in the Canary Islands' flora. In: Kunkel G. (ed) *Biogeography and ecology in the Canary Islands*. Dr W. Junk, The Hague, pp249-266
- Lamborn E and Ollerton J (2000) Experimental assessment of the functional morphology of inflorescences of *Daucus carota* (Apiaceae): testing the "fly catcher effect". *Funct Ecol* 14:445-454
- Loayza A, Rios R and Castillo C (1999) Actividad alimenticia de *Patagona gigas* en arbustos de *Nicotiana glauca* (Solanaceae). *Ecología en Bolivia* 33:75-79
- Lopezaraiza-Mikel ME, Hayes RB, Whalley MR and Memmott, J (2007) The impact of an alien plant on a native plant-pollinator network: an experimental approach. *Ecology Letters* 10: 539-550
- Marloth R (1901) Die ornithophilie der Flora Süd-Afrikas. *Berichte der Deutschen Botanischen Gesellschaft* 19: 176-179
- Memmott J and Waser NM (2002) Integration of alien plants into a native flower-pollinator visitation web. *Proceedings of the Royal Society of London B* 269: 2395-2399
- Moragues E and Traveset A (2005) Effect of *Carpobrotus* spp. on the pollination success of native plant species of the Balearic Islands. *Biological Conservation* 122: 611-619
- Morales CL and Traveset A (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecology Letters* 12: 716-728

- Nattero J and Cocucci AA. (2007) Geographic variation in floral traits of the tree tobacco in relation to its hummingbird pollinator fauna. *Biological Journal Linnean Society* 90: 657-667.
- Nattero J, Sérsic AN and Cocucci AA (2010) Patterns of contemporary phenotypic selection and flower integration in the hummingbird-pollinated *Nicotiana glauca* between populations with different flower-pollinator combinations. *Oikos*: 119: 852-863
- Nienhuis CM, Dietzsch AC and Stout JC (2009) The impacts of an invasive alien plant and its removal on native bees. *Apidologie* 40: 450-463
- Nienhuis CM and Stout JC (2009) Effectiveness of native bumblebees as pollinators of the alien invasive plant *Impatiens glandulifera* (Balsaminaceae) in Ireland. *Journal of Pollination Ecology* 1: 1-11
- Olesen JM, Eskildsen LI and Venkatasamy S (2002) Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distributions* 8: 181-192
- Ollerton J, Killick A, Lamborn E, Watts S and Whiston M (2007) Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon* 56: 717-728
- Ollerton J, Cranmer L, Stelzer R, Sullivan S and Chittka L (2008) Bird pollination of Canary Island endemic plants. *Naturwissenschaften* 96: 221-232
- Ortega-Olivencia A, Rodríguez-Riaño T, Valtueña FJ, López J and Devesa JA (2005) First confirmation of a native bird-pollinated plant in Europe. *Oikos* 110:578-590
- Padrón B, Traveset A, Biedenweg T, Díaz D, Nogales M, Olesen JM. (2009) Impact of alien plant invaders on pollination networks in two archipelagos. *PLoS One* 4: e6275
- Richardson DM, Allsopp N, D'Antonio CM, Milton SJ and Rejmanek M (2000) Plant invasions - the role of mutualisms. *Biological Reviews* 75: 65-93
- Rodger, JG, van Kleunen, M and Johnson, SD (2010) Does specialized pollination impede plant invasions? *International Journal of Plant Sciences* 171: 382-391
- Saini M.S. and Ghattor H.S. (2007) Taxonomy and food plants of some bumble bee species of Lahaul and Spiti valley of Himachal Pradesh. *Zoos' Print Journal* 22: 2648-2657

- Schurkens S and Chittka L (2001) The significance of the invasive crucifer species *Bunias orientalis* (Brassicaceae) as a nectar source for central European insects
Entomologia Generalis 25: 115-120
- Schueller, S. K. 2002. Hummingbird pollination and floral evolution of introduced *Nicotiana glauca* and native *Epilobium canum*: California island-mainland comparisons. Ph.D. dissertation, University of Michigan, Ann Arbor, Michigan, USA.
- Schueller SK (2004) Self-pollination in island and mainland populations of the introduced hummingbird-pollinated plant, *Nicotiana glauca* (Solanaceae).
American Journal of Botany 91: 672-681
- Schueller SK (2007) Island-mainland difference in *Nicotiana glauca* (Solanaceae) corolla length: a product of pollinator-mediated selection? *Evolutionary Ecology* 21: 81-98
- Simberloff D (1995) Why do introduced species appear to devastate islands more than mainland areas? *Pacific Science* 49: 87-97
- Skead CJ (1967) The sunbirds of southern Africa. Balkema, Cape Town.
- Stiles FG 1973. Food supply and the annual cycle of the Anna Hummingbird.
University of California Publications in Zoology 97: 1-116
- Stiles FG 1976. On taste preferences, color preferences, and flower choice in hummingbirds. *The Condor* 78: 10-26
- Stout JC, Parnell JAN, Arroyo J and Crowe TP (2006) Pollination ecology and seed production of *Rhododendron ponticum* in native and exotic habitats. *Biodiv and Conservation* 15:755-777
- Stout JC and Morales CL (2009) Ecological impacts of invasive alien species on bees. *Apidologie* 40: 388-409
- Tadmor-Melamed H (2004) The ecological role of secondary metabolites in floral nectar governing the interaction between *Nicotiana glauca* and *Nectarinia osea*. M.Sc. Thesis. The Hebrew University of Jerusalem, Israel.
- Tadmor-Melamed H, Markman S, Arieli A, Distl M, Wink M and Izhaki I (2004) Limited ability of Palestine Sunbirds *Nectarinia osea* to cope with pyridine alkaloids in nectar of Tree Tobacco *Nicotiana glauca*. *Functional Ecology* 18:844-850

- Theoharides, KA and Dukes, JS (2007) Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *New Phytologist* 176: 256-273
- Tillberg CV, Holway DA, LeBrun EG and Suarez AV (2007) Trophic ecology of invasive Argentine ants in their native and introduced ranges. *Proceedings of the National Academy of Sciences USA* 104: 20856–20861
- Valido A, Dupont YL and Olesen JM (2004) Bird-flower interactions in the Macaronesian islands. *Journal of Biogeography* 31: 1945-1953
- Vilá, M., Bartomeus, I, Dietzsch, AC, Petanidou, T, Steffan-Dewenter, I., Stout, JC, and Tscheulin, T. (2009) Invasive plant integration into native plant–pollinator networks across Europe. *Proceedings of the Royal Society series B* 276: 3887-3893
- Vogel S, Westerkamp C, Thiel B- and Gessner K (1984) Ornithophilie auf den Canarischen Inseln. - *Plant Systematics and Evolution* 146: 225-248
- Westerkamp, C. 1990. Bird-flowers - hovering versus perching exploitation. *Botanica Acta* 103: 366-371

Table 1: Reproductive output and mean stigma-anther distances of populations of *Nicotiana glauca*. Stigma-anther distances were measured to the nearest 0.1mm using a digital calliper. Sample sizes vary considerably and are available from the corresponding author on request. All means are \pm SD

Region	Locality	Status (I=Invasive; N = native)	Mean fruit set (%)	Mean minimum stigma-anther distance (mm)
Argentina and Bolivia (Nattero and Cocucci 2007, Nattero et al. unpublished data)	Tupiza	N	36 \pm 25	2.2 \pm 0.8
	Cuesta de Miranda	N	39 \pm 16	1.2 \pm 0.6
	Cochabamba	N	32 \pm 14	3.0 \pm 0.8
	Dique Los Sauces	N	28 \pm 17	2.6 \pm 0.8
	Potosí	N	47 \pm 10	3.6 \pm 1.1
	Sanagasta	N	48 \pm 26	1.3 \pm 0.8
	Sucre	N	67 \pm 16	2.6 \pm 1.5
	Paraná	I	67 \pm 7	2.5 \pm 0.9

	Costa Azul	I	41 ± 18	1.2 ± 0.6
	Bella Vista	I	56 ± 8	1.2 ± 0.8
Peru (Watts, unpublished data)	Urubamba	I	-	1.7 ± 0.4
California (Schueller 2004)	Santa Cruz Island	I	41 ± 49	1.6 ± 0.3
	Santa Catalina Island	I	75 ± 20	1.8 ± 0.2
	Sedgwick Reserve	I	26 ± 44	1.9 ± 0.5
	Starr Ranch	I	70 ± 46	2.1 ± 0.2
Israel (Izhaki, unpublished data)	Jezreel Valley	I	55 ± 8	1.8 ± 0.7
Tenerife (Ollerton et al., unpublished data)	South West	I	80 ± 21	1.4 ± 0.4
Greece (Schueller, 2002 and unpublished data)	Athens	I	Data un-quantified,	1.5 ± 0.5
	(Ano Illioupolis)		but fruit set high and seeds viable.	
	South-central Peloponese (Gerolimenas and	I	ditto	1.6 ± 0.3

	Gythio)			
	Crete, Agia Galini	I	ditto	1.4 ± 0.9
	Crete, Tympaki	I	ditto	1.9 ± 0.5
Ollerton (unpublished data)	Crete, Agios Nikolaos	I	42 ± 23	1.1 ± 0.2
South Africa (Geerts and Pauw unpublished data)	Buffelsrivier	I	61 ± 10	1.4 ± 0.3
	Leipoldtville	I	74 ± 9	-

Table 2: Flower visitors to *Nicotiana glauca* within its native range and in areas where it is introduced. “Legitimate flower visitors” are those which enter from the front of the flower and are the most likely pollinators; flower robbing visitors pierce holes at the base of the corolla to access the nectar, or make secondary use of previously excavated holes.

Range and locality	Legitimate flower visitors	Flower robbing visitors
<i>Native – within the range of specialist</i>		
<i>flower visiting birds</i>		
Argentina and Bolivia (6 sites - Nattero and Cocucci 2007, Nattero et al. 2010)	Hummingbirds (4 spp.)	<i>Xylocopa ordinaria</i> <i>Chlorostilbon aureoventris</i>
<i>Introduced – within the range of specialist</i>		
<i>flower visiting birds</i>		
Peru (3 sites within the Sacred Valley during February, June and August)	Hummingbirds (5 spp.)	<i>Bombus</i> sp., <i>Xylocopa</i> sp. and other native bees, <i>Apis mellifera</i> , Syrphidae, <i>Diglossopsis cyanea</i>

2002 - SW, unpublished data)

USA

California

Hummingbirds (3 spp.)

House finches and white-crowned sparrows

(4 sites - Schueller 2004)

Bees and Diptera (very infrequently)

observed pecking at flowers and usually

California, Sonora and Sinaloa (Stiles

Hummingbirds

destroying them or ripping corolla; also

1973, 1976)

occasional holes at base of corolla made by

unknown bee and frequently find ants in

flowers that consume a lot of the nectar, but

do not act as pollinators.

Israel

Palestine Sunbirds (60% of 274 visits

Palestine Sunbirds (40% of 274 visits were

(Tadmor-Melamed 2004, Tadmor-

were legitimate

nectar robbery)

Melamed et al. 2004, Cohen 2007)

Hummingbird Hawk-moth

Xylocopa pubescens

(*Macroglossum stellatarum*)

Apis mellifera (secondary nectar robber)

Seven ant species (Formicidae)

South Africa

Malachite sunbirds (*Nectarinia famosa*)

C. chalybea and *C. fuscus*

(Skead 1967, Knuth 1898-1905;
 Marloth 1901 Geerts and Pauw 2009) Dusky sunbirds (*Cinnyris fuscus*)
 Southern double-collared sunbirds
 (*Cinnyris chalybea*)

Australia White-fronted Honeyeater (*Phylidonyris*
 (Hobbs 1961) *albifrons*)*

México Hummingbirds (4 spp.) *Diglossa baritula* and *Xylocopa* sp.
 (1 site Hernández 1981)

Argentina Hummingbirds (3 spp.) *Xylocopa ordinaria*
 (3 sites - Nattero and Cocucci 2007)

*Introduced – outside of the range of specialist
 flower visiting birds*

Tenerife None observed None observed > 1000 flowers checked
 (5 sites in the arid south west -
 Ollerton et al. unpublished data)

Crete, Agios Nikolaos None observed None observed on c. 200 flowers

(Ollerton unpublished data)

Greece

(Schueller unpublished)

None observed on at least 18 large plants, over 8 sites, for at least 30 min each, including evening/dusk to check for hawkmoths. On one occasion a small bee was seen resting inside the corolla – no damage to corolla, not clear if it was an effective pollinator. None observed

*Hobbs (1961) does not indicate if visits were legitimate. The short, broad bill of this species suggests that it acts as a robber (B. Lamont pers. com.).