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Spatial and functional determinants of long-term fecundity in serotinous shrub communities

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I would like to dedicate my thesis to the late Brummer Olivier

Contents

Abstract

Understanding the dynamics of biological communities is a central aim of ecological research. Contemporary environmental change reinforced this interest: in order to predict how communities will react to environmental change, we have to understand the processes driving their dynamics. Ultimately, the dynamics of a community depends on the reproduction, mortality and dispersal of its component individuals, and on how these demographic processes are altered by environmental factors and biotic interactions.

A general understanding of biological communities is unlikely to arise from a 'species-specific' approach that attempts to quantify all pairwise interactions between species. Instead, it seems promising to pursue a 'trait-based' research program that quantifies how variation in the performance of species and individuals is shaped by the interplay of functional traits, biotic interactions and environmental factors. In this thesis, I investigated how functional plant traits determine plant-plant, plant-pollinator and plant-herbivore interactions in space and time, and how these spatiotemporal interactions affect the long-term fecundity of plants. In the South African Fynbos biome (a global biodiversity hotspot), I studied a species-rich, ecologically and economically important group of woody plants (genus *Protea*) and its interactions with pollinators and seed predators.

The objectives of this thesis were: (1) to combine plant traits and high-resolution maps of *Protea* communities in order to quantify the landscapes of nectar sugar and seed crops that plant communities provide for pollinators and seed predators, (2) to examine how sugar landscapes shape pollinator behaviour, and how pollinator behaviour and pollinator-mediated interactions between plants affect the reproduction of *Protea* individuals, (3) to study how the spatial structure of plant communities and seed crop landscapes determine direct and predator-mediated interactions between plants, and (4) to understand how the interplay of these biotic interactions shapes the dynamics of plant communities. I addressed these objectives by analysing spatially-explicit data and high-resolution maps from 27 sites of 4 ha each that contained 129,750 plants of 22 *Protea* species.

The results show that *Protea* plants and their pollinators interact on several spatial and temporal scales, and that these interactions are shaped by sugar landscapes. Within plants, inflorescences compete for pollination. At a neighbourhood scale, *Protea* reproduction benefits from nectar sugar of conspecific neighbours but not from heterospecific neighbour sugar. Seed set also increases with the

amount of nectar sugar at the scale of entire study sites. This corresponds with the finding that the abundance and the visitation rates of key bird pollinators strongly depend on phenological variation of site-scale sugar amounts. Nectar sugar furthermore influences the strength of interactions between *Protea* species and bird pollinators: *Protea* species that provide nectar of high sugar concentration depend more strongly on bird pollinators to reproduce. When foraging in sugar landscapes, these bird pollinators show both temporal specialization on single plant species and a preference for common plant species.

In addition to these pollinator-mediated interactions, the long-term fecundity of *Protea* individuals is reduced through both competition and apparent competition mediated by seed predators. Competition is stronger between conspecifics than between heterospecifics, whereas apparent competition shows no such differentiation. The intensity of competition between plants depends on their size and the intensity of apparent competition between plants depends on their seed crops. Moreover, competition has a stronger effect on plant fecundity than apparent competition.

These findings have interesting implications for understanding the dynamics of *Protea* communities and the maintenance of plant diversity in the Fynbos biome. The positive interspecific density-dependence resulting from pollinator-mediated interactions causes community-level Allee effects that may lead to extinction cascades. My analyses also imply that competition stabilizes the coexistence of *Protea* species (because intraspecific competition is stronger than interspecific competition), whereas apparent competition via seed predators does not have such a stabilizing effect. In summary, this study highlights the benefits of ‘community demography’, the demographic study of multiple interacting species. Community demographic studies have the potential to identify general determinants of biotic interactions that act across species and communities. In this thesis, I identified nectar sugar and seed crops as interaction currencies that determine how multiple plant species interact through shared pollinators and seed predators. In megadiverse systems such as Fynbos, such generalizations are urgently needed to understand and forecast community dynamics. The analysis of community dynamics with respect to such interaction currencies provides an alternative to the classical species-specific approach in community ecology.

Zusammenfassung

Räumliche und Zeitliche Interaktionen zwischen Pflanzen, Pflanzen und ihren Bestäubern und Räubern spielen eine Schlüsselrolle in der Dynamik von terrestrischen Gemeinschaften. Doch in der Ökologie fehlt weitgehend ein quantitatives Verständnis der Merkmale und Prozesse, die sowohl räumliche und zeitliche Interaktionen einschließen. Deshalb untersuchte ich in dieser Doktorarbeit, wie funktionale Pflanzenmerkmale Pflanzen-Pflanzen-Interaktionen und Pflanzen-Tier-Interaktionen in Raum und Zeit bestimmen, und wie diese räumlich-zeitliche Interaktionen die Pflanzenreproduktion beeinflussen. Ich studierte eine artenreiche, ökologisch und ökonomisch wichtige Gruppe von südafrikanischen Büschen (Gattung Protea) mit ihren wichtigsten Bestäubern (Cape Sugarbird, Promerops cafer and sunbird, *Anthobaphes violacea*) in der Cape Floristic Region (CFR). Durch die Kombination von Experimenten und Beobachtungen in verschiedenen *Protea* Gesellschaften die mit hoher Auflösung kartiert wurden, untersuchte ich wie die räumliche und zeitliche Verteilung von *Protea*-Ressourcen („Ressourcen-Landschaften") die Verhaltensweisen von Bestäubern prägt und wie sich Nachbarpflanzen auf die Verhaltensweisen von Bestäubern auswirken und im Umkehrschluss wie sich die Bestäuber auf die Reproduktion von *Protea* Individuen auswirkt. Darüber hinaus studierte ich, wie Ressourcen-Landschaften die Konkurrenz und die Prädationsrate beeinflusst und wie sich die Kombination von beiden auf die Dynamik der *Protea*-Gesellschaften auswirkt. Um dies zu tun, habe ich Pflanzenmerkmale durch raumzeitliche Interaktionen aus der Perspektive der beiden Pflanzen und Tiere quantifiziert und analysierte Interaktionen zwischen *Protea* Individuen (insgesamt 129750 *Protea* Positionen mit 22 Arten in 27 verschiedene Pflanzengesellschaften). Die Ergebnisse dieser Studie tragen zum Verständnis von demographischen Prozessen und Gesellschaftsdynamiken bei und zeigen die Auswirkungen auf die Erhaltung der biologischen Vielfalt in diesem „Biodiversitäts-Hotspot“.

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Synopsis

General introduction

Ecological research requires an understanding of plant community dynamics, particularly in relation to the maintenance of species diversity in speciose communities. Ultimately, the dynamics of a plant community is driven by intra- and interspecific mutualistic and antagonistic processes (e.g. Bronstein et al. 2003), which stresses abiotic and biotic interactions in multispecies communities (Hubbell 2001, Chase & Leibold 2003, Clark 2009). These interactions can depend on the spatial interplay among individuals within plant communities, and can either be mediated directly by the abiotic environment (e.g. through soil nutrients) or indirectly through other organisms, such as pollinators (Stoll & Weiner 2000, Brooker et al. 2008), or predators (e.g. Janssen et al. 1998). These interactions affect demographic processes including reproduction, recruitment and survival of plant individuals. Of these processes, reproduction is a key component and has a strong influence on plant community dynamics. Neighbouring plants may have positive or negative effects on plant reproduction. Abiotically-mediated interactions between neighbouring plants can be positive due to facilitation or can be negative due to competition over resources (e.g. nutrients or water) (Stoll & Weiner 2000, Brooker et al. 2008), while biotically-mediated interactions may be positive, for example pollinator-mediated interactions (Nottebrock et al 2013). In plant populations that are small or have low densities, lack of pollination can decrease reproductive success (Lamont et al. 1993, Roll et al. 1997, Groom 1998, Kery et al. 2000, Nottebrock et al. 2013) causing Allee effects (Allee 1931, Stephens et al. 1999). This positive density-dependence is an important component of understanding dynamics and interactions in plant communities (Courchamp et al. 2008), which can promote extinction cascades (Collwell et al. 2012). Conversely, predation may cause a negative biotic interaction (Sih et al 1985), where the plant reproduction is decreased via seed predation.

It is unlikely that a ‘species-specific’ approach, involving quantifying all pairwise interactions among species, will aid understanding of plant community interactions (McGill et al. 2006). A more promising approach is a 'trait-based' research programme that quantifies how variation in the performance of species and individuals is shaped by the interplay of biotic interactions, functional traits, and environmental factors (McGill et al. 2006, Adler et al. 2013). Trait-based research on plant-plant and plant-animal interactions has to consider spatial heterogeneity and spatial interactions. This is because the performance of plants strongly depends on intra and interspecific interactions with neighbouring plants (Stoll & Weiner 2000). Furthermore, sessile plants provide spatially heterogeneous resources that strongly affect the abundance, movement, and behavior of animals (Nathan et al. 2008). In turn, animal abundance, movement, and behavior can cause spatial variation in plant fitness (Bosch & Waser 2001, Knight et al. 2005). Such spatial feedbacks between animals and plants are particularly strong in specialized pollination systems in which pollinators rely on the resources provided by plants and plant reproduction depends on pollinators (Ghazoul 2005). In this thesis I developed a trait-based research programme by quantifying how functional traits determine plant-plant and plant-animal interactions in space, and how these spatial interactions determine plant fecundity. Specifically, I studied shrubs of the genus *Protea*, a species-rich group of plants dominating South African Fynbos shrublands (Rebelo 2001) and their interactions with pollinators and seed predators. Two major pollinators were focused on; the Cape sugarbird (*Promerops cafer*) and the orange-breasted sunbird (*Anthobaphes violacea*). In the following I provide a literature review on plant-plant and plant-animal interactions and an outline of the study design.

Spatial interactions between plants and plant traits

Spatial interactions between sessile plants can either be direct (mediated by the abiotic environment) or indirect (mediated by other organisms, Stoll & Weiner 2000, Brooker et al. 2008). Competition for abiotic resources (such as nutrients, water or light) causes negative interactions between neighbouring plants (e.g. Stoll & Weiner 2000). However, plant interactions can also be positive and neighboring plants may facilitate each other by increasing the availability of abiotic resources (Brooker et al. 2008). Eventually, the net interaction effect of a neighbour is the sum of its negative and positive effects (Stoll & Weiner 2000). A key trait determining competitive effects is plant size, because depletion of light and belowground resources increases with above- and belowground biomass and the competitive effects of large plants extend more widely than those of small plants (Stoll & Weiner 2000). A simple way of quantifying spatial interactions, such as competition, is to regress the performance of target plants against the density of neighbouring plants at a predefined spatial scale (e.g. in a circle of given radius around the target individual, Nottebrock et al. 2013). The problem with this is twofold; it requires a priori assumptions about the spatial scale of interactions and it ignores the fact that the neighbor-effect decreases with distance (Stoll & Weiner 2000). Statistical neighbourhood analyses circumvent these problems by analysing measures of plant performance (e.g. growth or fecundity) with respect to spatially-explicit maps of individual plant locations (Canham & Uriarte 2006). Neighbourhood analyses seem well suited to studying facilitative interactions (Brooker et al. 2008) but currently, they have only been applied to competitive interactions. However, as the number of interacting species increases, the number of parameters to be estimated by such species-specific neighbourhood analyses quickly becomes too large to be interpretable (McGill et al. 2006). Hence, complex species-specific analyses are being replaced by simpler trait-based neighbourhood analyses (Uriarte et al. 2004, Uriarte et al.

2010).These models use spatial neighbourhood interactions of species specific mean valuesof plant traits to understand demographic rates (e.g. plant reproduction).

Indirect plant interactions (pollinator-mediated)

Indirect plant interactions can involve animals and play a key role in understanding community dynamics (Werner and Peacor 2003). Plant-pollinator interactions can be mutualistic and plant-predator interactions can be antagonistic. Most terrestrial plant species rely on the services of pollinators for reproduction (Ollerton et al. 2011). Pollinators depend on the floral reward provided by individual plants and, in turn, by providing pollination service they are important for plant reproduction (Gahzoul 2006). Pollinator-mediated interactions are therefore pivotal to understanding ecological processes and these indirect interactions are necessary for plant species (Waser and Ollerton 2006, Mitchell et al. 2009). Hence, pollinator-mediated interactions might be responsible for the biodiversity of plants in many ecosystems (Sargent and Ackerly 2008, Pauw 2013, Greenspoon and M'Gonigle 2013). Most pollinator species visit more than one plant species (Waser et al. 1996, Waser and Ollerton 2006) which causes indirect plant-plant interactions, because co-flowering plant species can share pollinators and can even occur between species that do not flower at the same time (Rathcke 1983, Waser & Ollerton 2006, Sargent & Ackerly 2008, Hegland et al. 2009). In plant communities these indirect interactions may be important for shaping community dynamics and species coexistence (Sargent and Ackerly 2008, Mitchell et al. 2009, Pauw 2013). The outcome of such interactions can either be positive (i.e. facilitation, Rathcke 1983, Feldman et al. 2004, Moeller 2004, Ghazoul 2006, Hegland et al. 2009), negative (i.e. competition, Rathcke 1983, Hegland et al. 2009) or neutral (Hegland et al. 2009).

The model of Rathcke (1983), which provides theoretical support that the relation of pollinator visitation to floral density is density dependent, illustrates the positive effects of plant-pollinator interactions on plant reproduction. An increase in floral density increases the visitation rate of pollinators and, consequently, the reproductive success of plants. Another example of a positive pollinator-mediated interaction is the so-called “magnet effect” (Laverty 1992, Johnson et al. 2003, Moeller 2004). It denotes the phenomenon that a plant species offering high rewards attracts pollinators, which may then increase pollinator visits for neighboring species offering little floral reward. Conversely, the high offer of floral resources from heterospecifics could either result in strong competition for pollinators (Goulson 2000, Chittka and Schürkens 2001, Ghazoul 2005) and/or in pollen clogging (Brown et al 2002), which decreases the reproductive success of a focal plant due to wrong pollen. The spatial distribution of nectar rewards determines the distribution, density and movement patterns of pollinators (e.g Feinsinger et al. 1991). Yet, pollinator behavior is not only defined by floral density or species attractiveness (Chittka and Thomson 2001), but also by the spatial structure of plant communities (Goulson 1994, Morales and Vazquez 2008). Pollinators usually fly long distances to find resources (Hagen et al. 2011) and they tend to fly short distances to show constancy (Waser 1982). Animal pollinators tend to restrict their visits to a single species even when rewarding alternative flowers must be bypassed, which refers to flower constancy (Waser 1986). For example, spatially clumped species offer a locally increased availability of resources for visiting pollinators (Goulson 1994, Feldmann et al. 2004).

Pollinators' foraging movements depend on spatial floral patterns and on floral fidelity, which in turn is responsible for pollen transfer between co-flowering species (Duncan et al. 2004). Plants benefit from floral fidelity: plant reproduction increases due to the specialisation of pollinator behavior of short term plant-pollinator relationships and plants benefit that pollinators pursue flowers of a certain species. Flower constancy shows that pollinators prefer the same floral resource and is distinct from learned fidelity (Waser 1986). Thus, the transfer of conspecific pollen between individuals is largely determined by the amount of pollen deposition during subsequent flower visits (Richards et al. 2009). Therefore, plants in small populations often benefit from an increase in conspecific density which increases the quantity and quality of transferred pollen (Bosch & Waser 2001, Knight et al. 2005). Moreover, plants that share the same pollinators can facilitate each other by attracting more pollinators which then enhance seed production (Moeller 2004). Conversely, the relative strength of intra- and interspecific competition mediated by pollinators determines whether pollinators promote or hinder coexistence of plant species (Pauw 2013).

In summary, many studies discuss aspects of spatiotemporally distributed floral resources in communities (quality, quantity, composition and availability of nectar sugar for example) and show that they are important for understanding pollinator behavior and pollinator-mediated interactions (e.g. Kunin 1997; Ghazoul 2005, Gunton & Kunin 2009; Williams *et al.* 2012; Carvalheiro *et al.* 2014; Feldman & McGill 2014) but these studies yield conflicting results. Results show positive and negative effects of plant-pollinator interactions on demographic processes (plant reproduction). Then also analyses of spatially and temporally distributed floral resources show plant fitness is influenced to varying degrees byabundance, movement and behaviour of pollinators (Ghazoul 2005). Therefore the composition and distribution of floral resources, so called sugar landscapes, are important for a better understanding of pollinator-mediated interactions. This requires an integrative approach that quantifies different aspects of sugar landscapes at different spatial and temporal scales and investigates their relative importance for pollinator behaviour and plant reproductive success.

Competition and apparent competition mediated by predators

In ecology, competition and predation have received more attention than all other forms of species interaction, and they dominate studies of community dynamics, especially coexistence (Kotler and Holt 1989, Chesson and Kuang 2008, Garzon-Lopez et al. 2015). Both competition for resources and apparent competition mediated by predators depend on spatial structure and lead into reciprocal negative density effects of one species on another (e.g. Janzen 1970, Chase et al 2002). Negative density dependence prevents coexistence if density dependence does not differentiate between species. In contrast, stabilizing effects are essential for coexistence (Chesson 2000). Stabilizing mechanisms for coexistence is dependent on negative intraspecific density dependence being stronger than negative interspecific density dependence (Chesson 2000). Thus, an important distinction within species coexistence mechanisms is to analyse effects of niche differentiation, which distinguishes between effects of conspecifcic and heterospecific and their resource-use in space or time (Chesson 2000). However, understanding the maintenance of biodiversity through niche differentiation is contested by neutral ecological theory (Hubbell 2001). Hubbell (2001) assumes in his model that all individuals in a community are strictly equivalent regarding their prospects of reproduction and death. Hence, neutral theory relies on the ecological equivalence of different species and describes concisely the complexity of natural communities (Hubbell 2001, Chave 2004, Alonso and McKane 2004). Neutral theory, however, has been criticized by many studies because it excludes the mechanistic processes for niche differentiation (e.g. Chave 2004, Chesson 2008). In the megadiverse Cape Floristic Region (CFR), Latimer et al. (2005) tested neutral theory with a large data set of plant species abundances and found that rank abundance curves are quite well explained by neutrality. Yet, Etienne et al. (2006) criticized the statistical methodology used in this study and found, using correct parameters, that neutral theory is unlikely to explain the rank abundance of CFR communities. Despite a range of suggested mechanisms behind the high biodiversity in the CFR (Cowling et al. 1996), the stabilizing mechanisms to understand species coexistence remains still uncertain.

Finally, it is not known whether the mechanisms of competition and facilitation, as mediated by antagonistic or mutualistic interactions, maintain the biodiversity in highly diverse plant communities. There are many theoretical and empirical examples that illustrate the mechanisms maintaining diversity (e.g. Chesson and Kuang 2008, M'Gonigle & Greenspoon 2014). However, theoretical models have tended to neglect indirect interactions, such as pollinator-mediated or other seed predator-mediated interactions, to understand mechanisms of community dynamics and species coexistence (Sargent and Ackerly 2008). Therefore an integrated framework for understanding community dynamics needs to incorporate direct and indirect interactions between plants. This thesis attempts to evaluate biotic and abiotic species interactions in a biodiversity ‘hotspot’ which hosts a high diversity of plant species.

The Study System

Study region

The study region lies within the Fynbos biome, a biodiversity "hotspot" in the Cape Floristic Region (CFR). The CFR covers ca. 90000 km^2 in the south-western part of South Africa. Topographically, the CFR consists of a mosaic of plains and rugged mountainous areas. Elevations range from sea level to more than 2000 m (Linder 2003). The western part of the CFR has a Mediterranean-type climate with cool, wet winters and hot, dry summers. Mean annual rainfall ranges from 200 mm/yr on the leeward slopes of interior ranges to 2000 mm/yr on high coastal mountains (Goldblatt & Manning 2002). The two main soil types are nutrient-poor sands, and clays of intermediate nutrient status (Goldblatt & Manning 2002). However, much of the tremendous plant diversity is restricted to areas with nutrient poor soils. In total the CFR hosts ca. 9000 plant species of which roughly 70% are endemic and many can be locally abundant (Richardson et al. 2001, Goldblatt & Manning 2002, Linder & Hardy 2004).

Most of the CFR's plant diversity is found in the Fynbos biome. The Fynbos biome has the highest plant diversity in the world, more than 7000 plant species occur in 46,000 km^2, making it part of a global biodiversity hotspot (Myers et al. 2000). It is because of its extraordinary plant diversity, combined with its invertebrate richness, that it was granted World Heritage Site Status in 2004 by the IUCN (International Union for Conservation of Nature). Fynbos has four major vegetation types: mountain fynbos, foothill fynbos, lowland fynbos and grassy fynbos (Mucina et al. 2014). All these fynbos types are fire-prone sclerophyllous shrublands that are dominated by Ericaceae, Restionaceae, and Proteaceae, the latter being the focus of this study. The extraordinary species richness of fynbos is under threat from agriculture, urbanization and the spread of alien plants (Rouget et al. 2003, Latimer et al. 2004), and from climate change (Midgley et al. 2002a, 2003, Thomas et al. 2004).

Study species – *Protea* spp.

In the CFR more than 330 species of Proteaceae have been identified (Rebelo 2001), and the genus *Protea* are largely distributed throughout the south-western part of the fynbos biome (Cowling 1992). *Protea species* frequently dominate the overstorey of fynbos vegetation, and they are important not only for the functioning of this ecosystem (Stock & Allsopp 1992) and because of their high ecological and economic importance (Schurr et al. 2012) (Fig. 1). From a total of 22 *Protea species* in this study all are reseeders (nonsprouters) except three, which are resprouters; both resprouters and nonsprouters represent two distinct life history strategies in this fire driven ecosystem. The adults of nonsprouting species are killed by fire, whereas the adults of resprouting species have an underground rootstock or a thick bark that allows them to survive fires (Bond & van Wilgen 1996, Bond & Midgley 2001, 2003). After a fire, resprouters quickly resprout and produce flowers, whereas it takes seedlings at least 2-3 years of growth to produce their first flowers (Le Maitre & Midgley 1992, Rebelo 2001). Thus, populations of nonsprouters can go locally extinct if fire intervals are shorter than the time required to become reproductively mature. Apart from these differences in fire-adapted strategies, *Proteas* exhibit a large variety of different vegetative and floral traits (Fig. 2), hence their name being derived from Proteus, a Greek deity that could change his appearance (Rebelo 2001). *Protea* species grow in the nutrient poor soils of the fynbos (Bond 2010), which restrict plant growth, and the high levels of sun irradiance facilitates carbon fixation (Milewski 1983). In the winter rainfall season, when sufficient water is available, this carbon fixation leads to the production of large quantities of carbohydrate in the form of nectar (Milewski 1983, Collins & Rebelo 1987). Therefore, *Protea* plants are referred to as "sugarbushes" (Rebelo 2001). During their flowering season, amounts of nectar accumulate at the base of *Protea* inflorescences (flowerheads) (Collins & Rebelo 1987). The flowering phenology of co-occurring *Protea* species tends to overlap to some extent, but can also vary within sites (Rebelo 2001). Flowers of *Protea* species are arranged in Fibonacci spirals within large brush-type inflorescences (20–300 mm) surrounded by heavily furred involucral bracts (Rebelo 2001) which shield the nectar, even from heavy rain. Flowers are proteandrous: when the anthers dehisce, pollen is deposited on the upper part of the stigma (the area designated as pollen presenter) and the stigma tip becomes receptive to pollen after two to three days (Steenhuisen and Johnson 2012). All *Protea* species are hermaphroditic: within an inflorescence, flowers open sequentially from the outer to the inner circles and thus flowers in the male phase (pollen donor) and female phase (receptive to pollen) co-occur within an inflorescence. An inflorescence can be in flower for two to five weeks. Nectar abundance peaks halfway through flowering, when some flowers are receptive while others remain closed (Carlson and Holsinger 2013). All of the study species are serotinous: after successful fertilization, inflorescences develop into woody, fire-proof cones (mature cones) in which seeds are typically retained for many years until the mother plant burns, unless predation takes place beforehand (Rebelo 2001). Therefore, serotinous Proteaceae have a 'canopy seed bank', and do not seem to form persistent soil seed banks (Le Maitre & Midgley 1992, Bond & van Wilgen 1996). *Protea* inflorescences bear many individual florets, each of which contains a single ovule and can thus produce a single seed (Rebelo 2001). To set seed, it is still unclear if *Protea* species require pollinator visits to inflorescences. Members of the *Protea* genus have a large variety of pollinators such as birds (Wright 1994, Hargreaves et al. 2004), insects (Coetzee and Giliomee 1985, Johnson et al. 2012, Steenhuisen and Johnson 2012), and mammals (Wiens and Rourke 1978, Biccard and Midgley 2009). Among their major pollinators are Cape sugarbirds (*Promerops cafer*) and orange-breasted sunbirds (*Anthobaphes violacea*) (Figure 3). Coleoptera or Lepidoptera species are the major pre-dispersal seed predators of *Protea* (Wright and Samways, 1999). In summary, the Fynbos biom with overstorey *Protea* species is ideal to study demographic studies, because of its high diversity and the easy measurements of lifetime fecundity (Nottebrock et al. 2013).

Figure 1. *Protea cynaroides* with its large and striking inflorescences has been designated as South Africa's national flower.

Bird pollinators

In this thesis two major bird pollinators, the Cape sugarbird (*Promerops cafer* L., Fam. Promeropidae, Fig. 2) and the orange-breasted sunbird (*Anthobaphes violacea* L., Fam. Nectariniidae, Fig. 2) were studied predominantly. Both pollinators tend to be common foragers of *Protea* nectar in the south-western fynbos region during winter and are two major pollinators for *Proteas* (Rebelo 2001). Cape sugarbirds and orange-breasted sunbirds are both endemic to the Fynbos biome, but they differ in their dependence on *Protea* nectar (Rebelo 1987). The distribution of the Cape sugarbird appears strongly associated with the occurrence of *Protea* species (Collins and Rebelo 1987), whereas it is still poorly known for orange-breasted sunbirds. Sugarbirds have been reported foraging on 22 *Protea* species (Collins & Rebelo 1987), which cover most of their energetic requirements from nectar (Skead 1967, Cheke et al. 2001), and they synchronize breeding with the peak flowering time of certain *Protea* species (Collins and Rebelo 1987). Sugarbirds are territorial and male sugarbirds actively defend their territory (mean territory size vary from 207 to 25,880 m^2, Calf et al. 2003), regularly driving away the smaller, subordinated orange-breasted sunbirds when foraging on *Protea* inflorescences. Cape sugarbirds, with a body mass of 36.6g and 31.8g for males and females respectively (Tjørve and Scholtz 2007), are much heavier than the orange-breasted sunbird, 9.5 g (Williams 1993). Both are associated as key pollinators for *Protea* species (Rebelo 2001) and that sugarbirds and sunbirds use most of the nectar sugar as food resources. Allometric predictions show daily requirements of about 9.1 g sugar for

Cape sugarbirds and 3.8 g sugar for orange-breasted sunbirds (Nagy 2001). In conclusion, it is not clear whether floral resources provided by *Protea* communities determine the local abundance and movement behaviour of sugarbirds and sunbirds.

Fig. 2. Two key pollinators of *Protea* shrubs: the Cape sugarbird (*Promerops cafer* L., Fam. Promeropidae; left) and the orange-breasted sunbird (*Anthobaphes violacea* L., Fam. Nectariniidae; right)

Research questions and design

Protea species in the Fynbos biome are well-studied and because of its high beta-diversity, local plant communities host different combinations of species. Hence, the system is perfectly suited for studying interactions among multiple plant species, community dynamics and coexistence. Spatial distributions and local abundances of all southern African Proteaceae species were recorded by the Protea Atlas Project (Rebelo 2001). This massive mapping effort resulted in what Gelfand et al. (2005) identified as one of the largest and highest quality global datasets for studying biodiversity. This data set has been used for many studies to predict changes in community dynamics under climate change (e.g. Midgley at al. 2002, Hannah et al. 2005). These species distribution models showed marked declines in range sizes of many Proteaceae, and demonstrated the extinction risks associated with climate change. Yet, environmental change such as land transformation or climate change (Cowling et al. 2003, Midgley et al. 2006) are not the only threats to these highly diverse Fynbos communities; they may also be vulnerable to pollinator decline (Pauw 2007). All these changes increase the extinction risk of plant species, thus increasing the susceptibility of communities to extinction cascades (Colwell et al. 2012). Therefore, the study of population and community dynamics and the interactions between plants and animals may help conserve diverse Fynbos plant communities. Nevertheless, to grasp population and community dynamics, it is crucial to understand density dependence within and between species, which shapes the dynamics of communities and populations. These processes can occur at different spatial scales, thus to identify the relevant interactions within plant communities, it is important to quantify species interactions at different spatial scales (McGill 2010). This spatial complexity can be integrated in a framework that quantifies how variation in the performance of species and individuals is shaped by the interplay of biotic interactions, functional traits, and environmental factors (McGill et al. 2006, Adler et al. 2013). Consequently, for this study 27 sites of 4 ha each were selected (Fig. 3), containing a total of 127,993 individual plants representing 21 *Protea* species, all mapped to a high level of accuracy using dGPSs (Trmble GeoXH). The study sites contained between two and nine *Protea* species. Within these sites, I selected ca. 2500 focal plants for which I measured several plant traits and key components of plant fecundity and from ca. 1300 focal plants I used the observations of the frequency of bird visits to inflorescences and plants collected Baptiste Schmid. Together with Baptiste Schmid I sampled 6500 *Protea* individuals and counted at up to three different visits during the flowering season in 2011 and 2012 between March and December the number of inflorescences.

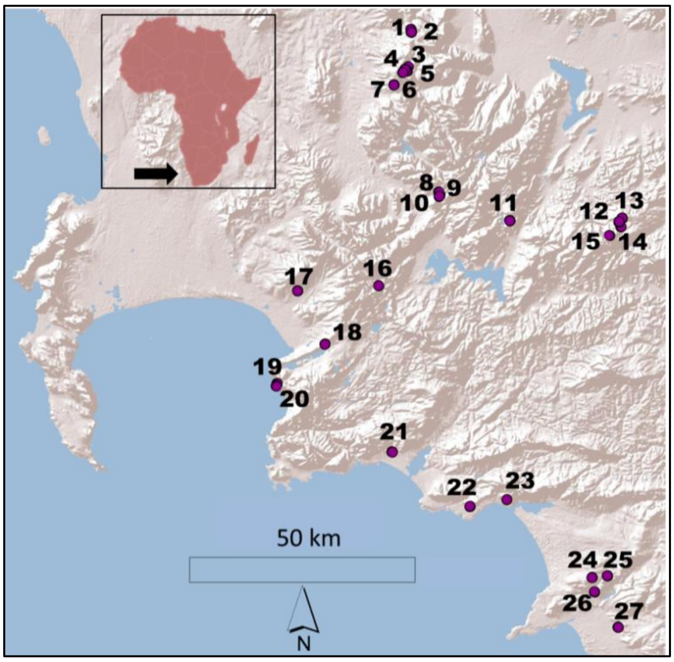

Figure 3. Location of study sites in the Fynbos biome of the Cape Floristic Region (CFR).

I used trait-based models to quantify, precisely, phenological variation in sugar amount per inflorescence and in the number of inflorescences per plant to predict the product of these two (standing sugar crops per plant) at different temporal and spatial scales. Thereafter these sugar landscapes were then related to pollinator abundance and to examine plant reproduction (seed set) and bird pollinator behavior. Moreover, I used these fine-scale maps to fit neighbourhood models predicting seed crop landscapes to quantify competition and apparent competition mediated by seed predation.

In summary, this thesis aimed to quantify the effects of spatial community structure on plant-plant interactions, the spatiotemporal variation in plant-based ‘resource landscapes’, and the effects of these landscapes on interactions between plants and mutualistic and antagonistic animals. To this end, it disentangles how different aspects of floral resource landscapes affect plant-pollinator interactions and how these interactions impact the dynamics of plant communities. Importantly, this thesis discusses how the spatial distribution of resources and traits affects the density and movement of pollinating animals, and how pollinator density and movement in turn affect pollen movement and ultimately plant reproduction. Furthermore, it studies whether the quality of floral resource provided by different *Protea* species predicts the effects of bird-pollinators on the reproduction of *Protea* species. Moreover this thesis studies interactions between competition and apparent competition mediated by seed predation and their role for plant coexistence. Finally, I speculate as to whether the pollinator-mediated and predator-mediated interactions, as well as direct plant-plant interactions can explain the the dynamics of Fynbos plant communities (Figure 4).

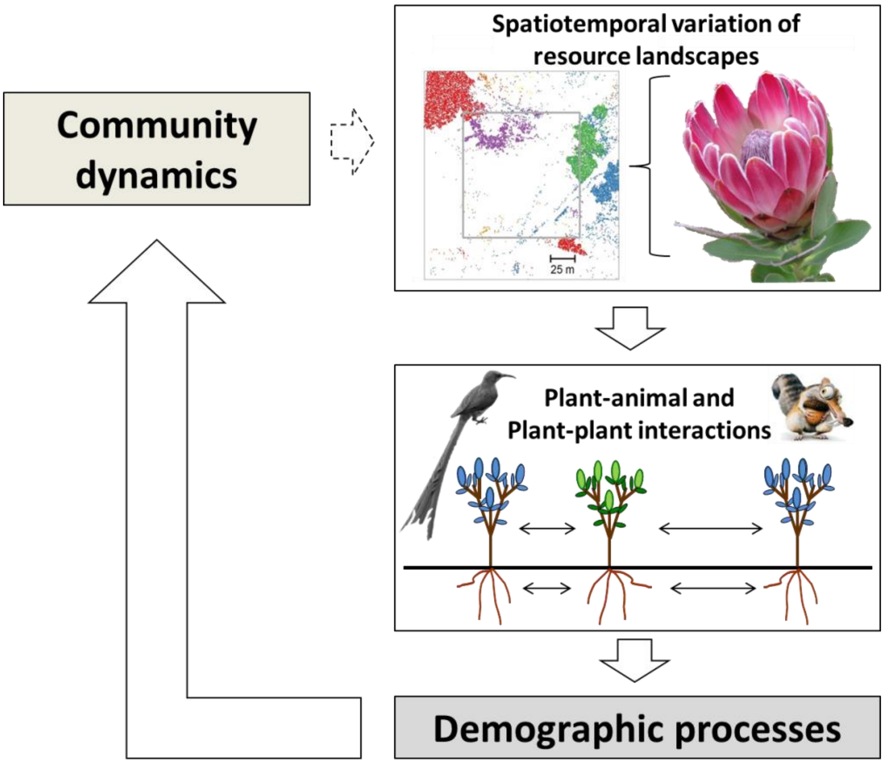

Figure 4. A conceptual diagram showing the research approach of this thesis. The quantification of resource landscapes at different spatial and temporal scales serves to understand plant-animal and plant-plant interactions that determine demographic processes and ultimately community dynamics.

All chapters 1-5 use trait-based neighbourhood analyses to examine plant-plant and plant-animal interactions. Chapter 1-4 deals with plant-plant and plant-pollinator interactions whereas chapter 5 highlights the role of interactions between competition and predation for species coexistence and its consequences for community dynamics. Finally, the discussion deals with methodological and ecological findings and their implications for nature conservation. This thesis is a thesis by publication and, as such, was part of a larger collaborative project that involved several researchers and another PhD student (Baptiste Schmid). The following chapters are publications and manuscripts that resulted from this collaborative project. In each of the following chapters I will therefore indicate when I was co-author or when I was first author. Measurements of nectar sugar and the prediction of sugar landscapes with trait-based neighbourhood analyses which are used in the first two chapters and are part of three chapters where I am second co-author have been developed over the course of my doctoral thesis. Additionally, I included in the Appendix my Diploma thesis (Appendix I) which has been published in the course of my doctoral studies and which was also one key study motivate this collaborative project. Data collected in my thesis were also used in another study which investigated the effects of large-scale environmental variation on demographic rates of Proteaceae (Appendix II).

Chapter 1 quantifies floral resource landscapes provided by *Protea* communities and their role for pollinator-mediated interactions among plants. The following chapters then investigate the role of floral resources for plant-pollinator interactions in more detail. Chapter 2 examines whether the quality of floral resources provided by different *Protea* species predicts the effect of bird pollinators on the reproduction of these species. Chapter 3 examines how floral resource landscapes determine the abundance and per-plant visitation of bird pollinators. Chapter 4 studies the role of floral resource landscapes for pollinator constancy. Finally, Chapter 5 studies the interaction between competition and seed predation and their role for species coexistence.

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Chapter 1

Sugar landscapes and pollinator-mediated interactions in plant communities*

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*This article with similar content has been published in Ecography:

Nottebrock H., Schmid B., Mayer K., Devaux C., Esler K.J., Pagel J., Böhning-Gaese K., Schurr F. and Schleuning M. Sugar landscapes and pollinator-mediated interactions in plant communities. *Ecography* doi: 10.1111/ecog.02441

Abstract

Pollinator-mediated interactions between animal-pollinated plants can play an important role for the dynamics of plant communities. Pollination services depend on the abundance and the foraging behaviour of pollinators, which in turn respond to the availability and distribution of floral resources (notably nectar sugar). However, it is still insufficiently understood how the ‘sugar landscapes’ provided by flowering plant communities shape pollinator-mediated interactions between multiple plant species and across different spatial scales. A better understanding of pollinator-mediated interactions requires an integrative approach that quantifies different aspects of sugar landscapes and investigates their relative importance for pollinator behaviour and plant reproductive success. In this study, we quantified such sugar landscapes from individual-based maps of *Protea* shrub communities in the Cape Floristic Region, South Africa. The 27 study sites of 4 ha each jointly comprise 127,993 individuals of 19 species. We analysed how recorded rates of visitation by key bird pollinators and the measured seed set of plants respond to the distribution of nectar sugar in space, and across plant species, inflorescences and flowering phenologies. We found that pollinator visitation rates strongly depended on phenological variation in site-scale sugar amounts. The seed set of plants increased with nectar sugar of conspecific neighbours and with site-scale sugar amounts. Seed set increased particularly strongly with site-scale sugar amounts that were provided by heterospecific plants with low sugar content per inflorescence. Our study indicates that nectar sugar is a common interaction currency that determines how multiple plant species interact via pollinators. The responses of pollinator-mediated interactions to different aspects of this interaction currency alter conditions for species coexistence in *Protea* communities and may cause community-level Allee effects that promote extinction cascades.

Introduction

Pollinators mediate indirect interactions between conspecific and heterospecific plants, thereby shaping the dynamics of plant communities (Ghazoul 2005; Sargent and Ackerly 2008; Pauw 2013). Within plant species, pollinator-mediated interactions can be positive when neighbouring plants attract pollinators and increase pollinator visitation rates, or negative when plants compete for shared pollinators (Rathcke 1983; Ghazoul 2005). Between plant species, generalist pollinators can also mediate both competitive and facilitative interactions (Moeller 2004; Sargent and Ackerly 2008; Mitchell et al*.* 2009). These interspecific interactions depend on the foraging behaviour of pollinators in multi-species plants communities, and on whether interspecific pollen transfer reduces plant reproductive success (Waser 1978). The relative strength of intra- and interspecific competition mediated by pollinators determines whether pollinators promote or hinder coexistence of plant species (Pauw 2013).

Energetic principles are critical to pollinator-mediated interactions (Heinrich and Raven 1972; Heinrich 1975; Tomlinson *et al.* 2014): pollinators take up energy resources provided by flowering plants (notably as nectar sugar) and partly use it for foraging movements that define their pollination services. Consequently, spatial variation in the floral resource landscape provided by a plant community should translate into spatial variation in pollinator foraging behaviour and pollinator-mediated interactions (Ghazoul 2005; Fig. 1a). Pollinator-mediated interactions also depend on flowering phenology because pollinators track temporal changes in resource landscapes (Hegland et al. 2009; Fig. 1a). Despite these simple principles, pollinator-mediated interactions among plant species can be complex. This complexity arises from spatial and temporal variation in floral resources and from the partitioning of these resources among plant species and individual inflorescences (Fig. 1).Pollinators can mediate interactions among plants at several spatial and temporal scales. Their small-scale foraging behavior affects interactions among inflorescences on the same plant (Goulson 2000; Devaux et al. 2014) whereas foraging movements determine interactions and pollen transfer among neighbouring plants (Seifan et al. 2014). At large spatial scales, pollinator abundance and pollination service respond to the quantity of floral resources within the community (Williams et al. 2012; Nottebrock et al. 2013; Schmid et al. 2015b). The sign of pollinator-mediated interactions can change with spatial scale: for instance, plants may benefit from pollinator attraction by close neighbours but suffer from competition for pollination over large scales (Gunton and Kunin 2009). Overall, the intensity of pollinator-mediated interactions between two individual plants should decrease with the spatial and temporal distance between them (Heinrich and Raven 1972, Elzinga et al. 2007; Devaux and Lande 2009, Fig. 1a). Yet, even plants that do not flower simultaneously may interact via pollinators: early-flowering species can contribute to high pollinator densities that benefit late-flowering species (Riedinger et al. 2014).

In behavioural ecology, it is well established that the quality of resource patches affects foraging decisions of animals. For a foraging pollinator, an inflorescence represents a food patch whose quality can be defined as the amount of floral resources available in a single visit (Pyke 1978). Hence, plant-pollinator interactions should not only depend on total resource amounts but also on whether these resources are split into a few high-quality inflorescences or into many low-quality inflorescences (Fig. 1b). Optimal foraging theory predicts that pollinators should respond to differences between the quality of a focal inflorescence and the quality of surrounding inflorescences: pollinators should prefer higher-quality inflorescences over lower-quality inflorescences (MacArthur and Pianka 1966) and they should spend more time visiting them (Charnov 1976; Pyke 1978). Higher-quality inflorescences can thus negatively affect pollinator visitation and reproductive success of surrounding plants with lower-quality inflorescences (Kandori et al. 2009). Conversely, higher-quality inflorescences could attract more pollinators, which then pollinate neighbouring plants with lower-quality inflorescences (Seifan et al. 2014). The net outcome of these opposite effects of higher-quality inflorescences on their surroundings remains unclear. Moreover, it is not obvious how quality differences between a focal inflorescence and other inflorescences should be evaluated, because the set of available inflorescences depends on the spatial scale at which pollinators take their foraging decision, which is generally poorly known (Ghazoul 2005).

Pollinator-mediated interactions between a focal plant and the surrounding floral resource landscape can also be affected by taxonomic ‘purity’ of floral resources, defined as the proportion of floral resources contributed by conspecifics (Fig. 1c, Ghazoul 2005). Positive effects of purity on pollinator efficiency and plant reproductive success result from increased intraspecific pollen transfer and reduced stigma clogging by incompatible heterospecific pollen (Waser 1978; Shore and Barrett 1984). Additionally, purity may increase reproductive success via positive effects on pollinator visitation (Ghazoul 2005) because pollinators preferentially visit common plant species or because they sequentially visit inflorescences of the same species (Chittka and Thomson 2001). Conversely, purity can reduce plant reproductive success if competition for pollinators is more intense among conspecifics than among heterospecifics (Pauw 2013). Furthermore, heterospecifics can increase pollinator visitation if different plant species with temporally staggered flowering phenologies facilitate each other by maintaining high pollinator densities (Riedinger et al. 2014). Hence, the purity of floral resources can have either positive or negative effects on plant reproductive success and the balance between these effects likely varies with the spatial and temporal scales at which floral resource purity is considered.

The spatial distribution, phenology, quality and purity of floral resource landscapes are thus expected to strongly influence pollinator-mediated interactions among plants. Previous studies considered these aspects individually and demonstrated their relevance for plant-pollinator interactions. However, the results of these previous studies seem conflicting (e.g. Kunin 1997; Ghazoul 2005, Gunton and Kunin 2009; Williams *et al.* 2012; Carvalheiro *et al.* 2014; Feldman and McGill 2014). We argue that progress in understanding the effects of floral resources on pollination requires an integrative approach that quantifies the different aspects of floral resource landscapes and analyses their relative importance for pollinator behaviour and plant reproductive success (Fig. 1). Here, we develop such an approach and apply it to 27 plant communities from the South African Fynbos biome, a global biodiversity hotspot (Myers et al. 2000). We construct high-resolution resource landscapes of the nectar sugar provided by these plant communities in order to (1) quantify how sugar landscapes vary in space, time, quality and purity, and (2) determine the relevance of these aspects of sugar landscapes for pollinator visitation and seed set.

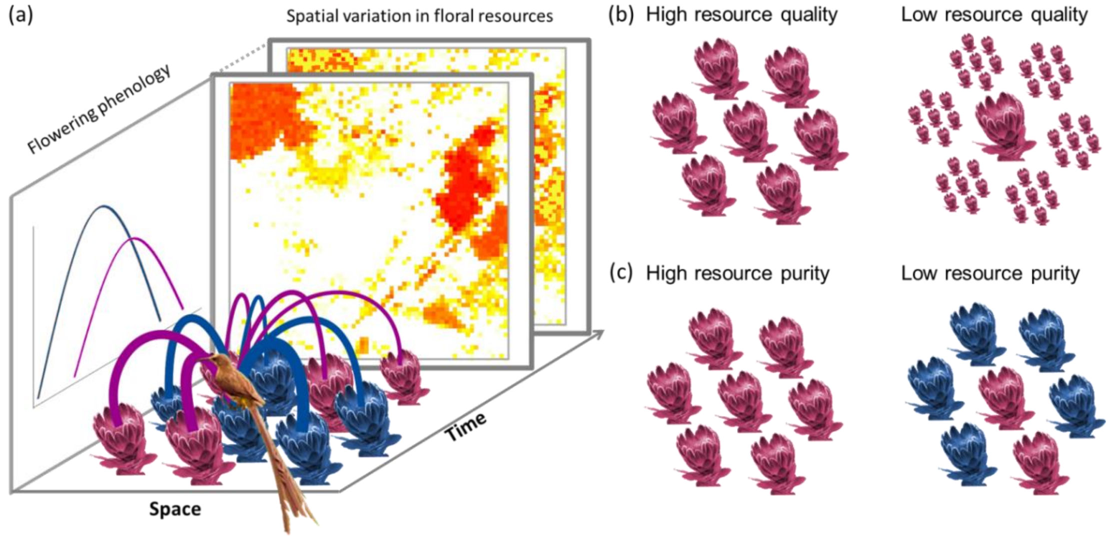


Figure 1: A conceptual framework for studying effects of floral resource landscapes on pollinator-mediated interactions among plants. (a) Effects of spatial and phenological variation in floral resource amounts: the strength of pollinator-mediated interactions experienced by a focal inflorescence depends on the resource amount, spatial and phenological proximity of other inflorescences (interaction strength indicated by line widths). (b) Effects of inflorescence quality: pollinator-mediated interactions depend on whether a given floral resource amount is split into a few high-quality inflorescences or into many low-quality inflorescences. In the example figures, the central inflorescence is surrounded by inflorescences of equal quality (left) or lower quality (right). (c) Effects of floral resource purity: pollinator-mediated interactions depend on the proportion of conspecific floral resources. The example figures show cases of high purity (left) and low purity (right).

Material and Methods

Study system and study design

We studied shrub communities dominated by the species-rich genus *Protea* that has high ecological and economic importance in the Fynbos biome (Schurr et al. 2012) and is well suited for studying plant-pollinator interactions. *Protea* species frequently dominate the overstorey of Fynbos shrublands and provide copious amounts of nectar accumulated at the base of their inflorescences (flowerheads) (Collins and Rebelo 1987). These inflorescences bear many individual florets, each of which contains a single ovule and can thus produce a single seed (Rebelo 2001). To set seed, *Protea* species require pollinator visits to inflorescences and many species are strongly dependent on pollination by nectarivorous birds, notably Cape sugarbirds (*Promerops cafer*) and orange-breasted sunbirds (*Anthobaphes violacea*, Schmid et al. 2015a). Since inflorescences (referred to as cones after flowering) are the functional unit of plant-pollinator interactions in our study, we measured standing nectar sugar crops, pollinator visitation and seed set at the level of inflorescences.

Protea meta-communities have a high beta-diversity, which allowed us to select 27 study sites that varied in species competition and density (Fig. 2a). Each site consisted of a 200x200 m² plot with a core zone of 120x120 m² surrounded by a 40 m wide buffer zone (Fig. 2b). To analyse the effects of sugar landscapes on pollinator-mediated interactions at these sites, we (1) generated fine-scale maps of all overstorey *Protea* individuals, (2) quantified sugar amount per inflorescence and phenological variation in the number of flowering inflorescences to predict sugar landscapes (Fig. 2d), (3) measured both visitation rates of key bird pollinators and seed set at the inflorescence level for a further subset of plants, and (4) and ran statistical analyses that quantify how pollinator visitation and seed set are shaped by sugar amounts at the plant, neighbourhood and site scale, and by the phenology, quality and purity of these sugar amounts.

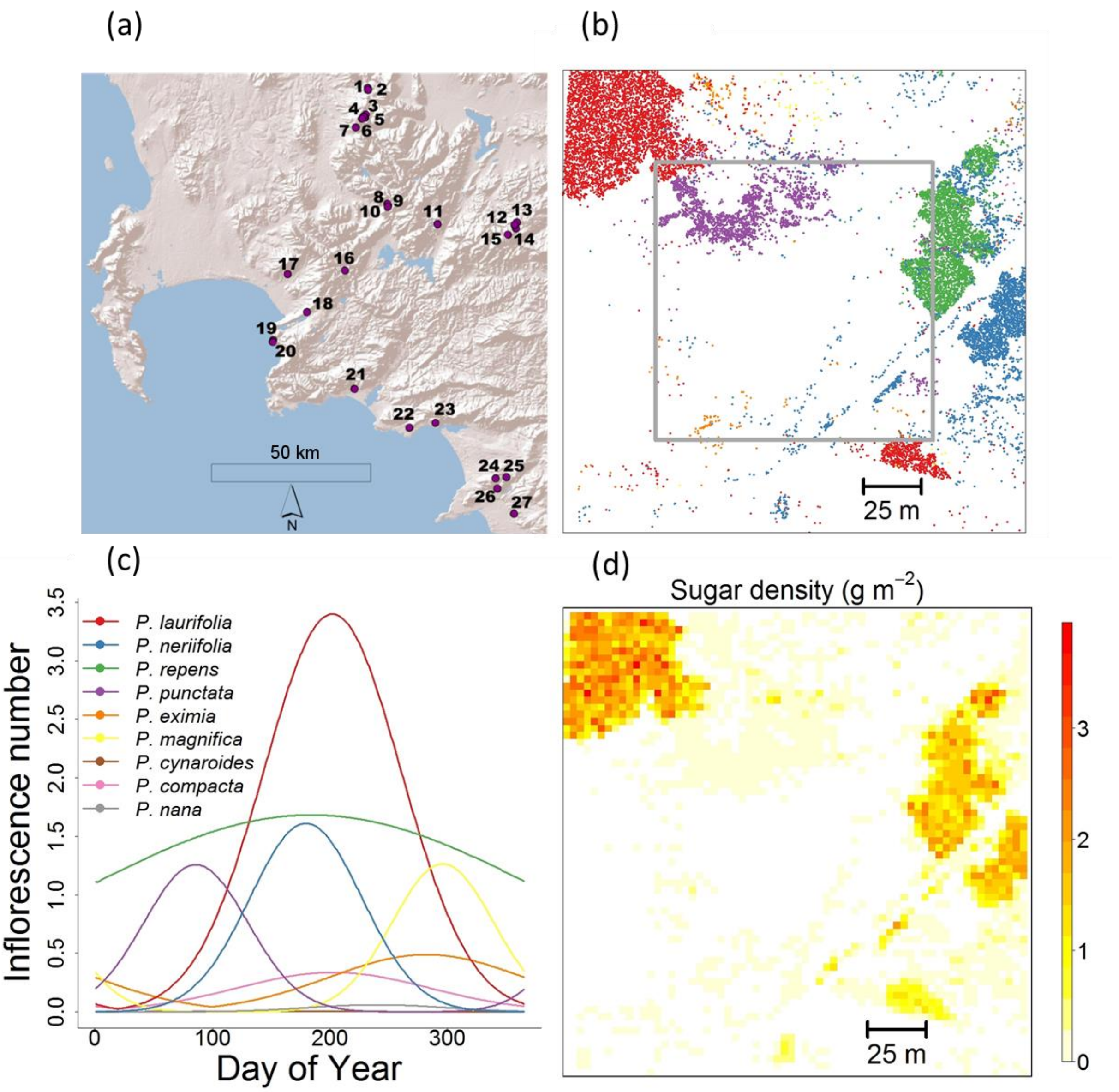


Figure 2: Quantifying the spatiotemporal dynamics of sugar landscapes. (a) Location of 27 study sites in the Fynbos biome, South Africa. (b) Map of 16,948 shrub individuals on study site 4 with colours indicating different *Protea* species (see legend in (c)). (c) Flowering phenologies of the nine *Protea* species on this site (shown as the number of flowering inflorescences of a median-sized plant). (d) Spatial distribution of nectar sugar on the site predicted for a given day (4 July). For a dynamic version of this figure see Supplementary Material Appendix 1, Video A1.

Fine-scale mapping

We mapped all overstorey *Protea* plants on the study sites using differential GPS (Trimble GeoXH; median accuracy 20 cm) and recorded their size (canopy height) and species identity. In very dense monospecific stands (>6 individuals per 2 m²), we mapped the stand outline, recorded plant density and then simulated plant locations within the stand according to a complete spatial random distribution with the observed density. The sizes of these simulated plants were drawn from a stand-specific gamma distribution estimated by a maximum likelihood fit to the sizes of 30 plants measured per stand. In total, the fine-scale maps comprise 127,993 individuals of 19 *Protea* species, with 318 to 48,602 individuals per species, 83 to 37,253 individuals per site, and 3 to 9 species per site.

Trait-based prediction of sugar landscapes

We monitored individual flowering phenologies for a subsample of 6,943 plants (51 to 1245 plants per species) by counting flowering inflorescences at up to three visits during the flowering seasons in 2011 (March to December) or 2012 (March to August). For a subsample of 850 plants in the core zones, (4 to 80 plants per species) we harvested two inflorescences, measured their size and the proportion of open florets, and extracted their nectar by centrifugation (Armstrong and Paton 1990). We measured nectar volume with microsyringes (0.05 mL precision) and nectar concentration with a hand refractometer (Bellingham and Stanley, reading range: 0-50 Brix). Nectar concentration in Brix was then converted into grams of sugar per litre and multiplied by nectar volume to obtain sugar amount per inflorescence.

To predict sugar landscapes, we fitted trait-based models of sugar amount per inflorescence and number of inflorescences per plant. As predictors for these trait-based models, we measured inflorescence size, cone mass, specific leaf area (SLA), and trunk length from the ground to the first branch for a subsample of 2,580 plants in the core zone (25 to 502 plants per species). Additionally, the models included resprouting ability as a species-level trait (Rebelo 2001). The model for inflorescence number also included a date-derived covariate to describe species-specific flowering phenologies. With these trait-based models we then predicted phenological variation in inflorescence number, sugar amount per inflorescence and their product, sugar amount per plant, for all 127,993 mapped plants (for details see Supplementary Material Appendix 1).

From these spatially explicit predictions, we derived sugar amount, purity and inflorescence quality at the neighbourhood and site scales. At the neighbourhood scale (within 40 m radius around each focal plant), we calculated sugar amounts using a neighbourhood index that accounts for the decline of neighbour effects with distance *d* from the focal plant (Uriarte et al. 2010): we summed the sugar amounts of all neighbours within 40 m weighted by 1/(1+*d*). At the site scale, we calculated the total sugar amount of all plants on the site (in g/ha). At both the neighbourhood and site scales, we also calculated purity and inflorescence quality. Purity was calculated as the proportion of the sugar amount at the respective scale that is contributed by conspecifics of the focal plant. As a relative measure of inflorescence quality at the neighbourhood and site scale, we subtracted the focal plant's sugar per inflorescence from the mean sugar per inflorescence at the respective scale.

Phenology was treated differently when characterizing sugar landscapes for analyses of pollinator visits and seed set, respectively (see below). For pollinator visits, we considered sugar landscapes at the respective day of observation. In contrast, seed set integrates over the entire flowering period of an inflorescence, thus seed set analyses included temporally averaged sugar variables that were weighted by the phenology of the focal plant (Supplementary Material Appendix 1).

Pollinator observations and seed set measurements

Pollinator visitation and seed set were measured on plants located within the core zones of the study sites. On up to three visits per site we counted legitimate inflorescence visits by nectarivorous Cape sugarbirds (*Promerops cafer*) and orange-breasted sunbirds (*Anthobaphes violacea*). We recorded the number of inflorescences probed by birds for 1,333 plants (1 to 346 plants per species) during 45 min sessions in the morning (8am – 10am, up to 10 plant-level observations per session). We only considered legitimate probing events, in which birds had contact with stigmas and thus potentially transferred pollen.

Seed set was measured for 1,717 plants (22 to 378 plants per species) by counting the number of fertile seeds ($W_{fertile}$) in up to five randomly harvested mature cones (Nottebrock et al. 2013). The seeds were cross-cut and then probed with a needle to identify fertile seeds containing a soft endosperm. Pre-dispersal seed predation rate was estimated as the proportion of the cross-sectional cone area consumed by predators. The total number of ovules per plant that could potentially set seed was calculated as $W_{potential}= (1- \pi_p) A_C / A_S$, where π_p is the estimated predation rate, A_C and A_S are the cross-sectional areas of cones and seeds (A_C was measured for each cone, A_S was determined as the mean of up to 50 seeds per population).

Analysing effects of sugar landscapes on pollinator-mediated interactions

To analyse how pollinator visits and seed set respond to different aspects of sugar landscapes, we used generalised linear mixed models (GLMMs, package lme4, Bates et al. 2014 in R 3.1.1, R Core Team 2015). We used Poisson errors for the number of pollinator visitations and binomial errors for seed set expressed as the ratio of fertile seeds to potential seeds ($W_{fertile}/W_{potential}$). The model for pollinator visitation controlled for the number of visible inflorescences per plant (included as an offset) in order to describe pollinator visitation rate per inflorescence.

As explanatory variables, the models for both response variables included measures of floral resources at three spatial scales: the number of inflorescences and sugar per inflorescence at the focal plant scale, and sugar amount at the neighbourhood and site scales. To describe how purity and inflorescence quality modify the effects of sugar amount at the neighbourhood and site scale, we included interactions of purity and quality with sugar amounts at the respective scale. We did not include main effects of purity and quality since purity and quality are irrelevant when sugar amounts are zero. To facilitate the interpretation of purity effects, we used impurity (1-purity), which is zero for a purely conspecific neighbourhood. Hence, the main effects of sugar amounts describe effects of 'pure' sugar landscapes in which all sugar is provided by conspecifics. By adding the impurity-interaction term to the corresponding main effect of sugar amount, one obtains the effect of sugar provided exclusively by heterospecifics with identical inflorescence quality. The further addition of the quality-interaction term describes the effect of sugar provided by heterospecifics with higher inflorescence quality.

Analyses of both pollinator visitation and seed set corrected for focal plant size and the seed set analysis additionally controlled for direct plant-plant interactions (such as competition for nutrients) by including the density of con- and heterospecific neighbours (using again the $1/(1+d)$ distance-weighting index). Lastly, we accounted for random variation in space, time and among species: for pollinator visits we included random effects of plant species and observation session (which encompasses site and day effects) and for seed set we included random effects of plant species and site.

To quantify the relevance of different aspects of sugar landscapes for pollinator visitation and seed set, we calculated the AIC difference between the full models (see above) and control models without the respective aspect. Control models for different spatial scales were obtained by dropping all sugar variables at the respective scale, whereas control models for purity and inflorescence quality omitted the respective interaction terms. In the control model for phenology, we replaced all phenology-weighted sugar variables by the respective annual mean.

Finally, we examined the relationship between seed set (response variable) and pollinator visitation (explanatory variable) for the 279 plants for which both data were available. We used a binomial GLMM with a fixed effect of visitation per inflorescence and random effects of species identity and site. Note that pollinator observations were conducted on single dates within the flowering season, but not necessarily at the plant’s peak flowering time. Pollinator visitation rates that were observed close to a plant’s peak flowering time can be expected to be more representative for the entire flowering period and thus more closely related to seed set than visitation rates observed towards the limits of the plant's flowering period. We therefore weighted each data point by $\exp((-\Delta t^2)/\sigma)$, where Δt is the time difference between the pollinator observation and the plant’s peak flowering time and σ is the standard deviation of the plant’s flowering phenology (Supplementary Material Appendix 1).

Results

Spatiotemporal variation of sugar landscapes

Trait-based models of flowering phenology and sugar amount per inflorescence quantify the spatiotemporal dynamics of sugar landscapes in the 27 study communities (Fig. 2, Supplementary Material Appendix 1, Video A1). At the plant scale, sugar per inflorescence varied between 0.01 g and 1.94 g, and the annual maximum of co-flowering inflorescences per plant varied between 0 and 44. The 19 study species showed considerable differences in flowering phenology: their peak flowering time varied from March to October and they ranged from seasonally-restricted to year-round flowering (Fig. 2c, Supplementary Material Appendix 1, Table A1). We calculated the average sugar landscape experienced by a flowering inflorescence by integrating sugar amounts and inflorescences over these flowering phenologies (see Supplementary Material Appendix 1). At the site scale, this phenology-integrated sugar amount was on average 388.9 g/ha (95% interquantile range: 11.1 – 1414.9 g/ha) with a mean purity of 52% (0 – 99%). The mean sugar amount of co-flowering inflorescences on the same site differed from an inflorescence's own sugar amount by an average quality difference of +0.008 g (-0.7 – +0.8 g). The summed sugar amount in the neighbourhood of flowering inflorescences (weighted by 1/(1+*d*)) was on average 18.3 g (0.4 – 103.3 g) with a mean purity of 63% (0 – 100%) and a mean quality difference of -0.003 g (-0.6 – +0.7 g).

Effects of sugar landscapes on pollinator visits and seed set

The spatial structure, quality, purity and phenology of sugar landscapes were of different relevance for pollinator visitation and seed set (Fig. 3). For pollinator visitation, the relevance of sugar variables increased from the plant over the neighbourhood to the site scale (Fig. 3a). Visitation rates depended strongly on the phenology of nectar sugar, and to a lesser extent on inflorescence quality, but the purity of the sugar landscape was of minor relevance for pollinator visitation (Fig. 3a). In contrast, seed set was mostly driven by sugar variables at the neighbourhood scale (Fig. 3b). Moreover, seed set was strongly affected by the purity of sugar landscapes, whereas inflorescence quality had intermediate relevance and phenology had relatively minor relevance for seed set (Fig. 3b).

Significant effects of sugar landscapes on pollinator visitation were only found at the neighbourhood and site scales, where the main effects of sugar amount were modified by interactions with inflorescence quality (Fig. 4a). Pollinator visitation increased with sugar amount at the neighbourhood scale if neighbouring inflorescences provided more sugar than the focal inflorescence (positive quality-sugar-amount interaction, $\chi^2_{1\ \mathrm{df}} = 4.33$, $P < 0.05$, Fig. 4a). Site-scale sugar amounts had a strong negative effect on pollinator visitation, which was particularly pronounced if site-scale sugar amounts were composed of higher-quality inflorescences (negative quality-sugar-amount interaction, $\chi^2_{1\,\mathrm{df}} = 6.93$, $P < 0.01$, Fig. 4a). In contrast, the purity of the sugar landscape did not alter the effect of sugar amount on pollinator visitation at either scale ($P > 0.05$).

Seed set showed significant responses to all aspects of sugar landscapes at all spatial scales (Fig. 4b). At the plant scale, seed set increased with sugar amount per inflorescence ($\chi^2_{1\,\mathrm{df}} = 22.6$, $P < 0.001$, Fig. 4b) and decreased with the number of inflorescences on the focal plant ($\chi^2_{1\,\mathrm{df}} = 96.7$, $P < 0.001$, Fig. 4b). At the neighbourhood scale, seed set increased with conspecific sugar amounts (positive main effect of neighbour sugar amount), but slightly decreased with entirely heterospecific sugar amounts (since the positive main effect of neighbour sugar amount was outweighed by the negative impurity-sugar-amount interaction, $\chi^2_{1\,\mathrm{df}} = 262.0$, $P< 0.001$, Fig. 4b). This negative effect was particularly pronounced if neighbouring inflorescences had lower quality than the focal inflorescence (positive quality-sugar-amount interaction, $\chi^2_{1\,\mathrm{df}} = 117.3$, $P < 0.001$, Fig. 4b). While sugar neighbourhoods had either positive or negative effects on seed set (depending on their purity and quality), the effects of neighbour plant density were consistently negative. The negative intraspecific density dependence of seed set was stronger than the negative interspecific density dependence ($\chi^2_{1\,\mathrm{df}} = 57.8$, $P < 0.001$). This negative effect of conspecific density was almost exactly compensated by the positive effect of conspecific sugar amounts (standardized regression coefficients for conspecific density and sugar amount were -0.33 and +0.33, respectively, Fig. 4b). At the site scale, we found a strong positive effect of sugar amounts, which was more positive if site-scale sugar was provided by heterospecific plants (positive impurity-sugar-amount interaction, $\chi^2_{1\,\mathrm{df}} = 100.3$, $P < 0.001$) and by lower-quality inflorescences (negative quality-sugar-amount interaction, $\chi^2_{1\,\mathrm{df}} = 165.4$, $P < 0.001$, Fig. 4b).

Finally, we found a positive relationship between pollinator visitation and seed set for the 279 focal plants on which we had measured both variables. The seed set of these plants showed a logistic response to pollinator visitation rate ($\chi^2_{1\,\mathrm{df}} = 9.7$, $P < 0.01$).

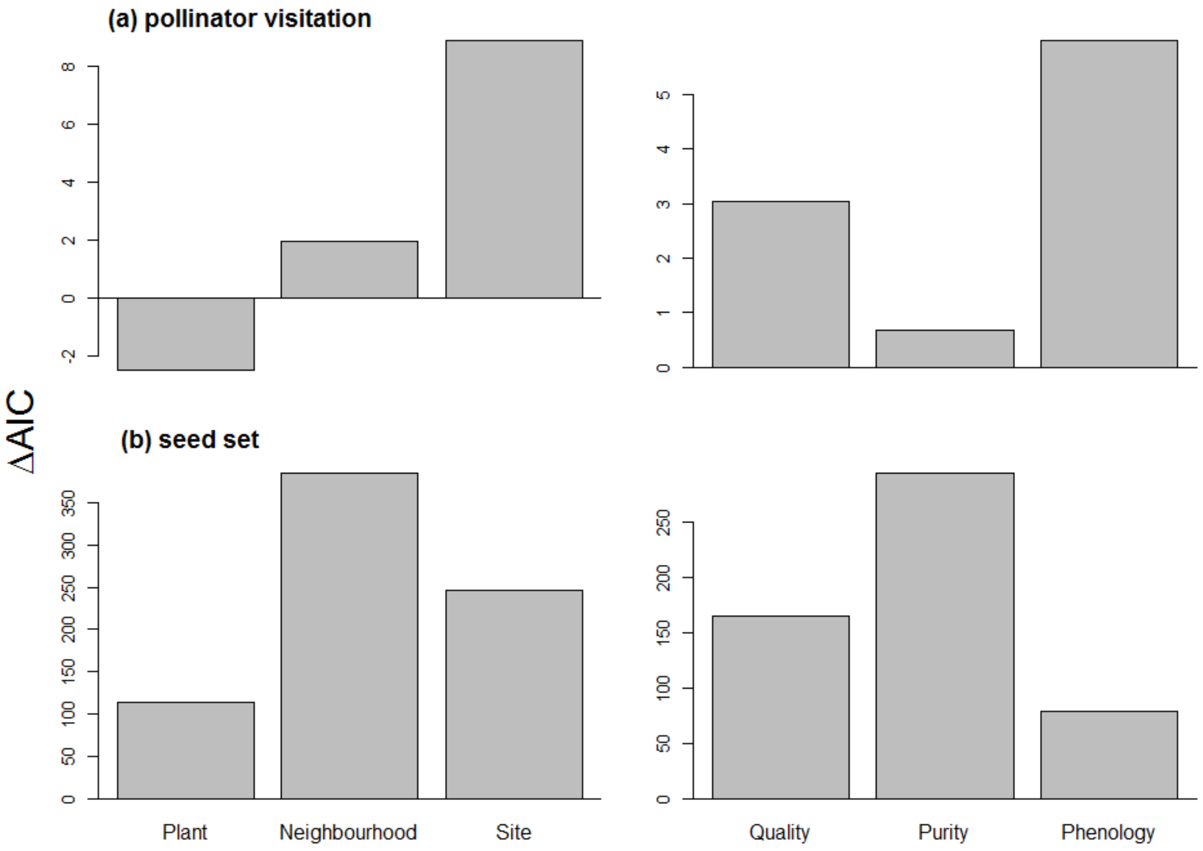
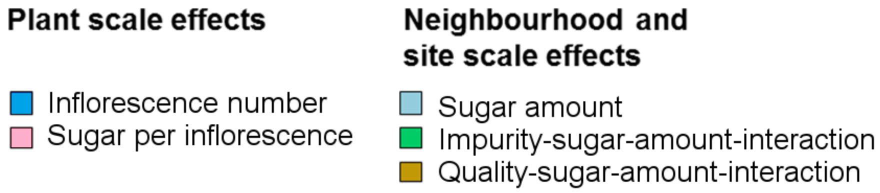
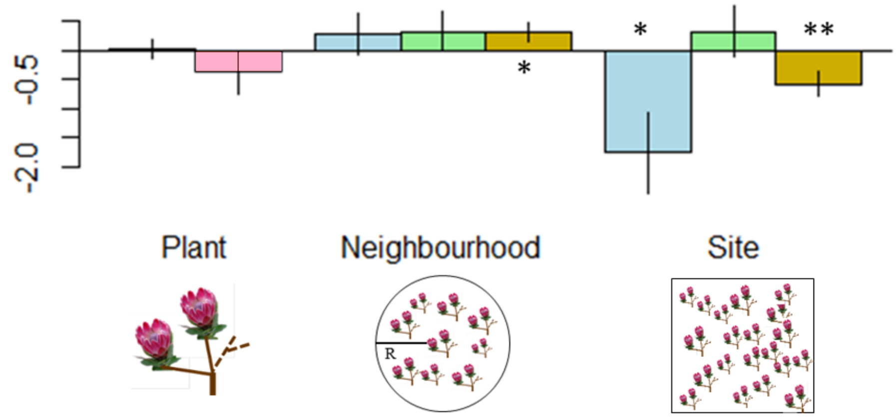


Figure 3: Relevance of different aspects of sugar landscapes for (a) pollinator visitation per inflorescence and (b) seed set per inflorescence. The left panels show the relevance of sugar variables at three spatial scales, the right panels show the relevance of inflorescence quality, purity and phenology. The relevance of a given aspect of sugar landscapes is measured as the AIC difference difference between a control model without the respective aspect and the full model (a positive value indicates better performance of the full model).



(a) Effects on pollinator visitation



(b) Effects on seedset

Figure 4: Effects of sugar landscapes at the plant, neighbourhood and site scale on (a) pollinator visitation and (b) seed set per inflorescence. Bars indicate standardized regression coefficients, whiskers the corresponding standard errors and stars the significance of effects (*: p < 0.05, **: p < 0.01, ***: p < 0.001). At the plant scale, bars show the effect of inflorescence number (dark blue) and sugar amount per inflorescence (pink). At the neighbourhood and site scale, light blue bars show main effects of sugar amount, green bars show interactions between impurity (proportion of sugar amount provided by heterospecific plants) and sugar amount, and brown bars show interactions between relative inflorescence quality (difference in sugar per inflorescence) and sugar amount. Light blue bars at the neighbourhood and site scale thus represent effects of purely conspecific sugar amounts, the addition of the corresponding green bars yields the effect of heterospecific sugar amounts with identical quality, and the addition of the corresponding brown bars shows how sugar effects are altered for heterospecifics with higher inflorescence quality.

Discussion

The high-resolution description of sugar landscapes for 27 plant communities enabled us to quantify how floral resources (nectar sugar amounts) vary in space and time, and how their partitioning among plant species and inflorescences causes differences in purity and inflorescence quality. The relevance of these aspects of sugar landscapes differed between pollinator visitation and seed set: pollinator visitation largely depended on site-scale sugar variables, whereas seed set was determined jointly by sugar variables at the plant, neighbourhood and site scale (Figs. 3 and 4). Below, we discuss the mechanisms causing these floral resource effects, and their consequences for the dynamics of plant communities.

Floral resource effects on pollination and seed set

Sugar amounts at the site scale had a strong negative effect on pollinator visitation per inflorescence but a strong positive effect on seed set (Fig. 4). While the negative response of pollinator visitation may seem surprising, it can be explained by the behaviour of bird pollinators. On the same study sites, bird pollinator abundance increases were less than proportional with site-scale sugar amounts (Schmid et al. 2015b), possibly due to territoriality of bird pollinators. This negative effect does, however, not propagate into seed set (Fig. 4b). The opposite response of seed set to site-scale sugar amounts could result from saturation of stigmas at relatively low levels of pollinator visits, above which more visits do not translate into higher seed set. We observed such a saturating effect in the logistic relationship between seed set and pollinator visitation. Importantly, any interpretation of the differential responses of pollinator visitation and seed set to site-scale sugar amounts must consider the different temporal scales at which pollinator-mediated interactions act: competition for pollination results mainly from the behavioural response of pollinators to instantaneous resource offers, whereas facilitation mainly results from the numerical response of pollinators to long-term resource availability (Gahzoul 2005; Riedinger et al. 2014). Facilitative effects caused by increased pollinator abundance thus likely dominate the positive effect of phenology-integrated sugar variables on seed set. In contrast, pollinator visitation was negatively related to sugar availability on the same day, which likely results from short-term competition for pollinator visits.

The purity of the sugar landscape had weak effects on visitation (Figs. 3a and 4a), which is consistent with the finding that the bird pollinators of our study species are generalists that visit all available study species (Schmid et al. 2015b). In contrast, seed set increased with the purity of neighbourhoods and decreased with the number of inflorescences on the focal plant (Fig. 4b), both effects are expected if seed set is limited by the availability of outcrossed conspecific pollen. The larger importance of phenology for pollinator visitation rather than seed set could arise because pollinator visitation depends on instantaneous sugar landscapes at the day of pollinator observation, whereas seed set integrates over phenological variation throughout the season. These different temporal scales could also explain why the positive effect of the site-scale sugar amounts on seed set increased with impurity (Fig. 4b) so that heterospecific sugar had a stronger facilitative effect than conspecific sugar. The flowering phenologies of our study species are displaced (Fig. 2b), which should reduce interspecific competition for shared pollinators (Devaux and Lande 2009). Conversely, facilitative effects via the maintenance of large pollinator populations through the season are enhanced by the staggering of flowering phenologies among species (Moeller 2004; Riedinger et al. 2014). Overall, the balance between competitive and facilitative effects on pollination visitation and seed set can thus be more positive for heterospecific nectar sugar than for conspecific nectar sugar.

The inflorescence quality (sugar per inflorescence) of focal plants had a positive effect on their seed set (Fig. 4). Moreover, pollinator visitation and seed set of plants with lower-quality inflorescences benefitted from higher-quality neighbours, which suggests that these neighbours attract pollinators and exert a ‘magnet effect’ (Moeller 2004; Seifan et al. 2014). In contrast, it is disadvantageous for a plant to offer inflorescences of lower quality than the site-scale average. This possibly arises because the large-scale foraging decisions of pollinators induce site-scale competition for pollination.

Floral resources and plant community dynamics

The role of floral resources and pollinator-mediated interactions for the dynamics of plant communities has received increasing attention in recent years (Sargent and Ackerly 2008; Pauw 2013; Greenspoon and M’Gonigle 2013). We found that both con- and heterospecific sugar amounts at the site scale have strong positive effects on plant reproductive success. Previously, Nottebrock et al. (2013) found positive effects of large-scale community density on seed set and lifetime fecundity of *Protea repens*. The present study of 19 *Protea* species in 27 communities suggests that such community-level Allee effects are a general feature of *Protea* communities and that they are mediated by nectar sugar. These community-level Allee effects can have profound consequences for plant population and community dynamics: decreased sugar amounts of certain plant species can increase the extinction risk of other plant species, thus increasing the susceptibility of communities to extinction cascades (Colwell et al. 2012).

Our findings also have interesting implications for species coexistence and the structure of diverse plant communities. We found that seed set in *Protea* communities is affected by negative direct effects of plant density and by predominantly positive effects of sugar amounts (Fig. 4b). The direct density effects reveal that intraspecific density-dependence is more negative than interspecific density-dependence, which should cause rare species to experience less competition than common species and should therefore stabilize coexistence (Chesson 2000). These stabilizing density effects are, however, counteracted by pollinator-mediated effects at the neighbourhood scale: conspecific sugar increases seed set whereas heterospecific sugar has much weaker effects (Fig. 4b). These sugar effects thus tend to neutralize intraspecific density-dependence while leaving interspecific density-dependence unaffected. Hence, an individual plant immigrating into a neighbourhood dominated by another species will have strongly reduced seed set compared to a member of the dominant species. This 'priority effect' should promote the formation of monospecific stands (M'Gonigle and Greenspoon 2014) that are a prominent feature of *Protea* communities (cf. Fig. 2a). The emergence of such monospecific stands reduces neighbourhood-scale coexistence but can facilitate larger-scale coexistence. This is because stable stand boundaries decrease large-scale competitive exclusion which led M'Gonigle and Greenspoon (2014) to state that it 'stabilizes coexistence'. In the classification of Chesson (2000), however, this effect is equalizing (reducing fitness differences between species) rather than stabilizing (favouring rare species). In contrast, the positive effects of site-scale sugar amounts on seed set (Fig. 4b) are stabilizing *sensu* Chesson (2000): site-scale facilitation is stronger between than within species, which favours species that are rare at the site scale.

Our results suggest that pollinator-mediated interactions contribute to the formation of monospecific stands, but cause interspecific facilitation across stand boundaries, which stabilizes site-scale coexistence. These effects can help to explain the typical spatial structure of plant communities in a biodiversity hotspot, which differs from other megadiverse systems (such as tropical forests) through the existence of monospecific stands at small scales, but high species richness at larger scales and thus high beta-diversity (Goldblatt and Manning 2002). Such multi-scale impacts of pollinator-mediated interactions on plant communities are not fully covered by existing single-scale theories (Sargent and Ackerly 2008; Pauw 2013; Greenspoon and M'Gonigle 2013).

Conclusions

This study shows that nectar sugar can act as a common 'interaction currency' (Kissling et al. 2012) that determines how multiple plant species interact via their shared generalist pollinators. Inflorescence number and sugar amount per inflorescence are key quantities that convert the spatial structure and phenology of individual plant species into the spatiotemporal dynamics, purity and quality of this common currency at the community level. Pollinator visitation and seed set respond to these multiple aspects of the 'sugar currency', with potentially important consequences for the dynamics and coexistence of plant species within communities. The identification of such interaction currencies is crucial both for developing a more general understanding of community dynamics and for predicting community dynamics in changing environments (McGill et al. 2006; Kissling et al. 2012). It is timely to test whether resource landscapes play similar roles in other pollination systems and for other types of generalized trophic interactions, such as plant-herbivore and plant-frugivore networks.

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Supplementary Material A1: Prediction of sugar landscapes.

This Appendix describes how plant functional traits were measured and how they entered trait-based models of sugar amount per inflorescence and flowering phenology, reports the trait effects detected by these models, and describes how the sugar landscapes predicted by these models were phenology-averaged for use in the seed set analyses.

Trait measurements

Plant size was measured as the aboveground canopy height. Trunk length to first branching was measured from the ground to the first branching node. To determine specific leaf area (SLA) we placed at least five fresh leaves per plant in plastic bags and scanned them with an area meter LI-COR LI 3100C. Thereafter we dried the leaves for 3 days in an oven at 60°C to determine the leaf dry mass with a high precision scale. Inflorescence length was measured from the base to the top of the inflorescences of a subsample of at least 20 individuals per species. For all inflorescences from which nectar was sampled, we estimated the proportion of open florets. Cone mass was measured with a high precision scale.

Trait based models of sugar amount per inflorescence and flowering phenology

The trait-based models for sugar amount per inflorescence and the phenology of flowering inflorescences included the following functional traits: resprouting ability (Rebelo 2001), plant size, trunk length to first branching, SLA, inflorescence size and cone mass. All traits were averaged per population except plant size (for which we used individual-level measurements) and resprouting ability (which is a species-level trait).

For analyses of sugar amount per inflorescence we used linear mixed-effects models with crossed random effects of site and species identity. All analyses were performed in R 3.0.1 (R Core Team, 2013) using package lme4 (Bates et al. 2013). To correct for temporal variation in sugar amounts, we included linear and quadratic effects of the proportion of flowering florets (as a measure of flowering status) and the hour of inflorescence sampling. This model was then simplified by stepwise-backward variable selection (Crawley 2007) omitting all variables with $P > 0.05$. .

Analyses of the phenology of inflorescence number used generalized linear mixed models with Poisson errors (R-package lme4, Bates et al. 2013). In addition to the interactions between plant size and the other functional traits mentioned above (which were used to predict maximal inflorescence number), the maximal model for inflorescence number included the interaction of species identity with the squared time difference between the day on which inflorescences were censused and the species' peak flowering day (thus describing species-specific flowering phenologies). The peak peak flowering day for each species (Table A1) was obtained from phenological information for populations in our study region that is provided by the Protea Atlas Project (Rebelo 2001).

Trait effects on sugar amount per inflorescence and flowering phenology

The minimal adequate model for sugar amount per inflorescence includes a positive effect of inflorescence size ($\chi^2_{1\ \mathrm{df}} = 29.1$, $p<0.001$) and a humped-shaped effect of the proportion of open florets per inflorescence, where young and old inflorescences had lower sugar content than middle-aged inflorescences ($\chi^2_{1\ \mathrm{df}} = 43.7$, $p<0.001$). The minimal adequate model of phenology (number of inflorescences per plant individual) estimated negative effects of squared time difference for all species and thus described hump-shaped flowering phenologies with widths varying between species (see Table A1). The model also included SLA as well as interactions of plant size with trunk length to first branching, sprouting ability and inflorescence size. Plant size had a positive effect and its interaction with trunk length to first branching and inflorescence size was also positive ($\chi^2_{1\ \mathrm{df}} = 37.6$, $p<0.001$; and $\chi^2_{1\ \mathrm{df}} = 22.2$, $p<0.001$, respectively), additionally the effect of interactions of plant size with sprouting ability had a negative effect ($\chi^2_{1\ \mathrm{df}} = 4.5$, $p<0.05$). In addition SLA shows a negative effect ($\chi^2_{1\ \mathrm{df}} = 4.8$, $p<0.05$). The validation of this phenology model with independent data on the sums of flowering inflorescences on focal plants per species and site showed that this model has high predictive power (Fig. S1). Hence, we used the phenology and inflorescence sugar models to predict temporal variation in plant sugar amounts of all mapped *Protea* plants.

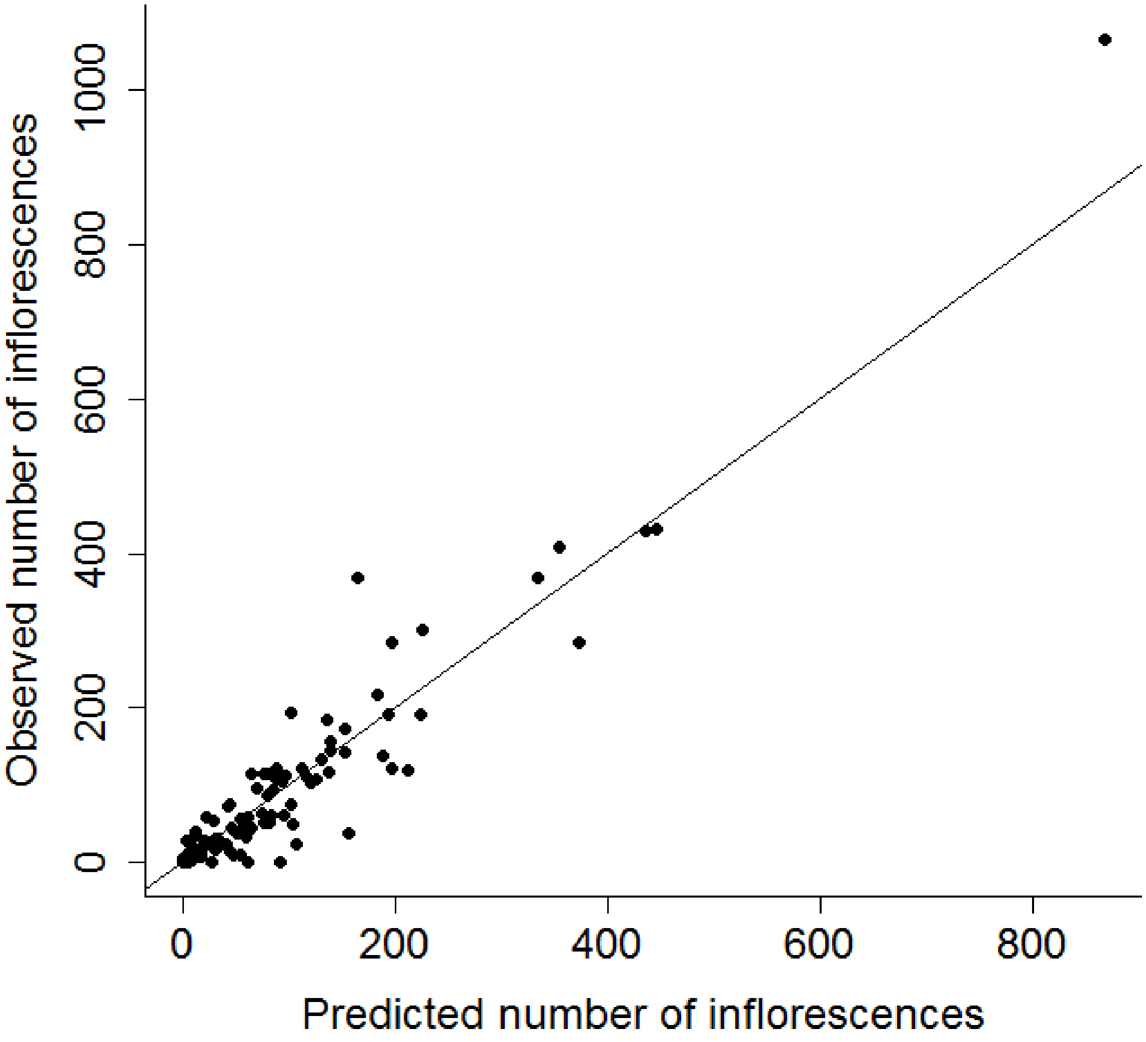

Figure A1: Validation of the trait-based model for flowering phenology. The figure plots observed sums of flowering inflorescences on focal plants per species, site and date of observation versus corresponding predictions of the phenology model. The line shows the 1:1 identity.

Table A1: Flowering phenology of the 19 studied *Protea* species as described by the trait-based model for the number of flowering inflorescences. This model describes the phenology of the number of flowering inflorescences as proportional to a normal probability density function with mean μ (the peak flowering day) and standard deviation σ (determining the extent of the flowering period).

Species	**Peak flowering day, μ (day of year)**	**Extent of flowering period, σ**
Protea acuminata	206	18.66
Protea burcchelii	215	59.41
Protea coronata	152	23.13
Protea compacta	203	81.99
Protea cynaroides	145	77.13
Protea eximia	282	82.60
Protea laurifolia	202	58.37
Protea lepidocarpodendron	188	60.61
Protea longifolia	189	44.04
Protea lorifolia	181	77.40
Protea magnifica	296	43.17
Protea mundii	108	27.53
Protea nana	240	57.15
Protea neriifolia	184	194.29
Protea nitida	197	28.86
Protea obtusifolia	192	80.64
Protea punctata	85	44.24
Protea repens	179	47.57
Protea susannae	110	66.71

Calculation of phenology-averaged properties of sugar landscapes

Seed set integrates over the entire flowering period of an inflorescence and thus over temporally varying floral resources X_j of another plant *j* in the community. For the seed set analyses, we thus calculated the floral resource amounts of plant *j* that are experienced by an average inflorescence of focal plant *i*, $E(X_j)$. To this end, we temporally averaged X_j weighting by the focal plant phenology $f_i(t)$.

Any property that is proportional to the flowering phenology of plant *j*, $f_j(t)$, (such as inflorescence number or sugar amount per plant) can be expressed as

$$X_j(t) = f_j(t)\frac{\max[X_j(t)]}{\max[f_j(t)]}.$$

The average of X_j weighted by the phenology of focal plant *i* is

$$E(X_j) = \frac{\int_0^{365} X_j(t)f_i(t)dt}{\int_0^{365} f_i(t)dt}$$

$$= \frac{\max[X_j(t)]}{\max[f_j(t)]}\frac{\int_0^{365} f_j(t)f_i(t)dt}{\int_0^{365} f_i(t)dt} \qquad \text{(Eq. 1)}$$

The phenology model (see above) describes flowering phenology as proportional to a normal probability density function $n(t;\mu,\sigma)$ with mean μ (the peak flowering day) and standard deviation σ (describing the extent of the flowering period). Projecting this phenology model (in which time is centred on the species-specific peak flowering day) to the time interval [0, 365] and assuming (without loss of generality) that $\mu \epsilon (-\frac{365}{2},\frac{365}{2})$, we obtain the phenology of each plant as a piece-wise combination of two normal PDFs

$$f(\mathrm{t}) = \begin{Bmatrix} n(t;\ \mu,\sigma) & t\,\epsilon\,[0,\mu+\frac{365}{2}) \\ n(t;\ \mu+365,\sigma) & t\,\epsilon\,[\mu+\frac{365}{2},365] \end{Bmatrix} \qquad \text{(Eq. 2)}$$

To calculate the integral $\int_0^{365} f_j(t)f_i(t)dt$ we make use of the fact that for any time *t* the product $f_i(t)$ $f_j(t)$ is a product of two normal probability density functions , which is a function $g(t)$ that itself is proportional to a normal probability density function.

Since for each plant, $f(t)$ is composed of two normal PDFs with different means (Eq. 2), the integral $\int_0^{365} f_j(t)f_i(t)dt$ in Eq. 1 has to be calculated as the piecewise sum of integrals over the functions g obtained for $\mu_i \ or \ \mu_i + 365$ and $\mu_j \ or \ \mu_j + 365$.

If $\mu_i \ < \ \mu_j$,

$$\int_0^{365} f_j\,(t)f_i(t)dt$$

$$= \int_0^{\mu_i+\frac{365}{2}} g(t;\mu_i,\mu_j)$$

$$+ \int_{\mu_i+\frac{365}{2}}^{\mu_j+\frac{365}{2}} g(t;\mu_i+365,\mu_j) + \int_{\mu_j+\frac{365}{2}}^{365} g(t;\mu_i+365,\mu_j+365)$$

and if $\mu_i >= \ \mu_j$,

$$\int_0^{365} f_j\,(t)f_i(t)dt$$

$$= \int_0^{\mu_i+\frac{365}{2}} g(t;\mu_i,\mu_j)$$

$$+ \int_{\mu_i+\frac{365}{2}}^{\mu_j+\frac{365}{2}} g(t;\mu_i,\mu_j+365) + \int_{\mu_j+\frac{365}{2}}^{365} g(t;\mu_i+365,\mu_i+365)$$

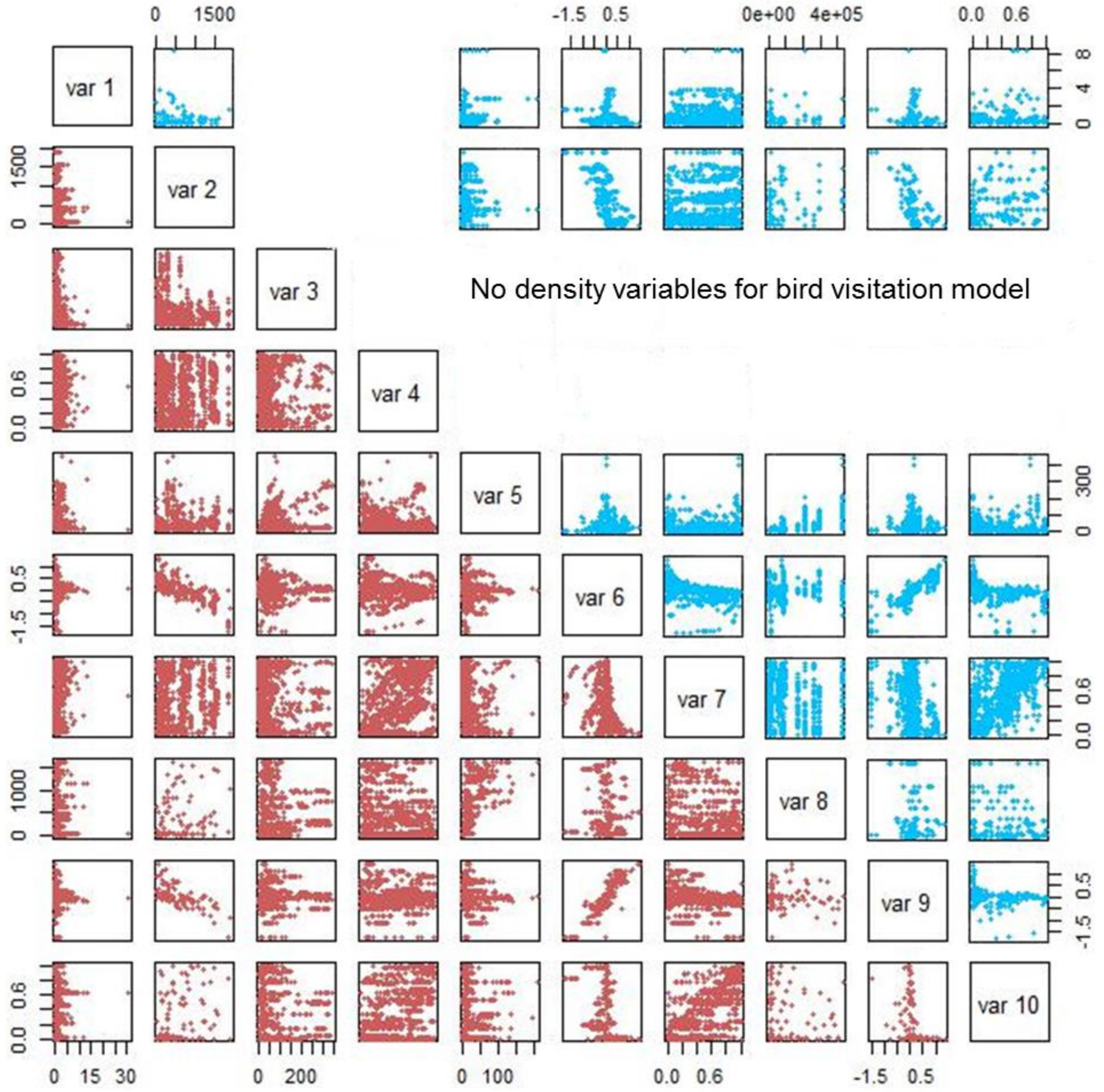

Figure A2: Relationship between variables describing sugar landscapes. Blue plots show variables used in the pollinator visitation model, red plots show phenology-averaged variables used in the seed set model. The variable names are: var 1= inflorescence number, var 2= sugar amount per inflorescence, var 3= neighbourhood-scale density, var 4= neighbourhood-scale plant purity, var 5= neighbourhood-scale sugar amount, var 6= neighbourhood-scale resource quality, var 7= neighbourhood-scale resource purity, var 8= site-scale sugar density, var 9= site-scale resource quality, var 10= site-scale resource purity.

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Chapter 2

Responses of nectar-feeding birds to floral resources at multiple spatial scales*

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Abstract

The responses of animal pollinators to the spatially heterogeneous distribution of floral resources are important for plant reproduction, especially in species-rich plant communities. We explore how responses of pollinators to floral resources varied across multiple spatial scales and studied the responses of two nectarivorous bird species (Cape sugarbird *Promerops cafer* L., orange-breasted sunbird *Anthobaphes violacea* L.) to resource distributions provided by communities of co-flowering *Protea* species (Proteaceae) in South African fynbos. We used highly resolved maps of about 125’000 *Protea* plants at 27 sites and estimated the seasonal dynamics of standing crop of nectar sugar for each plant to describe the spatiotemporal distribution of floral resources. We recorded avian population sizes and the rates of bird visits to >1300 focal plants to assess the responses of nectarivorous birds to floral resources at different spatial scales. The population sizes of the two bird species responded positively to the amount of sugar resources at the site scale. Within sites, the effects of floral resources on pollinator visits to plants varied across scales and depended on the resources provided by individual plants. At large scales (radii >25 m around focal plants), high sugar density decreased per-plant visitation rates, i.e. plants competed for animal pollinators. At small scales (radii <5 m around focal plants), we observed either competition or facilitation for pollinators between plants, depending on the sugar amount offered by individual focal plants. In plants with copious sugar, per-plant visitation rates increased with increasing local sugar density, but visitation rates decreased in plants with little sugar. Our study underlines the importance of scale-dependent responses of pollinators to floral resources and reveals that pollinators' responses depend on the interplay between individual floral resources and local resource neighbourhood.

Introduction

Since about 90% of angiosperms are dependent on animal pollination (Ollerton et al. 2011), studies on plant-pollinator interactions are of immense ecological importance (Heithaus 1974, Bond 1994, Dixon 2009, Menz et al. 2011). Floral energy rewards produced by plants play a crucial role in mediating plant-pollinator interactions (Heinrich and Raven 1972, Heinrich 1975) because foraging movements of pollinators are influenced by the spatially heterogeneous distribution of floral resources [Ghazoul 2005, see Carlo and Morales (2008) for a study on seed-dispersal interactions]. Pollinators tend to optimise their foraging movements in order to balance energy intake in relation to energy expenditure and, according to optimal foraging theory (Cody 1989), this may lead to an ideal free distribution of pollinators among resource patches (Fretwell and Lucas 1970).

Pollinator richness and abundance are often positively correlated with the richness and abundance of plant species (Potts et al. 2003, Biesmeijer et al. 2006, Dauber et al. 2010). However, pollinator abundance may not increase proportionally to the resource density provided by a plant community. A decrease in the pollinator to resource ratio may emerge because the size of the pollinator population is constrained by other environmental factors (Donaldson and Nänni 2002, Feldman and McGill 2014) or influenced by competition among pollinators (Ford and Paton 1982, Smith-Ramirez and Armesto 2003, Mac Nally and Timewell 2005). Under such circumstances, the pollination services are shared among more plants within a community (Goulson 2000) and pollinators become more selective in their visits to plants (Schmitt 1983), directing their foraging movements toward resource "hotspots" within plant communities.

Pollinators mediate pollen transfer between plants and these interactions are crucial for plant reproduction (Ollerton et al. 2011). Thus, the pollinators' response to the resource density of plants may influence the balance between pollinator-mediated facilitation and competition between co-flowering plants. An increase in plant density was often associated with a higher frequency of pollinator visits to plants, promoting facilitation of pollination services (reviewed in Ghazoul 2005, see also Essenberg 2012, Duffy et al. 2013). Within sites, however, plants with copious resources might act as "magnets" for pollinators, concentrating the shared pollination services and inducing competition among plants (Thomson 1981, Ohashi and Yahara 2001, Seifan et al. 2014). Both facilitation and competition for animal pollinators have been observed between co-occurring conspecific and heterospecific plants with shared pollinators. Many studies have reported a positive relationship between plant density and pollinator visitation per unit of time ("per-plant visitation rates" hereafter; Klinkhamer and de Jong 1990, Kunin 1997, Grindeland et al. 2005), while others have found no or negative effects (Aizen 1997, Bosch and Waser 2001, Sieber et al. 2011, Heystek and Pauw 2013). In order to explain these contradictory findings, a recent study underlined the importance of spatial scales

(Hegland 2014, see also Rathcke 1983). Hegland (2014) showed that high floral density at small scale (2.5 m) increased per-plant visitation rates of shared pollinators, but that high floral density at large scale (25 m) decreased per-plant visitation rates. Thus, facilitation effects of resource abundance on pollinator visits at small scale changed towards competition at large scale. Since plants differ in the amount of provided resources, the effects of neighbourhood resource density on pollinator visits may additionally depend on the resource amount provided by individual plants (Sargent 1990, Ohashi and Yahara 2001, Grindeland et al. 2005). Furthermore, the response of a pollinator to resource density is likely to vary among pollinator species (Essenberg 2013). Functional traits of pollinators, such as body size, may be related to the foraging requirements and the competitive ability of a pollinator species (Parker and Sutherland 1986). We therefore studied how pollinator responses depend on the resource amount of individual plants and how different animal pollinators respond to spatial resource distributions.

Although the importance of energetic rewards in pollination systems has been recognised for decades (Heinrich and Raven 1972, Heinrich 1975), we are aware of only one field study that has attempted to summarize the heterogeneous spatial distribution of floral resources provided by multiple plant species into distribution maps of floral resources (see Feldman and McGill 2014). Here we explore how pollinators respond to the distribution of nectar resources provided by plants across scales and discuss how the response may influence the balance between competition and facilitation for animal pollinators between co-occurring plants. We first tested whether the population size of two differently sized nectarivorous birds (Cape sugarbird *Promerops cafer* L. and orange-breasted sunbird *Anthobaphes violace* L.) were related to variation in resource density among sites. Within sites, we then tested whether per-plant visitation rates were related i) to bird species identity, ii) avian population size, and iii) the resource amount provided by individual plants and their resource neighbourhood at various spatial extents. Previous studies did not directly quantify the spatial distribution of floral resources, but rather used proxies such as densities of flowering plants (e.g. Gunton and Kunin 2009, Hegland 2014). Such approaches, however, neglect variation in individual resource availability, e.g. among plant individuals differing in species identity or size. In this study, we quantified floral resources across various scales using highly resolved spatial distributions of nectar resources provided by species-rich plant communities. The plant communities encompassed 19 species of the genus *Protea* (Family Proteaceae) from South African fynbos. *Protea* species produce abundant diluted nectar and are regularly visited by nectarivorous birds of the sugarbird (Promeropidae) and sunbird (Nectariniidae) families (Collins and Rebelo 1987).

We used optimal foraging theory to derive our hypotheses (MacArthur and Pianka 1966, Ohashi and Yahara 2001). We predicted A) a linear increase in avian population size with an increasing resource density at a site, but B) a slower increase of the smaller of the two bird species because of inter-specific competition among pollinator species, C) higher per-plant visitation rates on plants with copious nectar than on plants with little nectar; and D) increasing per-plant visitation rates at high nectar resource densities at small scale (i.e. facilitation predominant at the small scale), but decreasing per-plant visitation rates at high nectar resource densities at large scale (i.e. competition predominant at the large scale).

Methods

Study system and study site

We studied the responses of nectarivorous bird populations and per-plant visitation rates to the spatial distribution of nectar resources of *Protea* species (Fam. Proteaceae). The genus *Protea* is emblematic for the ecological diversity found in the fire-prone fynbos biome in the Cape Floristic Region, South Africa (Schurr et al. 2012). In the fynbos biome, *Protea* species are serotinous, i.e. release seeds after fire. Some *Protea* species resprout from the roots shortly after fire, while most are killed by fire and seedlings need about three years of growth to produce first flowers (Protea Atlas, Rebelo 2001). Flowers are grouped in large inflorescences surrounded by involucral bracts and flowers opens progressively from the outer to the inner circles (Rebelo 2001). *Protea* species vary greatly in floral traits and attract various flower visitors (Rebelo 2001). In our study, we focused on tall, overstorey *Protea* species that produce abundant diluted nectar (Collins and Rebelo 1987). Overstorey *Protea* species are considered to be primarily bird-pollinated (Rebelo 2001). We confirmed this notion for the majority of *Protea* species with bird-exclusion experiments, although insect pollination also contributed to reproduction of a few *Protea* species (Schmid et al. 2015). Pollination experiments further showed that all studied overstorey *Protea* species require animals to set seed, but are self-compatible (Schmid et al. 2015). Nectarivorous bird species visiting *Protea* species mainly encompass representatives of two families, the sugarbirds (Promeropidae) and sunbirds (Nectariniidae), mostly represented by two species. The large Cape sugarbird (males 36.6 g, females 31.8 g, Tjørve and Scholtz 2007) strongly depends on *Protea* nectar and its breeding season matches the peak in flowering time of *Protea* species (Rebelo 1987). The orange-breasted sunbirds (9.5 g, Williams 1993) mostly breed outside the peak flowering season of *Protea* species and depend mostly on Ericaceae species, especially during breeding (Rebelo 1987). Cape sugarbirds often chase and dominate the smaller orange-breasted sunbird (Wooller 1982, Rebelo 1987).

Our study was conducted in the winter-rainfall fynbos in the Western Cape. The study area (120 x 70 km) extended from Bainskloof Pass (33°37'S, 19°05'E) in the North-West to Gansbaai (34°36'S, 19°30'E) in the South-East, incorporating variation in elevation from 20–1500 m (Fig. 1A). Field work took place at 27 study sites on which *Protea* species dominated the overstorey vegetation (so called "proteoid fynbos"). The study sites varied largely in the number of *Protea* species (2–9 species) and plant density. Adjacent study sites were situated at least 300 m apart and were unlikely to host the same breeding birds. At each study site, we defined a 200 x 200 m plot (including a 110 x 110 m core zone). The dimension of the core zone is larger than the mean size of sugarbird territories (mean territory size = 0.8 ha, Calf et al. 2003). At each site, we mapped all overstorey *Protea* plants with a high accuracy Differential Global Positioning System (80–37'300 mapped plants per site). For all mapped plants, we recorded the species identity and the plant size (stem length along the main growth axis). For some large and dense monospecific patches (>10 plants per m^2), plants were not individually mapped. Instead, we mapped the outline of the patch, estimated the plant density in 2 x 2 m plots and measured the canopy height of a subsample of 30 plants. We then simulated plant locations in a patch according to a completely random spatial distribution with plant density given by the observed mean density. The sizes of these plants were drawn from a patch-specific gamma distribution estimated by a maximum likelihood fit to the measured plant sizes (the gamma distribution generally fitted better than alternative lognormal or Weibull distributions). Observations of bird activity took place during the flowering peaks of most *Protea* species from April to August 2012. Every study site was visited three times with two to five weeks between visits. During each visit, we recorded A) population sizes of nectarivorous birds and B) the visitation rates of birds on focal plants in the morning hours. For each focal plant, we recorded the exact location and the number of flowering inflorescences in the afternoon following the bird observations.

Resource maps and multiple-scale resource variables

In order to compare floral resource effects on avian pollinators across various spatial scales, we estimated floral resource distributions at multiple spatial scales, corresponding to site scale, neighbourhood scale and plant scale. The sugar amount of each focal plant (referred to as “plant sugar”) was given by the product of the observed number of inflorescences and the predicted mean sugar amount per inflorescence for each species and site (see Figure S1). To compute the resource distributions at site and neighbourhood scales, we estimated maps of resource distributions, given by the standing crop of nectar sugar for each mapped plant and for each day of observation (Fig. 1B-C; see details in Appendix, Fig. S1). Standing crops of nectar sugar are an accurate measure for nectar availability in *Protea* species that generally produce very large amounts of nectar that are rarely depleted (Nicolson and Thornburg 2007, Geerts and Pauw 2011).

We used different data sources to estimate standing crops of nectar sugar in time and space. We first collected information on flowering phenology for each *Protea* species, from the flowering status (binary variable) of 15,863 populations (48-4,145 per *Protea* species) provided by the Protea Atlas Project (Rebelo 2001). We further recorded the number of inflorescences on randomly selected plants (n = 6943 observations, 51-1,245 per species, 1-865 per populations) at our study sites between March 2011 and August 2012. For each of these plants, we measured plant size (stem length along the main growth axis), inflorescence length, trunk length and specific leaf area (see Fig. S1). We further used information on sprouting ability of each species, as provided by Rebelo (2001). For a subset of these plant individuals (n_{tot} = 484 individuals, 4-80 individual per species, 1-34 individual per population), we also extracted standing crop of nectar from harvested inflorescences (n_{tot} = 850 inflorescences) by centrifugation in the field (Armstrong and Paton 1990). We measured nectar volume (ml) using microsyringes (0.05 ml precision) and nectar sugar concentration (w/w) using handheld refractometers (Bellingham and Stanley, reading range: 0-50 Brix). The product of nectar sugar concentration (transformed into weight per volume) and nectar volume gave the sugar amount (mg) per inflorescence. For the same plant individuals, we additionally recorded the time of the day and the percentage of open flowers of the harvested inflorescences.

We combined these different sources of information to estimate the spatiotemporal distribution of standing crop of nectar sugar at the study sites for each day of observation (“resource maps”, Figure 1C and Figure S1 for analytical details). First, we used the extensive data on the flowering status of *Protea* populations at different times of the year (from the Protea Atlas Project, Rebelo 2001) to estimate the phenological peak of flower production for each species (“day of flowering peak”). Second, we described temporal variation in inflorescence number for each sampled plant (“individual plant phenology”) using a generalised linear mixed model (GLMM) with Poisson errors. We related the number of inflorescences per plant (response variable) to temporal distance from the flowering peak, plant size, and a set of plant functional traits, while treating site identity and species identity as random effects. Third, we used a linear mixed model to relate the standing crop of nectar sugar per inflorescence (“standing crop of nectar sugar”) to plant functional traits, while accounting for time of the day and percentage of open florets in the florescence (as fixed effects), as well as site identity and species identity as random effects. Finally, we used the predictions from the second and third model to estimate the standing crop of nectar sugar for each mapped plant and day of observation, given by the product of the number of inflorescences and standing crop of nectar sugar. This yielded spatially-explicit maps of standing crop of nectar sugar per plant at the days of bird observation (“resource maps”). The spatiotemporal variation in sugar densities depends on *Protea* densities and its species composition, as well as on the number of inflorescences produced by a *Protea* individual at a given time. Sugar densities are assumed to be independent from the foraging behaviour of pollinators.

With the estimated resource maps, we were able to calculate i) site sugar density in the 110 x 110 m core zone (“site sugar”, expressed as g sugar * ha^{-1}, Fig. 1C) and ii) neighbourhood sugar density in various radii from focal plants ("neighbourhood sugar”, Fig. 1C). Consistent with Hegland (2014), neighbourhood sugar density was calculated across concentric circles of different radii around a focal plant. We considered radii of 1 to 40 m (Fig. 1C), excluding focal plants from calculations of neighbourhood sugar. The spatial variation of site sugar among sites was much larger than temporal variation within sites across the four months of study (Figure S2A; between site variation (spatial): Sum of Squares = 276.1, within site variation (temporal): Sum of Squares = 8.0). Since the time intervals between repeated visits were similar across all sites (2-5 weeks), the temporal variability in site sugar at each site was not influenced by sampling bias.

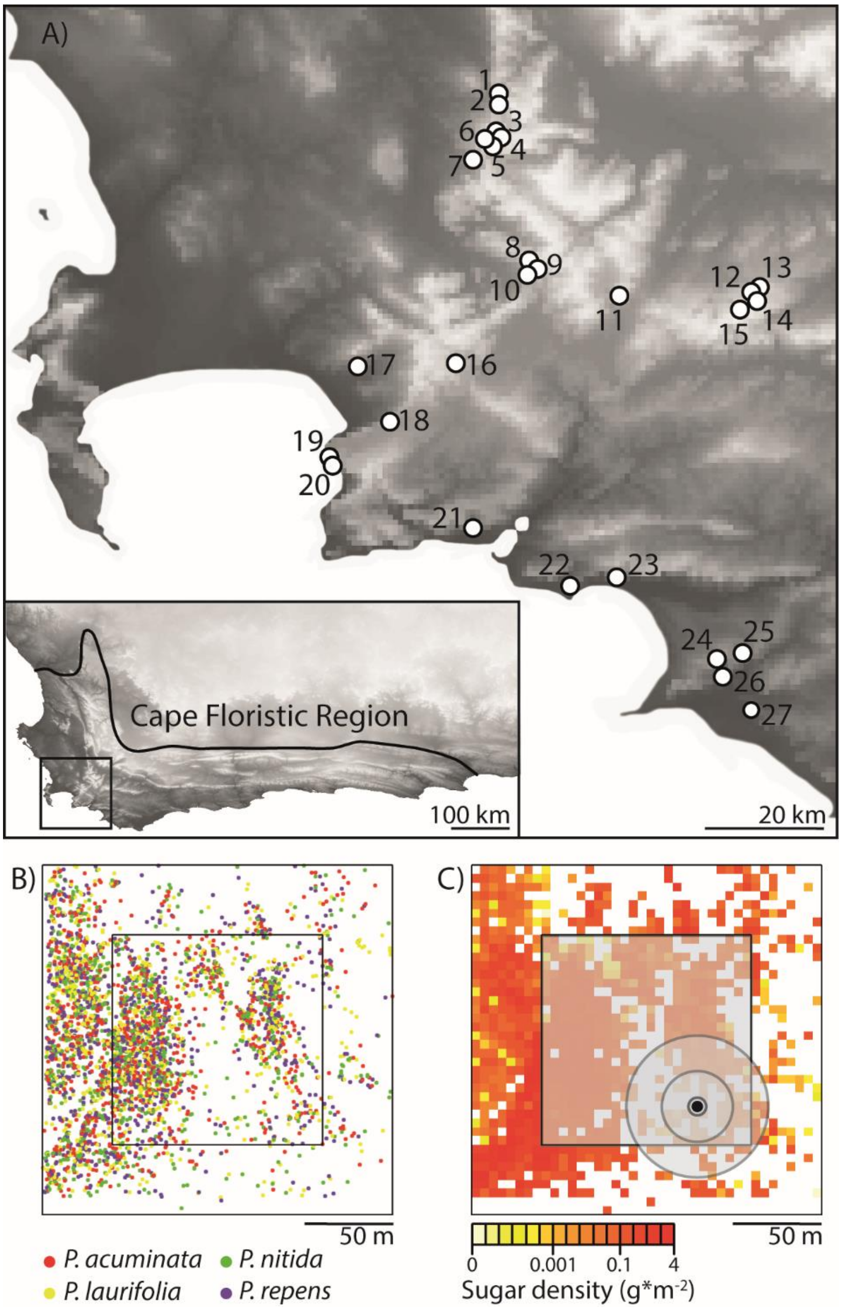

Fig. 1: Study area and resource maps of standing crop of nectar sugar in *Protea* communities. A) Study area in southern Africa within the Cape Floristic Region (CFR) as delimited by Goldblatt (Goldblatt 1978), black line). 27 study sites (white points) were situated in the south western part of the Fynbos Biome. The image was processed with the elevation grid of 30 arc-seconds (~1 km) provided by WorldClim (Hijmans et al. 2005). B) An exemplary 200 x 200 m plot (site nr. 11) and its 110 x 110 m core

zone showing the locations of all mapped plants of the four *Protea* species occurring at this site. C) Resource map for study site 11 on Julian day 181. Predictions of resource maps were derived by a combination of data from different sources and yielded predictions of the standing crop in nectar sugar for each mapped plant on each day of observation (see Figure S1 for analytical details). The grey-shaded areas on top of the resource map illustrate the scale-specific sugar densities: i) site sugar within the 110 x 110 m core zone (grey square) and ii) neighbourhood sugar around a focal plant (black dot) in various concentric radii (grey circles represent exemplary radii at radius 40 m, 20 m, and 5 m, respectively). Focal plant sugar (plant scale) was given by the product of the observed number of inflorescences and the estimated standing sugar crop for the species.

Avian populations

Populations of each nectarivorous bird species were assessed before 10:00 h with 10 minute point counts during three visits at each site. Two observers were situated in the middle of the plot and recorded bird activity in opposite directions. We included all observations of nectarivorous birds within 30 m distance from the observers to avoid biases in detection probability and used the maximal number of individuals recorded simultaneously to avoid double counts. The nectarivorous bird community in the study area included two families (sunbird family Nectariniidae and sugarbird family Promeropidae) and four other bird species. The number of observations varied strongly between species (n = 493, orange-breasted sunbird; n = 189, Cape sugarbird). We did not include the rare observations of the Southern double-collared sunbirds *Cinnyris chalybeus* and the Malachite sunbirds *Nectarinia famosa* in population measures because they represented less than 5% of all observations and were only observed on a few study sites (<4 sites). We observed Cape sugarbirds and orange-breasted sunbirds throughout the study area; population sizes of the two species were unrelated to the elevation of the study sites ($R^2 < 0.04$; $P > 0.05$ in both cases). We determined the number of simultaneously observed individuals of Cape sugarbirds and orange-breasted sunbirds separately for each site and visit and derived an overall measure of avian population size by summing the two species-specific estimates, referred to as “avian population size”. In addition, we recorded whether Cape sugarbirds were breeding at the site at the time of the visits and we estimated the number of defended territories.

Per-plant visitation rates

During the three visits at each site, we recorded visitation rates of birds on focal plants of 17 *Protea* species during 45 min sessions from 07:00 h to 12:00 h. Depending on the availability of flowering plants, we selected 1-11 focal plants per session, all situated in the core zone of each plot at least 20 m distant from the observer. We observed 6-346 (median = 46, 1333 in total) focal plants per species across all sites (see Figure S2B for the complete species list and sample sizes for each species). We considered only avian visits to focal plants with legitimate foraging events, i.e. birds made contact with the stigma and therefore potentially transferred pollen. Bird visitors frequently foraged on *Protea* inflorescences from the side (17% of all foraging events observed), and these illegitimate visits were excluded from the analysis. Including all bird visits in the analyses resulted in qualitatively identical results. We used per-plant visitation rates (number of avian visits per unit of time) for each nectarivorous bird species as response variables in further analyses. We only included bird visits from Cape sugarbirds and orange-breasted sunbirds to be consistent with the counts of avian population size, and because the two species represented more than 85 % of all visits.

Data analyses

We tested the relationships between avian population sizes of the two bird species (Cape sugarbird and orange-breasted sunbird) and main and interaction effects of bird species identity and site sugar (log-transformed) assuming a Poisson error distribution. We also tested the effects of site sugar on the presence or absence of Cape sugarbirds on the study site and the number of territories of Cape sugarbirds on the day of observation, assuming a binomial and a Poisson error distribution, respectively. We included site identity and date as random intercepts in these models.

We tested the relationships between per-plant visitation rate (number of legitimate bird visits per 45 minutes) and bird species identity (Cape sugarbird or orange-breasted sunbird), avian population size (from the point count survey conducted on the same day, but independent from the observation of bird visitation rates), focal plant sugar (log-transformed) and a sugar density variable (log-transformed) assuming a Poisson error distribution. The sugar density was either site sugar or neighbourhood sugar at a specific radius (Fig. 1). Sugar density variables were partly correlated between each other (Table S1). To account for collinearity, we sequentially tested each sugar density variable, including in the models either site sugar or one of the neighbourhood sugar variables. Because bird species might differ in their response to resources, we also included the two-way interactions of bird species with avian population size, focal plant sugar and the sugar density variable, respectively. Because the effect of sugar density could depend on focal plant sugar, we also added the two-way interaction of focal plant sugar and the respective sugar density variable. In order to account for spatial and temporal autocorrelation between focal plants, we included random intercepts of species identity and, nested in this order, session identity, date and site identity.

All statistical analyses were performed with R (version 3.0.2, R Core Team 2013). We used mixed effects models as implemented in the “lme4” package (version 1.0-4, Bates et al. 2013) and model averaging functions as implemented in the “MuMIn” package (version 1.9.5, Barton 2013). For each response variable, model selection among the full model and all possible subsets of predictor variables was performed according to the corrected Akaike Information Criterion (AICc). We considered all models with ΔAICc < 2 to be similarly supported, best models (Burnham and Anderson 2002). From these subsets of best models, we averaged the parameter estimates across the models where the respective parameter appeared, weighted by the relative importance (given by AICc) of each model (Johnson and Omland 2004). The approach of model averaging allows the quantification of the effect size of each variable across several equivalent models. To assess the significance of the main and interaction effects that were included across all models with ΔAICc < 2, we applied Wald's χ^2 tests. The significance level of each variable was tested independently of the sequence of main effects in the statistical model. To ensure comparability among predictor variables, all numerical predictors were scaled and centred prior to analysis.

Results

Avian population size and nectar resources

Avian population size (i.e., the number of simultaneously observed bird individuals in standardised point counts) increased with site sugar (full model including main and interaction effects of site sugar and bird species: marginal R^2 = 0.53, Table 1, Fig. 2; ΔAICc < 12 to all other models). Populations of orange-breasted sunbird increased continuously with site sugar and were also present at sites with low amounts of site sugar. In contrast, Cape sugarbirds were absent from sites with low amounts of site sugar. Sugarbird populations, however, increased at a higher rate than sunbird populations with increasing site sugar (Table 1, Fig. 2). Accordingly, the presence of breeding sugarbirds and the number of sugarbird territories were positively related to site sugar (presence: $\chi^2_{df=1}$ = 6.8, P = 0.009, marginal R^2 = 0.37; number of territories: $\chi^2_{df=1}$ = 13.9, P < 0.001, marginal R^2 = 0.34). Avian population size of the two species increased less than proportionally with increasing site sugar. For instance, while about 3000 g sugar per ha were required for the occurrence of a single Cape sugarbird, about 8000 g sugar per ha were required for an additional sugarbird individual (model estimates are given in Table 1).

Table 1: Effects (mean ± standard errors) of bird species and site sugar on avian population size (n = 84 point counts for each species). The full model including main and interaction effects of bird species and site sugar was better than all other models (ΔAICc < 12 in comparison to all other models).

	Estimates	$\chi^2_{df=1}$	*P*-value
Intercept	-1.26 ± 0.24		
Bird species (sunbird)	1.46 ± 0.25	21.3	< 0.001
Site sugar	1.00 ± 0.23	7.5	0.006
Bird species x site sugar	-0.82 ± 0.23	12.4	0.004

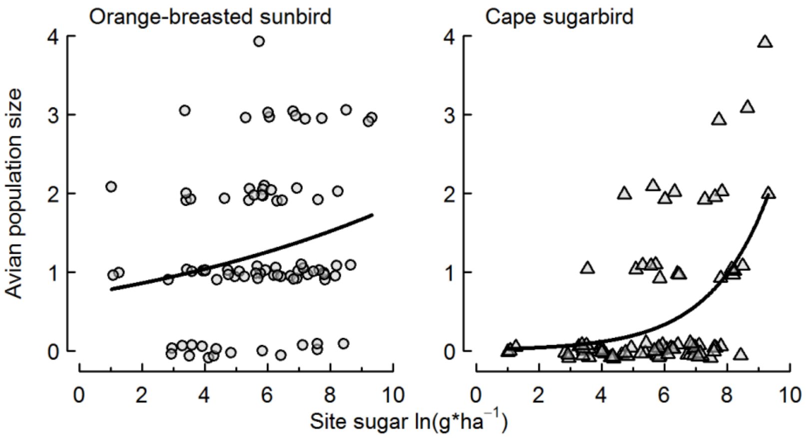

Fig. 2: Effect of site sugar (in g sugar ha^{-1}, log-normal transformed) on the avian population sizes of the two main nectarivorous bird species present in the study area: orange-breasted sunbirds *Anthobaphes violacea* (circles) and Cape sugarbirds *Promerops cafer* (triangles). Raw data (points) for the three visits on the 26 study sites are represented with predicted values of fixed effects (lines). We added jitter to the raw data to reduce overlapping data points.

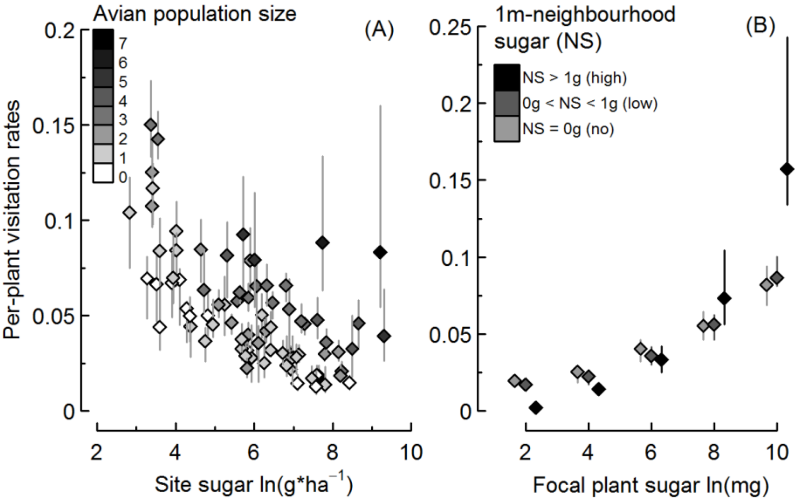

Fig. 3: Relationships between per-plant visitation rates and A) site sugar and avian population size (number of individuals) for every visit per site, and B) focal plant sugar and 1 m-neighbourhood sugar. We computed the predicted values from the averaged estimates of all fixed effects in the sets of best models with ΔAICc < 2 to draw the medians (points) and .05/.95 quartiles (lines).

Per-plant visitation rates within communities

On 1333 focal plants, we recorded a total of 239 visits of Cape sugarbirds and 242 visits of orange-breasted sunbirds. Per-plant visitation rates were positively related to avian population size (Table 2, Fig. 3A). This effect was stronger for sugarbirds than for sunbirds (Table 2), corroborating the stronger increase of sugarbird than sunbird populations with increasing site sugar (Table 1). Per-plant visitation rates were consistently positively correlated with focal plant sugar, i.e. focal plants with copious sugar showed higher per-plant visitation rates than focal plants with little sugar (Table 2, Fig. 3B). Effects of neighbourhood sugar on per-plant visitation rates varied with the radius at which neighbourhood sugar was calculated (Table 2, Fig. 4). At large scales (radii >25 m) and site scale, per-plant visitation rates decreased with sugar density (Table 2, Fig. 3A and Fig. 4) and there was no interaction between focal plant sugar and neighbourhood sugar, i.e. high sugar density at large scales had similarly negative effects on all plants (Table 2, Fig. 4). At small scales (radii <5 m), per-plant visitation rates increased with high sugar density, particularly in plants with copious sugar, but decreased in plants with little sugar (Table 2; Fig. 3B and Fig. 4). Models with neighbourhood sugar measured at 1, 2 and 3 m radii had similarly low AICc values, whereas all models with neighbourhood sugar measured at radii larger than 3 m showed ΔAICc values >2 compared to the model with the lowest AICc-value at 2 m-radius.

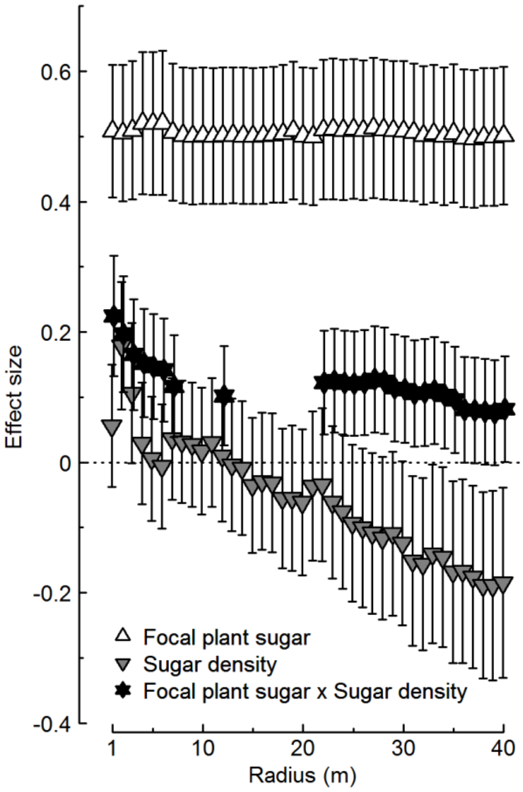

Fig. 4: Scale-specific effect sizes of three predictors on per-plant visitation rates: i) focal plant sugar (unfilled pointing-up triangles), ii) neighbourhood sugar density within various radii from focal plants (grey pointing down triangles) and iii) interaction between focal plant sugar and neighbourhood sugar density (black stars). Focal plant sugar was identical in all models, sugar density values changed according to the radius considered in each model. If the respective main or interaction effects were included in models with ΔAICc < 2, mean averaged estimates were drawn with standard errors, otherwise no effect size was drawn. Table 2 gives effect sizes of all predictors for the radial distances of 1 m and 40 m, respectively.

Table 2: Effects of bird species, avian population size, focal plant sugar and sugar density on per-plant visitation rates of avian pollinators. We show averaged parameter estimates (mean ± standard error) across subsets of best models for three models differing in the sugar density variable (log-transformed), while the other variables were identical. We present results for 1 m-neighbourhood sugar (marginal R^2 = 0.05), 40 m-neighbourhood sugar (marginal R^2 = 0.05), and site sugar (marginal R^2 = 0.08). All numerical predictors were scaled and centred. Significant effects ($P \leq 0.05$) are written in bold. Asterisks denote the level of significance of the respective predictor term with Wald's χ^2-test: * for $P \leq 0.05$; ** for $P \leq 0.01$ and *** for $P \leq 0.001$.

	Sugar density variables		
	1m-neighbourhood sugar	40m-neighbourhood sugar	Site sugar
Bird species (sunbird)	0.13 ± 0.10	0.11 ± 0.11	0.12 ± 0.10
Avian population size	0.36 ± 0.18	0.42 ± 0.18	**0.60 ± 0.18 ****
Focal plant sugar	**0.51 ± 0.10 *****	**0.50 ± 0.10 *****	**0.50 ± 0.11 *****
Sugar density	0.06 ± 0.10	-0.19 ± 0.15	**-0.56 ± 0.21 ****
Bird species x avian population size	**-0.28 ± 0.10 ****	**-0.30 ± 0.11 ****	**-0.31 ± 0.10 ****
Bird species x focal plant sugar	-0.07 ± 0.10	-0.12 ± 0.10	-0.13 ± 0.10
Bird species x sugar density	-0.18 ± 0.09	-0.12 ± 0.09	-0.04 ± 0.10
Focal plant sugar x sugar density	**0.23 ± 0.09 ***	0.08 ± 0.08	0.08 ± 0.09

Discussion

We explore how responses of pollinators to floral resources varied across spatial scales and discuss how this scale-dependence may influence the balance between competition and facilitation for animal pollinators between co-occurring plants. Both Cape sugarbirds and orange-breasted sunbirds responded positively to the amount of sugar resources at the site scale, especially the large-bodied Cape sugarbirds. Within sites, the effects of sugar resources on pollinator visits to plants varied across scales and depended on the individual properties of plants. At large scales (radii > 25 m and site sugar), high sugar density decreased per-plant visitation rates, i.e. plants competed for animal pollinators. At small scales (radii < 5 m), our results suggest that both competition and facilitation for avian pollinators occur between neighbouring plants, depending on the sugar amount offered by the focal plants. At high sugar density, plants with copious sugar attracted more pollinators, whereas plants with little sugar were less frequently visited. Our findings, thus, show that effects of floral resource on plant-pollinator interactions vary across scales and depend on the variability in individual floral resources. This finding has important implications for pollinator effects on plant reproduction.

Avian populations

In line with our hypothesis, the local abundance of nectar resources strongly influenced the population sizes of the two bird species. Our results suggest a causal link between the density of floral resources and the size of pollinator populations. This may be explained by the high mobility of birds that enables them to track nectar resources over large distances (Fraser 1997a). Consequently, nectarivorous birds aggregate and attempt to breed where nectar resources are abundant (Ford 1979, Calf et al. 2003). Nonetheless, local environmental conditions, for instance those related to elevation and temperature, may also influence the number of nectarivorous birds at a site (Feldman and McGill 2014). Feldman and McGill (2014) reported only weak relationship between abundance of nectar resources and hummingbird populations. The effect of floral resources on birds may indeed vary among years (Herrera 1998) and bird species (Mulwa et al. 2013).

In our study, bird species differed in their responses to sugar density. The large-bodied Cape sugarbirds were almost always absent from study sites below a threshold sugar density of about 3000 g sugar per ha. Cape sugarbirds strongly depend on sugar resources of *Protea* species and their breeding cycle coincides with the flowering peak of *Protea* species during winter rainfalls (Rebelo 1987, Geerts 2011). Establishing appropriate breeding territories thus requires Cape sugarbirds to track *Protea* resources over large spatial scales (Fraser 1997a). In contrast, the smaller-bodied orange-breasted sunbirds were commonly observed at low resource density and showed a continuous increase in population size with increasing nectar resources. Even though orange-breasted sunbirds regularly forage on *Protea* species, their distribution is more closely related to the presence of Ericaceae species, especially during their breeding season (Rebelo 1987), and they are less likely to track *Protea* resources over large spatial extents (Fraser 1997b). During the austral winter, however, only few *Erica* species flower and orange-breasted sunbirds therefore frequently forage on *Protea* species during that time of the year. The distinctive response of the two bird species to sugar density might be further explained by different daily energy requirements related to difference in body mass, given that Cape sugarbirds (males 36.6 g; females 31.8 g; mean = 34.1 g, Tjørve and Scholtz 2007) are substantially heavier than orange-breasted sunbirds (9.5 g, Williams 1993). Assuming that sugarbirds and sunbirds absorb almost 100% of the nectar sugar they take up (Lotz and Nicolson 1996, Jackson et al. 1998), the allometric equation for nectarivorous birds (Nagy 2001) predicts daily sugar requirements of about 9.1 g for Cape sugarbirds and 3.8 g for orange-breasted sunbirds. Higher flight costs at low resource density and higher daily energy requirements of sugarbirds compared to sunbirds might explain the smaller proportion of sugarbirds compared to sunbirds observed at low resource density (Buchmann et al. 2012).

We observed lower pollinator-to-resource ratios at high sugar density compared to low sugar density. One explanation for the slow increase of avian population size with increasing sugar resources might be competition among avian pollinators (Ford 1979, Mac Nally and Timewell 2005), especially due to the aggressive territorial behaviour of Cape sugarbirds during their breeding season (Wooller 1982, Seiler and Rebelo 1987). The low pollinator-to-resource ratio at high resource density, as observed in this study, lends support to the hypothesis that avian pollinators mediate the negative effect of plant density on *Protea* seed set at high plant density, as has been reported in previous studies on *Protea* species (Esler and Cowling 1990, Nottebrock et al. 2013).

Per-plant visitation rates

Per-plant visitation rates decreased with increasing site sugar, indicating competition between plants for avian pollinators at high sugar density. These results are in line with expected dilution effects of pollinator visits to plants at low pollinator-to-resource ratios (Goulson 2000, Otway et al. 2005). Under this scenario, it has been hypothesised that pollinators concentrate on highly rewarding plants within plant communities (Thomson 1981). Our results corroborate this hypothesis in two ways. First, we observed more frequent bird visits to focal plants with copious sugar. Second, high neighbourhood sugar density at small scales significantly increased the visitation rates of plants with copious sugar, but decreased the visitation rates of plants with little sugar. Plants with little sugar only contribute marginally to the sugar density at small scales and thus become less likely to be visited by pollinators. Plants with copious sugar therefore experienced a competitive advantage over plants with little sugar. Our findings are therefore the first to show that floral resources of focal plants interact with the local resource neighbourhood in their effect on pollinator visitation. The only other study that formally tested the interacting effects between individual properties and local floral neighbourhood on pollinator visits found a positive correlation between visitation rates and individual display size and neighbouring floral density (Grindeland et al. 2005). However, this study only detected additive effects of display size and local neighbourhood on visitation rates of pollinators and no synergistic interaction as found in our study.

Increased per-plant visitation rates on plants with copious sugar and the interspecific variation in focal plant sugar among *Protea* species (Figure S2B) may promote interspecific competition for avian pollinators. Interspecific competition for animal pollinators may depend on the local context, since the study sites varied in plant species assembly. That is, a plant species with a medium sugar amount might show competitive advantages in a community where most species produce little sugar, but competitive disadvantages in communities where most plants produce copious sugar. To reduce competition for animal pollinators within plant communities, different non-exclusive mechanisms have been proposed. For instance, competitively inferior species may displace their flowering phenology, thereby avoiding synchronous flowering with highly competitive species (Waser 1978, Silvertown et al. 2005). Additionally, plants may adapt their pollination system and decrease their dependency on the pollinators shared with the competitive plant species (Waser 1983). Hence, variation in flowering phenology among *Protea* species (Protea Atlas, Rebelo 2001) and a low dependency on avian pollination of some *Protea* species (Coetzee and Giliomee 1985, Schmid et al. 2015) might contribute to reduce interspecific competition for avian pollinators among *Protea* species.

Per-plant visitation rates generally increased with increasing avian population size. This positive effect was particularly strong for Cape sugarbirds, because they only occurred on the most sugar rich sites, whereas orange-breasted sunbirds were more widespread. At high sugar density, sugarbirds may displace sunbirds towards alternative nectar resources, due to their competitive dominance. Pollination services of orange-breasted sunbirds may nevertheless be important for *Protea* communities that do not host breeding Cape sugarbirds. At low *Protea* density, sunbirds may buffer the lack of pollination services provided by sugarbirds. Such buffering effects of displaced pollinators may increase the stability of plant communities (Ghazoul 2006, Verdú and Valiente-Banuet 2008).

Conclusions

Albeit the prediction of floral resources from species traits is not as accurate as repeated resource measurements on all plant individuals, our approach is more efficient and offers the unique opportunity to estimate the spatial and temporal variability in floral resources among and within plant communities. The consumer-resource relationships detected by our analysis corroborate the strength of this trait-based approach for studying plant-animal interactions in natural communities. Here, we have shown that variation in floral resource availability among sites influences the population density of nectarivorous birds and that within-site variability in floral resources shapes variation in pollinator visitation among plants. The effects of neighbourhood resources on per-plant visitation rates of pollinators varied across scales, suggesting competition between plants for pollinators at large scales and both facilitation and competition at small scales. Furthermore, the interacting effects of individual and neighbourhood resources on visitation rates underline the importance of integrating individual resource variability into resource-based pollination studies. Future studies of resource effects on animal pollinators should therefore account for the scale- and context-dependence of consumer-resource relationships in species-rich communities.

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Supplementary Material

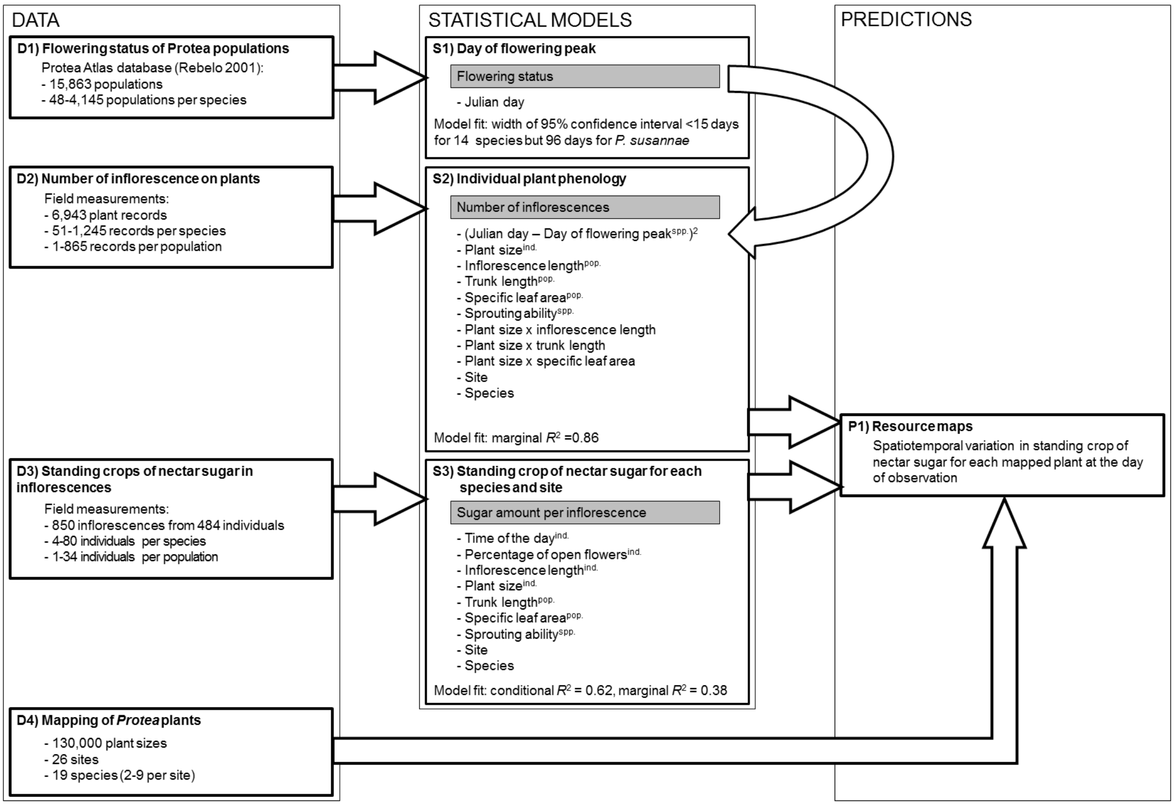

Figure S1: Detailed procedure used to predict the resource maps from statistical models (S) fitted to datasets (D). We used data on the flowering status of *Protea* populations in the study region (D1, from the Protea Atlas database, Rebelo 2001) to predict the day of flowering peak for each species (S1). Observations of the number of inflorescences on individual plants in the study sites (D2) served to predict individual plant phenology (S2). These analyses included the squared temporal distance between the day of inflorescence observation and the day of flowering peak. Data on standing crops of nectar sugar in inflorescences on individual plants in the study sites (D3) served to predict standing crop of nectar sugar per inflorescence for each species and site (S3). For each mapped plant (D4) and the days of bird observation, we predicted the number of inflorescences multipled by the standing crop of nectar sugar per inflorescence. This yielded spatially-explicit predictions of “resource maps” (P1) for each day of bird observation. For each statistical model, we give the response variable (grey shaded area), the explanatory variables at individual ($^{ind.}$) population ($^{pop.}$) and species ($^{spp.}$) levels, and we provide measures of model quality (R^2 and width of 95% confidence intervals, respectively).

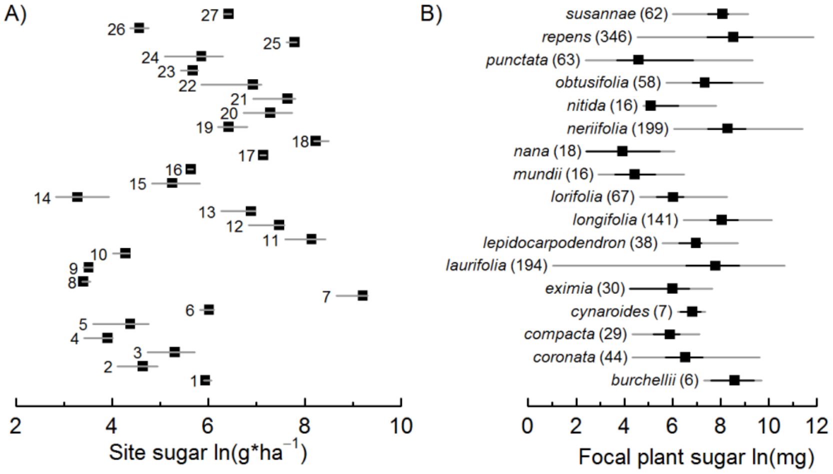

Figure S2: Resource variables. Panel A) shows the temporal variation of site sugar (g*ha^{-1}, log-transformed) for each site on three visits (2-5 weeks interval in between visits); median site sugar (points) and the range across the three visits (grey lines) are given. Panel B) shows the distribution of focal plant sugar (mg, log-transformed) for each *Protea* species with the range (grey lines), first/third quartiles (black lines) and median (point); species name and number of focal plants per species are given above each line. Focal plant sugar was the product of the observed number of inflorescences and the predicted sugar amount per inflorescences for each species.

Table S1: Pearson correlations of site sugar and r m-neighbourhood sugar with r = 1, 2, 5, 20 or 40 m.

	40 m	20 m	10 m	5 m	2 m	1 m
Site sugar	0.83	0.70	0.58	0.47	0.30	0.19
40 m	-	0.89	0.75	0.62	0.43	0.28
20 m		-	0.90	0.76	0.54	0.37
10 m			-	0.88	0.64	0.44
5 m				-	0.75	0.52
2 m					-	0.71
1 m						-

Chapter 3

Reward quality predicts effects of bird-pollinators on plant reproduction in African *Protea* spp.*

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Abstract

Pollination syndromes predict the principal animal pollinators of plant species from floral traits. However, the existence of discrete pollination syndromes and the relevance of these syndromes for the effects of animal pollinators on plant reproduction are controversial and a quantitative, trait-based approach may refine the concept of pollination syndromes. Thus far, however, very few studies have simultaneously analysed the importance of signalling, accessibility and reward traits of flowers for the effects of animal pollinators on plant reproduction of multiple species. The genus *Protea* is an ideal study system because the members of this genus exhibit remarkable variation in functional traits and *Protea* species are visited by several guilds of animal pollinators, such as birds and insects. We experimentally investigated seven *Protea* species from the South African fynbos to assess variability in reproduction (seed set, seed mass, germination) in response to pollinator exclusion (full-exclusion, bird-exclusion) and hand-pollination (self-pollen, cross-pollen). To generalize the findings of the experiment, we conducted a meta-analysis of the effects of bird-exclusion on seed set of 14 *Protea* species, associating effect sizes of bird-pollination with signalling (inflorescence length and diameter), accessibility (inflorescence and style length) and reward traits (length of pollen presenter, nectar volume and sugar concentration). All *Protea* species were self-compatible, but only two species were to a small degree capable of autonomous selfing. *Protea* species varied in their dependence on animal pollinators, including species depending exclusively on birds, insects, or both for seed production. *Protea* species exhibited continuous variation in the effects of bird-pollinators on reproduction, challenging the accuracy of categorical pollination syndromes for predicting pollinator effects on plant reproduction. The meta-analysis showed that the quality of floral rewards, especially nectar sugar concentration, predicts the dependence of *Protea* reproduction on bird pollinators, whereas the signalling and accessibility traits tested in this study were not important. Beyond revealing new insights into the pollination biology of *Protea* species, our findings underscore the usefulness of experimental approaches on multiple plant species to test the effects of pollinator guilds on plant reproduction and contribute to a trait-based understanding of the functional determinants of plant-pollinator interactions.

Introduction

Many plant species have evolved floral traits adapted to specific groups of animal pollinators (Faegri and Van der Pijl, 1966; Schiestl and Johnson, 2013). Suites of floral traits might thus enable the prediction of guilds of animal pollinators that are associated with particular plant species, as formulated by the pollination syndrome hypothesis (Faegri and Van der Pijl, 1966). Even though the pollination syndrome hypothesis has received much support over the past decades (Fenster et al., 2004; Johnson, 2013; Rosas-Guerrero et al., 2014), the accuracy of this hypothesis for predicting pollinator effects on plant reproduction is controversial (Johnson and Steiner, 2000; Ollerton et al., 2009). While the pollination syndrome hypothesis proposes a qualitative and nomenclatural approach to classify plant-pollinator interactions, a quantitative, trait-based approach that accounts for the phylogenetic relatedness among plant species may help to refine and revitalize the classical concept of pollination syndromes (Rosas-Guerrero et al., 2014).

Because flower visitors greatly vary in their efficiency to pollinate (King et al., 2013), the pollination syndrome hypothesis proposes that flowers should adapt their traits to the most frequent and efficient pollinators (Stebbins, 1970) that contribute most to plant reproduction. Rather than quantifying the number of floral visits, it is therefore crucial to quantify pollinator effects on plant reproduction, notably on seed set and seed quality (King et al., 2013). In cases where pollinator guilds differ in body size or activity periods, their effects on plant reproduction can be estimated experimentally by selective exclusion (Kearns and Inouye, 1993). Exclusion experiments on multiple plant species with differences in functional traits, thus, allows to test which traits mediate the effects of particular pollinators on plant reproduction. However, studies with exclusion experiments on multiple species have mostly been qualitative or have only used categorical measurements of traits (Hargreaves et al., 2012; Lange et al., 2000; Liu and Huang, 2013; Steenhuisen et al., 2012b). It is therefore timely to quantify the relationship between continuous floral traits and pollinator effects on plant reproduction, i.e. seed set, seed mass and germination, across an array of related species with diverse floral traits.

Pollination biologists face the challenge to identify plant traits that mediate the effects of animal pollinators on plant reproduction. For instance, the pollination syndrome hypothesis predicts that plant species that exhibit vivid floral displays, emit little scent and contain large quantities of diluted nectar are primarily bird-pollinated (Brown et al., 2011; Cronk and Ojeda, 2008; Johnson and Nicolson, 2008), whereas flowers that produce easily accessible, odour-rich nectar and generous pollen rewards are primarily insect-pollinated (e.g. Steenhuisen and Johnson, 2012a). The attraction of particular pollinators and the exclusion of others occur in two stages that are mediated by different sets of plant traits. The first array of traits is associated with the approach of pollinators to floral resources. These traits include *signalling traits* to attract animal pollinators, like the size of floral display (Nattero et al., 2011), colour (Hopkins and Rausher, 2012), and scent (Wright and Schiestl, 2009). This array of traits also includes morphological *accessibility traits* that determine the ease of pollinator access to the floral resources, such as the depth to the nectaries (Castellanos et al., 2004; Stang et al., 2009; Thompson et al., 2013). The second array of traits relates to plant rewards for animal pollinators. *Reward traits*, especially pollen and nectar, positively influence visitation rates and duration of pollinator visits (Cresswell, 1999; Kudo and Harder, 2005; Leiss and Klinkhamer, 2005), increase pollen deposition by pollinators and may ultimately increase plant reproduction (Bolstad et al., 2010; Hodges, 1995; Thomson and Plowright, 1980).

In this study, we investigated the importance of animal pollination (birds and insects) in *Protea* species (Family Proteaceae) and experimentally tested the association between floral traits and the effects of avian pollinators on plant reproduction. In the Cape Floristic Region in southern Africa, the genus *Protea* includes many ecologically and economically important species (Schurr et al., 2012). It is an ideal study system because *Protea* species exhibit remarkable variation in floral traits and are visited by different guilds of animal pollinators, such as birds (Hargreaves et al., 2004; Wright et al., 1991), insects (Coetzee and Giliomee, 1985; Johnson et al., 2012; Steenhuisen et al., 2012b) and mammals (Biccard and Midgley, 2009; Wiens and Rourke, 1978). Nectarivorous birds are frequent visitors on tall-growing *Protea* shrubs producing large, scent-less inflorescences offering diluted nectar (Collins and Rebelo, 1987; Hargreaves et al., 2004; Wiens et al., 1983). While birds are generally considered to be the main pollinators of these species (Steenhuisen et al., 2012a), some *Protea* species produced seeds when bird-pollinators were excluded from inflorescences (Coetzee and Giliomee, 1985; Wright et al., 1991), questioning the general importance of bird pollinators for *Protea* reproduction (Steenhuisen and Johnson, 2012a, 2012b).

In order to identify floral traits that drive the effect of bird pollinators on *Protea* reproduction, we selectively excluded bird-pollinators from inflorescences of seven *Protea* species and quantified effects of bird pollinators on plant reproduction (seed set, seed mass, germination). Additional self- and outcross-treatments served to measure how plant reproduction depends on pollen transfer by animal pollinators. To generalize our findings, we conducted a meta-analysis comprising a total of 14 *Protea* species to test whether the effect of bird-pollinators on reproduction is primarily associated with signalling, accessibility or reward traits.

Methods

Study system

The genus *Protea* has a centre of diversity in the Cape Floristic Region (CFR) in South Africa. We selected seven overstorey *Protea* species present in the CFR (*P. laurifolia*, *P. magnifica*, *P. eximia*, *P. compacta*, *P. repens*, *P. punctata*, *P. longifolia*). All species are thus far assumed to be primarily bird-pollinated (Steenhuisen et al., 2012a), but require a reassessment of their breeding system (see Steenhuisen and Johnson, 2012b and references therein). Flowers of *Protea* species are grouped in large inflorescences surrounded by involucral bracts (Rebelo, 2001). Flowers are proteandrous: when the anthers dehisce, pollen is deposited on the upper part of the stigma (the area designated as pollen presenter) and the stigma tip becomes receptive to pollen after two to three days (Steenhuisen and Johnson, 2012b). Within an inflorescence, flowers open sequentially from the outer to the inner circles and thus flowers in male phase (pollen donor) and female phase (receptive to pollen) co-occur within an inflorescence. The abundant nectar produced often form a pool of nectar at the bottom of the inflorescence (Steenhuisen and Johnson, 2012a). Each flower can develop a single hard-coated seed and the presence of endosperm distinguishes fertilised from unfertilised seeds.

Our study area extended from Bainskloof Pass (33°37'S, 19°06'E) in the north-west to Gansbaai (34°36'S, 19°30'E) in the south-east of the Western Cape region of South Africa. The multispecies experiments were conducted from May to September 2011 on 15 study sites with different *Protea* communities. During the flowering season, we mostly observed two pollinating bird species on the study sites: Cape sugarbird (*Promerops cafer)* and orange-breasted sunbird (*Anthobaphes violacea)*. In addition to nectarivorous birds (Rebelo, 1987; Skead, 1967), several insect guilds have been reported to visit or reside in inflorescences of the studied *Protea* species, including bees (e.g. Cape honey bee *Apis mellifera capensis*), butterflies (e.g. mountain pride butterfly *Aeropetes tulbaghia*), flies (e.g. nemestrinid fly *Prosoeca longipennis*), ants (Collins and Rebelo, 1987; Lach, 2013) and beetles [e.g. cetoniine beetles, Familiy Cetoniinae, such as the protea beetle *Trichostetha fascicularis* (Cetoniini) or smaller monkey beetles (Hopliini); Collins and Rebelo, 1987; Johnson et al., 2012; Mostert et al., 1980].

Pollination treatments

We carried out three different experimental treatments: A) pollinator exclusion experiments, B) hand-pollination experiments and C) resource re-allocation controls (Fig. 1). For each species we selected groups of three plant individuals of similar size growing in the same neighbourhood and randomly assigned each plant to one of the three treatments (hereafter referred to as 'exclusion plants', 'hand-pollination plants' and 'resource re-allocation plants'). The different *Protea* species were studied on one to five sites per species. Depending on the availability of flowering individuals, we selected 6-18 plant individuals per site and species (1-5 sites per species; for exact sample sizes of inflorescences, see Fig. 2).

The exclusion plants were used to test the effect of bird pollination and autonomous-selfing on seed production. On each exclusion plant, we randomly assigned (i) one inflorescence to the bird-exclusion treatment using a green plastic cage (20 x 20 mm mesh size) that excluded birds, but not small-sized insects, (ii) one inflorescence to the full-exclusion treatment using a bag (1 x 1 mm mesh, reinforced by a wire to avoid contact with the stigma) that excluded all pollen vectors (birds and insects, irrespective of their size), and (iii) two inflorescences that were tagged but remained open and accessible to pollinators ('control treatment'). The cages or bags were installed on the inflorescences prior to the onset of flowering. Bird-exclusion and control inflorescences of a species flowered during the same time period at a given site. Visual inspection of flowering inflorescences during the study revealed no differences in insect occurrence between bird-exclusion and control treatments. Butterflies were rare in the rainy winter season during which the experiments were conducted. The largest beetles (Cetoniine) that were observed in the inflorescences did not exceed 10 mm of body width and about 20 mm of body length and were able to pass through the bird-exclusion cages.

On each hand-pollination plant, we randomly assigned (i) one inflorescence to a self-treatment, using pollen originating from the same bagged inflorescence or closed flowers of another inflorescence of the same plant (flowers were opened with a slight pressure on the anthers), (ii) one inflorescence to a cross-treatment, using a pool of pollen originating from 3 conspecific plants situated on the same study site but at least 10 m away from the focal plant, and (iii) two inflorescences that were tagged, but remained open and accessible to pollinators ('control treatment'). All hand-pollinated inflorescences were bagged (1 x 1mm mesh size, as above) to exclude all pollinators prior to the onset of flowering. Self- and cross-pollen were collected in clean petri-dishes using toothpicks and were applied with a paint-brush within two hours of collection. Immediately before the application of cross-pollen, the self-pollen of cross-pollinated inflorescences was removed with toothpicks and cotton-buds (Figure 1C). Because *Protea* flowers open sequentially from the edge to the interior of the inflorescence (Rebelo 2001), hand-pollinations were repeated weekly during the two to four week flowering period of an inflorescence. Hand-pollination experiments comprised the same species as the exclusion experiments except *P. eximia* where hand-pollination failed due to plant mortality and cone predation.

The experimental modification of seed set (through exclusion or hand-pollination experiments) can induce resource re-allocation within an individual, from inflorescences with experimentally reduced pollination success towards inflorescences with experimentally increased pollination success. In fact, inflorescences with a high number of fertile ovaries may monopolise energy reserves originally allocated to inflorescences with few fertile ovaries (Wesselingh, 2007; Zimmerman and Pyke, 1988). Our experimental design therefore included unmanipulated resource re-allocation plants, on which we randomly tagged two inflorescences ('control treatment'). Effects of resource re-allocation can be quantified by comparing the seed set of control treatments between control inflorescences of resource re-allocation plants and manipulated exclusion and hand-pollination plants.

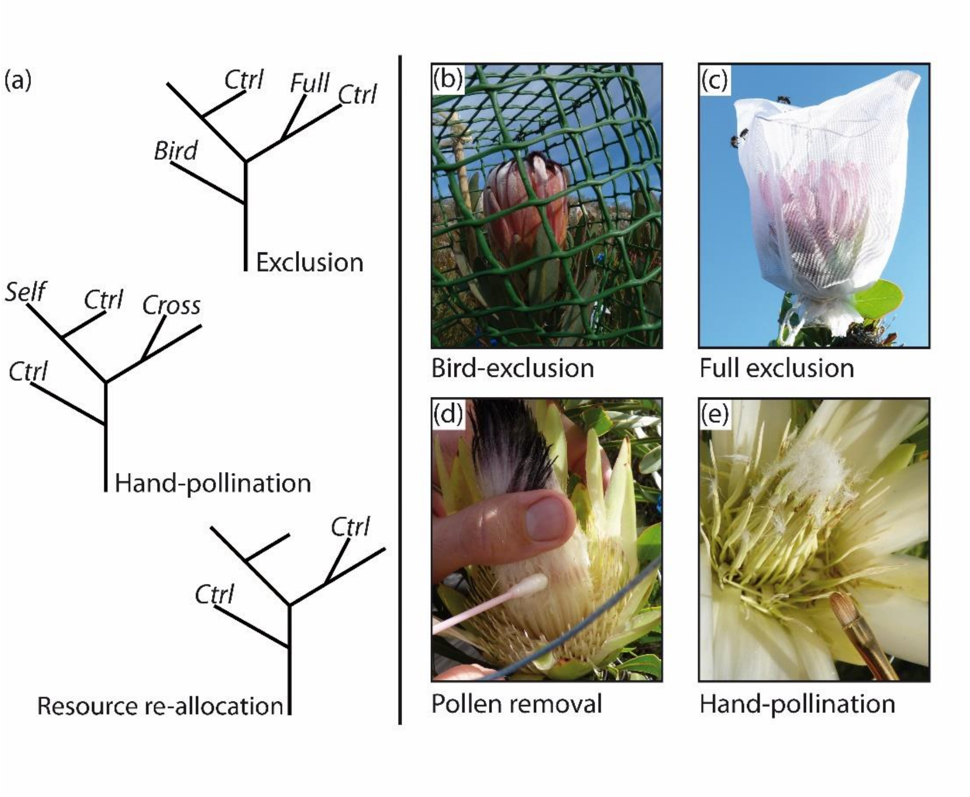

Figure 1: Illustration of the experimental design. (a) Experimental treatments were Bird: bird-exclusion; Full: full-exclusion of flower visitors; Ctrl: unmanipulated control on exclusion plants; Self: self-hand-pollination; Cross: cross-hand-pollination; Ctrl: unmanipulated control on hand-pollination plants and two Ctrl: unmanipulated controls on resource re-allocation plants. Pictures illustrate the (b) bird-exclusion on *P. laurifolia*, (c) full-exclusion treatment on *P. compacta*, (d) pollen removal before cross-hand-pollination on *P. longifolia* and (e) hand-pollination on *P. repens*.

Seed set and seed quality

Infructescences were harvested in March 2012, at least six months after the end of the experiment. The infructescences were then oven-dried at 70°C for 24 hours to trigger seed release. Seed set was defined as the proportion of fertile seeds in respect to the total number of non-preyed seeds (fertile plus infertile seeds). Fertile seeds were distinguished from infertile seeds by their plump appearance due to the presence of endosperm and by the smooth texture of the seed coat. The predators found in the infructescences were determined at family level. We found three Coleoptera families (Cerambycidae, Nitidulidae and Tenebrionidae) and two Lepidoptera families (Cossidae and Pyralidae). The predation rate was defined as the number of preyed seeds divided by the total number of seeds (fertile plus infertile plus preyed seeds, equalling the total number of flowers per inflorescence); the fertility of preyed seeds could not be defined. We observed no sign of predation in 265 out of the 829 infructescences (32.0%), whereas 30 infructescences (3.6%) were preyed upon 100% and were excluded from further analyses. We controlled for differences in predation rates among infructescences in the statistical analyses.

We used seed mass and germination rate as indicators of seed quality between treatments within species. Up to 30 fertile seeds per infructescence, depending on availability, were evenly distributed in one to three petri-dishes, each containing one to 10 seeds (1 to 5 seeds were pooled in one petri dish, 6 to 20 seeds were distributed in two petri-dishes, 21 to 30 seeds were distributed in three petri-dishes). We manually removed the hairs from the seeds and weighed the seed pool of every petri dish. As a measure of seed mass, we divided the mass of all seeds by the number of seeds in each petri dish. To reduce bacterial and fungal infections we placed the seeds briefly in an ethanol (EtOH) and a chloride solution [1 min 96% EtOH, 3 min 1.5%-chloride solution, 3 sec in 96% EtOH, 5 min double distilled water ($_{dd}H_2O$); (Mueller et al., 2004)]. Smoke is known to trigger the seed germination of many fire-prone serotinous plant species, such as *Protea* (Van Staden et al., 2000), but no smoke treatment was necessary for the studied species (Brown and Botha, 2004). The seeds were placed on filter papers humidified with doubly-distilled water, arranged at least one centimetre apart from each other. The petri dishes were randomly placed in a growth cabinet under alternating temperatures of 10 / 20°C and alternating 14h-dark / 10h-light daily cycles. We recorded radical emergence weekly. We quantified germination rate per petri dish as the proportion of seeds for which the radical emerged during the 16 week observation period. Very few *P. repens* and *P. magnifica* seeds germinated and these two species were not included in the analyses. At the end of the germination trials, we cut open the non-germinated seeds of all species to check for the presence of endosperm and whether non-germinated seeds were falsely recorded as fertile seeds at first assessment. We recorded the number of seeds without endosperm (*false* fertile seeds) and controlled for this by correcting the number of fertile seeds in the analyses of seed set and germination and by an additional covariate in the analysis of seed mass.

Floral traits and bird-pollination

To test whether the effect of bird-pollination on seed set can be explained by plant traits, we compiled published and unpublished information on bird-exclusion experiments and functional traits for 14 *Protea* species (all data and references available in Table S2, supporting information). We used seed set from control and bird-exclusion treatments from this study (n = 7 species) and from published data (n = 7 species), including species from Fynbos and grassland habitats, to estimate the effect size of bird pollination on plant reproduction in the year of the respective experiment. In contrast to the Fynbos species that are considered to be primarily pollinated by birds, seed production of grassland *Protea* species depend mostly on beetles (Steenhuisen and Johnson, 2012c; Steenhuisen et al., 2012a). The 14 *Protea* species represented a large fraction of floral trait variation in overstorey *Protea*, with the exception of the small globose inflorescences of “rose sugarbushes“ (e.g. *P. accuminata*). For the 14 species, we further gathered information on signalling, accessibility and reward traits. As signalling traits, we considered the length and diameter of the inflorescence because inflorescence size influences the visibility of floral structures for animal pollinators (Kugler, 1943; Mulligan and Kevan, 1973). We did not measure inflorescence colour because of intra-specific colour polymorphisms in several *Protea* species and the absence of bird preferences for specific colours in polymorphic populations (Carlson and Holsinger, 2010), making it unlikely that inflorescence colour is a crucial trait mediating pollinator effects in this system. Due to incomplete data and methodological constraints, we also could not consider floral scent in this study, despite its importance for the attraction of beetles (Steenhuisen et al., 2013, 2012a). Accessibility traits were given by inflorescence length and style length. Both variables describe the depth of the nectaries and are associated with the accessibility of nectar rewards for pollinators. Reward traits were pollen quantity, nectar quantity and nectar sugar concentration. Due to incomplete data and methodological constraints, we also could not consider the chemical composition of the nectar in this study, despite its importance for the diet of animals (Nicolson and Wyk, 1998). The length of the pollen presenter was measured as the length of the portion of the style where anthers shed pollen which is a good measure for interspecific variation in pollen production among Proteaceae (Turner, 1984). Pollen quantity may attract insect pollinators and thus also influence the relative importance of bird pollination for the respective species. Nectar volume (“standing crop”) was measured by centrifuging the inflorescences (in the case of Fynbos species; H. Nottebrock, University of Potsdam, Germany, unpublished data) or by sampling a few individual flowers with calibrated micropipettes and multiplying the averaged nectar volume by the number of flowers (in the case of grassland species; Steenhuisen and Johnson, 2012a). Because between-species differences in nectar volume are very large (see Fig. 3e), both methods are accurate for resolving between-species differences although the nectar volumes derived from a subsample of open flowers may overestimate the nectar volume of the inflorescence since not all flowers produce nectar at the same time. Data on nectar production rates were not available for all studied species, but nectar production rates and standing crop of nectar are usually closely correlated (Cresswell and Galen, 1991; Zimmerman, 1988), especially in species with large, rarely depleted nectar crops, such as *Protea*. Nectar sugar concentration (w/w) was determined with handheld refractometers in all studies. The product of nectar sugar concentration (transformed into weight per volume) and nectar volume gave the sugar amount (g) per inflorescence. All floral traits were measured on numerous individuals per *Protea* species to ensure that among-species differences were captured accurately. Traits were correlated to varying degrees (Table S3). The strongest correlations were found between inflorescence size and nectar volume ($n = 14$ species; length, $r = 0.73$; diameter, $r = 0.75$) and sugar amount ($n = 14$ species; length, $r = 0.85$; diameter, $r = 0.47$). Sugar concentration was rather weakly correlated to all other traits ($n = 14$ species; $r < 0.56$ in all cases; Table S3, supplementary information).

Data analyses

All statistical analyses were performed with R (version 3.1.0, R Core Team, 2014). We tested the effects of experimental treatments on seed set (angular-transformed), seed mass (log-transformed) and germination rate (angular-transformed) with linear-mixed-effect models as implemented in the package "lme4" (Bates et al., 2014). Small sample size corrected Akaike information criterion (AICc) was used to compare models that included pollination treatments (exclosure treatments or hand-pollination treatments, respectively, including their main effect and interaction term with species identity) with reduced models omitting these two treatment effects. All models always included species identity and a different set of covariates as fixed effects. For models of seed set, we always included predation rate as covariate and individual identity nested in study site as random intercepts. For models of seed mass, we included the ratio between cut-open seeds without endosperm and all seeds in the petri dish as covariate and inflorescence identity nested in individual identity and study site as random intercepts. For the models of germination rate, we included no covariates but inflorescence identity nested in individual identity and study site as random intercepts. Since only few seeds were produced under the full-exclusion treatment, this treatment was not included in the analyses of seed mass and germination rates.

For the meta-analysis, we combined the result from our study with published data on exclusion experiments [all data and references available in Table S2, supporting information]. We included all other published experiments on overstorey *Protea* species with the exception of experiments conducted on *P. roupelliae* because of lack of data (missing data were number of fertile and non-fertile seeds as well as standard deviations of means, Hargreaves et al., 2004). We focused on measurements of seed set because measurements on seed mass and germination rates were not equally available from previous studies. To quantify the effect of bird pollination on plant reproduction, we computed unbiased Hedges' *d* as the standardized difference in the means (using mean number of fertile seeds, its standard deviation and sample size, i.e. number of inflorescences, as indicated in Table S2) of the two groups (control and bird-exclusion treatments). We additionally computed the log odds ratio (i.e. the ratio between the number of fertile and the sum of fertile and non-fertile seeds, as indicated in Table S2) of the two groups. Since experimentally treated inflorescences rather than individual seeds represent the independent replicates in these studies, we consider comparisons of Hedge's *d* more appropriate for this meta-analysis. Nevertheless, we related Hedges' *d* and log odds ratio to the respective floral traits (inflorescence length, inflorescence diameter, style length, length of the pollen presenter, nectar volume and nectar sugar concentration) in a formal meta-analysis that was performed with the R-package "metafor" (Viechtbauer, 2010). We fitted mixed effects models, accounting for random unobserved variation among studies, and additionally included random effects of species identity and a correlation matrix of pairwise phylogenetic distances among *Protea* species, using the phylogeny by Schnitzler et al. (Schnitzler et al., 2011), to account for potential phylogenetic effects. We computed parameter estimates with a restricted maximum likelihood approach (Gurevitch and Hedges, 2001) and applied Wald's Chi^2-test to assess whether the variance explained by the floral trait is greater than the residual variance.

Results

Pollination experiments

Full-exclusion of pollinators strongly reduced seed production in all species (Fig. 2a, Table S1, supporting information). Only *P. compacta* and *P. repens* regularly produced a few seeds under full-exclusion (Fig. 2a). Exclusion of bird-pollinators affected *Protea* seed set and these effects differed among *Protea* species (Fig. 2a, Table 1, Table S1, supporting information). Bird exclusion reduced seed set in *P. laurifolia*, *P. magnifica, P. eximia, P. compacta* and *P. repens*, but *P. compacta* and *P. repens* were still able to produce numerous seeds without bird-pollinators. Seed set was not reduced by bird exclusion in *P. longifolia* and *P. punctata* (Fig. 2a). We observed no significant effect of bird-exclusion treatments on seed mass and germination rate (Table 1).

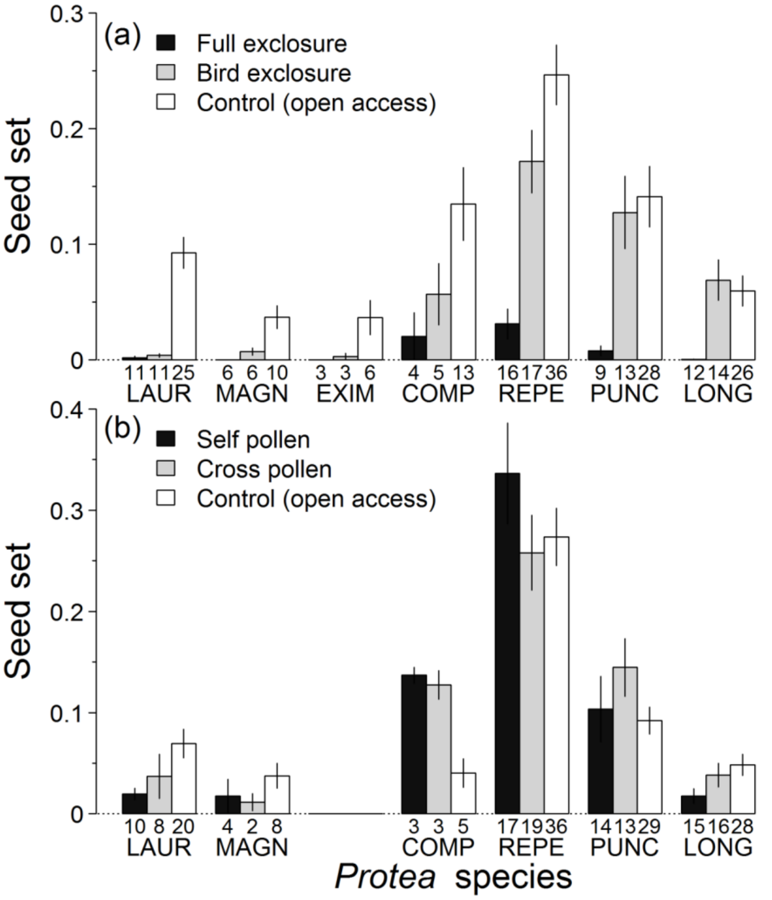

Figure 2: Effects of (a) pollinator exclusion and (b) hand-pollination on seed set (i.e. the proportion of fertile seeds in respect to total number of non-preyed seeds) of *Protea* species. Bars and whiskers indicated mean values ± standard error, numbers below each bar indicate the respective sample size (number of inflorescences). Species acronyms are LAUR: *P. laurifolia*, MAGN: *P. magnifica*, EXIM: *P. eximia*, COMP: *P. compacta*, REPE: *P. repens*, PUNC: *P. punctata*, LONG: *P. longifolia*. Tests statistics of the species-specific effects of exclusion experiments on seed set (a) are presented in Supplementary information.

Table 1: Effects of pollinator exclusion (EX), hand-pollination (HP) and resource re-allocation (RA) on seed set, seed mass and germination rate of *Protea* species. Models including treatment effects were compared against reduced models including only species identity and model-specific covariates, but omitting the respective treatment effects. Differences between treatment models and reduced models are indicated by ΔAICc-values for (A) main effects of treatments and (B) main and interaction effects of treatments with species identity. ΔAICc-values which were smaller than –2 indicate substantial effects of the respective treatment and are highlighted in bold.

	Seed set			Seed mass			Germination rate		
	EX	HP	RA	EX*	HP	RA	EX*	HP	RA
A) Treatment	-82.7	11.9	11.3	2.6	12.7	10.7	2.6	8.3	7.5
B) Treatment × species identity	-47.6	45.4	35.0	14.7	44.0	24.9	2.2	17.3	6.8

All models used Gaussian error structure and maximum likelihood parameter estimation.

* Seeds from full-exclusion treatments were not included in this model.

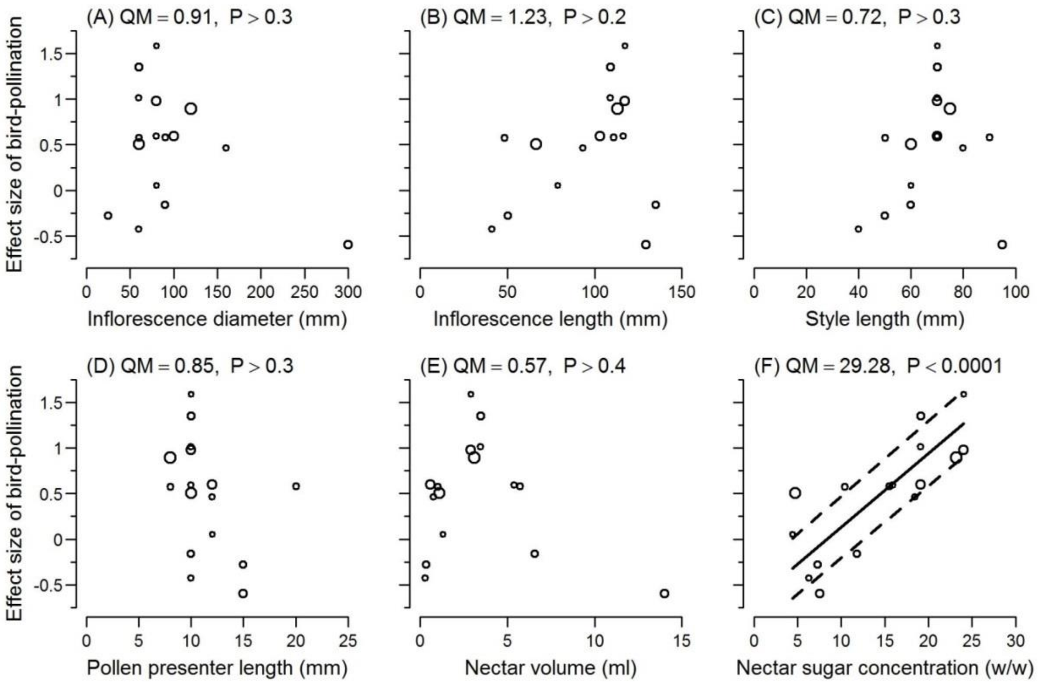

Figure 3: Phylogenetically controlled relationships between plant traits and the effect size of bird-pollination on seed set of 14 *Protea* species. Effect size is measured as Hedges' *d* comparing the number of fertile seeds relative to the total number of seeds between open-pollination and bird-

exclusion treatments. We show the test of the respective floral trait (Q_M) assuming normally distributed effect sizes. The model prediction and the 95% confidence intervals are shown for the significant effect of nectar sugar concentration on the effect size of bird pollination.

Experimental hand-pollination with different pollen sources (self- vs. cross-pollen) did not influence any of the three measures of reproduction (Table 1). Only *P. compacta* produced more seeds with hand-pollination treatments than in the control treatment (Fig. 2b). We did not find differences in reproduction between plants treated experimentally and plants that served as a control for resource re-allocation (Table 1).

Plant traits and bird pollination

In the phylogenetically controlled meta-analysis of the relationship between effects of bird-pollination on seed set (Hedges' *d*) and floral traits, the effect size of bird-pollination increased with nectar sugar concentration (test of moderator: $Q_M = 29.3$, $P < 0.0001$; residual heterogeneity: 26.2, $P = 0.02$; Table 2; Fig. 3f). Relationships between the effect size of bird-pollination and all other traits were not significant ($Q_M < 1.23$, $P > 0.2$; Table 2; Fig. 3a-e). Sugar amount per inflorescence was also not statistically associated with the effect size of bird-pollination on seed set ($Q_M = 0.8$, $P > 0.3$; Table 2). Results were qualitatively identical in the analysis of log odds ratios instead of Hedges' *d* (results details in Table 2 and Figure S1).

Discussion

We found continuous variation in the dependence of *Protea* species on animal pollinators and a close association between the effect of avian pollinators on plant reproduction and nectar sugar concentration. These results underscore the usefulness of experimental approaches on multiple plant species to test the effects of different pollinator guilds on plant reproduction and contribute to a trait-based understanding of the functional determinants of plant-pollinator interactions. Multispecies experiments in a range of taxa will aid to develop the classic hypothesis of categorical pollination syndromes into a trait-based concept describing the continuous variability in the effects of animal pollinators on plant reproduction. Below, we briefly summarize new insights into the pollination biology of *Protea* species and discuss the importance of these findings for pollination ecology.

Pollination biology of Protea

Hand-pollination showed that pollen source (self- or cross-pollen) had very weak effects on reproduction, as measured by seed set, seed mass and germination. In addition to previous and often ambiguous studies of *Protea* breeding systems (Horn, 1962 , criticised in Steenhuisen and Johnson, 2012c), our study provides the first evidence of self-compatibility in *P. laurifolia*, *P. magnifica*, *P. compacta*, *P. punctata* and *P. longifolia*. This contradicts earlier findings that suggested low incidence of self-compatibility, e.g. in *P. compacta* and *P. longifolia* (Horn, 1962 ; but see van der Walt, 1995). Many *Protea* species thus show a high flexibility to the origin of deposited pollen. As suggested by Steenhuisen and Johnson (2012b), our results demonstrate that shifts to self-compatibility are hence not restricted to *Protea* species that occur outside the Cape Floristic Region. The high selfing capability and the absence of inbreeding depression until germination suggest a rather high tolerance of *Protea* species against isolation from conspecifics. Due to the low autonomous selfing capability observed in our study, *Protea* individuals will nonetheless require animal pollinators to set seed.

Our results provide evidence for continuous variation in the effects of bird-pollination on seed set in *Protea* species. This corroborates other studies that found continuous variation in the effects of particular groups of animal pollinators on plant reproduction (e.g. Smith et al., 2008). Of the species studied in our experiment, three were dependent on bird-pollination, as indicated by strongly reduced seed set under bird-exclusion (*P. laurifolia*, *P. magnifica*, *P. eximia*). Our results confirm the dependence of *P. magnifica* on bird-pollination and are in line with the low seed set of *P. laurifolia* under bird-exclusion (Wright et al., 1991). Four species produced a substantial number of seeds under bird-exclusion. The very low degree of autonomous selfing further ascertained the contribution of insects to pollination in these *Protea* species (*P. compacta*, *P. longifolia, P. punctata*, *P. repens*). Our results, thus, confirm earlier findings that some overstorey *Protea* species from the Cape Florisitc

Region depend mostly on insect pollination (Coetzee and Giliomee, 1985). In addition to beetles (Steenhuisen and Johnson, 2012a), long-proboscid insects are also suggested to effectively pollinate particular *Protea* species, such as *P. punctata* (Johnson et al., 2012). This is consistent with the high seed set of *P. punctata* under bird-exclusion. Rodents were also excluded by the bird-exclusion treatment, but their general role as pollinators for the studied overstorey species is uncertain. Thus far, only anecdotal observations of nectar feeding events have been reported [striped mouse *Rhabdomys pumilio* on *P. longifolia* (personal observations) and on *P. obtusifolia* (J. Carlson, Nicholls State University)]. Our study provides experimental evidence for a high incidence of self-compatibility in *Protea* species and continuous variation in the dependence of *Protea* reproduction on bird-pollination, with a number of species exhibiting mixed bird-insect pollination systems.

Floral traits and bird-pollination

Our study highlights the importance of reward traits for predicting effects of animal pollinators on plant reproduction. Although nectar quantity in *Protea* inflorescences can increase the duration of avian visits (Carlson and Holsinger, 2013), interspecific variation in nectar reward quality, rather than in nectar quantity, was statistically associated to the effect size of bird-pollination on plant reproduction. Interspecific variability in nectar rewards is often associated with the physiological constraints and energetic needs of the plants' primary pollinators (Bolten and Feinsinger, 1978; Hainsworth and Wolf, 1976; Odendaal et al., 2010; Wolf et al., 1972). Accordingly, the evolution of reward traits has been associated with shifts in pollinator guilds (reviewed in Fenster et al., 2004). Since we controlled our meta-analysis for the phylogenetic relatedness among *Protea* species, it is likely that the association between nectar sugar and bird pollination has evolved by trait convergence in several *Protea* lineages. In general, the studied *Protea* species never exceeded 25% of nectar sugar concentration, which is consistent with the observation that bird-pollinated plants offer rather diluted nectar compared to most insect-pollinated plant species (Pyke and Waser, 1981; Wiens et al., 1983). *Protea* species that offer nectar sugar exceeding 25% of concentration are probably primarily pollinated by mammals (Wiens et al., 1983). The lack of bird-pollination for *Protea* species at the lower range of sugar concentrations (<10%) is an interesting and unexpected finding. Several plant species that offer nectar with low sugar concentration are pollinated by opportunistic bird pollinators (Johnson and Nicolson, 2008; Martén-Rodríguez et al., 2009). However, opportunistic bird pollinators, such as the Cape weaver *Ploceus capensis* in the *Protea* system (Odendaal et al., 2010), were also excluded by the cages and did not contribute to the pollination of these species. Therefore, it is likely that *Protea* species with low sugar concentration were primarily pollinated by beetles, especially by the numerous hopliine beetles. Beetle-pollination for *Protea* species offering low sugar concentration has been previously demonstrated for grassland *Protea* species that were also included in our meta-analysis (Steenhuisen and Johnson, 2012a). In these *Protea* species, beetles consume large amounts of pollen and might depend less on nectar resources (Steenhuisen and Johnson, 2012a). In general, the role of beetle pollination appears to be largely underestimated and might be of great ecological importance in the Cape Floristic Region.

We observed no significant relationships between the effect size of bird-pollination and the signalling and accessibility traits tested in this study. Although we could not formally test the effects of inflorescence colour and scent, it is unlikely that these signalling traits were related to the observed variability in bird effects on *Protea* reproduction (Carlson and Holsinger, 2010). We can advance some, non-exhaustive explanations for the weak relationships between signalling and accessibility traits and bird-pollination. First, signalling and accessibility traits might have evolved in adaptation to other evolutionary drivers (Smith et al., 2008). For instance, the accumulation of red pigments in particular plant tissues may primarily function as predator repellent (Carlson and Holsinger, 2010; Pellmyr and Thien, 1986). Second, because we restricted our meta-analyses to closely related, overstorey *Protea* species, our meta-analyses did not encompass the full range of floral trait values in the Fynbos flora. The investigation of a greater diversity of plant species may shed more light into importance of signalling and accessibility traits in mediating plant-pollinator interactions. Our study nonetheless stresses the importance of the quality of floral rewards in determining the strength of interactions between bird-pollinators and plants. This is in line with the findings of early studies on plant-hummingbird interactions (Hainsworth and Wolf, 1976; Pyke and Waser, 1981) and calls for a reconsideration of these seminal papers in current studies of trait-based pollination biology.

Towards a trait-based understanding of plant-pollinator interactions

This study demonstrates the benefits of analysing multispecies pollination experiments with a trait-based perspective. It contributes to the broader agenda of trait-based community ecology (McGill et al., 2006) which aims to replace nomenclatural concepts, such as pollination syndromes, by quantitative relationships, such as the effect of sugar concentration on bird-pollination. This parallels developments in seed dispersal research where the traditional focus on dispersal syndromes, as mostly derived from seed morphology (Van der Pijl, 1982), is being replaced by the quantitative analysis of generalized dispersal systems in terms of plant traits and properties of dispersal vectors (Nathan et al., 2008). For our study system, we have identified nectar sugar concentration as a key trait predicting the dependence of *Protea* species on bird pollinators. The identification of such easily measurable functional traits (Lavorel and Garnier, 2002) will be crucial for a better mechanistic understanding of the sensitivity of plant species to pollinator extirpation (Bond, 1994). While the classical concept of pollination syndromes has inspired many important studies on plant-pollinator interactions, we propose that it is timely to develop this nomenclatural concept into a continuous framework describing the ultimate effects of animal pollinators on plant reproduction.

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Supplementary Material

Table S1: Results from linear mixed effects models of exclusion treatments on seed set in seven *Protea* species. We show main and interaction effects of exclusion treatments with species identity. Exclusion effects were partitioned into two orthogonal contrasts: (C1) the mean effect of animal pollinators on seed set relative to full exclusion, and (C2) the effect of bird-pollination on seed set relative to bird-exclusion. The reference species in the model was *P. laurifolia*. Predation rate was included as a covariate, and random intercepts were defined by the identities of plant individuals nested in study site. Significant terms ($P < 0.05$) are highlighted in bold.

	Estimate	Standard error	t-value	Pr(>|t|)
(Intercept)	0.119	0.034	3.46	**<0.001**
Predation rate	-0.064	0.028	-2.24	**0.026**
C1 (mean pollinator effect)	0.049	0.018	2.76	**0.006**
C2 (bird-pollination effect)	0.125	0.027	4.60	**<0.001**
P. compacta	0.151	0.061	2.50	**0.013**
P. eximia	-0.047	0.075	-0.62	0.536
P. longifolia	0.049	0.048	1.01	0.311
P. magnifica	-0.025	0.058	-0.43	0.669
P. punctata	0.127	0.047	2.70	**0.007**
P. repens	0.234	0.041	5.68	**<0.001**
C1 x *P. compacta*	0.011	0.034	0.34	0.737
C2 x *P. compacta*	-0.062	0.048	-1.28	0.203
C1 x *P. eximia*	-0.013	0.038	-0.33	0.742
C2 x *P. eximia*	-0.058	0.059	-0.99	0.324
C1 x *P. longifolia*	0.028	0.025	1.11	0.268
C2 x *P. longifolia*	-0.141	0.037	-3.82	**<0.001**
C1 x *P. magnifica*	-0.008	0.030	-0.27	0.790
C2 x *P. magnifica*	-0.075	0.047	-1.59	0.112

C1 x *P. punctata*	0.043	0.026	1.66	0.099
C2 x *P. punctata*	-0.113	0.037	-3.06	**0.003**
C1 x *P. repens*	0.061	0.023	2.66	**0.008**
C2 x *P. repens*	-0.085	0.035	-2.41	**0.017**

Table 2: Summary table of the relationships between the effect size of avian-pollination on plant reproduction and floral traits. We show the slope with 0.05 – 0.95 confidence intervals (95%-CI) and Wald Chi^2-test of variance (in bold for P < 0.05).

	Standardised mean differences				Log odds ratio			
	Slope	95%-CI	Q_M	*P*	Slope	95%-CI	Q_M	*P*
Inflorescence diameter	-0.0027	-0.0082 – 0.0028	0.91	0.34	-0.0032	-0.0076 – 0.0012	2.01	0.15
Inflorescence length	0.0061	-0.0047 – 0.0169	1.23	0.27	0.0030	-0.0080 – 0.0140	0.29	0.59
Style length	0.0098	-0.0128 – 0.0325	0.72	0.40	0.0032	-0.0188 – 0.0252	0.08	0.78
Pollen presenter length	-0.519	-0.1621 – 0.0584	0.85	0.36	-0.0667	-0.1716 – 0.0382	1.55	0.21
Nectar volume	-0.0382	-0.1372 – 0.0608	0.57	0.45	-0.0410	-0.1238 – 0.0419	0.94	0.33
Nectar sugar concentration	**0.0809**	**0.0516 – 0.1102**	**29.28**	**<0.0001**	**0.0454**	**0.0016 – 0.0891**	**4.13**	**0.04**
Sugar amount	0.0004	-0.0005 – 0.0012	0.80	0.37	0.0002	-0.0007 – 0.0010	0.18	0.67

Table S2: Floral and plant reproduction traits under bird-exclusion and control treatment for 14 *Protea* species used in the meta-analyses. Sample sizes for sugar concentration and nectar volume traits are indicated in brackets next to traits values.

	Floral traits						**Plant reproduction traits**			
							Bird-exclusion treatment		**Control treatment**	
***Protea* species names**	**Inflorescence length (mm)**	**Inflorescence diameter (mm)**	**Style length (mm)**	**Pollen presenter length (mm)**	**Sugar concentration (w/w)**	**Nectar volume (ml) (sample size)**	**Mean proportion of fertile seed ± standard deviation (sample size)**	**Mean number of fertile, non-fertile seeds**	**Mean proportion of fertile seed ± standard deviation (sample size)**	**Mean number of fertile, non-fertile seeds**
caffra	78.70[*]	80.00[¶]	60.00[¶]	12.00[¶]	4.40 (37)[*]	1.30 (62)[*]	0.268 ± 0.131 (56)[3]	44.05, 116.02[3]	0.276 ± 0.133 (113)[3]	42.60, 106.29[3]
compacta	103.00[§]	100.00[¶]	70.00[¶]	12.00[¶]	19.10 (22)[§]	0.57 (22)[§]	0.057 ± 0.060 (5)[1]	4.80, 61.60[1]	0.126 ± 0.118 (34)[1]	6.73, 60.82[1]
cynaroides	129.56[§]	300.00[¶]	95.00[¶]	15.00[¶]	7.53 (8)[§]	14.01 (8)[§]	0.070 ± 0.064 (15)[4]	16.50, 238.20[4]	0.032 ± 0.061 (15)[4]	11.10, 293.2[4]
dracomontana	66.40[*]	60.00[¶]	60.00[¶]	10.00[¶]	4.70 (52)[*]	1.10 (52)[*]	0.101 ± 0.120 (6)[2]	14.00, 111.33[2]	0.177 ± 0.155 (5)[2]	24.40, 110.53[2]
eximia	113.13[§]	120.00[¶]	75.00[¶]	8.00[¶]	23.22 (31)[§]	3.11 (31)[§]	0.003 ± 0.005(3)[1]	0.33, 128.33[1]	0.031 ± 0.032 (15)[1]	4.07, 122.53[1]
laurifolia	109.03[§]	60.00[¶]	70.00[¶]	10.00[¶]	19.10 (67)[§§]	3.45 (67)[§]	0.004 ± 0.006 (11)[1]	1.09, 285.18[1]	0.084 ± 0.063 (71)[1]	20.70, 219.61[1]
							0.010 ± 0.016 (45)[4]	2.48, 233.33[4]	0.057 ± 0.064 (45)[4]	12,54, 205.00[4]
longifolia	135.03[§]	90.00[¶]	60.00[¶]	10.00[¶]	11.8 (35)[§]	6.58 (35)[§]	0.069 ± 0.065 (14)[1]	12.86, 152.93[1]	0.058 ± 0.064 (88)[1]	10.14, 137.43[1]
magnifica	117.37[§]	80.00[¶]	70.00[¶]	10.00[¶]	24.02 (18)[§]	2.89 (18)[§]	0.007 ± 0.008(6)[1]	2.33, 292.17[1]	0.034 ± 0.029 (31)[1]	10.16, 260.26[1]
							0.054 ± 0.011 (70)[4]	17.80, 292.50[4]	0.074 ± 0.012 (70)[4]	21.7, 280.50[4]
neriifolia	116.54[§]	80.00[¶]	70.00[¶]	10.00[¶]	15.94 (49)[§]	5.39 (49)[§]	0.017 ± 0.013 (50)[4]	2.60, 230.30[4]	0.024 ± 0.007 (50)[4]	6.90, 253.30[4]
nitida	93.37[§]	160.00[¶]	80.00[¶]	12.00[¶]	18.44 (4)[§]	0.76 (4)[§]	0.105 ± 0.042 (50)[4]	23.90, 205.50[4]	0.132 ± 0.068 (50)[4]	28.330, 182.50[4]
punctata	50.45[§]	25.00[¶]	50.00[¶]	15.00[¶]	7.33 (38)[§]	0.35 (38)[§]	0.127 ± 0.114 (13)[1]	12.46, 89.69[1]	0.098 ± 0.105 (87)[1]	8.17, 80.33[1]
repens	110.83[§]	90.00[¶]	90.00[¶]	20.00[¶]	15.48 (84)[§]	5.71 (84)[§]	0.171 ± 0.112 (17)[1]	12.53, 46.18[1]	0.264 ± 0.164 (110)[1]	17.11, 45.18[1]
simplex	41.00[*]	60.00[¶]	40.00[¶]	10.00[¶]	6.30 (20)[*]	0.30 (20)[*]	0.271 ± 0.153 (52)[2]	19.46, 52.62[2]	0.210 ± 0.134 (68)[2]	14.88, 55.57[2]
welwitschii	48.30[*]	60.00[¶]	50.00[¶]	8.00[¶]	10.40 (35)[*]	1.00 (35)[*]	0.081 ± 0.061 (28)[2]	6.71, 78.14[2]	0.123 ± 0.078 (42)[2]	10.19, 73.91 [2]

[§] H. Nottebrock, University of Potsdam, Germany, unpublished data; [¶] Rebelo 2001; [*] Steenhuisen and Johnson 2012a; [1] This study; [2] Steenhuisen and Johnson 2012c; [3] Steenhuisen et al. 2012b; [4] Wright et al. 1991

Table S3: Matrix of Pearson’s correlation coefficients between floral traits (species means).

Inflorescences diameter						
0.517	Inflorescences length					
0.727	0.753	Style length				
0.227	0.104	0.470	Pollen presenter length			
0.757	0.687	0.667	0.338	Nectar volume		
0.038	0.559	0.423	-0.206	-0.033	Sugar concentration	
0.467	0.852	0.695	0.199	0.821	0.434	Sugar amount

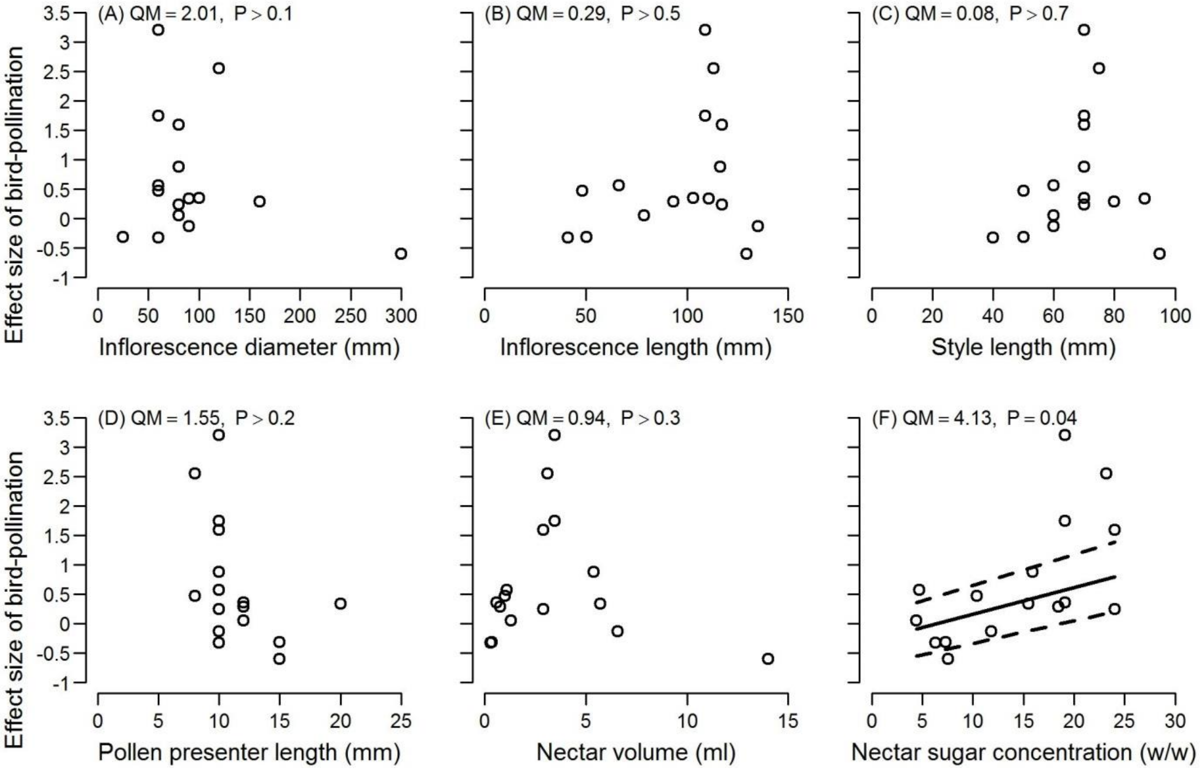

Figure S1: Relationship between plant traits and the effect size of bird-pollination on seed set of 14 *Protea* species. Effect size is measured as the log odds ratio between the proportion of fertile seeds under open-pollination and bird-exclusion treatments. We show the test of the moderator (Q_M) assuming normal distribution. The model prediction and the 95% confidence intervals are shown for the significant effect of nectar sugar concentration on the effect size of bird pollination.

Chapter 4

A bird pollinator shows positive frequency dependence and constancy of species choice in natural plant communities *

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Abstract

Animal pollinators mediate plant reproduction. Positive frequency-dependent foraging (a preference for common resources) and flower constancy (a temporary specialisation on specific resources) of pollinators ensure the transfer of pollen between conspecific plants, but have never been simultaneously tested in natural plant communities. We investigated the foraging behaviour of Cape sugarbirds (*Promerops cafer*), the main avian pollinator of *Protea* plants, using high-resolution maps of the spatiotemporal distribution of flowers in *Protea* communities (2-7 *Protea* species). Our analysis provides first evidence for flower constancy acting simultaneously with positive frequency-dependent foraging in natural plant communities. These results suggest that i) positive frequency-dependent foraging of avian pollinators favours common plant species and that ii) flower constancy improves pollen transfer between conspecific plants, being particularly important for rare species. The individual variability in pollinators' foraging decisions may therefore be a key mechanism favouring the coexistence between common and rare species in species-rich plant communities.

Introduction

Animal pollinators forage on plant resources optimising their net energy intake (Stephens and Kerbs 1986) while simultaneously providing essential services to plant species in ecological communities (Waser and Ollerton 2006). According to optimal foraging theory (MacArthur and Pianka 1966), foraging choices of animal pollinators change according to the distributions of plant resources and the local context (Bateson *et al.* 2003; Hersch and Roy 2007; Brosi and Briggs 2013). This plasticity in the foraging behaviour of animal pollinators can influence plant reproduction (Rathcke 1983; Brosi and Briggs 2013) and thus the dynamics of plant communities (Kunin and Iwasa 1996; Song and Feldman 2014).

Two mechanisms of context-dependent foraging behaviour that ought to facilitate conspecific pollen transfer have been proposed, positive frequency-dependent foraging and flower constancy (Waser 1986; Smithson 2001). Positive frequency-dependence of pollinators occurs when a pollinator visits common plant species at higher frequency than expected from a random choice among co-occurring plant species (Krebs 1989; Fründ *et al.* 2010). Flower constancy describes the foraging behaviour of individual pollinators that continue to forage on the same species, bypassing other rewarding species (Waser 1986). Flower constancy thus describes a temporary specialisation of pollinator individuals (Chittka *et al.* 1999), a foraging behaviour that differs from the widespread traplining behaviour of pollinators along regular foraging routes (Thomson *et al.* 1997). A possible mechanism generating these two foraging behaviours is the cognitive limitation of pollinators to efficiently forage on multiple flower types simultaneously (Chittka *et al.* 1999; Gegear and Laverty 2001; Menzel 2001; but see Grüter and Ratnieks 2011). For instance, foraging efficiency improves when pollinators activate search images to accelerate and refine the detection of suitable flowers (Goulson 2000; Smithson 2001). Controlled experimental studies demonstrate that animal pollinators show both positive frequency-dependent foraging (Levin 1972, reviewed in Smithson 2001) and flower constancy (Chittka *et al.* 1999), although other studies suggest that avian pollinators show no flower constancy, due to their high cognitive abilities (Waser 1978; Meléndez-Ackerman *et al.* 1997; Hersch and Roy 2007).

Positive frequency-dependence and constancy have rarely been studied in natural, species-rich plant communities (de Jager *et al.* 2011), and to our knowledge they have never been studied simultaneously. Evidence for frequency-dependent foraging from field studies is ambiguous (discussed in Smithson 2001, see also Eckhart *et al.* 2006), and evidence for flower constancy is rare because it is difficult to account for non-random distributions of plants at spatial scales corresponding to pollinator movements (Waser 1986; de Jager *et al.* 2011). We investigated the foraging behaviour of Cape sugarbirds (*Promerops cafer*), a key pollinator for *Protea* species in the Cape Floristic Region. We mapped all plants of 2 to 7 co-flowering *Protea* species at 14 study sites

(Fig. 1A) and tested frequency-dependent foraging of sugarbirds with observations of foraging events on focal plants (Fig. 1B). We additionally determined foraging constancy by recording sugarbird movements between foraging events (Fig. 1B) and applying step-selection functions to compare observed with random encounter probabilities between pollinators and plants (Fig. 1C, see Thurfjell *et al.* 2014).

We hypothesised that (1) Cape sugarbirds adjust their foraging choices in response to the relative abundance of flowering plants, preferring common over rare species, as observed in other pollinators (Levin 1972; Smithson 2001), and that (2) Cape sugarbirds show no foraging constancy during subsequent foraging events, like observed in other bird pollinators (Waser 1978; Meléndez-Ackerman *et al.* 1997; Hersch and Roy 2007). Because male and female sugarbirds differ in morphology and behaviour (Seiler and Rebelo 1987), we also explore (3) whether the two sexes differ in frequency dependence and flower constancy. We show for the first time that flower constancy acts simultaneously with positive frequency-dependent foraging in natural, species-rich plant communities. These results suggest that i) positive frequency-dependent foraging of avian pollinators favours common plant species and that ii) flower constancy improves pollen transfer between conspecific plants, being particularly important for rare species.

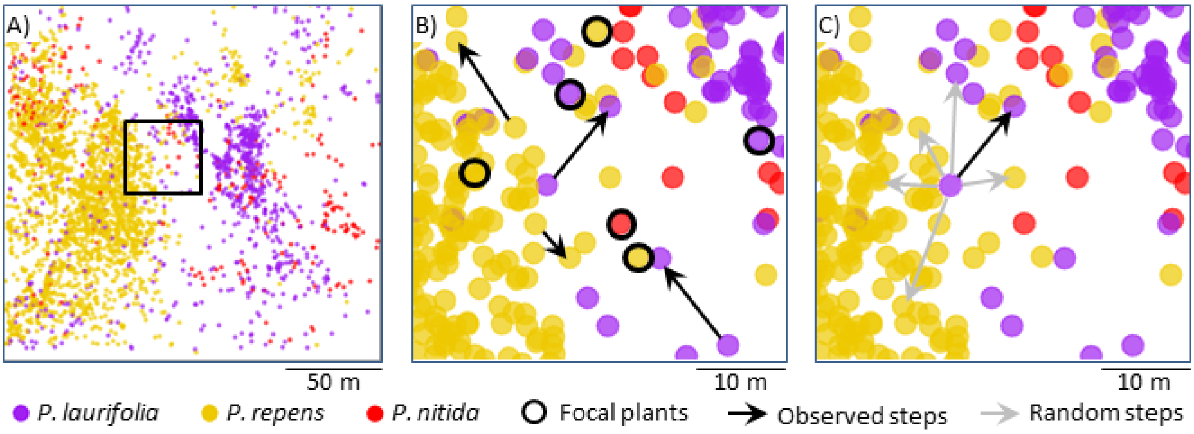

Fig. 1: Observations of foraging Cape sugarbirds in *Protea* communities. A) Example for the mapped spatial distribution of individual plants of three *Protea* species; the square indicates the area displayed in panels B) and C). B) Example of randomly selected focal plants (black circles) used to study frequency-dependent foraging, and observed bird movements between consecutive foraging events (black arrows) used to investigate foraging constancy. C) For each observed movement step (black arrow), we sampled 50 random steps (grey arrows) that share the same start-plant, but can differ in end-plants. End-plants of random steps are selected at distances that correspond to the lengths of the observed movement steps (Fig. S2).

Methods

Study system

We investigated the foraging plasticity of a generalist avian pollinator, the Cape sugarbird (*Promerops cafer*, Fam. Promeropidae). During the breeding season, Cape sugarbirds depend on nectar resources provided almost exclusively by a variety of *Protea* species (Rebelo 1987) and are considered key pollinators for many of these species (Collins and Rebelo 1987; Schmid *et al.* 2015a). Sugarbirds' morphology and behaviour differ between sexes. Males are territorial and have larger bodies and longer beaks than females (Seiler and Rebelo 1987; Tjørve and Scholtz 2007).

We studied interactions between sugarbirds and *Protea* species in the fynbos vegetation of the Western Cape region, South Africa. To this end, we selected 14 study sites where *Protea* species dominated the overstorey vegetation. At each site, we defined a 200 x 200 m plot, extending the size of individual sugarbird territories (Calf *et al.* 2003), and mapped all overstorey *Protea* plants with a high accuracy Differential Global Positioning System. The study plots varied in the number of co-flowering *Protea* species (2-7 species) and overall plant abundance (650-15,500 plants). We observed bird foraging activity during the breeding season of sugarbirds and flowering peak of *Protea* species from April to August 2012 and repeated observations three times per study plot every 2-5 weeks. During each visit, we recorded A) the number of sugarbird visits to 711 randomly selected focal plants and B) the movements of foraging sugarbirds within the study plot. We restricted our analyses to the ten *Protea* species for which we recorded >10 sugarbird visits across plots (*P. coronata*, *P. cynaroides*, *P. eximia*, *P. laurifolia*, *P. longifolia*, *P. neriifolia*, *P. nitida*, *P. obtusifolia*, *P. repens*, *P. susannae*).

Spatiotemporal distribution of flowering plants

To estimate the spatiotemporal distribution of flowering *Protea* plants in the study plots for each day of observation, we estimated the flowering phenology for each mapped plant (detailed procedure in Supplementary information, Fig. S1). We first used information on the flowering status of 15,863 populations (48-4,145 per species) provided by the Protea Atlas Project (Rebelo 2001) to estimate the phenological peak of flower production for each species (day of flowering peak, see Fig. S1 for model fit). Second, we recorded the number of inflorescences on focal plants in the study plots (n = 6943 observations, 51-1,245 per species, 1-865 per plot), including measurements of plant size (stem length along the main growth axis), inflorescence length, trunk length and specific leaf area (see Fig. S1 for details). We also used information on the sprouting ability of each species, as provided by Rebelo (Rebelo 2001).

We then combined these different sources of information (Fig. S1). We described the temporal pattern in inflorescence number for each mapped plant (individual plant phenology), using a generalised linear mixed model (GLMM) with Poisson errors, and related the number of inflorescences per plant to the temporal distance from the flowering peak, plant size, and a set of plant functional traits (for details see Fig. S1). Finally, we used the predictions from this model to estimate the number of inflorescences for each mapped plant and day of observation. This yielded spatially-explicit maps of flowering plants at the days of bird observation (Fig. S1). From these predictions, we also computed the number of flowering plants per species for each plot and visit and calculated the mean relative abundance of all flowering *Protea* species for each plot.

Nectar traits

We used field measurements on 850 inflorescences from a total of 484 plants (4-80 per species, 1-34 per population) to estimate nectar sugar concentration (mg*ml^{-1}, converted from w/w) and standing crop sugar amount per inflorescence (mg) for each species and plot. To estimate these two variables, we fitted mixed effects models to account for variation in the flowering age of the sampled inflorescences and for random effects of species and plot identity (nectar sugar concentration conditional R^2 = 0.35, marginal R^2 = 0.11; sugar amount conditional R^2 = 0.62, marginal R^2 = 0.38). We used the product of the predicted sugar amount per inflorescence and the observed number of inflorescences on the focal plants to compute the standing crop of mean nectar sugar amount per focal plant. Standing crops of nectar sugar are an accurate measure for nectar availability in *Protea* species that generally produce very large, rarely depleted, amounts of nectar (Nicolson and Thornburg 2007; Geerts and Pauw 2011).

Frequency-dependence foraging

We assessed frequency-dependent foraging of male and female sugarbirds on each study plot. We observed 1,122 focal plants (12-358 focal plants per *Protea* species, 1-8 study sites per species, depending on the availability of flowering plants, Fig. 1A). We observed focal plants from a distance of at least 20 m during 45 min sessions in the morning from 07:00 h to 12:00 h. We considered only bird visits to focal plants with legitimate feeding events, i.e. birds that were in contact with the reproductive parts of an inflorescence and potentially transferred pollen. Results of analyses including legitimate and illegitimate bird visits were qualitatively identical. As proposed by Fründ *et al.* (2010) and based on Krebs' forage ratio (Krebs 1989), we calculated relative preference indices for each species s on each study plot p, pooling the observations from repeated visits. The preference index ($PI_{s,p}$) ranges between 0 and 1, and was calculated for each sugarbird sex as:

$$PI_{s,p} = P_{obs\ s,p} / \left(P_{obs\ s,p} + P_{null\ s,p}\right) \quad \text{(eq. 1)},$$

where $P_{obs\ s,p}$ is the proportion of visits on species s among all visits on plot p, and $P_{null\ s,p}$ is the proportion of focal plants of species s among all focal plants observed on plot p. We thus control for the number of focal plants observed for each *Protea* species. Increasing $PI_{s,p}$ denotes increasing foraging preferences; $PI_{s,p}$ rarely approaches 1 since the maximum value of $PI_{s,p}$ depends on $P_{null\ s,p}$.

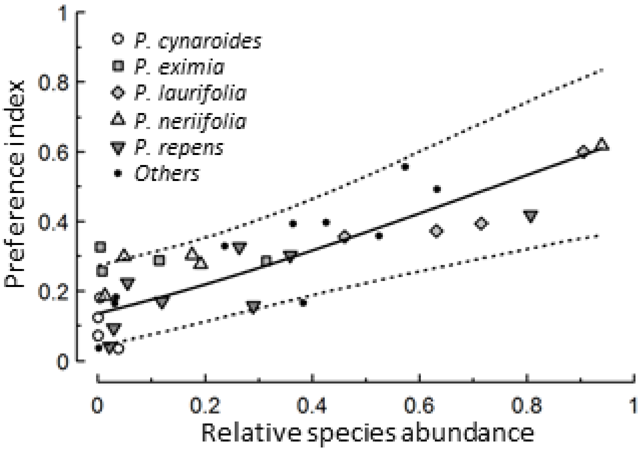

Fig. 2: Relationship between indices of sugarbird foraging preferences for 10 *Protea* species and the relative abundances of the respective species on the plots. *Protea* species include (number of study sites in brackets): CYNA: *P. cynaroides* (4); EXIM: *P. eximia* (4); LAUR: *P. laurifolia* (4); NERI: *P. neriifolia* (5); REPE: *P. repens* (8); Others (with less than four study sites) include *P. coronata* (2), *P. longifolia* (2), *P. nitida* (1), *P. obtusifolia* (3), *P. susannae* (2). We computed posterior values (points) and predicted values of fixed effects (solid line; dotted lines represent the 0.025/0.975 confidence intervals) using models that included only relative species abundance as fixed effect, since other variables showed no significant effects (see Table S1; intercept: 0.53 ± 0.07, relative species abundance: 0.16 ± 0.05; marginal $R^2 = 0.24$).

Flower constancy

We conducted direct observations of movement paths of bird individuals and used bird movements between consecutive foraging events to examine flower constancy. For the examination of flower constancy, we compared the observed proportion of consecutive foraging events on two conspecific plants with the respective proportions expected from random pollinator movements (Gegear and

Laverty 2005; de Jager *et al.* 2011). In contrast to former studies on flower constancy (Gegear and Laverty 2005; de Jager *et al.* 2011), we explicitly integrated pollinator movements to compute random encounter rates with plant species (Fig. 1B). This approach resembles case-control designs in studies of habitat selection of moving animals (Fortin *et al.* 2005; Thurfjell *et al.* 2014). We define a step as the Euclidian distance between consecutive foraging events, geographically defined by the location of a foraging event (start-plant) and the consecutive foraging event (end-plant). We coupled each case step (i.e. observed foraging step; n = 257) with 50 random steps that shared the same start-plant, but could differ in end-plants. Random end-plants were selected from the predicted spatial distribution of flowering plants (see Fig. S1). We drew case step distances (with replacement) to select the random end-plants matching the observed distance between start- and end-plant (Supplementary Information: Fig. S2; Fortin *et al.* 2005). It is possible that the detection probability of foraging events decreases with increasing distance of the bird from the observer. However, density distributions of distances from the observer to case end-plants were similar to those to random end-plants and thus we did not find any evidence for biases, due to changes in detection probability (Supplementary Information: Fig. S2). Because we were also interested in sex difference in foraging choice, we drew step distances for each sex separately.

Data analyses

Positive frequency-dependent foraging

We tested the relationship between preference indices (range: 0-1; angular-transformed) per species and plot and i) relative plant species abundance, ii) nectar traits (mean sugar amount per plant (log-transformed) and sugar concentration), and iii) sex of sugarbirds, assuming Gaussian error distributions. Because of the relatively small sample size (n = 77), we tested the effects of the two nectar traits in separate models. The effects of nectar traits could depend on relative species abundance. We therefore added the two-way interaction of relative species abundance and the respective nectar trait to the model. Because bird sexes might differ in their foraging preferences, we also included the two-way interactions of bird species and relative species abundance and nectar traits, respectively. We included random intercept effects of plot identity and *Protea* species identity to account for potential spatial and taxonomic autocorrelation in preference indices. To ensure comparability among predictor variables, all numerical predictors were scaled and centred. We applied Wald's χ^2 tests to assess the level of significance for each predictor variable; each predictor variable was tested with all other variables in the statistical model, accounting for their respective effects. We used mixed effects models as implemented in the lme4-package (version 1.0-6, Bates *et al.* 2014) of R (version 3.1.0, R Core Team 2014).

Foraging constancy

We used mixed logistic regression models to test whether the probability of conspecific foraging sequences in observed movement steps differed from the probability of conspecific foraging sequences in random steps. The response variable was a binary variable that indicated for each foraging step whether the start-plant and the end-plant belonged to the same species. Predictor variables included the relative species abundance of end-plants. For each case step and its coupled random steps, we computed the relative species abundance of end-plants in the 50 random steps (sampling procedure of random steps described above). We further included as predictor the categorical variables 'step mode' (observed step or random step) and bird sex (male or female). The main effect of relative species abundance of end-plants accounted for encounter probabilities that are due to the relative frequency of randomly drawn end plants; the main effect of step mode (observed versus random step) indicated whether the foraging choices of birds deviated from the random expectation given by the relative species abundance of end-plants. To explore whether foraging constancy of observed birds might be influenced by bird sex, we also included bird sex as main and interaction effects with step mode in the model. A significant interaction effect of sex with step mode would indicate whether the observed probabilities of foraging constancy differed between sexes. In all models of foraging constancy, we also included random intercepts of the species identity of end-plants to account for between species differences, and nested random intercepts of step identity, movement path identity, date and plot identity to account for spatial and temporal autocorrelation. We repeated the sampling and analyses 1,000 times to estimate the range of parameter values. To ensure comparability among predictor variables, all numerical predictors were scaled and centred. We used mixed effects models as implemented in the lme4-package (version 1.0-6, Bates *et al.* 2014) of R (version 3.1.0, R Core Team 2014). We could not use two-steps conditional logistic models (R package “TwoStepCLogit”; version 1.2.3, Craiu *et al.* 2014) because it does not support crossed random effects, such as species identity, site identity, and date.

Results

Positive frequency-dependent foraging

Cape sugarbirds of both sexes showed positive frequency-dependent foraging. For 10 *Protea* species, we observed 190 bird visits (female sugarbirds, n = 69; male sugarbirds, n = 121) during 115 hours of observation on 711 focal plants. Preference indices ranged between 0 and 0.84 and increased with the relative abundance of flowering plants of the respective species (Fig. 2, Table S1). Preference indices were independent of pollinator sex and nectar traits (sugar amount per plant, sugar concentration), and the two-way interactions between species abundance, bird sex and nectar traits (Table S1).

Flower constancy

Observed movements between consecutive foraging events were in most cases directed to conspecific plants (79 %, n = 257 observations). The odds that birds showed flower constancy were 2.39 (median log-odd = 0.87, parameter range after 10^3 permutations: 0.71–1.07) times greater in the observed movement steps than expected from the random encounter frequencies (Fig. 3). As expected, the random steps indicated that increasing relative species abundance increased the probability that birds continued to forage on the same species. That is, birds showed higher odds to foraging on the same species when a species was relatively common (median log-odd = 2.98, parameter range: 2.75–3.25; Fig. 3). However, the effect size of flower constancy (the ratio of observed against random encounters) was much higher for rare than for common plant species (Fig. 3, Fig. S3). We observed no differences in flower constancy between bird sexes (observed movements of females contrasted to the observed movements of males: median log-odd = 0.31, parameter range: -0.05–0.71).

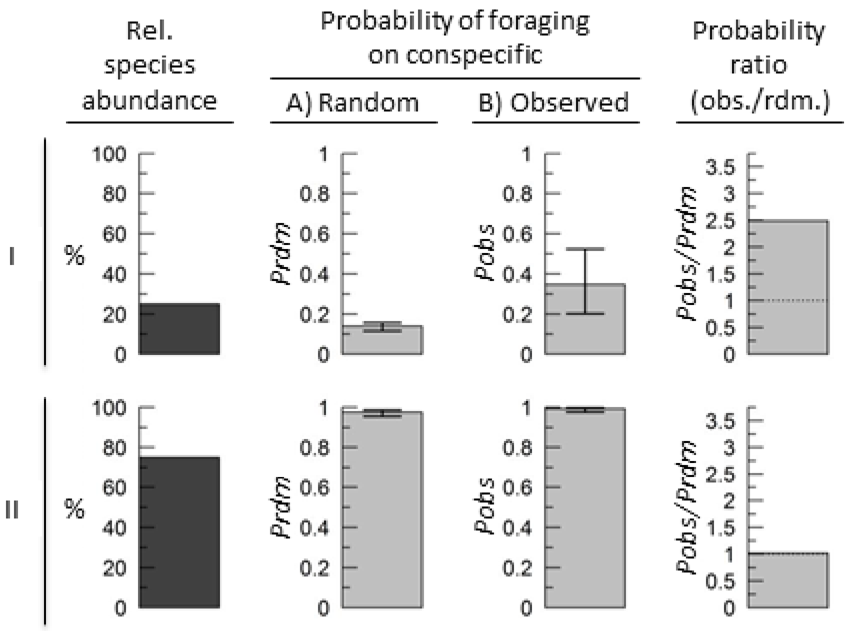

Fig. 3: The probability of foraging on a conspecific plant for (I) a rare and (II) an abundant plant species: A) according to random steps, and B) according to the observed bird movements (case steps). The ratio of the probabilities of B) relative to A) indicates the effect size of flower constancy for a given species abundance. We used the median and the 0.025/0.975 intervals of the parameter values of the 1,000 models to compute predicted means and error bars. The effects of relative species abundance on foraging probabilities deviate from linearity because of the effects of the random intercepts included in the statistical model; variance and standard deviation of random effects (median across 1000 permutations): species identity = 14.63 ± 4.64; plot identity = 10.80 ± 3.29; date = 1.19 ± 1.08; path identity = 2.88 ± 1.70; step identity = 2.97 ± 1.72). An animated figure with varying relative species abundances is given in the Supplementary Information (Fig. S3).

Discussion

We studied the plasticity in foraging behaviour of an avian pollinator in natural, species-rich plant communities. We provide first evidence for flower constancy of avian pollinators under natural conditions and additional evidence for positive frequency-dependent foraging of pollinators in species-rich plant communities. Morphological and behavioural differences among bird sexes did not influence foraging choices. These results highlight the importance of individual variation in animal foraging choices for plant-animal interactions.

Positive frequency-dependent foraging

Cape sugarbirds, regardless of their sex, preferred to forage on common plant species. According to optimal foraging theory (MacArthur and Pianka 1966), foraging on common species may optimise the foraging efficiency of animals (Smithson 2001). Positive frequency-dependent foraging may occur when pollinators develop search images for common species (Levin 1972; Chittka and Thomson 1997). The search-image hypothesis has received much support in predator-prey interactions (e.g. Dawkins 1971), but the importance of search images in plant-pollinator interactions is contentious, due to contradictory findings of foraging studies under field conditions (reviewed in Smithson 2001, see also Eckhart *et al.* 2006). Smithson (2001) suggested that the discrepancy in the incidence of frequency-dependent foraging between experimental and field studies might be explained by the comparatively small floral polymorphism of plant species under natural conditions. Large inter-specific variation in floral traits among *Protea* species (Rebelo 2001) might therefore be an important factor promoting frequency-dependent foraging in the *Protea*-sugarbird system.

We observed no effects of nectar traits (mean sugar amount per plant and nectar sugar concentration) on the foraging preferences of sugarbirds. In contrast to our results, floral rewards for pollinators have been positively associated with visitation frequencies of pollinators in several studies (Meléndez-Ackerman *et al.* 1997; Ohashi and Yahara 1999; Bateson *et al.* 2003). The absence of foraging preferences of sugarbirds towards the most rewarding species might arise because of high intra-specific variation in nectar traits, such as sugar amount per plant (Schmid *et al.* 2015b). Weak effects of nectar traits on sugarbird foraging may also reflect the dietary niche breath of this generalist pollinator (Rebelo 1987) and underlines the crucial role of floral abundance in influencing pollinators' foraging choices.

Flower constancy

Cape sugarbirds prefer to continue foraging on the same species, bypassing other plant species. We detected this floral constancy of sugarbirds, in models that also accounted for frequency-dependent foraging. Our study is the first that explicitly considers the spatial distribution of resource plants at a spatial resolution directly related to the range of pollinator movements, generating accurate random encounter frequencies with co-occurring species (Waser 1986).

The significant flower constancy of sugarbirds is surprising since pollinators with high cognitive abilities, such as birds (Henderson *et al.* 2006), are expected to be able to identify different resources and are therefore expected to show low incidences of flower constancy (Meléndez-Ackerman *et al.* 1997). Flower constancy of sugarbirds can be explained by several, non-mutually exclusive explanations. First, limitations in processing simultaneous information have been observed also in organisms with high cognitive abilities, such as humans (discussed in Goulson 2000). Avian pollinators may improve foraging efficiency by the use of search images, especially in species-rich plant communities with large variation in floral traits (Gegear and Laverty 2001, 2005). Thus, variation in floral traits among *Protea* species (Rebelo 2001) may promote flower constancy of sugarbirds. Second, plant species may differ in the timing of nectar production rates within a day and pollinators may thus specialise temporarily on the most rewarding species. While information on the inter-specific variability in nectar production rates is lacking, *Protea* species indeed differ in the daily pattern of flower opening (Wiens *et al.* 1983). Third, differences in the competitive ability and energy requirements of individual pollinators within a population can lead to individualistic foraging choices and high degrees of individual foraging specialisation (Thomson and Chittka 2001; Brosi and Briggs 2013). This might also be true for breeding sugarbirds whose males interact strongly with conspecific males during courtship and territorial defence (Seiler and Rebelo 1987). Further studies with individually marked bird individuals would be required to thoroughly disentangle the underlying mechanisms of flower constancy in avian pollinators.

Potential effects of foraging behaviour on plant reproduction

The plasticity in the foraging behaviour of Cape sugarbirds may strongly influence plant reproduction in *Protea* communities. Positive frequency-dependent foraging can increase the reproductive success of common plant species to the detriment of rare species (Palmer *et al.* 2003). Consequently, rare species may experience reduced fitness because of decreased conspecific pollen transfer (Thomson and Plowright 1980; Rathcke 1983; Flanagan *et al.* 2009). The local extinction risk of rare plant species might be reduced by pollinator interactions and behaviour in two ways. First, pollinator species that differ in competitive ability may show distinct frequency-dependent foraging preferences, with subordinate pollinators showing preferences for rare species (Eckhart *et al.* 2006). For instance, orange-breasted sunbirds (*Anthobaphes violacea*) also regularly forage on *Protea* species, but are often chased away from *Protea* inflorescences by dominant Cape sugarbirds (Wooller 1982; Rebelo 1987). Thus, subordinate Orange-breasted sunbirds may prefer rare plant species unattended by sugarbirds. Second, the flower constancy of individual sugarbirds may improve the efficiency of conspecific pollen carry-over and mitigate indirect competition for pollinators between plants. Flower constancy may thus be particularly important to enhance the reproduction of rare plant species. Our findings demonstrate the prevalence of flower constancy in natural plant communities and suggest that individual foraging behaviour of animal pollinators may be an important mechanism for the coexistence between common and rare plant species.

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Supplementary information

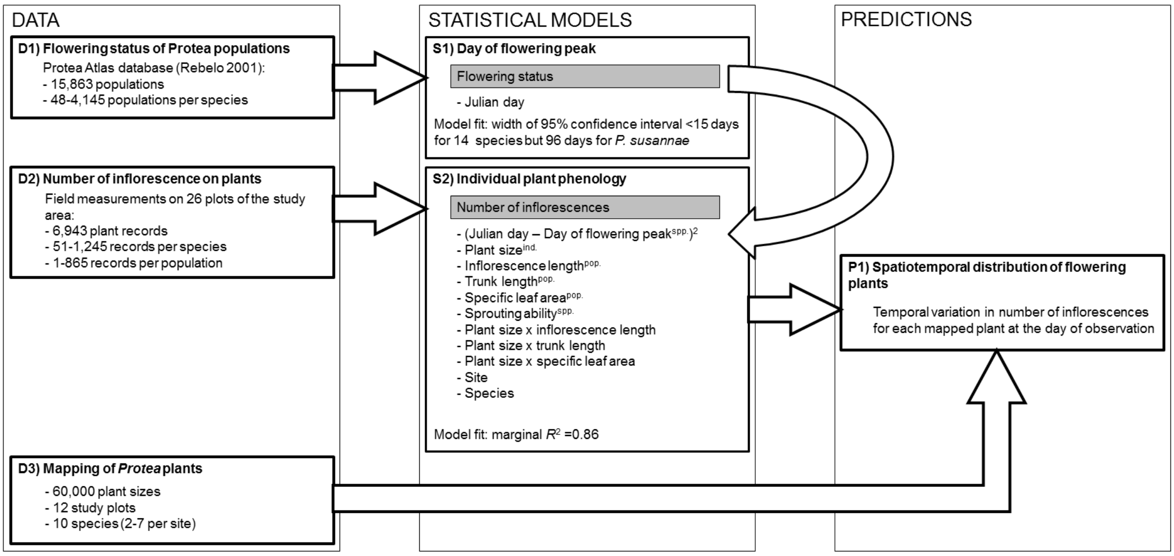

Fig. S1: Chain of analyses yielding predictions (P) of the spatiotemporal distribution of flowering plants from statistical models (S) fitted to datasets (D). We used data on the flowering status of *Protea* populations in the study region (D1, from the Protea Atlas database, Rebelo 2001) to predict the day of flowering peak for each species (S1). Observations of the number of inflorescences on individual plants in the study sites (D2) served to predict individual plant phenology (S2). These analyses included the squared temporal distance between the day of inflorescence observation and the day of flowering peak. For each mapped plant (D3) and the days of bird observation, we predicted the number of inflorescences. This yielded spatially-explicit predictions of flowering plants (P1) for each day of bird observation. For each statistical model, we give the response variable (grey shaded area), the explanatory variables at individual ($^{ind.}$) population ($^{pop.}$) and species ($^{spp.}$) levels, and we provide measures of model quality (R^2 and width of 95% confidence intervals, respectively).

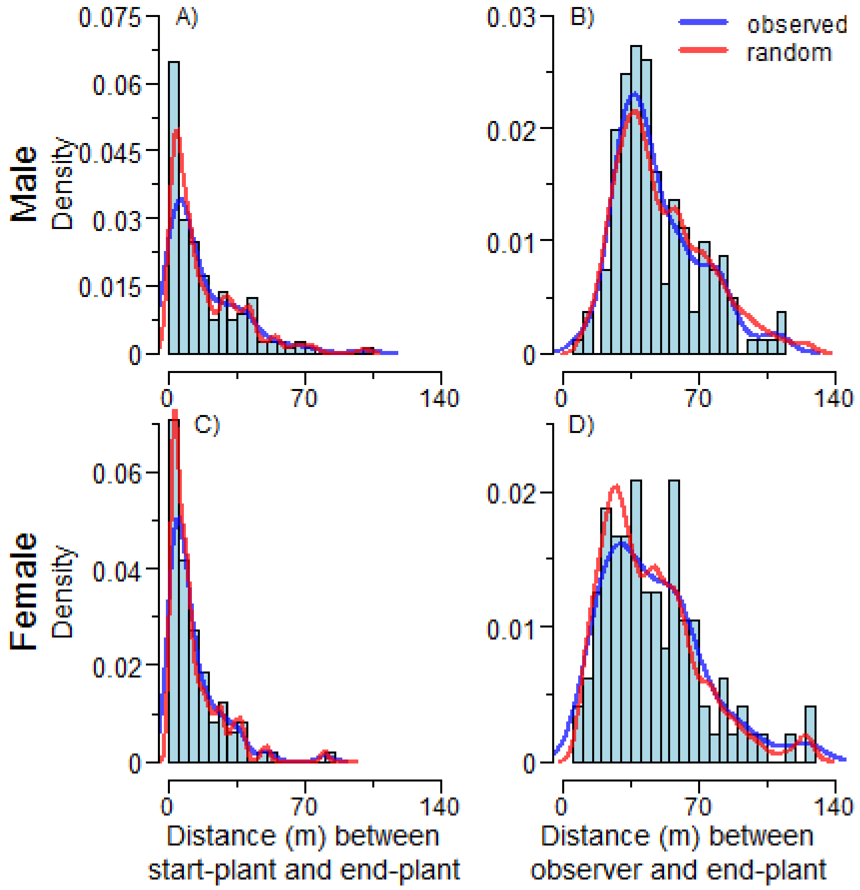

Fig. S2: Comparison of density distributions of observed (blue bars and lines) and randomly sampled (red lines) distances for: A) distances between the start- and the end-plants (step length) of male sugarbirds, B) distances between the observer and the end-plants for male sugarbirds' foraging movements, C) distances between the start- and the end-plants (step length) of female sugarbirds and D) distances between the observer and the end-plants for female sugarbirds' foraging movements. The lengths of observed steps ranged from 0 to 99.3 m, and tended to be slightly longer for male than for female sugarbirds (mean ± SD: male = 17 ± 18 m, n = 161; female = 12 ± 29 m, n = 96; Wilcoxon rank sum test: *W* = 6636, *P* = 0.06).

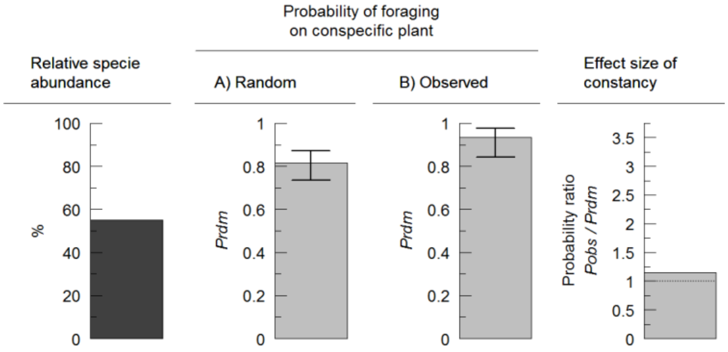

Fig. S3 (animated): The probability of foraging on a conspecific plant in relation to the relative abundance of plant species: A) according to random steps, and B) according to the observed bird movements (case steps). The ratio of the probabilities of B) relative to A) indicates the effect size of flower constancy for a given species abundance. We used the median and the 0.025/0.975 intervals of the parameter values of the 1,000 models to compute predicted means and error bars.

Table S1: Effects of relative species abundance, nectar traits and sex of sugarbirds on indices of Cape sugarbirds' foraging preferences. Preference indices (angular transformed) are given by the relative number of bird visits to focal plants of a species among all focal plants on a study site. We show model coefficients (mean ± standard error) of two models that varied in the nectar trait variable, while the other variables were identical in both models. We present results for mean sugar amount per plant (log-transformed, mg sugar; marginal R^2 = 0.31) and nectar sugar concentration (mg sugar * ml^{-1}; marginal R^2 = 0.28). All numerical predictors were scaled and centred. Significant effects ($P \leq 0.001$) according to Wald's χ^2 tests are written in bold.

	Nectar traits					
	Mean sugar amount per plant			Sugar concentration		
	Mean ± SE	χ^2	*P*-value	Mean ± SE	χ^2	*P*-value
Relative species abundance	0.162 ± 0.063	13.09	0.0003	0.128 ± 0.067	11.39	0.0007
Nectar trait	0.086 ± 0.073	3.18	0.075	0.017 ± 0.076	0.01	0.96
Sex (female)	-0.055 ± 0.076	0.6	0.45	-0.061 ± 0.083	0.53	0.47
Relative species abundance x nectar trait	0.046 ± 0.041	1.24	0.26	-0.054 ± 0.049	1.19	0.28
Relative species abundance x sex	0.012 ± 0.075	0.02	0.88	0.046 ± 0.086	0.29	0.59
Nectar traits x sex	0.056 ± 0.075	0.55	0.46	-0.017 ± 0.088	0.04	0.85

Chapter 5

Coexistence of plant species in a biodiversity hotspot is stabilized by competition but not by seed predation*

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*This chapter with similar content has been published in Oikos

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Abstract

Understanding the mechanisms that enable species to coexist is a key task for ecology. Ecological theory predicts that both competition and predation (which causes apparent competition among prey) can either promote or limit species coexistence. Both mechanisms cause negative interactions between individuals, and each mechanism promotes stable coexistence if it causes negative interactions to be stronger between conspecifics than between heterospecifics. However, the relative importance of competition and predation for coexistence in natural communities is still poorly known.

We study how competition and apparent competition via pre-dispersal seed predators affect the long-term fecundity of shrubs of the genus *Protea* in the fire-prone Fynbos biome (South Africa). These plants store all viable seeds produced since the last fire in fire-proof cones. Competitive effects on cone number and pre-dispersal seed predation reduce their fecundity and can thus limit recruitment after the next fire.

In 27 communities comprising 49,990 shrubs of 22 *Protea* species, we measured cone number and per-cone seed predation rate of 2,154 and 1,755 focal individuals, respectively. Neighbourhood analyses related these measures to individual-based maps to test whether (1) competition and apparent competition are stronger between conspecifics than between heterospecifics, (2) the size and seed crop of interacting plants determine the intensity of competition and apparent competition, respectively, and (3) competition and apparent competition have equally strong effects on the long-term fecundity of plants.

Conspecific neighbours had stronger competitive effects on cone number than heterospecific neighbours. In contrast, apparent competition via seed predators was not stronger between conspecifics than between heterospecifics. This indicates that competition stabilizes coexistence of *Protea* species, whereas pre-dispersal seed predation does not.

Larger neighbours had stronger competitive effects and neighbours with large seed crops exerted stronger apparent competition. For 97% of the focal plants, competition reduced fecundity more than apparent competition.

Even in communities of closely related and ecologically similar species, intraspecific competition can be stronger than interspecific competition. On the other hand, apparent competition through seed predators need not have such a stabilizing effect. These findings illustrate the potential of ‘community demography’, the demographic study of multiple interacting species, for understanding plant coexistence.

Introduction

All species compete for resources with other species and they are themselves resources for predators (such as herbivores, carnivores, pathogens or parasites). Hence, it is not surprising that competition and predation are the most frequently investigated interactions in ecology (Chesson and Kuang 2008). Importantly, both competition and predation can play a key role for the coexistence of species on the same trophic level (Chase et al. 2002, Chesson and Kuang 2008).

Competition can either limit or promote stable coexistence of species on the same trophic level. The principle of competitive exclusion (Hardin 1960) states that if multiple species compete for one limiting resource in a closed, homogeneous environment, only the species that is able to maintain a stable population at the lowest resource level will survive in the long-term (Tilman 1982). However, competition can also promote the stable coexistence of multiple species. This is the case if intraspecific competition is stronger than interspecific competition, so that each species suppresses its own population growth more than that of other species (Chesson 2000). Stronger intraspecific competition results if conspecifics use more similar resources than heterospecifics or if their resource use overlaps more in space or time (Chesson 2000).

In plant communities, understanding species coexistence is particularly challenging. This is because most plant species use a small number of limiting resources (notably water, nutrients and light) in seemingly similar ways (Silvertown 2004). Various mechanisms have been proposed to explain how plant species can coexist in a local community by subdividing the commonly used resources (see Silvertown 2004). For instance, the resource-ratio hypothesis states that plant species coexist if they have a trade-off in their requirements for two essential resources and if the ratio at which these resources are supplied varies in space (Tilman 1982). Environmental variation at small spatial scales also plays a key role in the segregation of plant species along hydrological niche axes of water logging and drought stress (Silvertown et al. 1999; Araya et al. 2011). A prominent mechanism by which temporal variation in the environment can promote coexistence is the storage effect (Chesson 1994). The storage effect arises if species respond differentially to temporal variation, so that intraspecific competition is temporally more aggregated than interspecific competition and if species have means to store reproductive output during unfavourable times, for example as long-lived adult plants or in seed banks (Chesson 2000). These and other mechanisms of stable coexistence, such as the competition-colonization trade-off (Tilman 1994), have in common that they cause intraspecific competition to be stronger than interspecific competition (Chesson 2000). In contrast, neutral models assume that competition is equally strong between conspecifics and heterospecifics. Under this assumption, stable coexistence is impossible and local diversity can only be maintained by immigration of species from the meta-community (Hubbell 2001).

The effects of predation on coexistence are in principle very similar to the effects of competition. This is because predation on prey individuals increases predator abundance which decreases the abundance of other prey. The resulting negative feedback has consequences for the population and community dynamics of prey that are largely analogous to those of competition for resources; hence, this predator-mediated feedback is termed 'apparent competition' (Holt 1977). As in the case of competition, predation can either promote or limit the coexistence of prey species (Chesson and Kuang 2008). Predators that specialize on single prey species tend to promote stable coexistence because they cause apparent competition within species, but not between species. A prominent example is the Janzen-Connell hypothesis which states that the high diversity of tropical rainforest trees is due to spatial variation in the abundance of species-specific antagonists of seeds and seedlings (Janzen 1970; Connell 1971). However, even generalist predators shared by multiple prey species can stabilize coexistence as long as the resulting apparent competition is stronger within than between prey species (Chesson and Kuang 2008).

In plant communities, apparent competition within and between species can be mediated by herbivores, pathogens and seed predators (Hersh, Vilgalys and Clark 2012; Hulme 1996; Hulme 1998). Seed predation before or after seed dispersal is particularly important because it directly reduces plant reproductive output. Given that many plant populations are seed-limited (Turnbull, Crawley and Rees 2000), seed predation can have important effects on community dynamics. Hence, seed predators are likely to play an important role in restricting or enhancing coexistence in plant communities.

Community ecology has long regarded competition for resources as the primary interaction determining coexistence, with predation secondarily modifying the effects of resource competition (Chase et al. 2002). However, theoretical models suggest that competition and predation should be treated symmetrically. Coexistence can be either competition-based (if intraspecific competition is stronger than interspecifc competition), predation-based (if predator-mediated apparent competition is stronger within than between species) or jointly based on competition and predation (Chesson and Kuang 2008). Yet, there is a lack of studies quantifying the relative importance of competition and predation for intra- and interspecific interactions in natural communities.

Analyses of competition and apparent competition among plants have to account for the spatial and size structure of plant communities. Spatial community structure is important because the strength of interactions in sessile plant communities generally decreases with the distance between plants, so that plants mostly interact with their close neighbours (Stoll and Weiner 2000). Moreover, many plant communities comprise individuals of very different sizes, which are likely to have very different effects on their competitors and seed predators. Larger plants are stronger competitors because they take up more resources (e.g. Schenk 2006). Similarly, plants with larger seed crops offer more resources for predators and are thus expected to exert stronger apparent competition (e.g. Schnurr, Ostfeld and Canham 2002). Statistical neighbourhood models (Canham and Uriarte 2006) provide a flexible framework for quantifying how interactions among plants depend on plant traits (such as size and seed crop) and on the spatial distance between them. These models use spatially-explicit maps of communities and an explicit description of spatial interactions among plants to predict the performance of focal plants. Neighbourhood models have been used widely to study the effect of competition on the growth and morality of long-lived plants (e.g. Uriarte et al. 2010, Kunstler et al. 2011).In communities of long-lived plants, it is particularly challenging to measure the relative importance of competition and predation. This is because competition and seed predation typically impact these communities on very different timescales: competition acts throughout the life cycle of a plant whereas seed predation is restricted to a relative short period of the life cycle. To compare the relative importance of competition and seed predation for coexistence, one therefore has to quantify their respective contributions to the lifetime fecundity of interacting plants. For most communities of long-lived plants this is a formidable task.

Here, we study how the long-term fecundity of long-lived plants is affected by competition and predator-mediated interactions within and between species. We studied these interactions in fire-prone shrubland communities in the South African Fynbos biome (a global biodiversity hotspot; Myers et al. 2000). These communities are dominated by serotinous shrubs of the genus *Protea* (Proteaceae) that form canopy seed banks, but no long-lived soil seed banks (Bond 1984; Rebelo 2001). Fire triggers seed release from fire-proof cones and subsequent seedling recruitment. Hence, the total fecundity of a plant since the last fire can be measured as the product of cone number, seed set per cone and seed predation rate per cone (Fig. 1a; Nottebrock, Esler and Schurr 2013). The majority of overstorey *Protea* species cannot survive fire as adults (Rebelo 2001); for members of these species, the total fecundity since the last fire is thus identical to their current lifetime fecundity (Nottebrock, Esler and Schurr 2013). Moreover, present-day community maps represent the long-term interaction neighbourhood of *Protea* plants because recruitment is limited to a short period after fire and the inter-fire mortality of successful recruits is generally very low (Bond, Maze and Desmet 1995). This facilitates analyses quantifying how conspecific and heterospecific neighbours affect the long-term fecundity of *Protea* individuals (Nottebrock, Esler and Schurr 2013). Making use of these features of serotinous shrub communities, we mapped 27 *Protea* communities at high spatial resolution and analysed the resulting maps of 49,990 shrubs of 22 *Protea* species with neighbourhood models (Fig. 1). These analyses served to test four hypotheses: (1) competition is stronger between conspecifics than between heterospecifics; (2) apparent competition mediated by seed predators is stronger between conspecifics than between heterospecifics; (3) the size and seed crop of interacting plants determine the intensity of competition and apparent competition, respectively; and (4) competition and apparent competition have equally strong effects on the long-term fecundity of plants.

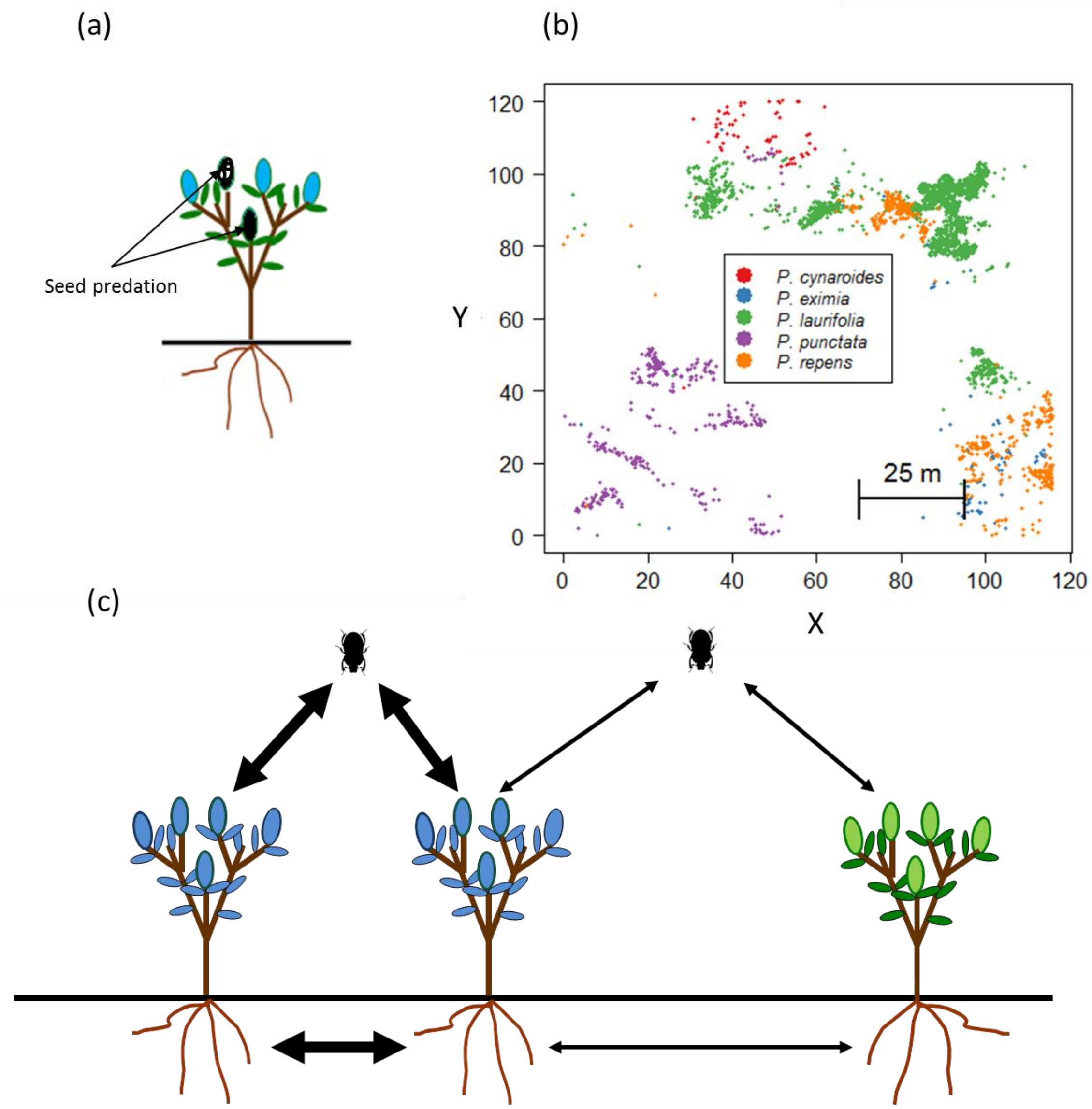

Figure 1. Effects of competition and apparent competition mediated by predators on the fecundity of serotinous *Protea* individuals. (a) The long-term fecundity of an individual depends on cone number and the rate of seed predation per cone. (b) Map of all *Protea* individuals on one of 27 study sites. (c) The fecundity of these individuals is affected by competition and apparent competition with conspecific and heterospecific neighbours; arrow sizes indicate hypothesized values of interaction strength between con- and heterospecific neighbours.

Methods

Study system

The genus *Protea* has high ecological and economic importance in the Fynbos biome (Schurr et al. 2012). The overstorey *Protea* species studied here are all serotinous, broadleaf evergreen shrubs. They typically dominate on nutrient-poor soils (Groom and Lamont 2010) where they compete for soil nutrients and water rather than light (Cramer et al. 2014). Competition for soil resources is generally size-symmetric: larger plants take up more resources, but they do not have a disproportional advantage over smaller plants (other than in competition for light; Schenk 2006). A previous study by Nottebrock et al. (2013) suggests that resource competition among serotinous Fynbos Proteaceae primarily acts to decrease cone numbers per plant.

Pre-dispersal predation of the canopy seed banks of *Protea* is mostly caused by insects. Endophagous beetle and butterfly larvae, notably *Sphenoptera* spp. (Buprestidae), *Genuchus hottentottus* (Scarabaedidae), *Argyroploce* spp. (Olethreutidae), *Tinea* spp. (Tineidae) are major predators of *Protea* seeds (Coetzee and Giliomee, 1987a, Coetzee and Giliomee, 1987b and Wright and Samways, 1999).

Study sites and mapping

We selected 27 study sites of 120 m x 120 m that are dominated by *Protea* shrubs (Fig. 1b). Focal individuals for which we measured cone number and seed predation were located in the 100 m x 100 m core zones that were surrounded by 10 m wide buffer zones. We mapped all overstorey *Protea* plants (>30 cm height) on the study sites using differential GPS (Trimble GeoXH; median accuracy 20 cm), and recorded their size (aboveground canopy height) and species identity. In very dense monospecific stands (>6 individuals per 2 m²), we mapped the outline of the stand, recorded the plant density and then simulated plant locations within the stand according to a completely random spatial distribution with the observed densities. The sizes of these simulated plants were drawn from a stand-specific gamma distribution estimated by a maximum likelihood fit to the sizes of 30 plants measured per stand. In total, the resulting maps of our study communities describe the spatial location and size of 49,990 individuals from 22 *Protea* species. The species composition and density of *Protea* plants varied widely both within and between the study sites (2-9 species per site, site-scale density: 0.003-0.767 plants/m^2, density within 10 m around focal plants: 0-2.2 plants/m^2). In 19 of 22 study species, adults cannot survive fire (so-called ‘nonsprouters’; Rebelo 2001). For members of these species, the size of the canopy seed bank is therefore a direct measure of lifetime fecundity.

Canopy seed banks of focal plants

We counted the number of mature closed cones (*N*) on 2,154 focal individuals within the core zone of the study sites. For 1,755 cone-bearing focal individuals, we harvested up to five mature cones per plant and cut the cones open with secateurs. Callipers were used to measure the cross-sectional diameter of each cone and the cross-sectional diameter of ca. 50 seeds per species and site. The total number of ovules per cone that could potentially set seed was then calculated by dividing the cross-sectional areas of cones by the cross-sectional area of a seed (determined as the mean per species and site population). To calculate seed crops (see below), we also measured the mass of ca. 500 1,014 seeds per species using a high precision scale. The seed predation rate per harvested cone (*P*) was measured as the mean proportion of the cross-sectional cone area that had been consumed by seed predators (see Nottebrock, Esler and Schurr 2013).

Neighbourhood analyses

For neighbourhood analyses of the cone number and per-cone seed predation rate of focal plants, we used extensions of linear mixed models (package lme4, Bates et al. 2014) in R 3.1.1 (R Core Team 2013). We assumed Poisson errors for analyses of cone number and normal errors for analyses of logit-transformed seed predation rates (Warton and Hui 2011). The mixed models described interactions among plants by including neighbourhood indices as explanatory variables. For each focal plant *i*, these neighbourhood indices had the general form

$$c_{\mathrm{i}}(\Omega, \mathrm{X}) = \sum_{j \in \Omega} x_j \exp\left(-\frac{d_{i,j}^2}{\sigma^2}\right)$$

(eqn. 1)

where Ω defines the set of neighbours considered, e.g. all conspecific neighbours in a given radius around the focal plant. In the present analysis, we always considered neighbours in a radius of 10 m. x_j is a trait of neighbour plant *j* (its size or potential seed crop). Alternatively, one can set x_j=1 for all neighbours, in which case the neighbourhood model describes simple density-dependence. Finally, the exponential term is a Gaussian interaction kernel that describes how neighbour effects decline with distance $d_{i,j}$ between the focal and the neighbour plant. The unknown spatial scaling parameter σ is the distance at which the effect of a neighbour drops to 37% of the maximal value (this maximum is reached for $d_{i,j}$=0).

A model that includes such neighbourhood indices as explanatory variables is nonlinear in σ. Hence, it cannot directly be fitted with package lme4. We therefore used a two-level fitting procedure: one-dimensional optimization was used to find the value of σ that maximizes the conditional log-likelihood of the data given σ. For each value of σ, this conditional log-likelihood was obtained by calculating the neighbourhood variables and fitting the respective generalized linear mixed model.

To test our hypotheses, we fitted alternative neighbourhood models that contained different neighbourhood indices. First, we tested whether conspecific neighbours had a stronger competitive effect on the cone number of focal plants than heterospecific neighbours. To this end, we fitted a density-dependent model (setting all x_j=1 in eqn. 1) which included two separate neighbourhood indices that were calculated for all conspecific and all heterospecific neighbours, respectively. We compared this model to a ‘neutral’ density-dependent model describing equal effects of con- and heterospecific neighbours. This neutral model contained a single neighbourhood index that summed over all neighbours (irrespective of whether they were con- or heterospecific). Both models additionally included random effects of site and focal species identity on the intercept. Second, we fitted analogous density-dependent models for seed predation rate to test whether conspecific neighbours exert stronger apparent competition than heterospecific neighbours. Third, we tested whether accounting for traits of interacting plants (size or potential seed crop) improved models for cone number and seed predation, respectively. To this end, we fitted trait-dependent neighbourhood models that included an effect of the focal plant trait and that used the trait values of neighbours to calculate trait-dependent neighbourhood indices (eqn. 1). These trait-dependent neighbourhood models also contained random effects of site and focal species identity on the intercept and the focal trait slope. For each response variable, we fitted both a trait-dependent model describing differential effects of con- and heterospecific neighbours and an alternative neutral model describing equal effects of all neighbours (see above). For trait-dependent analyses of seed predation, we calculated the potential seed crop of each plant as a measure of resource availability to seed predators. This potential seed crop was calculated as the product of cone number (predicted by the cone number model with the lowest AIC), potential seed set per cone and seed mass (see *Canopy seed banks of focal plants* above). Fourth, we used the best model for each response variable (the model with the lowest AIC) to test whether competition and apparent competition differ in their effect on the long-term fecundity of *Protea* plants. The following section describes how these effects were quantified.

Quantifying effects of competition and predation on long-term plant fecundity

The total fecundity of a plant since the last fire, F, is given by

$$F = N * S * (1 - P), \qquad \text{(eqn. 2)}$$

where N is the number of closed cones, S is seed set per cone, and P is the seed predation rate. Since this expression is multiplicative, any change in cone number (N) and in the proportion of seeds that are not consumed by predators ($1 - P$) causes a proportional change in fecundity. For each focal plant, we therefore used the best model for cone number to quantify the summed effect of competition on fecundity as $1 - N_n / N_0$, where N_n and N_0 are predicted cone numbers in the presence and absence of neighbours, respectively. Analogously, we calculated the summed effect of apparent competition mediated by predators as $1 - (1 - P_n) / (1 - P_0)$, where P_n and P_0 are predation rates in the presence and absence of neighbours, as predicted by the best model for predation rate.

Results

The neighbourhood analyses of cone number and per-cone seed predation rate detected clear signs of both competition and apparent competition mediated by seed predators. All neighbourhood models for cone number estimated that neighbour effects decrease the cone number of focal plants, and all models for seed predation estimated neighbours to increase the predation rate of focal plants (for examples see Fig. 2).

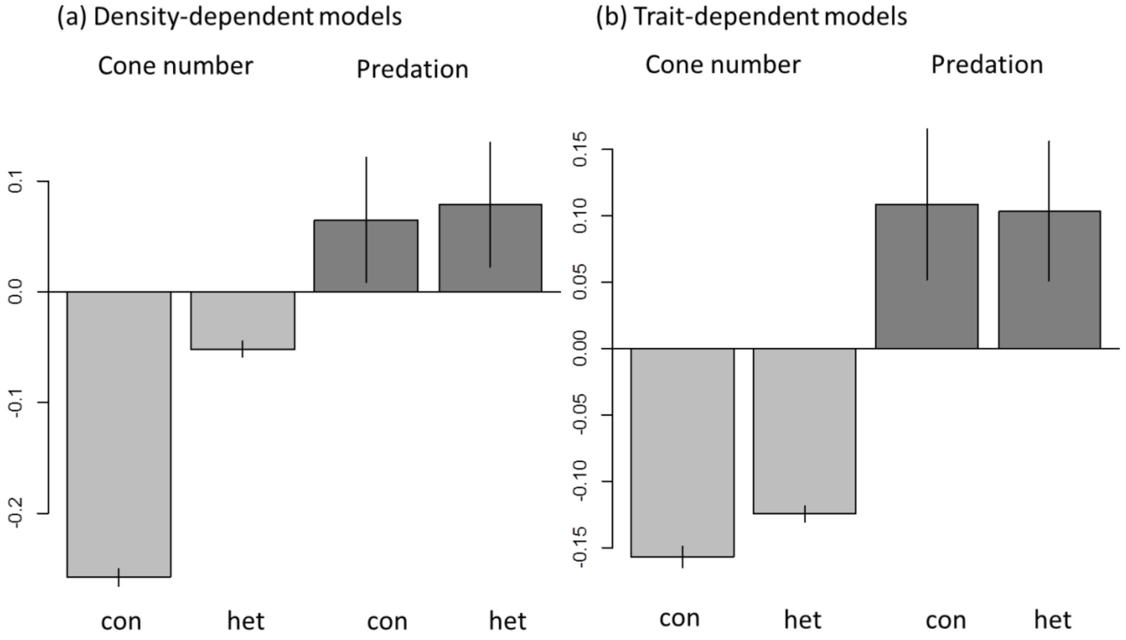

Figure 2. Effects of conspecific and heterospecific neighbours on cone number and seed predation of *Protea* individuals. The barplots show standardized neighbour effects and associated standard errors for (a) density-dependent and (b) trait-dependent neighbourhood models of cone number (light grey bars) and seed predation rate (dark grey bars). The trait-dependent models for cone number and seed predation accounted for the size and potential seed crop of interacting plants, respectively.

As hypothesized, a density-dependent model for cone number estimated that conspecific neighbours have a much stronger competitive effect on cone number than heterospecific neighbours (Fig. 2a). This model, describing differential effects of con- and heterospecific neighbours, performed markedly better than an alternative model of neutral density-dependence in which con- and heterospecifics had the same competitive effect (Likelihood ratio test: $\chi^2_{1\,df} = 414.8$, $p < 0.001$; Table 1a).

Contrary to our expectation, however, density-dependent models for seed predation showed that apparent competition was not stronger among conspecifics than among heterospecifics. A model in which the strength of apparent competition could differ between con- and heterospecifics estimated very similar effect sizes (Fig. 2a). Accordingly, this model did not perform better than the alternative model of neutral apparent competition ($\chi^2_{1\,df} = 0.03$, $p > 0.85$; Table 1b).

Accounting for the size and potential seed crop of interacting plants improved neighbourhood models for both cone number and seed predation (Table 1). According to the best model for cone number, larger plants produced more cones, larger neighbours had a stronger competitive effect, and conspecific neighbours were stronger competitors than heterospecific neighbours (Fig. 2b). The best model for seed predation predicted that per-cone seed predation rates increased with the seed crop of focal plants and with the seed crop of their neighbours, but that neighbour effects did not differ between conspecifics and heterospecifics (Fig. 2b). These best, trait-dependent models for cone number and seed predation thus confirmed the findings of the simpler density-dependent models: conspecific neighbours had a stronger competitive effect on cone number than heterospecifics, but the effect of conspecifics on seed predation was comparable to that of heterospecifics. Overall, alternative models for seed predation showed smaller AIC differences than alternative models for cone number (Table 1). The best models of cone number and seed predation produced very similar estimates of the spatial scaling parameter σ, which suggests that competition and apparent competition act at similar spatial scales (Fig. 3).

Table 1. Comparison of alternative neighbourhood models for (a) cone number and (b) seed predation rate. Neighbour identity indicates whether models describe different effects of con- and heterospecifcs or whether they describe identical effects of con- and heterospecifics. Neighbour effect indicates whether the strength of a neighbour’s effect depends on plant size and potential seed crop, respectively, or whether the model describes density-dependence independent of plant or crop size. The best model (with lowest AIC) is highlighted in italics.

(a) Models for cone number

Neighbour identity	**Neighbour effect**	**df**	**ΔAIC**
Con- vs. heterospecific	***Size-dependent***	***12***	***0***
Neutral	Size-dependent	11	6.7
Con- vs. heterospecific	Density-dependent	5	24953.6
Neutral	Density-dependent	4	25356.6
No neighbour effects		10	900.9

(b) Models for seed predation rate

Neighbour identity	**Neighbour effect**	**df**	**ΔAIC**
Con- vs. heterospecific	Seed-crop-dependent	12	2.0
Neutral	***Seed-crop-dependent***	***11***	***0***
Con- vs. heterospecific	Density-dependent	6	3.3
Neutral	Density-dependent	5	1.4
No neighbour effects		10	4.2

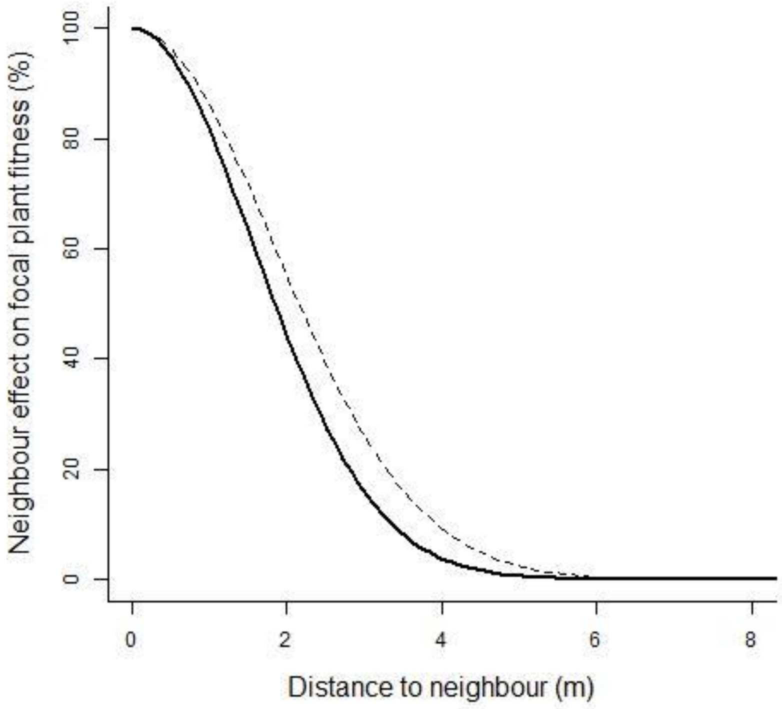

Figure 3. Distance dependence of neighbour effects estimated by the best neighbourhood models of cone number (solid line) and seed predation rate (dashed line). Neighbour effects are expressed relative to the maximal effect (as for a distance of 0 between a focal plant and its neighbour).

We compared how competition and apparent competition affected the fecundity of those focal plants that were included in neighbourhood analyses of both cone number and seed predation rate. This comparison showed that plants suffering from strong competition also experienced strong apparent competition (one-sided Spearman's rank correlation test, ρ=0.84, p<0.001, Fig. 4). However, competition had markedly stronger effects than apparent competition (mean fecundity reductions: 10.7% and 2.6%, respectively; one-sided paired Wilcoxon signed rank test, V = 4566, p<0.001). In fact, competition effects were stronger than predation effects for 97% of the 1,620 plants (Fig. 4).

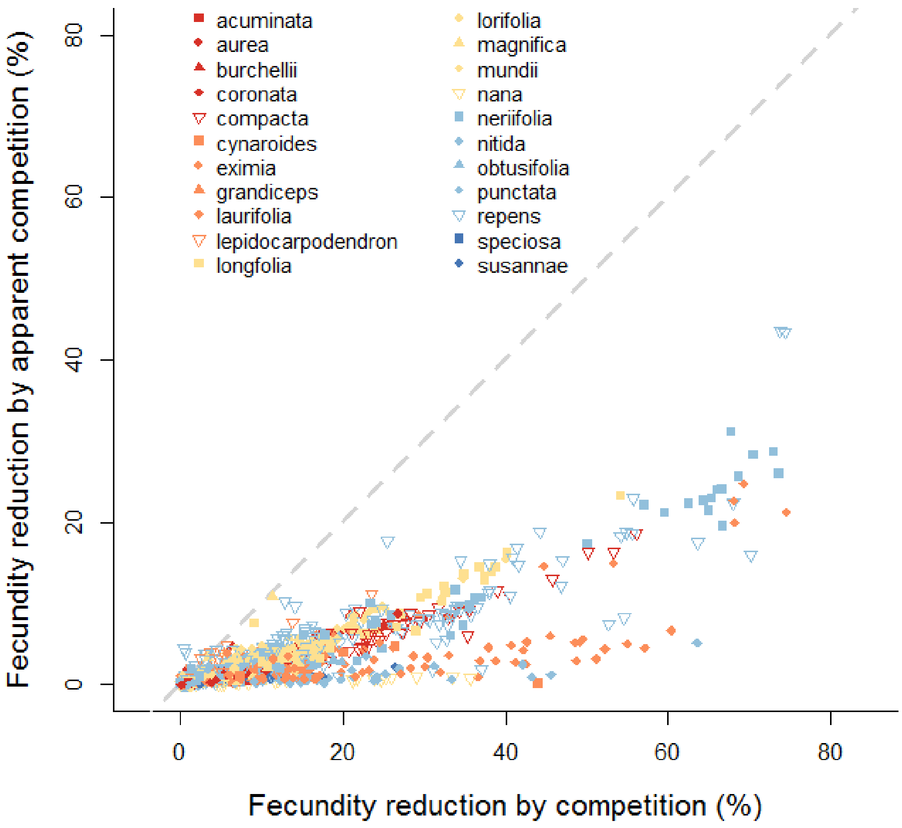

Figure 4. Relationship between the effects of competition and apparent competition on the fecundity of 1,620 focal plants of 22 *Protea* species. These effects were calculated as the relative reduction of fecundity that is caused by all neighbouring plants (as predicted by the best neighbourhood models for cone number and seed predation, respectively; see Table 1). The dashed grey line is the 1:1 identity.

Discussion

Our results show that neighbouring plants can reduce the long-term fecundity of *Protea* individuals both through direct competition and through apparent competition mediated by seed predators. As hypothesized, we found that conspecific neighbours have stronger competitive effects on cone number than heterospecific neighbours (Fig. 2). In contrast, we could not confirm the hypothesis that pre-dispersal seed predators cause apparent competition to be stronger between conspecifics than between heterospecifics (Fig. 2). This suggests that competition promotes the stable coexistence of *Protea* species, whereas pre-dispersal seed predation does not.

We also found support for the hypothesis that plant traits predict the intensity of interactions among plants. The competitive effects of neighbours increased with their size (as a proxy for resource acquisition; Schenk 2006) and predator-mediated effects of neighbours increased with their potential seed crop (as a measure of resource availability to seed predators). Simple traits measuring resource use by plants and resource availability to seed predators can thus explain the strength of biotic interactions in our study system. Finally, we rejected the hypothesis that competition and apparent competition via seed predators have similar effects on the fecundity of *Protea*. Instead, the effects of competition were much stronger than the effects of apparent competition (Fig. 4). It should be noted that the quantification of competitive effects was based on the best model for cone number which controlled for effects of focal plant size. The competitive effects on cone number estimated by this model do thus not incorporate competitive effects on focal plant size. Hence, the model may even underestimate the total amount of competition.

In the following, we first discuss mechanisms that may shape competition and predator-mediated interactions in *Protea* communities, and briefly highlight other processes that might affect the coexistence of *Protea* species. We end with a brief outlook on future studies in community demography.

Mechanisms shaping competition and predator-mediated interactions in Protea communities

Even though our study species are closely related and ecologically similar, we found that intraspecific competition is stronger than interspecific competition. This suggests that these species use resources at different spatial locations or at different times (Chesson 2000; Silvertown 2004). This result cannot be explained by simple spatial segregation of species (Pacala 1997), since our neighbourhood models detect stronger intraspecific competition while controlling for spatial community structure. However, fine-scale hydrological niche segregation may help to explain why conspecifics compete more intensively than heterospecifics. For another plant group in the Fynbos biome (Restionaceae), Araya et al. (2011) demonstrated fine-scale differentiation of hydrological niches that may reflect partitioning of space or water use over small scales via differences in rooting depth or phenology. In fact, phenological differentiation may play a key role for nutrient partitioning among *Protea* species. This is because the Mediterranean-type climate of Fynbos causes a temporal separation of optimal light and temperature conditions from peak water and nutrient availabilities, thereby creating opportunities for phenological differentiation in nutrient acquisition (Cramer et al. 2014). Interestingly, our study species show substantial phenological differentiation in flowering and cone production (Rebelo 2001, Nottebrock et al. submitted). This might explain why we found that conspecifics have a stronger effect on cone numbers than heterospecifics. Alternatively, the temporal displacement of flowering phenologies is often interpreted as a consequence of interspecific competition for generalist pollinators (Devaux and Lande 2009). It will be interesting to explore to what extent phenological differentiation in flowering and cone production is driven by competition for nutrients, competition for pollinators and feedbacks between these two potential mechanisms.

While we found competition for resources to be stronger for conspecifics, the strength of apparent competition did not differ between conspecifics and heterospecifics. Although our large-scale analysis did not distinguish between different species of seed predators, this finding suggests that the main pre-dispersal seed predators of *Protea* are rather generalists that indiscriminately attack several host plant species. In fact, an extensive study of seed predator assemblages in *Protea* cones from six sites found nine predator species, which all attack a wide range of *Protea* species (Wright and Samways 1999).

The trait-based neighbourhood analyses suggest an interesting interaction between competition and seed predation: competition decreased individual seed crops in dense stands, which may reduce apparent competition. Nevertheless, we found a positive correlation between the strength of competition and apparent competition (Fig. 4). This is because on our study sites the effects of competition on seed crops were not overcompensating: the overall seed crop of dense stands was not smaller than that of sparse stands, even though individual seed crops were smaller. Interestingly, overcompensating density-dependence of seed crops has been documented in some *Protea* populations (Bond, Maze and Desmet 1995). It will be interesting to test whether the correlation between competition and apparent competition becomes negative under these circumstances.

Alternative processes contributing to coexistence of Protea species

The stabilizing effect of resource competition on coexistence of *Protea* species might be reinforced by other processes. A first option is interactions mediated by shared pollinators (Pauw 2013). Yet, an analysis of pollinator-mediated interactions in our study communities suggests that these interactions do not stabilize local coexistence (Nottebrock et al. under review). Second, coexistence may be promoted through post-dispersal seed predation (Hulme 1998). However, the seeds of our study species are simultaneously dispersed in vegetation-free post-fire landscapes (Schurr et al. 2005) where they face seed predation by generalist rodents (Bond 1984). While post-dispersal seed predation is thus unlikely to promote coexistence, it is conceivable that pathogens with a high host-specificity cause apparent competition to be stronger within than between species. Indeed, Proteaceae host a large number of fungal pathogens, some of which are assumed to be highly host-specific (Crous et al. 2011). In addition to these biotic interactions, a trade-off between reproduction and survival may enhance coexistence. The genus *Protea* is a prime example for this trade-off since it comprises both resprouters (with high fire survival and low fecundity) and nonsprouters (with low fire survival and high fecundity). However, models for Proteaceae communities suggest that local coexistence between resprouting and nonsprouting species only occurs under a restricted set of conditions (Groeneveld et al. 2002; Higgins, Flores and Schurr 2008). Finally, regional-scale processes might contribute to the maintenance of species richness in *Protea* meta-communities. Notably, resprouters and nonsprouters represent a competition-colonization trade-off (Schurr et al. 2007, Higgins, Flores and Schurr 2008), yet it remains to be tested whether this trade-off is tight enough to enable the regional-scale coexistence of a large number of species (Tilman 1994). Regional-scale processes also figure prominently in the neutral theory (Hubbell 2001). However, fits of the neutral theory to rank-abundance data from Fynbos communities can yield biologically unrealistic parameter estimates (Etienne et al. 2006). Our study adds to this by showing that competition in Fynbos communities is not neutral.

Outlook

This study highlights the potential and challenges of ‘community demography’, the demographic study of multiple interacting species. One of these challenges is the joint analysis of spatial and temporal variation in demography and biotic interactions. It seems particularly promising to combine the explicit description of demographic variation between individuals and years (Clark 2010) with the explicit description of direct and indirect spatial interactions between individuals (this study). Still, even without explicitly resolving variation between individuals and years, our neighbourhood analyses of long-term fecundity components lead to testable hypotheses. For instance, the hypothesized stabilizing effect of phenological differentiation in nutrient acquisition (Cramer et al. 2014) can be examined by combining field experiments and observations in *Protea* communities with tests for phenological overdispersion among neighbouring plants. Additionally, community demographic studies have the potential to identify determinants of biotic interactions that act across species and communities. For instance, we have identified the potential seed crop as an interaction currency (Kissling et al. 2012) that determines apparent competition between multiple plant species via shared seed predators. The identification of interaction currencies provides an alternative to the classical, yet idiosyncratic and unrealistic description of community dynamics in terms of interactions among species pairs (McGill et al. 2006; Clark 2010; Kissling et al. 2012). In megadiverse systems such as Fynbos, such generalizations across communities of different species compositions are urgently needed to understand and forecast community dynamics (Yates et al. 2010).

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General Discussion

Discussion

In the final part of my thesis I summarize the basic methodological and ecological findings of the previous chapters, and discuss their implications for the maintenance of *Protea* species diversity and the conservation of *Protea species.* Finally, I discuss potential directions for further research.

Methodological findings

In this thesis, I used a trait-based approach to analyse spatiotemporal species interactions in *Protea* communities to understand key components of the lifetime fecundity of *Protea* plants (sensu McGill et al. 2006). The trait-based approach included analyses of data from a wide range of spatial (inflorescence to 4 ha sites) and temporal (pollinator visits to the lifetime of *Protea* plants) scales. Functional plant traits were collected at the individual, population and species levels and were used in analyses of plant-plant and plant-animal interactions of all Chapters. I used trait-based analyses, which quantify how these traits impact neighborhood interactions. Trait-based approaches are increasingly used in community ecology, because they offer opportunities for a deeper understanding of community dynamics (McGill et al. 2006, Garnier et al. 2011).

Using differential GPSs (dGPSs), highly accurate, spatially explicit data of 22 overstorey *Protea* species from 27 sites were collected, which enabled the generation of detailed maps of individual *Protea* positions. In addition all (non-Proteaceae) shrubs or trees taller than 100 cm were mapped and identified (at least to genus level). In total ca. 210,000 plants were mapped, of which ca 130,000 plants were *Protea* species. To estimate trait-based neighbourhood models, differential GPS (dGPS) was used to collect highly accurate data of plant positions to analyse aspects of community dynamics, plant-animal interactions (behaviour) and species coexistence in this mega-diverse system (sensu McGill et al. 2006).

I generated explanatory variables of different spatial and temporal scales for all mapped *Protea* individuals (Chapter 1). These enabled me to develop landscapes of floral resources at different spatial and temporal scales. As an example I generated predictions for the composition, distribution and availability of plant sugar amounts of all *Protea* species. This prediction of plant sugar in space and time defines sugar landscapes. These sugar landscapes were then used in “resource-based” neighbourhood models to understand plant-plant and plant-animal interactions and processes. I analysed plant reproduction and pollinator behaviour in Chapter 1 and pollinator behaviour in Chapter 2 and 4 with spatiotemporal sugar landscapes. In Chapter 5 I used the same trait-based neighbourhood models to create resource landscapes of traits, to understand the interaction of competition and apparent competition mediated by seed predators. Chapter 3 used a (local) trait-based model without neighbourhood interactions to understand drivers of plant reproduction, and established that sugar concentration matters for pollination success. The development of such a trait-based neighbourhood framework into a ‘resource-based’ neighbourhood framework on several hierarchical levels, which incorporated many different spatial and temporal scales, enabled me to show how the species, trait and resource composition of *Protea* communities affects the dynamics of these communities.

In summary, the major objective of this thesis was to quantify plant-plant and plant-animal interactions and their roles in structuring plant communities and facilitating species coexistence. The results suggest that trait-based and ‘resource-based’ neighborhood models can indeed help to understand and predict the spatial dynamics of plant-plant and plant-animal interactions at various spatiotemporal scales. Such an integrative approach shows that species interactions occur on several scales and helps to explain the species diversity in the Fynbos biodiversity hotspot. These are interesting implications for quantifying the dynamics of highly diverse plant communities in the Cape Floristic Region (CFR). This quantification might be useful to understand and predict the dynamics of plant communities under threat of global change (Yates et al 2010). The development of trait-based neighbourhood models into resource-based neighbourhood models shed new light on the identification of “interaction currencies” in plant communities (Kissling et al. 2012, this thesis). The integration of these interaction currencies, which can quantify plant-plant and plant-animal interactions, are helpful to distanle community dynamics under climate change. I hope that the presented trait- and resource-based neighbourhood models at different spatiotemporal scales will be a useful addition to the methodological toolbox of ecologists.

Ecological findings

This thesis shows how the spatiotemporal distribution of floral resources affects pollinator behaviour and in turn, how pollination services affect plant reproduction. Additionally, this thesis shows how the spatial distribution of resources explains key components of plant fecundity (number of cones and seed predation).

Using trait- and resource-based neighbourhood models I was able to demonstrate in the first chapter that interactions between *Protea* plants are mediated by pollinators. I quantified the floral resource landscapes that plant communities provide for pollinators, and studied how these landscapes affect pollinator behaviour and plant reproductive success. The study shows that floral resources can act as a common ‘interaction currency’ that explains pollinator-mediated interactions within and among plant species (Kissling et al. 2012). This ‘interaction currency’ can create surprising interactions in plant communities since its effects vary in the nectar sugar distribution, purity, quality and phenology at different spatial scales. Importantly, the impacts of floral resources on plant communities can alter conditions for species coexistence and can cause community-level Allee effects that promote extinction cascades (Colwell et al. 2012).

In the second chapter Baptiste Schmid and I used the generated floral resource landscapes (Chapter 1, Appendix 3) to understand the abundance and per-plant visitation rate of two major nectarivorous bird pollinators (Cape sugarbird *Promerops cafer* L., orange-breasted sunbird *Anthobaphes violacea* L.) at different spatial scales. The population sizes of both bird species responded positively to the amount of sugar resources at the site scale. However, high resource density decreased per-plant pollinator visits: i.e. pollinators show territoriality. At a smaller scale we observed that *Proteas* either compete for bird pollinators or they facilitate each other to attract pollinators depending on the sugar amount offered by focal *Proteas*. At the small scale bird pollinators visited plants with high sugar rewards, rather than those with low sugar. This study shows the importance of focusing on different spatial scales to understand the pollinator response to resource availability. Chapter 3 highlights the pollination syndrome of *Proteas* where Baptiste Shcmid and I aimed to test if *Proteas* are fully self-compatible or incompatible. We experimentally investigated seven *Protea* species from the South African fynbos to assess variability in reproduction (seed set, seed mass, germination) in response to pollinator exclusion (full-exclusion, bird-exclusion) and hand-pollination (self-pollen, cross-pollen). The results show that the 14 observed *Protea* species were all self-compatible and observed to rely on animal-pollination. A trait-based analysis indicated that nectar sugar concentration is a good predictor of *Protea* plant reproduction. In summary, the findings contribute to a resource-based understanding of the functional determinants of plant–pollinator interactions.

Chapter 4 has interesting implications on pollinators’ constancy in *Protea* plant communities. By constructing resource landscapes and by observations of bird pollinator behaivoir Baptiste Schmid and I were able to show the foraging behaviour of Cape sugarbirds (*Promerops cafer*) on *Protea* plants. Our analysis provides the first evidence for flower constancy (where pollinators show preference for common resources) acting simultaneously with positive frequency-dependent foraging (where pollinators temporally specify on specific resources) in natural plant communities. We show that positive frequency dependence of Cape sugarbirds foraging on common *Protea* species and flower constancy improve pollen transfer between conspecific plants. This behavior is important for rare species: pollinators' foraging decisions might be a key mechanism favoring the coexistence between common and rare species in the species-rich CFR.

In Chapter 5, I generated seed crop landscapes to compute variables of conspecific and heterospecific resource availability in order to explain levels of competition and predation and their role for species coexistence (sensu Chesson and Kuang 2008). I show with neighbourhood analyses that competition, rather than predation, stabilizes species coexistence in *Protea* communities. *Protea* plants compete more with conspecifics than with members of other species, which creates a stabilizing effect, whereas apparent competition mediated by seed predators is not stronger between conspecifics than between heterospecifics and is therefore not stabilizing. Moreover, I found that competition and predation are strongly positively correlated. Thus *Protea* plants suffer from competition and apparent competition at the same time. The stabilising effects may arise because conspecific *Protea* plants compete for similar resources at the same time (Cramer et al. 2014) and the neutral effect of apparent competition may arise because species share the same generalist predators that seem to feed largely indiscriminately on *Protea* species (Wright and Samways, 1999).

In summary, this thesis has demonstrated that a quantification of spatial community structure, coupled with trait-based models can determine the variation in the performance of plant species and individuals, and that this performance is shaped by the interplay of biotic interactions and functional traits at different spatial and temporal scales. Specifically, I show that *Protea* individuals experience not only direct competition from other plants but also experience indirect competition and facilitation mediated by pollinators and seed predators. The trait-based neighbourhood framework might therefore be helpful to understand the maintenance of plant species diversity in the Fynbos biome. In the following, I will discuss if the results could contribute to the maintenance of species diversity and if the results could have implications for the conservation of the biodiversity hotspot fynbos.

Implications for the maintenance and conservation of species diversity

This project focused on a trait-based rather than a species-specific understanding of Fynbos communities. Such a general trait-based understanding is indispensable if one want to predict the future fate of the megadiverse Fynbos flora under environmental change (Yates et al. 2009). For instance, the results of this project can be integrated into existing models for the community and range dynamics of Proteaceae under climate and land-use change (Higgins et al. 2008, Cabral and Schurr 2010, Appendix 2). Furthermore our findings of stabilizing mechanisms, which show niche differentiation in *Protea* communities and that coexistence is stabalized, might be interesting contributions for conservation management and planning (Chesson and Kuang 2008). I suggest, for example, managing *Protea* communities so that *Protea* species should form dense stands that can potentially stabilise coexistence. In addition, the composition of *Protea* species with different phenologies would provide nectar sugar for bird pollinator (e.g. Sugarbird) populations over extended flowering seasons. Concretely, I show that competition and –pollinator mediated interactions are not neutral, whereas apparent competition mediated by seed predation seems to be neutral. This neutral effect of seed predation might fit Hubbells (2001) neutral ecological theory where all species in a community are assumed to be equivalent. Neutral theory might describe the complexity of natural communities in a very concise way (Hubbell 2001, Chave 2004, Alonso and McKane 2004), where only two numbers characterize a meta-community: the fundamental biodiversity number (the potential species richness of the community) and the immigration parameter (its degree of isolation). Yet, neutral theory ignores the complexity of natural communities by lumping them into these stochastic terms (Clark et al 2009). I found complex interactions in natural *Protea* communities, which show niche differentiation (stabilizing effects) and that apparent competition mediated by seed predation does not contradict this effect. This suggests that a unified framework of niche and neutral theory may aid in translating ecological theory to conservation practice (Holt 2006), which has different implications for biodiversity conservation. For example conservation should focus on conserving entire *Protea* communities (including common species) not just rare species and rather considering the role of entire *Protea* communities in an ecosystem (Chesson and Kuang 2008). This can be seen in our findings that pollinator-mediated facilitation occurs at large spatial scales (Chapter 1). Moreover, the findings of Chapter 5 of stabilizing species coexistence and in Chapter 1 the Allee effect, which show a potential for extinction cascades, may mean that one has to conserve multiple plant species in an entire communitiy. The composition of *Protea* communities and the availability of '*Protea*-based' resources in space and time are important factors for pollinator- or predator-mediated interactions. These interactions drive the dynamics of plant communities and need to be considered in their conservation, which could also implement to not only focus on a species' own conservation but rather considering its role in an ecosystem to conserve multiple plant species in a community (Chesson and Kuang 2008).

In summary, this thesis shows that pollinators can mediate the maintenance of plant species diversity on a large scale (Chapter 1), the constancy behavior of bird species might facilitate rare plant species (Chapter 4) and that competition rather than apparent competition mediated by predators stabilizes plant coexistence (Chapter 5). All these processes have to be considered jointly to understand their effects on diversity maintenance. Beyond these applied perspectives, this thesis could be the starting point for a longer-term study into the physiological, energetic, and genetic mechanisms underlying spatial plant-plant and plant-animal interactions and the role of these mechanisms for the maintenance of diverse communities and for demographic processes at larger spatial scales (e.g. Appendix II).

Outlook

A next step could be to use the trait-based neighbourhood models, developed here, to predict the dynamics of potentially novel communities under future conditions e.g. of climate change (Ackerly and Cornwell 2007). There is still a lack of strong and coherent theoretical and empirical foundation to incorporate species interactions and traits into climate change research (Deutsch et al 2008). Novel communities composed of new combinations of species under climate change, are predicted to result from changing biotic and abiotic conditions, and from differential range shifts of species. The findings on general determinants of biotically- and abiotically-mediated interactions might help to predict the dynamics of these novel communities and (Brooker 2006).

Concretely, predictions of the trait-based neighbourhood model could predict dynamics of communities to virtually generate novel communities. These predictions could be used to test how the dynamics of communities will change if species are virtually removed from communities. This would help to assess how the local extinction of species due to climate change could change the dynamics of communities. Nevertheless, it is still a challenge to determine whether the responses of organisms are determined by particular species traits and how species interactions and community dynamic are determined (Lurgi et al. 2012).

These findings might not only help to predict future community dynamics under climate change but also help managing conservation areas. The findings of biotic interactions on different scales suggest that conservation should view the whole interaction system rather than conserving single species (Simberloff 1998). A next step for future research to understand community dynamics would be to include a more simplified model, where species are removed by their trait difference between individuals in a community. The trait difference is calculated by the spatial difference of plant traits of each individual and neighbouring plants. These trait differences could explicit distinguish between abiotic resource-use of co-flowering individuals in a community. Furthermore the incorporation of species specific flowering phenologies could correct for resource-use at different times in a year, because plants might use more resources during peak flowering time (Cramer et al. 2014). Finally, it is timely to test whether resource landscapes play similar roles in other pollination systems and for other types of generalized trophic interactions, such as plant-herbivore and plant-frugivore networks.

Conclusions

The results of this thesis show that resource landscapes can quantify plant-plant, plant-animal interactions and the pollinator behaviour in *Protea* communities. Resources landscapes appear to be a common ‘interaction currency’ (Kissling *et al.* 2012) that determines how multiple plant species interact via their shared generalist pollinators or via seed predators. The identification of such interaction currencies is crucial for both developing a more general understanding of community dynamics and predicting community dynamics in changing environments (McGill *et al.* 2006; Kissling *et al.* 2012). Moreover the thesis shows that a trait-based understanding of the functional determinants of plant-plant and plant–animal interactions reveals new insight for the dynamics of plant communities. The stabilising mechanism at site scale mediated by pollinators (Chapter 1), the constancy behaviour of bird pollinators (cape sugarbird) acting simultaneously with positive frequency-dependent foraging on *Protea* species (Chapter 4) and the stabilizing effects of competition and equalizing effects of seed predation at the neighbourhood scale (Chapter 5) might help to explain species coexistence in the Cape Floristic Region (CFR). This thesis shows that a 'trait-based' research program quantifies the variation in the performance of multiple species and individuals, which sheds new light on the dynamics of communities in a biodiversity ‘hotspot’.

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Curriculum vitae

Academic education

02/2016	Oral defence of Thesis at University of Hohenheim
10/2015	Doctoral Thesis: *Spatial and functional determinants of long-term fecundity in serotinous shrub communities.*
04/2014 – 02/2016	PhD student at the Institute of Landscape and Plant Ecology, University of Hohenheim (Germany) supervised by Dr. Frank M. Schurr: *Spatial determinants of animal pollination and plant fecundity in South African Fynbos: the Protea-bird system.*
02/2012 - 12/2015	PhD student at ISEM (Institut des Sciences de l'Evolution de Montpellier, France) in the metapopulation group supervised by Dr. Frank M. Schurr: *Spatial determinants of animal pollination and plant fecundity in South African Fynbos: the Protea-bird system.*
02/2011 - 08/2012	Research in different Nature Reserves of the Western Cape Region, South Africa permitted by CapeNature and affiliated student at the Department of Conservation Biology and Entomology, Stellenbosch University (South Africa) in collaboration with Dr. Karen J. Esler. Field work and laboratory work included plant mapping, plant trait sampling and analyses and measurements of various plant traits: responsible for six assistants.
02/2011	Start of my doctoral research project financed by the German Research Foundation (DFG) at the Institute of Plant Ecology and Nature Conservation, University of Potsdam (Germany).
10/2002 - 02/2011	Diploma (Master) in Geoecology, University of Potsdam (Germany) with specialisations in Conservation Ecology and Statistical Modelling. Thesis supervised by Dr. Frank M. Schurr and Dr. Karen J. Esler: *Effects of intra- and interspecific density on the lifetime fecundity of long-lived shrubs.*
2007/2008	Affiliated student at the Department of Conservation Biology and Entomology, Stellenbosch University (South Africa): research in different Nature Reserves of the Western Cape Region, South Africa and internship within the BIOTA project (Biodiversity Monitoring Transect Analysis in Africa) in collaboration with SANBI (South African National Biodiversity Institute), Cape Town, South Africa.
07/2001	Abitur (A levels), specialised in Biology, Chemistry, Economy and Literature, Wilhelm-Busch-Gymnasium Stadthagen (Germany).

Professional experience

2009 - 2011	System administrator and laboratorial assistant at the Institute of Plant Ecology and Nature Conservation at University of Potsdam (Germany) • Construction and coordination of an information system • Literature research and documentation, and laboratory work
2007 - 2009	Creation, conception and sale of embroidery textiles, ‘Stickbar’ in Berlin (Germany)
2005 - 2007	Shop assistant at an ergonomic furniture shop ‘Sitz-Art’ in Berlin (Germany) • Client service: Consulting , sale and delivery
Before 2005	Numerous seasonal work (botanical garden, Post office, hardware store)
2001 - 2002	Civil service at the old-aged house ‘Saint Elisabeth-Stift’ (Berlin, Germany)

Grants

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Skills

Software: R (professional): Tinn-R, RStudio, SIG (professional): QGIS, ArcMap, ArcGIS, SAGA GIS

Statistica and SPSS (linear models and assumptions)

Basic knowledge of programming languages: S, SQL, C, C++

Excellent knowledge of Microsoft Office (Word, Excel, Power Point, Access)

Basic knowledge of Adobe Photoshop and Illustrator

Languages

German: mother tongue

English: fluent

French: fluent

Interests

Sport: active handball, hiking and biking

Member of the Ecological Society of Germany, Austria and Switzerland (GfÖ)

Member of TRY (Plant Trait Database)

Publications

Nottebrock H., Schmid B., Treurnicht M., Esler K.J., Pagel J., Böhning-Gaese K., Schleuning M. & Schurr F. Coexistence of plant species in a biodiversity hotspot is stabilized by competition but not by seed predation. *Oikos.* doi: 10.1111/oik.03438

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Nottebrock H. (2015) Spatial and functional determinants of long-term fecundity in serotinous shrub communities. Oral thesis presentation at ISEM, Montpellier (France), 02. November 2015

Nottebrock H. (2013) Candy land: who can resist? Predicting plant fecundity from spatiotemporal variation in sugar landscapes. Oral presentation, seminar series at ISEM, Montpellier, 16. Décembre 2013

Nottebrock H., Schmid B., Esler K. J., Pagel J., Böhning-Gaese K., Schurr F., Schleuning M. (2013) Predicting plant fecundity from spatiotemporal variation in sugar landscapes. Oral presentation, 43nd Annual Meeting of the Ecological Society of Germany, Austria and Switzerland (GfÖ) in Potsdam (D), 09 – 13 September 2013

Nottebrock H., Schmid B., Esler K. J., Böhning-Gaese K., Schleuning M., Schurr F. (2012) How do plant-plant and plant-pollinator interactions affect the fecundity of Protea individuals? Oral presentation, Fynbos Forum in Cape St-Francis (South Africa), 17 – 19 July 2012

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APPENDIX I

Effects of intraspecific and community density on the lifetime fecundity of long-lived shrubs*

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*This article includes data and similar content of the Diploma thesis of Henning Nottebrock, but has been modified during the cours of this doctoral thesis and has been published in Perspectives in Plant Ecology, Evolution and Systematics:

Nottebrock H., Esler K.J. and Schurr F. (2013) Effects of intraspecific and community density on the lifetime fecundity of long-lived shrubs. *Perspectives in Plant Ecology, Evolution and Systematics.* 15: 150–161.

Abstract

Intra- and interspecific density dependence has profound consequences for plant population and community dynamics. In long-lived plants, however, lifetime patterns and mechanisms of density dependence are difficult to study. Here, we examine effects of intraspecific and community density on the lifetime fecundity of two long-lived shrub species from South African Fynbos: *Protea repens* (animal-pollinated, hermaphroditic) and *Leucadendron rubrum* (wind-pollinated, dioecious). Both species are serotinous, retaining seeds in cones until fire kills the mother plant. We measured lifetime fecundity as the product of cone number, proportion of cones that are not damaged by predation and seed set (fertile seeds per intact cone). Intraspecific and community densities were quantified by counting individuals of target species and all Proteaceae in small- and large-scale neighbourhoods (10 m and 50 m radius) around each focal individual. Additionally, we determined the age and size of focal individuals. We found that lifetime fecundity of the wind-pollinated *L. rubrum* is density independent. In contrast, the lifetime fecundity of the animal-pollinated *P. repens* increases with large-scale intraspecific density and shows a hump-shaped relationship to large-scale community density. Community density has a hump-shaped effect on seed set (probably through absence of pollinators at low and competition for pollinators at high densities) and negatively affects cone number per individual. For both species, plant age decreases seed set while increasing lifetime fecundity. These qualitative differences in the density dependence of lifetime fecundity may arise from differences between animal and wind pollination. In particular, interactions with generalized animal pollinators may cause community-level Allee effects with profound consequences for the future dynamics of long-lived plant populations and communities.

Introduction

The density dependence of fecundity and reproduction strongly shapes the dynamics of populations and communities. Negative density dependence determines the carrying capacity of populations, and the strength of negative density dependence affects the intrinsic stability of population dynamics (May, 1974). Moreover, the relative strength of negative intra- vs. interspecific density dependence is important for community dynamics and species co-existence (Tilman, 1994; Chesson, 1994). However, density dependence is not necessarily negative: there is now substantial evidence that reproductive success decreases at low intraspecific densities (Lamont et al*.,* 1993; Roll et al., 1997; Groom, 1998; Kery et al., 2000; Hackney and McGraw, 2001). The resulting positive density dependence at low densities (a so-called Allee effect) can reduce population persistence, and has substantial consequences for the conservation and management of small populations (e.g. Courchamp et al*.,* 2008).

Sessile plants interact over limited spatial scales (e.g. Silander, 1978; Law and Dieckmann, 2000; Gunton and Kunin, 2007). The dynamics of plant populations and communities thus depend on the spatial scales over which intra- and interspecific density effects act (Kunin, 1997; Groom, 1998; Bolker and Pacala, 1999; Moeller, 2004; Schurr et al*.,* 2008). To understand and predict the dynamics of plant communities, we have to determine the magnitude, spatial scale and direction (positive or negative) of intra- and interspecific density dependence. This requires an understanding of the mechanisms that generate different types of density dependence.

A major mechanism causing negative density dependence of plant reproduction is competition for abiotic resources such as nutrients or light (e.g. Stoll and Weiner, 2000). However, negative density dependence can also arise from plant-animal interactions, for instance if pre-dispersal seed predators are attracted to high-density stands or if plants compete for flower visits by pollinating animals. At the same time, biotic and abiotic pollination is also a common cause for positive density dependence: plants in small populations often have reduced reproductive success because of decreased pollination quantity or quality (Ghazoul, 2005).

At low intraspecific densities, both wind- and animal-pollinated plants may show positive density dependence of pollination because the presence of conspecifics increases pollen production and reduces pollen loss, which may decrease pollen limitation (Ghazoul, 2005). In animal-pollinated plants, this positive density dependence may be further enforced if pollinators are absent from low-density populations (Sih and Baltus, 1987; Kunin, 1997). Above a certain intraspecific density, however, wind- and animal-pollinated plants may differ in the density effect on pollination: wind pollination is likely to become density-independent (unless conspecifics lower wind speeds, Kuparinen et al*.,* 2007; Schurr et al*.,* 2008), whereas animal-pollinated plants may increasingly compete for pollinator visits (Goulson, 2000; Ghazoul, 2005). Moreover, animal and wind pollination are expected to respond differently to the density of other plant species: heterospecific neighbours may either have no effect on wind pollination or they may decrease wind pollination by intercepting airborne pollen (Kuparinen et al*.,* 2007) or enhancing interspecific pollen transfer (Friedman and Barrett, 2009). In contrast, pollination by animals can show complex responses to the density of other plant species. Animal-pollinated plants may suffer from competition for pollinators against more common flowering species (Levin and Anderson, 1970; Waser, 1978; Rathcke, 1988) or from interspecific pollen transfer (Ghazoul, 2005). However, they may also benefit from co-occurring plant species that attract shared pollinators, thereby increasing pollinator visits and enhancing seed production (Moeller, 2004; Ghazoul, 2006). In summary, wind pollination is expected to be independent of interspecific density and to show positive or no responses to intraspecific density, whereas animal pollination may show more complex responses to both intra- and interspecific density.

While experiments and observational studies have thus established multiple mechanisms of density dependence in plant communities, it is largely unclear how these different mechanisms interact to shape the lifetime fecundity or fitness of plants. In particular, we know very little about the density dependence of lifetime fecundity in long-lived plants. This is because the lifetime fecundity of long-lived species is typically difficult to measure. Our knowledge about the density dependence of fecundity thus originates largely from short-lived plants (especially annuals) or from short-term studies of long-lived plants (Ghazoul, 2005). However, the population and community dynamics of long-lived plants ultimately depends on lifetime rather than short-term effects on fecundity and reproduction. Hence, we need to know the density dependence of lifetime fecundity if we want to understand how interactions shape the population and community dynamics of long-lived plants.

Here we present a study of the density dependence of lifetime fecundity in two long-lived shrub species (*Protea repens* (L.) L. and *Leucadendron rubrum* Burm. f.) from the South African Cape Floristic Region (CFR). The serotinous habit of these common members of the Proteaceae family enables direct measurements of lifetime fecundity (Bond et al*.,* 1995): because the study species form canopy seed banks but no long-lived soil seed banks (Bond et al*.,* 1984; Rebelo, 2001), the total fecundity of an individual can be measured as the product of cone number, the proportion of intact, undamaged cones, and seed set per intact cone. Fire triggers seed release from the canopy and subsequent seedling recruitment while killing adult plants. Hence, the total fecundity of a plant is identical to the plant’s lifetime fecundity given that it burns before the next flowering season (Bond et al. 1995). These long-lived shrubs are thus ideally suited to quantify how lifetime fecundity and its components vary with intraspecific and community (intra- and interspecific) density at different spatial scales.

Methods

Study system and study species

Protea repens and *Leucadendron rubrum* are two common species of the Proteaceae family that co-occur in the Fynbos biome, a fire-prone Mediterranean-type vegetation in the CFR. Proteaceous shrubs frequently dominate the overstorey of Fynbos ecosystems and play an important role for their functioning (Stock and Allsopp, 1992). Both species produce inflorescences that consist of many individual flowers. Each flower contains a single ovule, so that – after successful fertilization – each fruit contains a single seed (Rebelo 2001). Selfing is impossible in the dioecious *L. rubrum* and seems to be rare in the hermaphroditic and protandrous *P. repens* (Colins and Rebelo, 1987). Moreover, both species are serotinous: after flowering, their inflorescences develop into woody, fire-proof cones in which seeds are typically retained for many years until the mother plant burns or except when predated. In contrast, the survival and establishment probabilities of seeds released between fires are very low. Hence, the study species have long-lived canopy seed banks but no persistent soil seed banks (Bond et al. 1984; Rebelo, 2001). Pre-dispersal predation of the canopy seed bank is mostly caused by insects: for *Protea repens* it has been shown that endophagous beetle and butterfly larvae (*Sphenoptera spp*. (Buprestidae), *Genuchus hottentottus* (Scarabaedidae)*, Argyroploce spp.* (Olethreutidae), *Tinea spp.* (Tineidae)) are major seed predators (Coetzee and Giliomee 1987a, b; Wright and Samways, 1999).

Fire not only triggers seed release from cones, it also kills the adult plants of both study species (Rebelo, 2001). Hence, the total number of seeds that an individual retains at the time of a fire is a close proxy of the individual's lifetime fecundity, and has direct consequences for rates of post-fire population growth (Bond et al*.,* 1995; Bond and van Wilgen, 1996; Rebelo, 2001; Schurr et al*.,* 2005, 2007).

Our study species have a very similar life history which they share with many other species of CFR Proteaceae (Rebelo, 2001). Yet, they differ in sexual system (*L. rubrum* is dioecious whereas *P. repens* is hermaphroditic, see above) and in pollination syndrome: *L. rubrum* is wind-pollinated, whereas *P. repens* is animal-pollinated (Rebelo, 2001). One of the most prominent pollinators of *P. repens'* showy inflorescences is the Cape Sugarbird (*Promerops cafer*), but sunbirds (*Nectarinia* spp) and large flying insects such as beetles (*e.g****.*** *Trichostheta spp*., *Hopliniidae*) also act as pollinators (Collins and Rebelo, 1987).

Study design

We studied 87 and 78 seed-bearing focal individuals of *P. repens* and *L. rubrum*, respectively. These focal individuals belonged to 30 and 15 populations, respectively, that were situated in an area of ca. 6000 km^2 (extending from 33°41'23"S to 34°31'34"S and from 18°30'39"E to 19°27'36"E) in the winter-rainfall area of the south-western Fynbos vegetation with a dominant Proteaceae overstory. The mean post-fire age of our study populations (~12 years) and minimum of 5 and maximum of 19 years falls well within the range of mean fire return intervals reported for Fynbos (10-20 years, Bond and van Wilgen, 1996; Le Maitre, 1998). Hence, the mean size of individual seed reserves is a realistic measure of seed reserves at the expected time of the next fire and thus of expected lifetime fecundity.

For each focal individual, we counted the number of conspecific individuals and the number of individuals from overstory Proteaceae species in circles of 10 m and 50 m radius (Fig. 1a). The 10 m radius (subsequently termed the small-scale neighbourhood) was chosen to reflect the area within which Proteaceae individuals interact for abiotic resources such as nutrients, water and light (10 m is approximately three times the average height of our study species, Rebelo, 2001). The 50 m radius (subsequently termed the large-scale neighbourhood) was chosen to reflect the pollination neighbourhood of the animal-pollinated *P. repens* (a circle of 50 m radius is close to the average territory size of Cape Sugarbirds, Calf et al., 2003) and seems a reasonable proxy for the pollination neighbourhood of the wind-pollinated *L. rubrum*. For the dioecious *L. rubrum* we initially distinguished between male and female conspecific neighbours. However, since male and female densities were highly correlated (Spearman's correlation coefficient between male and female density was 0.93 for both 10 m and 50 m radius), we only used the overall conspecific density for further analyses. In addition, we verified that the explicit inclusion of male and female density did not improve models for seed set and seed predation, respectively. This underlines that overall conspecific density is a sufficient proxy for sex-specific densities. The sum of conspecific and heterospecific density at each scale was our measure of community density.

For each focal plant, we determined individual age from node counts (Bond 1985), quantified canopy volume from measures of canopy height and lateral extent, and counted the number of closed cones. Subsequently, we randomly harvested five closed cones of different ages. The harvested cones of *Protea repens* were cut open with secateurs to count the numbers of fertile and infertile seeds (Fig. 1b), whereas the harvested cones of *Leucadendron rubrum* were bagged individually and placed in an oven at 50°C for 5 days until they had released all seeds. Furthermore we checked the cones for signs of pre-dispersal seed predation. These signs of predation were infestation by beetle or stem-boring lepidopteran larvae and we distinguished between heavily damaged cones by predation and “intact” cones classified as heavily damaged never contained fertile seeds. Hence, the proportion of predation-damaged cones is a good measure of pre-dispersal seed predation. Seemingly infertile seeds of L. rubrum were then cut open to check whether they contained living embryonic tissue. Since each flower produces a single seed (Rebelo, 2001), the sum of fertile and infertile seeds per intact cone equals the number of flowers produced per inflorescence. Moreover, we measured seed set per intact cone as the mean number of fertile seeds. In summary, we quantified three fecundity components for each focal individual: cone number per individual, the proportion of intact cones with no sign of predation, and seed set per intact cone. The lifetime fecundity of each individual was then determined as the product of these three components (Fig. 1b).

Statistical analyses

To analyse the density- and age- dependence of plant size, lifetime fecundity and its three components we used linear mixed-effects models (LME, package nlme, Pinheiro et al*.,* 2007) in R 2.10.1 (R Development Core Team, 2009) with random effects of population that account for environmental variation between populations. We log-transformed all response variables except cone predation rate which was arcsine-square-root transformed. The maximal models for fecundity and its components included log-transformed plant age as well as linear and quadratic effects of intraspecific and community density in 10 m and 50 m radius. The maximal model for canopy volume included log-transformed plant age and its two way interactions with these density effects. Note that this maximal model did not include main effects of density; this is because cohort density may affect plant growth but is not expected to affect the initial size of seedlings. Following the procedure described in Crawley (2007), we simplified the maximal model by removing non-significant model terms ($P>0.05$) in a stepwise backward manner to obtain a minimal adequate model for each response variable. Main effects were not removed as long as the corresponding variable was still contained in an interaction (Venables and Ripley, 2002). In particular, linear density terms were not removed as long as the corresponding quadratic term was retained in the model. Minimal adequate models with linear and quadratic density terms can describe hump-shaped density dependence whereas models containing only linear density terms describe monotonic negative or positive density dependence.

Results

The sampled individuals showed considerable variation in neighbourhood density and composition. Local-scale neighbourhoods (10 m radius) of the sampled *L. rubrum* plants contained 2 - 173 Proteaceae individuals, and their large-scale neighbourhoods (50 m radius) comprised 64 - 868 Proteaceae individuals. Local-scale neighbourhoods of *P. repens* held 0 - 105 Proteaceae, and large-scale neighbourhoods contained 0 - 420 Proteaceae. In both species, the proportion of conspecific neighbours varied widely between 0% and 100%. Moreover, the considered density measures did not show a clear relationship to plant age: with the exception of a weakly significant positive correlation between age and large-scale community density for *L. rubrum* (Spearman correlation, $P=0.04$), the density measures were not significantly correlated with age ($P>0.05$).The detected variation in density has direct consequences for plant growth. For both species, the minimal adequate models for canopy volume contain an interaction between plant age and local-scale community density (*L. rubrum*: $\chi^2_{1df} = 19.7$, $P<0.001$; *P. repens*: $\chi^2_{1df} = 13.2$, $P<0.001$, Table 1). Plants with more small-scale neighbours thus had a lower growth rate.

Density dependence of fecundity in L. rubrum

Despite commonalities in the density dependence of growth rate, the two study species differ considerably in the density dependence of lifetime fecundity and its components (cone number, cone predation rate and seed set). In *L. rubrum*, fecundity and its components show relatively weak and monotonically negative density dependence (Table 1, Figs. 3 and 4). The minimal adequate model for cone number contains a negative effect of local-scale intraspecific density ($\chi^2_{1df} = 6.3$, $P<0.05$, Fig. 3a) and a positive effect of plant age ($\chi^2_{1df} = 22.9$, $P<0.001$). Note, however, that alternative models in which local-scale intraspecific density was replaced by one of the three other density measures provide similarly good explanations of variation in cone numbers (ΔAIC < 1.2 for all three alternative models). The proportion of predation damaged *L. rubrum* cones is generally low (median 0%, mean 6%) and independent of all density measures and age ($P>0.2$ for all explanatory variables). The same holds for the overall number of flowers per cone ($P>0.15$ for all explanatory variables). Seed set (the number of fertile seeds per cone) is also density-independent ($P>0.12$ for all density measures) but decreases significantly with plant age ($\chi^2_{1df} = 5.1$, $P<0.05$). Consequently, the lifetime fecundity of *L. rubrum* as the product of cone number, proportion of undamaged cones and seed set shows a weak, non-significant negative response to local-scale community density ($\chi^2_{1df} = 3.6$, $P=0.06$, Fig. 4h). However, the minimal adequate model for *L. rubrum*'s lifetime fecundity only contains a positive effect of plant age ($\chi^2_{1df} = 16.9$, $P<0.001$).

Density dependence of fecundity in P. repens

In contrast to the relatively simple patterns of density dependence observed for *L. rubrum*, the lifetime fecundity of *P. repens* and its components show rather complex responses to intraspecific and community density (Table 1, Figs. 5 and 6). Cone number per individual increases with large-scale intraspecific density (χ^2_{1df} = 7.9, P<0.01, Fig. 5b), decreases with local-scale community density (χ^2_{1df} = 9.0, P<0.01, Fig. 6a) and increases with plant age (χ^2_{1df} = 39.8, P<0.001). In contrast to *L. rubrum*, *P. repens* experienced a relatively high cone predation rate (median 20%, mean 19%) which showed a unimodal response to large-scale intraspecific density (χ^2_{1df} = 5.4, P<0.05, Fig. 5d). As in *L. rubrum*, however, the number of flowers per *P. repens* cone is independent of plant age and all density measures (P>0.3 for all explanatory variables). Yet, the number of fertile seeds per *P. repens* cone shows a unimodal response to large-scale community density (χ^2_{1df} = 11.4, P<0.001, Fig. 6f) and decreases with plant age (χ^2_{1df} = 6.2, P<0.05). For the lifetime fecundity of *P. repens*, we found a unimodal effect of large-scale community density (χ^2_{1df} = 5.5, P<0.05, Fig. 6h), and positive effects of large-scale intraspecific density (χ^2_{1df} = 4.1, P<0.05, Fig. 5h) and plant age (χ^2_{1df} = 23.2, P<0.001).

Differences in the density dependence of lifetime fecundity

The two study species differ not only in that *P. repens* had significant density effects retained in the minimum adequate model for lifetime fecundity (Table 1); they also differ in the magnitude of the estimated density effects. In maximal models with scaled fecundity, density and age variables, the estimated effect of large-scale intraspecific density was 3.8 times larger for *P. repens* than for *L. rubrum*, and the linear and quadratic effects of large-scale community density were 2.6 and 2.3 times larger, respectively.

The lifetime fecundity of *P. repens* individuals is thus estimated to vary substantially as a function of large-scale intraspecific and community density (Fig. 7). In the absence of conspecific neighbours, the minimal adequate model predicts lifetime fecundity to increase1.6-fold as large-scale community density increases from 0 to an optimal density of 146 individuals in 50 m radius. As community density increases further, lifetime fecundity is predicted to decrease: for the maximum community density observed in our study (420 individuals) it drops to 31% of the zero-density value. If all neighbours are conspecifics, the positive density dependence of fecundity is even more pronounced: in this case, lifetime fecundity increases 4.4-fold as large-scale density increases from 0 to an optimal density of 260 conspecifics in 50 m radius. Beyond this optimal density, fecundity is then extrapolated to decrease (Fig. 7, but note that this is an extrapolation as we did not sample conspecific densities above 244 individuals in 50 m radius).

Discussion

Our detailed analysis of lifetime fecundity in two species of long-lived Fynbos Proteaceae reveals both commonalities and striking differences between the study species. In both species, lifetime fecundity and its components show the same qualitative response to plant age (Table 1). Moreover, canopy volume and cone number of both species show similar negative density dependence. Yet, the two species differ markedly in the density dependence of pre-dispersal seed predation, seed set and lifetime fecundity (Table 1, Figs. 3-6). In the following, we first discuss which mechanisms are likely to cause these differences and commonalities, before highlighting some consequences of our findings for population and community dynamics.

Mechanisms of density dependence

A non-manipulative observational study like this cannot unequivocally identify mechanisms of density dependence. Yet, the presented detailed analysis of lifetime fecundity and its components together with the good understanding of the species' life history enables us to narrow down the suite of possible mechanisms underlying the observed patterns of density dependence.

In both species, individuals that experience a high small-scale community density grow less (Fig. 2), suggesting that they had fewer resources available for growth. Since the study species inhabit relatively infertile soils and Mediterranean climates with dry summers, they are likely to compete for nutrients and water. At high densities, small-scale competition for abiotic resources seems to lower not only the growth but also the cone production of both species: in *P. repens*, we found for cone number the same negative response to local-scale community density as for plant growth (Figs. 2, 6, Table 1). In *L. rubrum*, growth and cone production also decrease with small-scale community density, although the minimal adequate model for cone production contains small-scale intraspecific rather than local-scale community density (Figs. 2, 3, Table 1). However, due to the similar explanatory power of alternative density measures (see Results), we cannot conclude whether cone production of *L. rubrum* responds more strongly to intraspecific rather than heterospecific competition. In addition to such small-scale competitive effects, the cone production of *P. repens* also increases with large-scale intraspecific density (Fig. 5, Table 1). This could in principle result from variation in habitat quality: higher quality sites might both support higher densities and enable each individual to produce more cones. Yet, this explanation seems questionable since the growth of *P. repens* (as an alternative measure of plant performance) does not increase with large-scale intraspecific density (estimated effect of age-density interaction = -0.0001, P=0.74).Thus, further research is needed to elucidate the mechanisms driving the positive relationship between cone number and large-scale intraspecific density of *P. repens.*

Since the number of flowers per inflorescence is age- and density-independent in both species (Table 1), age- and density dependence of seed set per cone is likely to arise from age and density effects on fertilization success and/or the survival of fertile seeds. In *P. repens*, seed set variation may additionally be shaped by a plant’s allocation to pollen versus seed production. In both species we found average seed set per cone to decrease with plant age. This is a well-known phenomenon in serotinous Proteaceae: since seeds experience mortality in the canopy seed bank, older plants - in which mean cone age is higher - have less viable seeds per cone (Bond and van Wilgen, 1996).

Despite these similarities, the two species differ markedly in the density dependence of seed set per cone and cone predation: both fecundity components show unimodal density dependence in *P. repens* but are density-independent in *L. rubrum*. This is remarkable because the difference in sexual system should act in the opposite direction: in the dioecious *L. rubrum* low density neighbourhoods are likely to be dominated by a single sex, which should cause positive density dependence of seed set and cone predation. Interspecific differences in sexual system thus cannot explain the differential density dependence of these fecundity components. In contrast, a likely explanation for the difference is that in *P. repens* both fecundity components are more strongly shaped by interactions with animals than in *L. rubrum*: *P. repens* not only depends on animals for pollination (Rebelo, 2001), its cones also harbour more insect seed predators (Roets et al*.,* 2006), and consequently suffer from threefold higher rates of cone predation (Figs. 3-6). Possibly, the unimodal response of *P. repens’* seed set to large-scale community density could arise from interactions with generalized wide-ranging pollinators (notably the Cape Sugarbird) that visit many nectar-producing Proteaceae (Collins and Rebelo, 1987), whereas the unimodal dependence of *P. repens'* cone predation rates to large-scale intraspecific density may be caused by specialized seed predating insects.

Generally, the per-plant effect of plant-animal interactions may show unimodal density dependence if two conditions are met: (1) when plant stands are relatively sparse, an increase in plant density causes a disproportionate increase in animal density, e.g. because the presence of animals requires a minimum level of plant-related resources that cannot be provided by a single plant, and (2) at high plant densities, animal density is limited by resources that do not increase linearly with plant density. Both conditions are likely to be fulfilled for the interaction between animal-pollinated Proteaceae and their prominent pollinator, the Cape Sugarbird. Cape Sugarbirds are typically absent from low-density Proteaceae stands in which they cannot meet their daily energy requirements (Collins and Rebelo, 1987). However, in high-density Proteaceae stands that produce abundant nectar the density of Cape Sugarbirds seems to be limited by territorial behavior rather than nectar amounts (Calf et al*.,* 2003) so that bird density should increase more slowly than the density of nectar-producing Proteaceae. Hence, the unimodal response of *P. repens*’ seed set to community density may arise because at low Proteaceae densities, animal pollination (notably birds) of an individual plant is first facilitated by neighbouring Proteaceae that help to attract e.g. Sugarbirds, whereas - as Proteaceae density increases further - individuals increasingly compete for pollinator visits, although Sugarbirds can play an important role and are likely to describe that mechanism rather than other small pollinators (notably insects). Similar mechanisms could also explain the unimodal density dependence of cone predation in *P. repens*: seed-predating insects may require a minimum seed density to build up viable populations, whereas in high density stands their density may be limited by resources other than seeds. Yet, this explanation is speculative because our knowledge about the seed predators of *P. repens* and their resource requirements are much more limited than the respective knowledge about Cape Sugarbirds.

While the unimodal density dependence of both seed set and cone predation could potentially shape the lifetime fecundity of *P. repens*, we only detected a unimodal response of lifetime fecundity to large-scale community density. This suggests that *P. repens*' interactions with pollinators are more important for the density dependence of lifetime fecundity than its interactions with seed predators. In summary, differences in pollination seem to be a key mechanism that causes our study species to differ in the density dependence of lifetime fecundity. In particular, animal pollination is likely to cause unimodal responses of *P. repens'* fecundity to community density. We speculate that this complex density dependence is not unique to *P. repens* but holds for many animal-pollinated serotinous Proteaceae and thus for the bulk of the Cape Proteaceae (only 3% of the serotinous species in this family are wind-pollinated, Rebelo, 2001). In the following, we explore how these differences in density dependence may affect the dynamics of serotinous Proteaceae populations and communities.

Consequences for population and community dynamics

For both study species, we found no evidence that lifetime fecundity is more negatively affected by conspecifics than by heterospecifics. Yet, this finding cannot directly be interpreted with respect to the coexistence of Proteaceae communities: to assess whether these communities can coexist locally (Esther et al*.,* 2008), it will be necessary to quantify whether each species depresses its own population growth more than it depresses the population growth of other species (Chesson, 2000). Moreover, it seems promising to study whether the detected community-level Allee effects broaden the range of conditions under which multiple species can coexist in diverse Fynbos shrublands.

The detected interspecific differences in the density dependence of lifetime fecundity may not only affect species coexistence - they are particularly likely to affect the response of our study species to environmental change. The absence of positive density dependence in *L. rubrum* suggests that this species is relatively robust to reductions in population or community density at the scales studied here. In particular, we found no positive density dependence of seed set which supports findings that wind-pollinated plants are generally less pollen-limited than animal-pollinated species (Friedman and Barrett, 2009). Potentially, the pollen limitation of *L. rubrum* could even be lowered further if global warming increases the probability of long-distance pollen transport by wind (Kuparinen et al*.,* 2009). In contrast, our results suggest that the lifetime fecundity of *P. repens* is likely to be reduced if factors such as increased fire frequency (e.g. Schurr et al*.,* 2007; Wilson et al*.,* 2010) or land transformation (Rouget et al*.,* 2003) lower either population or community density below certain critical levels (Fig. 7). This may even hold if these factors do not directly impact *P. repens* but rather lower the density of other Proteaceae. Moreover, since the community-level Allee effect seems to be caused by the response of pollinators to nectar resources, it seems likely that similar reductions of fecundity would also result from the commercial harvesting of Proteaceae inflorescences which can strongly reduce nectar provision in local Fynbos communities (e.g Cabral et al*.,* 2011).

Population and community-level Allee effects can have profound consequences for large-scale biogeographical dynamics (e.g. Keitt et al*.,* 2001; Holt, 2009; Cabral and Schurr, 2010; Schurr et al., in press). In fact, using a top-down approach Cabral and Schurr (2010) concluded that intraspecific Allee effects shape abundance variation across the geographical range of several Fynbos Proteaceae. This study now provides bottom-up evidence for the existence of such Allee effects in *P. repens*. Yet, to relate relatively small-scale studies like ours to large-scale models for biogeographical dynamics (e.g. Cabral and Schurr, 2010; Huntley et al*.,* 2010), we need further research on the scaling of density dependence (Schurr et al., in press). This seems worthwhile since results like ours may help to predict the future dynamics of novel communities arising due to environmental change (Thuiller et al. 2008; Kissling et al., 2011). Predictions for such novel communities are particularly challenging in extremely species-rich systems like the CFR (Yates et al*.,* 2010). So far studies of Fynbos Proteaceae only considered competitive effects on migration, showing that interspecific competition can markedly slow down migration rates under climate change (Higgins et al. 2008). Yet, our results suggest that pollinator-mediated facilitation by other species (Fig. 7) may promote the immigration of animal-pollinated species into areas that are already occupied by other animal-pollinated Proteaceae. In contrast, community-level Allee effects may slow down expansion into areas that are currently not occupied by nectar-producing Proteaceae.

Conclusions

The study of serotinous shrubs can provide insights into the patterns and mechanisms of density dependence, which are otherwise very difficult to obtain for long-lived plants. For two species of serotinous Proteaceae, we found striking differences in how lifetime fecundity depends on the density of conspecifics and all Proteaceae at two spatial scales. The decomposition of lifetime fecundity into its individual components (cone number, cone predation and seed set per cone) suggests that these differences mainly arise from differences in how strongly these species interact with animals (notably pollinators). While fecundity of the wind-pollinated *Leucadendron rubrum* is density-independent, interactions between *Protea repens* and its generalized pollinators (e.g. the Cape Sugarbird) seem to cause a unimodal response of lifetime fecundity to large-scale community density. The resulting community-level Allee effects may have profound consequences for the dynamics of Proteaceae communities. Understanding the mechanisms that cause such complex density dependence seems indispensable for predicting how plant communities will respond to future environmental change.

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L. rubrum	Intraspecific density (ind.)		Community density (ind.)		ln Plant age (a)
Response Variables	10 m	50 m	10 m	50 m	
ln Growth rate	n.s.	n.s.	-*** L: :-0.0040	n.s.	+ L: 3.761
ln Cone number	-* L: -0.006	n.s.	n.s.	n.s.	+*** L: 3.5912
arcsine Cone predation	n.s.	n.s.	n.s.	n.s.	n.s.
ln Flowers per cone	n.s.	n.s.	n.s.	n.s.	n.s.
ln Seed set per cone	n.s.	n.s.	n.s.	n.s.	-* L: -0.5264
ln Lifetime fecundity	n.s.	n.s.	n.s.	n.s.	+*** L: 3.6501

P. repens	Intraspecific density		Community density		Plant age (a)
Response Variables	10 m	50 m	10 m	50 m	
ln Growth rate	n.s.	n.s.	-*** L: -0.0048	n.s.	+ L: 3.434
ln Cone number	n.s.	+** L: 0.0048	-** L: -0.0110	n.s.	+*** L: 2.5351
arcsine Cone predation	n.s.	∩* L: 0.00395 Q: -0.00002	n.s.	n.s.	n.s.
ln Flowers per cone	n.s.	n.s.	n.s.	n.s.	n.s.
ln Seed set per cone	n.s.	n.s.	n.s.	∩*** L: 0.00510 Q: -0.00001	-* L: -0.4393
ln Lifetime fecundity	n.s.	+* L: 0.0050	n.s.	∩* L: 0.00639 Q: -0.00002	+*** L: 2.2412

Table 1. Linear mixed-effects models for the density- and age-dependence of canopy volume, fecundity components and lifetime fecundity in the wind-pollinated *L. rubrum* and the animal-pollinated *P. repens*. For terms retained in the minimal adequate models, the table indicates the type of the effect (+: positive, -: negative, ∩: unimodal), and the associated parameter estimates for linear (L) and quadratic (Q) effects. Parameter estimates refer to the natural log of all response variables except cone predation (for which parameters refer to arcsine-square root transformed proportions). For models of canopy volume the entries in the density columns refer to density-age interactions, and significance values of age effects are not given since age interacts with density.*: p< 0.05, **: p< 0.01, ***: p< 0.001, n.s.: not significant (p> 0.05)

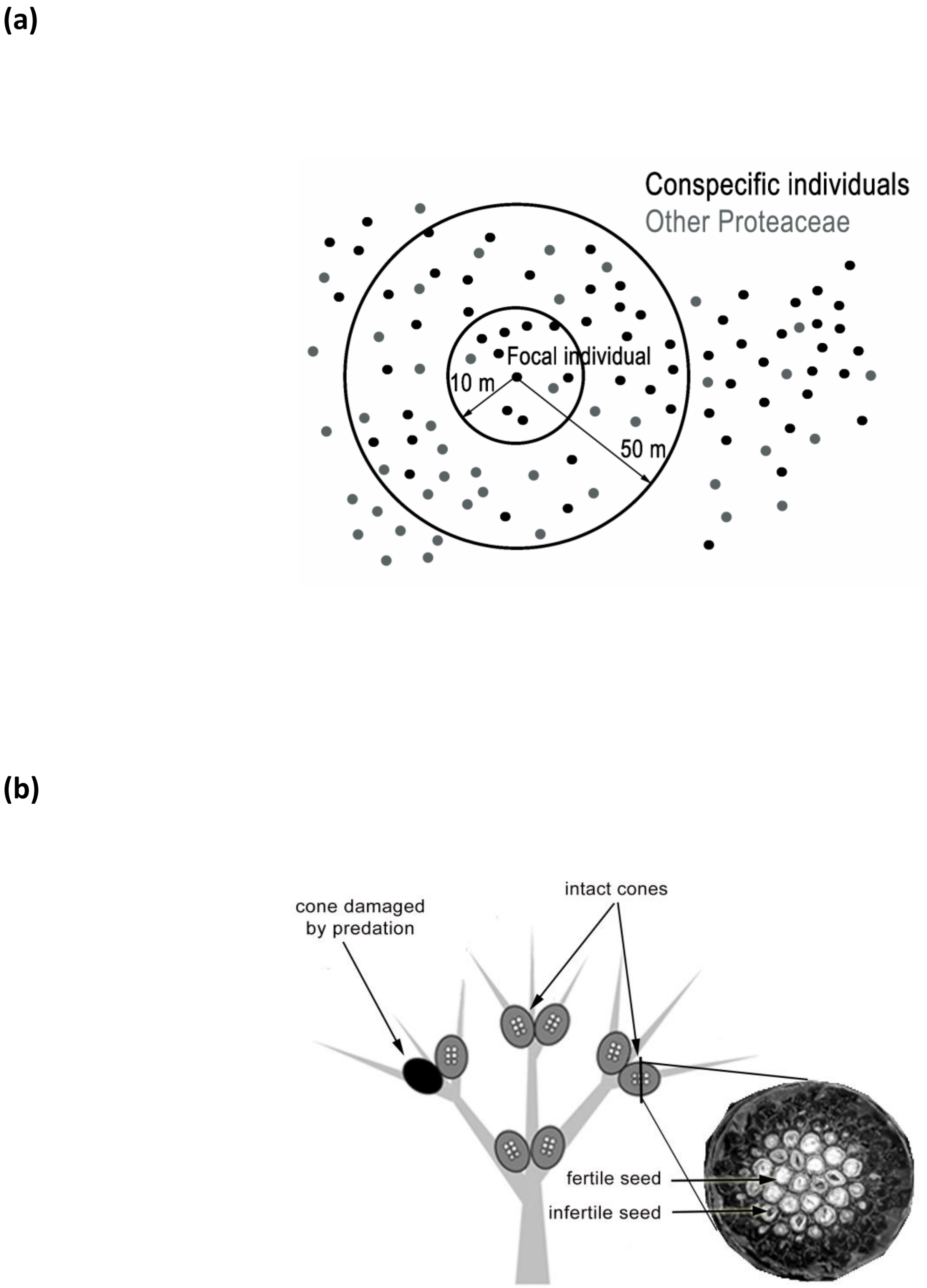

Fig. 1. The study design used to quantify the density-dependence of fecundity in serotinous Proteaceae from South African Fynbos. (a) The density of conspecifics and all overstored Proteaceae was quantified in 10 m (local-scale) and 50 m (large-scale) radii around focal individuals. (b) Fecundity components that were used to quantify the lifetime fecundity of focal individuals belonging to two species of serotinous Proteaceae. The photograph on the bottom right shows a cross-sectioned *Protea repens* cone with fertile and infertile seeds.

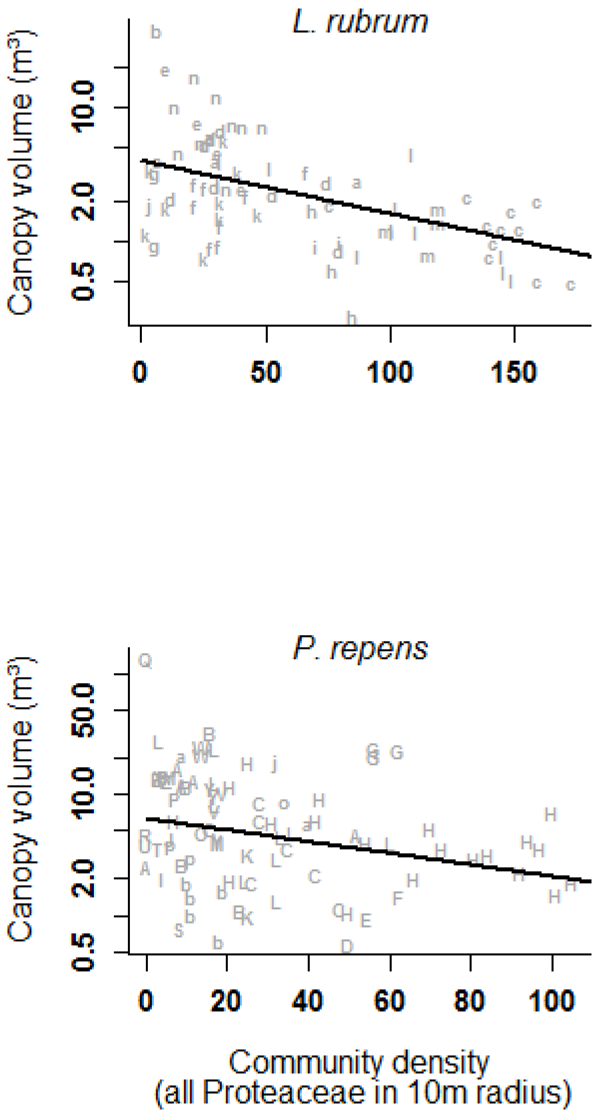

Fig. 2. Relationships between the canopy volume of *Protea repens* and *Leucadendron rubrum* individuals and local-scale community density around these focal individuals. Letters indicate different populations, and lines show predictions of minimal adequate models. Note that the y-axes are scaled logarithmically.

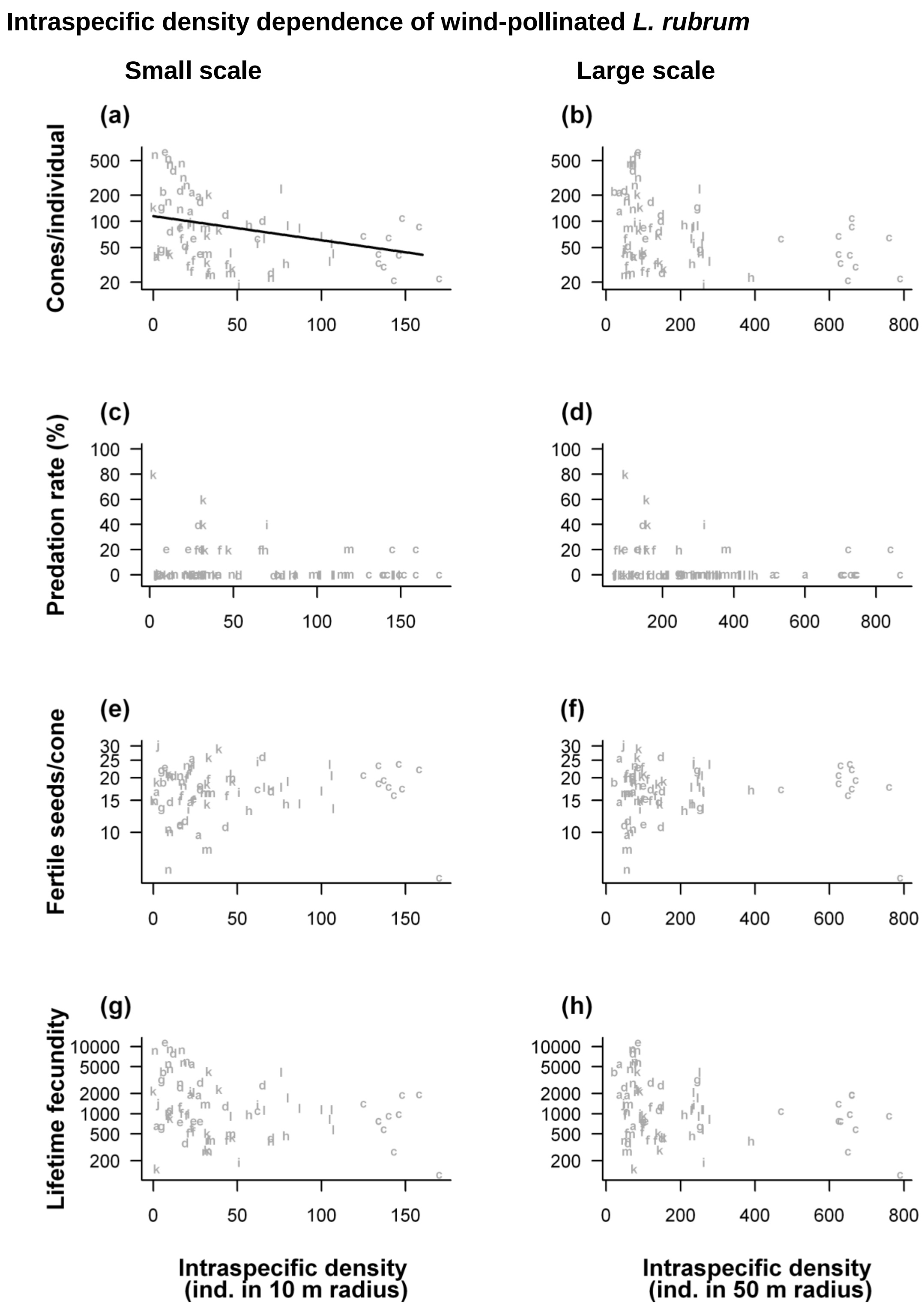

Fig. 3. Cone number, cone predation rate, seed set and lifetime fecundity of 78 wind-pollinated *L. rubrum* individuals against intraspecific density on two spatial scales (10 m and 50 m radius). Letters indicate different populations, and lines represent LME predictions for terms retained in the minimal adequate model including mean of plant age. Note that all y-axes except those in (e) and (f) are scaled logarithmically.

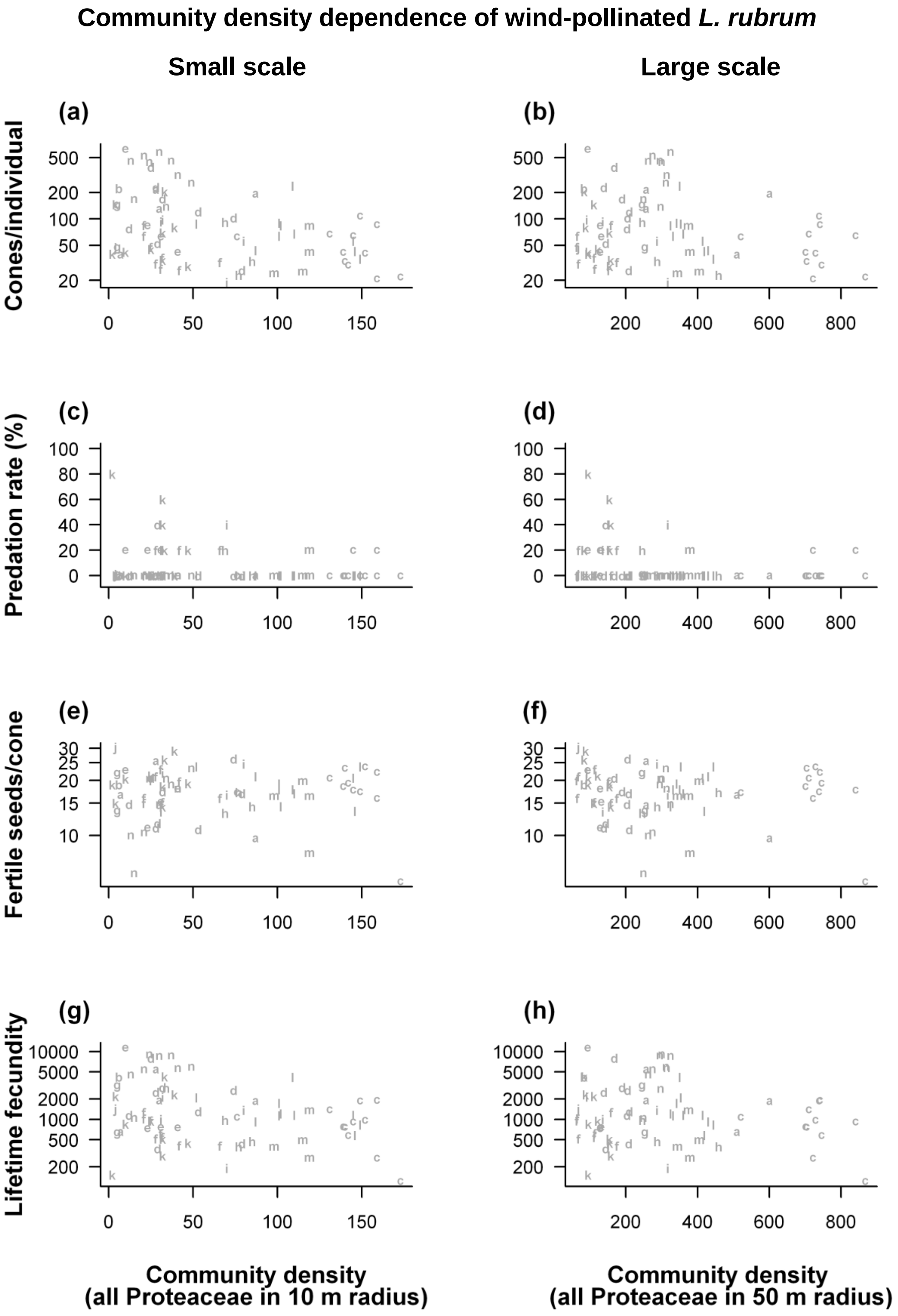

Fig. 4. Cone number, cone predation rate, seed set and lifetime fecundity of 78 wind-pollinated *L. rubrum* individuals against community density on two spatial scales (10 m and 50 m radius). For further details see Fig. 3.

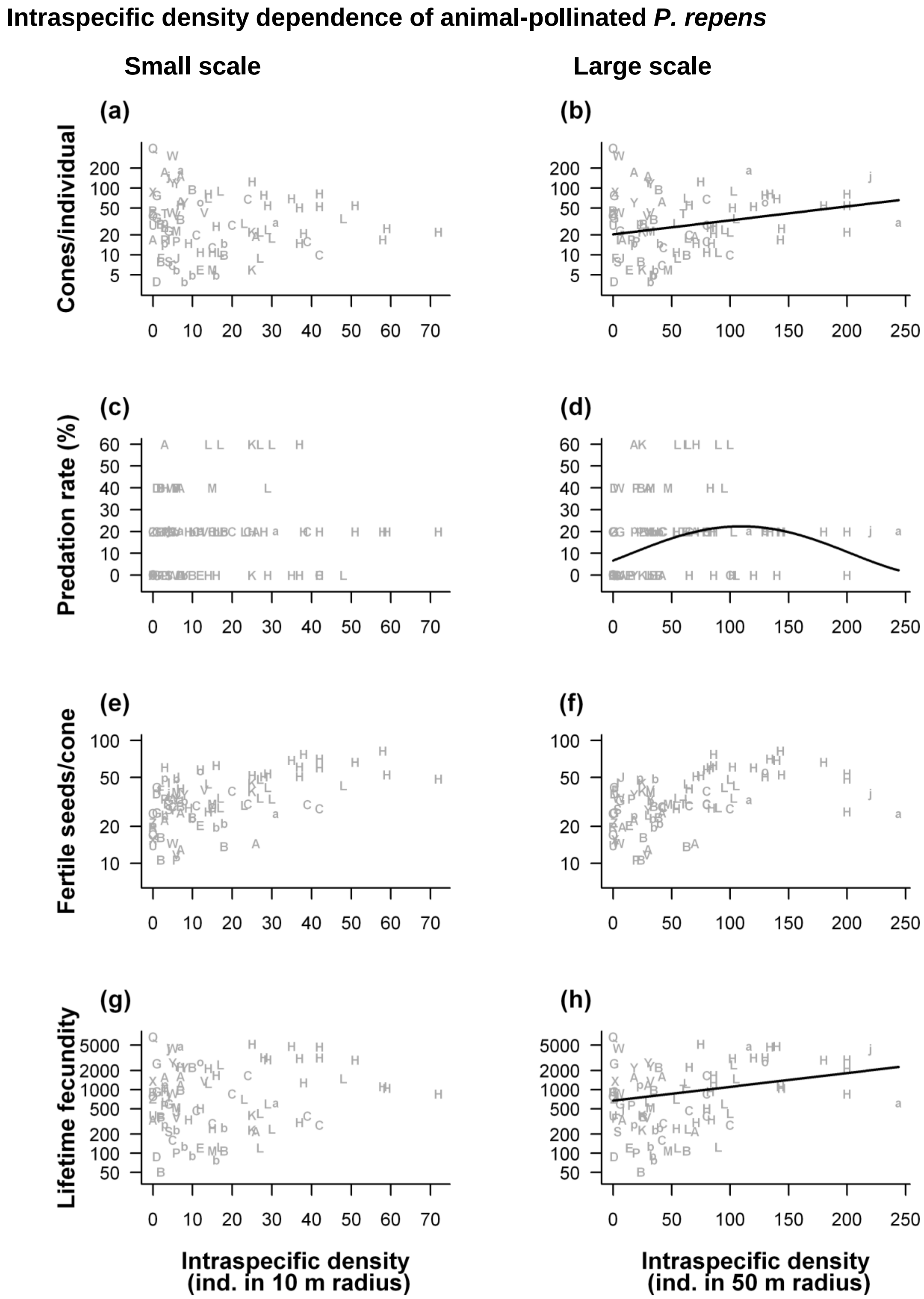

Fig. 5. Cone number, cone predation rate, seed set and lifetime fecundity of 87 animal-pollinated *P. repens* individuals against intraspecific density on two spatial scales (10 m and 50 m radius). For further details see Fig. 3.

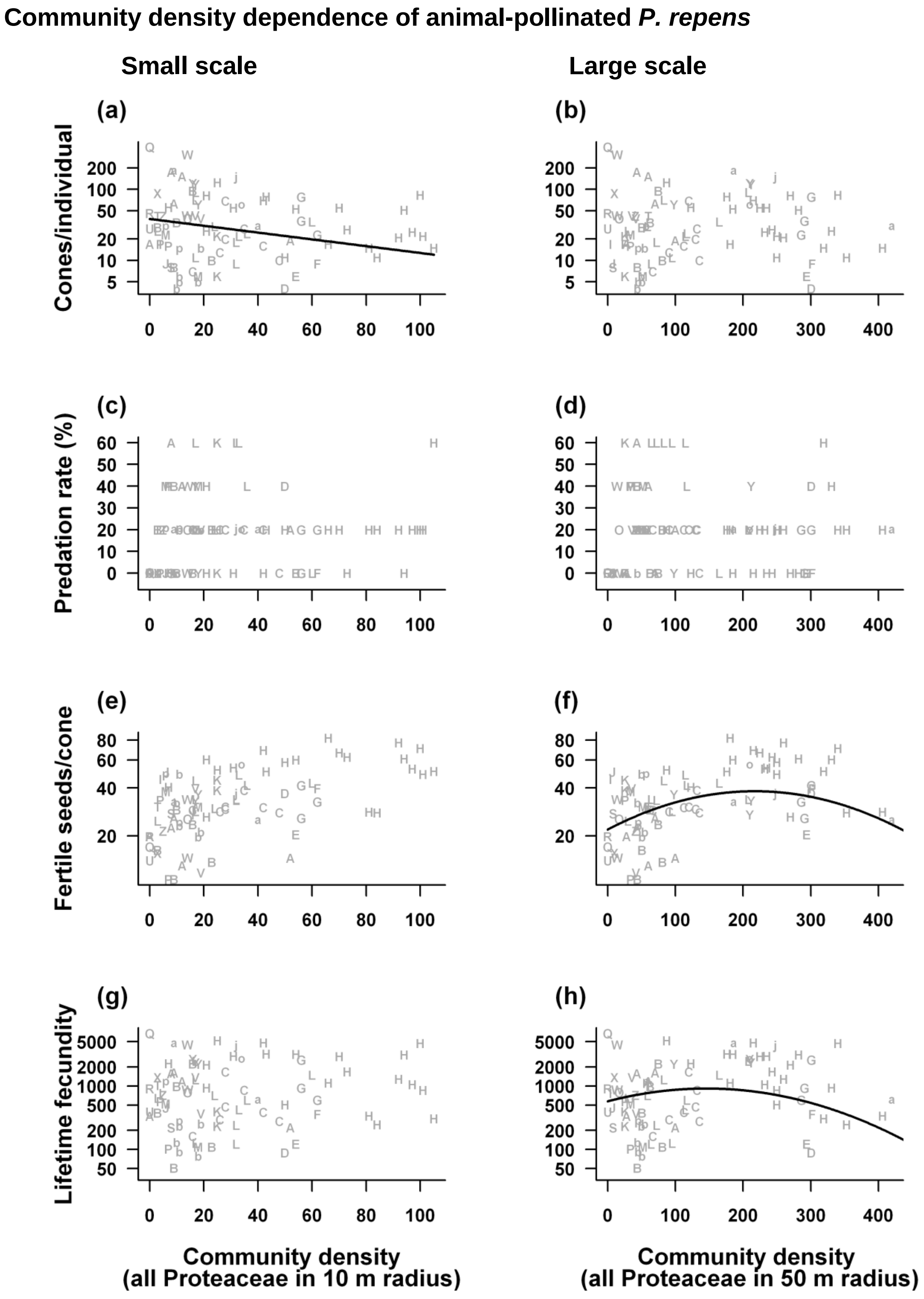

Fig. 6. Cone number, cone predation rate, seed set and lifetime fecundity of 87 animal-pollinated *P. repens* individuals against community density on two spatial scales (10 m and 50 m radius). For further details see Fig. 3.

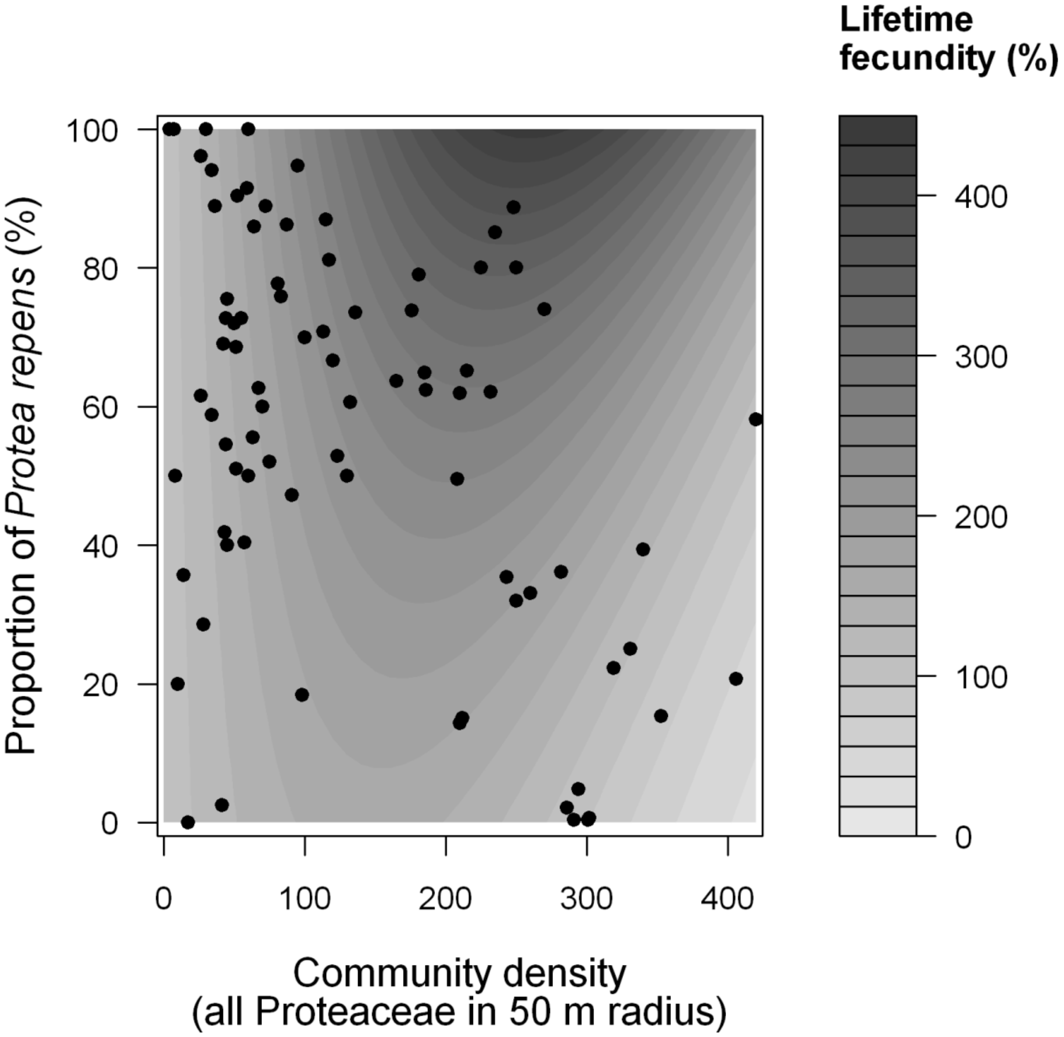

Fig. 7. Density dependence of lifetime fecundity in the animal-pollinated *P. repens*. Shades of grey indicate predictions of the minimal adequate model that describes how the fecundity of an individual varies with community density in 50 m radius and with the proportion of conspecifics in this radius. Fecundity is expressed relative to the fecundity of an individual growing at zero density. Points indicate observed variation in the density and composition of Proteaceae communities around *P. repens* individuals.

APPENDIX II

Environmental drivers of demographic variation across the global geographical range of 26 plant species*

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*This article is part of the doctoral thesis of Martina Treuernicht and similar content has been published in Journal of Ecology:

Treurnicht M., Pagel P., Esler K.J., Schutte-Vlok A.L., **Nottebrock H.,** Kraaij T., Rebelo A. and Schurr, F.M., Environmental drivers of demographic variation across the global geographical range of 26 plant species. *Journal of Ecology*. 104: 331–342. doi: 10.1111/1365-2745.12508

Abstract

Understanding how rates of reproduction and mortality respond to environmental variation across species' geographical ranges is a key task for both basic and applied ecology. So far, however, environmental drivers of range-wide demographic variation were only studied in a few plant species. Moreover, these studies did not control for potentially confounding effects of population density on demographic rates.

We present a large-scale demographic study of 26 shrub species (Proteaceae) in the Cape Floristic Region. All study species have a fire-linked life cycle and are serotinous: they exclusively form a canopy seedbank which contains the seeds produced since the last fire. Fire triggers seed release from the canopy so that recruitment is largely limited to a short period after fire.

Across the global geographical range of study species, we collected 3454 population-level records of total fecundity (size of individual canopy seed banks) since the last fire, per-capita recruitment (ratio between post-fire recruits and pre-fire adults) and adult fire survival. We used linear regressions to quantify how population density, fire interval, climate and soil fertility affect demographic variation.

Adult fire survival rates showed little intraspecific variation but a clear dichotomy between resprouting and nonsprouting species (with and without fire-protected buds, respectively): mean survival rates for these two functional groups were 97% and 2%, respectively.

Range-wide variation in fecundity was dominated by fire interval whereas recruitment was mostly climate-driven. Population density and soil fertility generally had smaller effects but were important for the fecundity and recruitment of several species. Effects of fire interval on fecundity were consistent across species but other demography-environment relationships showed substantial interspecific differentiation.

The comprehensive quantification of range-wide demographic variation demonstrate that multiple environmental drivers affect the life cycle of 26 Proteaceae species. Range-wide variation in fecundity showed a clear response to fire interval and affected species more consistently. In contrast, range-wide variation that was driven by climate, particularly in recruitment, differed among species. This identification of drivers of large-scale demographic variation is a necessary step for understanding the functional and macro-evolutionary determinants of species' niches and to better predict species' geographic ranges.

Introduction

The balance between demographic processes (or vital rates), notably reproduction and mortality, profoundly determine the local dynamics of populations in the absence of dispersal. What causes variation in these fundamental demographic processes is thus a central question in ecology. In particular, the Hutchinsonian niche of species can be defined as the set of environments in which reproduction exceeds mortality at low population density (Maguire 1973; Hutchinson 1978; Holt 2009). This long-standing interest in drivers of reproduction and mortality is increasingly expanding to the scale of species' geographical ranges. From a fundamental perspective, large-scale variation in reproduction and mortality impacts the geographical distribution and range dynamics of species as central quantities in ecology, biogeography and evolutionary biology (Pulliam 2000; Gaston 2009; Schurr *et al.* 2012). From an applied perspective, identifying the environmental drivers of large-scale variation in key demographic processes, such as reproduction and mortality, is essential for assessing and forecasting how global change will impact on population performance and the dynamics of species' ranges (Schurr *et al.* 2012; Normand *et al.* 2014; Ehrlén and Morris 2015).

While large-scale variation in reproduction and mortality is central to both fundamental and applied ecology, the drivers of this variation are still poorly understood. This is due to a dearth of data on large-scale demographic variation (Ehrlén and Morris 2015) across multiple populations (Salguero-Gomez *et al.* 2015). Given this lack of demographic data, it is not surprising that many studies resorted to other ways of inferring large-scale variation in species' performance. Notably, species distribution models (SDMs) which relate the occurrence probability of species to environmental variation (Guisan and Thuiller 2005) are commonly interpreted as describing large-scale variation in species performance. This interpretation of SDMs is, however, problematic since spatial population dynamics (Pagel and Schurr 2012; Schurr *et al.* 2012) and interspecific interactions (Svenning *et al.* 2014) can cause mismatches between the niche and the geographical distribution of species. Moreover, SDMs cannot unravel how variation in occurrence arises from the response of individual vital rates to environmental variation (Lavergne *et al.* 2010). It is thus not surprising that recent studies found unclear relationships between occurrence probability predicted by SDMs and estimates of intrinsic population growth (Thuiller *et al.* 2014; Bin *et al.* 2015). Hence, recent reviews have called for a demographic research agenda that identifies the environmental drivers of demographic variation across the geographical ranges of species (Schurr *et al.* 2012).

Plant ecology has long identified climate, soil conditions, disturbances and biotic interactions as the main drivers of small-scale variation in plant reproduction and mortality. Key climatic drivers of plant reproduction and mortality are heat, frost and drought (McDowell *et al.* 2011; Bykova *et al.*

2012). Soil conditions, notably nutrient availability, are also important drivers of population dynamics (Thuiller 2013) and, for example, in more fertile soils individual growth rates may be higher (Dahlgren and Ehrlén 2009). Major disturbances caused by floods, storms or fire alter demographic rates periodically and characteristics of the disturbance regime like fire return intervals can strongly affect population viability (e.g. Evans, Holsinger and Menges 2010). Biotic interactions with competitors, mutualists and antagonists can shape plant demography in multiple ways (e.g. Svenning *et al.* 2014). In particular, plant demography has long established that reproduction and mortality of plants depend on intraspecific density and that this density dependence can be negative (Stoll and Weiner 2000) or positive (Courchamp, Berec, Gascoigne 2008).

While determinants of small-scale demographic variation are thus reasonably well understood, only a few studies have identified environmental drivers of range-wide variation in key plant demographic rates (Angert 2009; Doak and Morris 2010; Merow *et al.* 2014). Angert (2009) showed increased demographic performance at high elevation sites for two plant species. Doak and Morris (2010) emphasised that compensation in individual demographic rates along a latitudinal gradient may buffer species against the adverse effects of climate warming. Merow *et al.* (2014) presented a multiple regression approach and showed that the interaction of climate variables (e.g. summer soil moisture stress) and large-scale disturbances may limit population growth and the geographical distribution of their study species. These studies jointly cover only a handful of plant species, which prevents generalizations to other species. Moreover, Ehrlén and Morris (2015) pointed out that previous studies of large-scale demographic variation did not control for density-dependence. This is important because density is likely to be correlated with environmental drivers of demographic variation. For instance, in environments that enable high intrinsic population growth rates, population density is likely to be high and intense intraspecific competition is likely to decrease observed rates of reproduction and survival. Analyses of demographic responses to environmental variation thus need to account for such potentially confounding effects of density-dependence (Thuiller *et al.* 2014; Ehrlén and Morris 2015).

The scarcity of data on range-wide demographic variation currently limits our ability to test assumptions that are frequently made in biogeographical theory and analyses. For instance, it is commonly assumed that the geographical ranges of plant species are more strongly limited by climate than by soil conditions or disturbance (but see Thuiller 2013; Merow *et al.* 2014). Moreover, niche theory commonly assumes that demographic rates respond to range-wide environmental variation in a monotonic or unimodal fashion (e.g. Maguire 1973, Pulliam 2000) and lack of data for multiple species prevents comparative analyses examining whether the shape of demographic responses is consistent across species. In summary, there is a need for large-scale demographic studies that (1) comprise multiple species, (2) span their entire geographical range, (3) cover their full life cycle, and (4) account for population density (Gaston 2009; Schurr *et al.* 2012; Ehrlén and Morris 2015).

We aim to close this gap by studying large-scale variation in reproduction and survival of 26 shrub species in the Cape Floristic Region (CFR; Bond and Goldblatt 1984), South Africa. The CFR is a global biodiversity hotspot (Myers *et al.* 2000) that is expected to be particularly threatened by global climate change (Malcolm *et al.* 2006; Yates *et al.* 2010a). However, current climate change biodiversity assessments in the CFR are largely based on SDMs and thus involve substantial uncertainty (Yates *et al.* 2010a). The notable exception is the study of Merow *et al.* (2014), who analysed range-wide demographic variation in one shrub species of the Proteaceae family. Proteaceae species frequently dominate the overstorey of fire-prone vegetation in the CFR (Rebelo 2001) and often have a fire-linked life cycle that simplifies demographic data collection. In light of this, we assembled in total 3454 population-level records of fecundity, post-fire recruitment and adult fire survival across the global geographical ranges of 26 Proteaceae species. We analysed this dataset to assess (1) the relative importance of multiple environmental drivers (population density, fire interval, climate and soil fertility) for range-wide variation in key demographic rates, and (2) whether different species show consistency or differentiation in demographic responses to environmental variation.

Materials and Methods

Study area and study species

The CFR is a geographically confined region (~91 000 km^2; Bond and Goldblatt 1984) that covers much variation in climatic and topographic conditions. The region generally experiences a Mediterranean-type climate with cool, wet winters and hot, dry summers and is characterised by largely nutrient-poor soils (Allsopp, Colville and Verboom 2014). The vegetation of the CFR is dominated by fire-prone shrublands, often with a tall Proteaceae overstorey (Bond and Van Wilgen 1996; Rebelo 2001). Fires are typically large in spatial extent; burn most aboveground biomass, i.e. crown-fires (Bond and Van Wilgen 1996), and occur on average every 10 – 21 years (Kraaij and Van Wilgen 2014). We studied 26 Proteaceae species of the genera *Leucadendron* and *Protea* (Table S1) which are serotinous (=bradysporous) and endemic to the CFR (Rebelo 2001).

Fire plays an important role in the life cycle of serotinous Proteaceae species (Fig 1a). Serotinous plants accumulate seed in fire-protected woody cones and exclusively form long-lived canopy seed banks (Bond, Vlok and Viviers 1984; Lamont *et al.* 1991). The size of the canopy seed bank is a measure of the total fecundity between two fires given that the plant would burn at the time of sampling (Bond, Maze and Desmet 1995; Nottebrock, Esler and Schurr 2013). Seed release from cones and dispersal are triggered by fire (Bond and Van Wilgen 1996; Rebelo 2001) and the establishment of new seedlings (or recruits) is generally confined to the immediate post-fire environment (Bond, Vlok and Viviers 1984; Lamont *et al.* 1991). Germination follows after a cool, wet winter and once the established recruits are about three years old, plants have a low mortality risk until the next fire (Lamont *et al.* 1991; Manders and Smith 1992; Bond and Van Wilgen 1996). Since a fire usually destroys large amounts of the above ground biomass, plants are often killed by fire. However, some Proteaceae species have the ability to resprout from meristems that are protected underground or by thick bark and adults of these species (‘resprouters’) are more likely to survive a fire than adults of species (‘nonsprouters’) that lack such traits (Bond and Van Wilgen 1996; Rebelo 2001). This fire-linked life cycle permits direct measurements and quantification of primary demographic rates in the field.

Demographic sampling

We collected data on the total fecundity of adult plants since the last fire (size of individual canopy seed banks), per capita post-fire seedling recruitment (ratio between post-fire recruits and pre-fire adults) and adult fire survival. These three demographic rates span the entire life cycle of our study species (Fig. 1a). Study sites for demographic sampling were selected to cover major environmental gradients in the geographical distribution of each study species and constituted 3454 population level records overall (Fig. 1b; Table S1).

Fecundity was measured as the number of fertile seeds in a plant's canopy seedbank. We determined the number of intact, closed cones for five individuals per population (restricted to female individuals for dioecious *Leucadendron* species). From each individual, we randomly selected and harvested (up to) five intact cones. The harvested cones were cut open with secateurs (cutting across seeds, for *Protea* species) or dried until seeds were released (for *Leucadendron* species) to determine the number of viable seeds per cone (e.g. Nottebrock, Esler and Schurr 2013). The product of total cone number (counted on each focal plant) and seed set per cone (from counting number of viable seeds per cone) yields an estimate of an individual's total fecundity since the last fire (Nottebrock, Esler and Schurr 2013). These individual fecundity values were then averaged at the population level and comprised 1575 populations. At every site, we also estimated the density of conspecific plants by counting the number of adults in a defined area (at least 100m^2).

Recruitment was measured as the number of seedlings per adult (recruit:parent ratio) in at least five transects of 20m x 2m on a recently burnt site. On each transect, we counted the number of living post-fire recruits (seedlings) and the number of pre-fire adults (comprising both fire survivors and fire-killed plants, which are identifiable as skeletons carrying burnt leaves or cones that opened after fire (Bond, Vlok and Viviers 1984; Bond, Maze and Desmet 1995). Adult population density was estimated as the density of conspecific (parent) plants counted per transect. The majority of recruitment sites (89.9%) were sampled less than 3 years after the last fire (Bond 1980; Manders and Smith 1992). In a few exceptional cases recruitment was measured on sites up to 6 years post-fire since it was still possible to measure recruitment and estimate adult density. Comparable data on post-fire recruitment measurements have long been collected through both local (CapeNature) and national (SANParks) conservation organisations' fire monitoring protocols (for description see Bond, Vlok and Viviers 1984). In our analysis, we integrated these existing datasets collected by CapeNature from 1979 – 2011 (CapeNature unpublished data) and by SANParks from 2007 – 2012 (Kraaij *et al.* 2013). Additionally, we also included post-fire recruitment data from Heelemann *et al.* (2008; 2011) and other unpublished data (W.J. Bond; R.M. Cowling; F.M. Schurr respectively). Combining all these data sources, our recruitment data consisted of 1308 populations.

Finally, on a subset of the recently burnt sites sampled for the recruitment data we recorded fire survival by counting the number of living (fire survivors) and dead (fire-killed) pre-fire adult plants in the sampling transects. Fire survival rates for in total 571 populations on these sites were then calculated as the proportion of survivors.

Environmental drivers

From reviewing the literature we assembled an initial subset of ecologically meaningful environmental variables expected to be main determinants of the performance and survival of serotinous Proteaceae in the hot, seasonally dry, nutrient-poor and topographically diverse CFR and are also used in building species distribution models for serotinous Proteaceae (Latimer *et al.* 2006; Yates *et al.* 2010a; Merow *et al.* 2014). This subset of major environmental factors represented effects of precipitation and drought, heat and temperature, frost and soil fertility. We however avoided combinations of highly correlated variables (>0.5) and retained: summer SMD (soil moisture stress days in the month of January, i.e. % days with soil moisture stress), summer heat units (accumulation of mean temperatures exceeding a threshold value of 10°C from October - March) and frost days (average number of days with frost per annum). As an edaphic variable we used a 'soil fertility score' (ranging from 0 (no fertility) to 5 (highest fertility)). The soil variable and all climate variables (averaged from 1950 – 2000) were extracted from the South African Atlas of Climatology and Agrohydrology (Schultze 2007) and have a resolution of 1′ × 1′ (1.55 × 1.85 km). Additionally, we calculated an 'altitude anomaly' as the difference between our site-recorded altitude measurement (by GPS) and the average grid cell altitude (Schultze 2007) to account for the influence altitude has on climate variation at smaller scales (see Table 1).

To investigate the effects of fire return intervals we recorded the time since last fire on each study site based on two types of information. Firstly, we always recorded the age of the vegetation at the time of demographic sampling: serotinous Proteaceae shrubs can be aged by counting the number of branches and/or internodes on the tallest stem; an established proxy for plant age (Bond, Maze and Desmet 1995; Carlson, Holsinger and Prunier 2011). Secondly, for most study sites we had also direct information on the fire history either from landowners and conservation managers, from a historical fire database (De Klerk 2008) or from satellite observations (MODIS, Roy *et al.* 2008). We also used these data for cross-checking with the vegetation age estimated from node counts in order to validate the use of node counts as a proxy for time since the last fire on sites without direct information on the fire history. For recruitment and mortality sites, such data sources on the fire history were also used for information on the length of the previous fire interval, i.e. vegetation age at the time of the recent fire. Notably, for the analysis of fecundity the effect of fire return interval is represented by the time since last fire, whereas for recruitment and adult fire survival the effective fire return interval is the length of the previous fire interval (see Table 1). The median sampled fire return interval was 11 years (range 1-48 years) and 14 years (range 2-66 years) across all study populations for fecundity and for recruitment and fire survival respectively.

Statistical analyses

Analyses were carried out with R.3.1.2. (R Development Core Team 2014) using the package MuMIn (Barton 2015). For each species, we estimated linear regressions that describe how fecundity and reproduction respectively respond to variation in population density, fire interval, climate and soil fertility (see Table 1). For all response variables we applied a log(x+1) -transformation. The explanatory variables were scaled and in the maximal models we included all linear and quadratic terms. Since intraspecific variation in fire survival was very low (Fig. 2), we did not analyse environmental effects on fire survival.

In the models for recruitment we used the number of parents as model weights (weights the contribution from each data point by the number of parents on a site). Note that the fire interval in the recruitment model was not represented by time since last fire but by the previous fire interval (see Table 1). The time since last fire was used, however, to account for seedling mortality that occurs up to three years after fire (Manders and Smith 1992). An exploratory data analysis suggested a negative log-linear relationship between seedling number and time since fire in the first three years. In the model we thus included a log transformed variable of time since last fire t as max[log(t/3), 0]. Hence the other explanatory variables in the model describe the per-capita number of recruits after the self-thinning phase which is a good proxy of the per-capita reproductive rate (Bond, Maze and Desmet 1995).

We used automated model selection (R package MuMIn; Barton 2015) among all combinations of explanatory variables, but excluded models that contained quadratic terms without the respective linear terms. The best model for each demographic rate per species was determined according to the lowest sample size corrected Akaike's Information Criterion (AICc; Burnham and Anderson 2002). The best models were then evaluated to show the relevant environmental determinants for each demographic rate across all 26 study species. We quantified the proportion of variance explained by each group of environmental determinants (population density, fire interval, climate, soil) as the difference between the coefficient of determination (R^2) of the selected best model and the R^2 of a reduced model without the respective explanatory variables. Based on whether the best model contained a quadratic effect of an environmental variable and on the sign of the coefficients, we classified and recorded the shape of the respective effects (i.e. positive, negative, unimodal or u-shaped) for each retained variable.

Results

Adult fire survival shows a clear dichotomy with almost complete fire mortality in nonsprouting species (mean fire survival rate 2%) compared to high fire survival in resprouters (mean fire survival rate 97%) and no species with intermediate fire survival (Fig. 2). Therefore, fire mortality is high in adult nonsprouters, whereas resprouters tend to survive fire, with very little variation within species. For nonsprouters, fecundity thus represents a close proxy of life-time fecundity and per-capita recruitment approximates fitness (the number of offspring contributed to the next generation).

Environmental variables explained on average 52% of the range-wide variation in fecundity and 49% of the range-wide variation in recruitment of study species (multiple R^2 of the best models). Examples of the estimated demographic responses are shown in Fig. 3 for one species (*Protea punctata*), whereas Figs. S1 and S2 in the supplementary material provide a complete depiction of the demographic response functions for all 26 species and relevant environmental drivers. Across our 26 study species the relative contribution of environmental drivers, notably climate and fire interval, differed between fecundity and recruitment (Fig. 4).

Environmental drivers of range-wide variation in fecundity showed a clear hierarchy (Fig. 4a): fecundity was predominantly driven by fire interval (median partial R^2 = 0.328) which remained in the best models of 96% of our study species (n = 25) and had either unimodal (n = 20) or positive (n = 5) effects (Fig. 5a; Fig. S1). To a small extent, fecundity was driven by climate (median partial R^2 = 0.067; Fig. 4a). Summer SMD, heat units and frost days affected 38% of our study species respectively with summer SMD and heat units having mostly negative and unimodal effects on fecundity (Fig. 5a; Fig. S1). Overall, fecundity-climate responses are more differentiated compared to the consistent unimodal and positive effects of fire interval (Fig. 5a). Population density had smaller, predominantly negative effects on fecundity (Fig. 4a; Fig. 5a); note also that u-shaped density effects effectively described negative density dependence (see Fig. S1). Soil fertility remained in the best models for several species but the overall variance explained was small (Fig. 4a; Fig. 5a).

Recruitment (as a proxy of per-capita reproductive rate) was driven foremost by climate (median partial R^2 = 0.199; Fig. 4b) where summer SMD and heat units affected the largest proportion of study species and effects were mostly negative (Fig. 5b; Fig. S2). Frost days and the altitude anomaly affected slightly less species and particularly frost effects were dissimilar compared to summer SMD and heat effects (Fig. 5b). Population density was the second most important driver of recruitment (median partial R^2 = 0.050; Fig. 4b) with largely negative density effects (Fig. 5b; Fig. S2). The fire interval did not explain much variation in recruitment (Fig. 4b) but mostly had unimodal effects for a few individual species (Fig. 5b). The overall effect of soil fertility on recruitment was low, but had a strong effect for a few species (Fig. 4b).

Discussion

This study quantified variation in primary demographic rates across the global geographical distribution of 26 species. By relating key demographic rates (fecundity and recruitment) to multiple environmental determinants of plant fitness (climate, fire disturbance, soil fertility, and population density) we quantitatively show that the importance and relative contribution of environmental drivers is different per demographic rate (Fig. 4). Variation in fecundity is mainly driven by the fire return interval and to a lesser extent by climate (Fig. 4a). Recruitment is primarily driven by climate and to a lesser extent by population density (Fig. 4b). The role of soil fertility was minor in both demographic rates. The quantification of adult fire survival rates show a clear distinction between nonsprouters and resprouters which have almost no or practically complete fire survival and little intraspecific variation, respectively (Fig. 2). Although it is widely accepted that adult nonsprouters are killed by fire, whereas adult resprouters tend to survive fire (Bond, Maze and Desmet 1995; Bond and Van Wilgen 1996), our demographic data is the first to quantify fire survival rates for a substantial number of nonsprouting and resprouting species. Overall, our findings show how multiple environmental drivers and population density interact to shape range-wide variation in plant demographic rates and significantly extend the taxonomic and geographic cover of previous studies.

Mechanisms underlying environmental effects on demography

Fecundity is strongly driven by the length of the fire return interval and relationships are largely unimodal across study species (Fig. 4a; Fig. 5a). Serotinous Proteaceae require several years to reach reproductive maturity and build a fertile canopy seedbank which explains why fecundity gradually increases over time. In fact, if fires occur during juvenile life stages, populations may fail to reproduce and even be at risk of local extinction (Lamont *et al.* 1991; Kraaij *et al.* 2013). The decrease in fecundity at longer fire intervals that we found for the majority (n = 20) of our study species (see Fig. S1) highlights that reproductive senescence is a common phenomenon in serotinous Proteaceae. Reproductive senescence comes about because older plants have older cones which contains fewer viable seeds because maintaining mechanical support and vascular supply to cones is costly (Midgley 2000). Thus the annual rate of cone opening and seed decay exceeds the annual rate of cone and seed production (Bond and Van Wilgen 1996). Also, cone bearing branches break off due to architectural instability in large plants (Midgley and Kruger 2000).

Effects of both extremely short and long fire return intervals on fecundity can translate into low post-fire recruitment success (Bond 1980), if populations are seed limited (Maze and Bond 1996). Bond (1980) suggested that low seed production in the aged parent generation (40 year old) was responsible for post-fire recruitment failure. However, across our notably larger taxonomic sample of study species recruitment was largely independent of the fire interval (Fig. 5b). In light of this, two aspects require clarification. Firstly, sampling explicitly at the shorter end of fire return interval, i.e. the juvenile phase (<5 years), could likely yield a stronger effect of the fire return interval in recruitment (Kraaij *et al.* 2013). Secondly, it is well known that fire season, fire intensity and the spatial extent of fires also affect recruitment rates (Bond, Vlok and Viviers 1984; Heelemann *et al.* 2008). Since our analyses only considered one element of the fire regime, i.e. the fire interval, we are likely to underestimate the overall importance of fire for range-wide variation in recruitment.

Climate had the strongest effect on post-fire recruitment (Fig. 4b) which is almost equally controlled by summer soil moisture stress and summer heat units with both having largely negative effects (Fig. 5b). Firstly, from a drought perspective, sufficient soil moisture is a critical requirement for germination, seedling emergence and survival (Lloret, Penuelas and Estiarte 2005; Mustart *et al.* 2012). Young seedlings require 2-3 years to establish a taproot (Manders and Smith 1992) and are most vulnerable to drought in their first summer and initial post-fire years (Lamont *et al.* 1991; Allsopp, Colville and Verboom 2014). However climate factors other than drought also impact on recruitment. For one, there is a strong signal of heat desiccation on recruitment. In fact, heat regulates all underlying processes of recruitment (i.e. germination, seedling emergence, growth and survival, Lloret, Penuelas and Estiarte 2005) and since the post-fire environment is bare and shade-deprived, soil surface heating and solar radiation may adversely affect physiological processes, damage young plant tissue and kill seedlings through direct overheating (Yates *et al.* 2010b). Contrary to summer soil moisture and heat effects, frost has predominantly positive effects on recruitment (Fig. 5b). Frost generally kills seedlings in evergreen plants (Marcante *et al.* 2012) which should translate into frost sensitivity but our results show the opposite. One plausible explanation of positive frost-responses may arise from the suppression of interspecific competitors during the winter growing season – allowing seedlings to be opportunistic during this time. This is also a likely explanation for other positive climate relationships where the trade-off between competitive ability and stress tolerance (the stress gradient hypothesis, Callaway 2007) allows species with higher tolerance to benefit from competitive release.

Climate is a less important driver for adult fecundity than for recruitment (Fig. 4). Hot, dry summers are defining features of the Mediterranean-type climate of the CFR (Allsopp, Colville and Verboom 2014) and specific plant traits may allow adult plants to be more tolerant to climatic variation. For example, Proteaceae shrubs have deep root systems which ensure access to underground-water during dry summer months (Manders and Smith 1992) and their often small, narrow leaves may allow cooling under excess heat (Yates *et al.* 2010b). The accumulation of leaf litter and/or understorey cover (which both increase during post-fire succession, Bond and Van Wilgen 1996) may also buffer adult plants against soil moisture stress and/or soil surface heating (Allsopp, Colville and Verboom 2014). This resilience of adult plants to climatic stress was also observed by West *et al.* (2012) who did not detect adverse effects of a 6 month experimental summer drought on adult plant growth, mortality or cone production in *Leucadendron laureolum*.

Intraspecific density affected fecundity and/or recruitment in the majority of our study species, predominantly through negative density-dependence (Figs. 5, S1 and S2). This supports the recent call to account for density-dependence in large-scale demographic analyses (Ehrlén and Morris 2015), especially since the form of density dependence can have profound consequences for the range dynamics of Proteaceae (Cabral and Schurr 2010). The finding that recruitment showed a stronger density-dependence than fecundity (Fig. 4) can be explained when considering how density affects cone number per plant and seed set per cone as the two components of fecundity, as well as per-seed establishment probability as the additional determinant of recruitment rate. Cone number is expected to decrease with density due to competition among adults for limiting resources such as space, water and nutrients (Esler and Cowling 1990; Bond, Maze and Desmet 1995; Nottebrock, Esler and Schurr 2013). In contrast, seed set per cone can show positive density-dependence because both pollen availability and densities of animal pollinators are higher in dense stands (Nottebrock, Esler and Schurr 2013). These potentially counteracting effects of density on the two components of fecundity may explain why a number of our study species showed no or weak density-dependence of fecundity. However, except in the case of over-compensatory density-dependence, denser adult stands will produce higher seedling densities (Bond, Maze and Desmet 1995) that are subject to more intensive density-dependent thinning of seedlings (Lamont, Witkowski and Enright 1993). This additional density-dependence at the seedling stage may explain why recruitment shows more pronounced density responses than fecundity.

Compared to other environmental drivers, soil fertility plays a smaller role for fecundity and recruitment of most study species (Fig. 4). There are several plausible explanations for this observation. Relative to soil fertility, climate effects may be more important for large scale variation in the performance of Proteaceae (see also Latimer *et al.* 2009; Merow *et al.* 2014). Moreover, soil properties other than soil fertility may determine demographic rates. For example, one study showed that soil depth defines interspecies boundaries by modifying soil water budgets (Mustart and Cowling 1993). Finally, the relatively low spatial resolution (1′ × 1′) of soil fertility information in the CFR may preclude the detection of demographic responses to fine-scale edaphic variation (see Latimer *et al.* 2006; Carlson, Holsinger and Prunier 2011; Thuiller 2013).

Interspecific consistency and differentiation of demographic responses

A striking result is that fecundity responses to the fire interval are remarkably similar across species whereas demographic climate-responses show greater interspecific variation (Fig. 5). A likely explanation for the consistency of fecundity-fire relationships is that the size of the serotinous canopy seedbank is strongly constrained by slow plant growth under the nutrient-poor and seasonally dry conditions of the CFR (Allsopp, Colville and Verboom 2014) and by the architectural requirements of serotiny (Harris and Pannell 2010). In contrast, despite a general sensitivity to climate variables (notably summer soil moisture stress and summer heat units), we concurrently detect differentiated interspecific responses to climate. Firstly, the predominant explanation for positive responses to soil moisture stress, heat units and frost days is that climatic stress exclude species that are competitively dominant to reduce interspecific competition (the stress gradient hypothesis, Callaway 2007). Secondly, differences in functional traits among CFR Proteaceae seem to enable more differentiated responses to climatic variation (Yates *et al.* 2010b). This is particularly true for recruitment which integrates over a larger part of the life cycle than fecundity and is thus more equally influenced by several environmental factors, offering more opportunities for trait-based differentiation among species. The clear difference in fire survival between resprouters and nonsprouters and the recent comparative study of Adler *et al.* (2014), highlight the potential of trait-based studies of interspecific variation in demography. A key question for the emerging field of functional biogeography (Violle *et al.* 2014) is to quantify the extent to which functional traits explain interspecific variation in range-wide demographic responses. The decomposition of species' niches into environmental responses of individual demographic rates may also provide new opportunities for the study of niche macroevolution. In particular, it may help to explain why species niches are evolutionarily conserved along certain axes and more labile along others (Wiens *et al.* 2010).

Combined environmental effects under global change

There is sufficient evidence that climate change alters fire regimes (Westerling *et al.* 2006). In the last decades, the CFR has become hotter and drier (MacKellar, New and Jack 2014) which has led to a shortening of fire intervals by an average of 4 years throughout the region (Wilson *et al.* 2010). The fire interval strongly determines the seed store in our study species (Fig. 5a) and shorter fire return intervals will almost certainly impact on fecundity rates. Enright *et al.* (2014) showed that resprouters can potentially buffer against the effect of shorter fire intervals by increasing their recruitment success but it is unlikely that nonsprouters will be able to do so, given their long juvenile periods of 4-9 years (Bond 1980; Kraaij *et al.* 2013). Even if less favourable conditions for post-fire recruitment occur (as currently predicted for the CFR; MacKellar, New and Jack 2014) the effects on the life cycle of our study species may be exacerbated since for a number of species the expected effects of changes in climate and fire both follow negative directions. The significance of studying multiple environmental drivers is a recurrent theme from our study and although we did not explicitly quantify such impacts, our life cycle- and species-level demographic approach provides insights on what can be expected under global change. The interplay between climate and fire will almost certainly impact in a combined fashion on serotinous Proteaceae and it is urgent to quantify the effects of such synergies (as in Enright *et al.* 2014).

Potential and challenges of large scale demographic studies

This study quantifies range-wide variation in plant demography and directly contributes to a demographic research agenda for biogeography (Schurr *et al.* 2012): showing that the large-scale collection of demographic data is feasible and demonstrates the value of longstanding data collection by conservation organisations (Kraaij *et al.* 2013). Simultaneously it also highlights the challenges of large-scale demographic studies. Observational studies can clearly only measure demography in the environmental conditions where the species occurs (i.e. the niche space currently occupied, Schurr *et al.* 2012) therefore studying natural variation in the field comes with obvious limitations (Ehrlén and Morris 2015). The feasibility of studying demographic variation in the field at all possible combinations of environmental factors is indeed an exceptional challenge. For example, Proteaceae shrubs generally occur on a limited range of soil fertilities (Rebelo 2001) and to effectively detect soil fertility effects may be challenging (similar to Latimer *et al.* 2009). In a few cases our analyses also show that it is particularly difficult to detect environmental limits in species with small geographic ranges (see Table S1; Figs S1 and S2) where distributions naturally cover less environmental variation and hamper our ability to detect species' thresholds or environmental limits. To overcome, these challenges, species distribution data should be used to augment demographic approaches, only then can we better forecast species responses to environmental change (Schurr et al. 2012; Ehrlén and Morris 2015).

Finally, there are several unique features of demographic response functions. Firstly, our findings provide clear evidence that fire frequency is an important demographic driver across almost all species. Merow *et al.* (2014) showed how such large scale disturbances like fire can indeed be included in demographically driven predictions to understand and model species distributions. Our range-wide demographic analyses greatly extend the taxonomic cover of previous studies and advocate strongly for the inclusion of temporal drivers like fire frequency if we are to accurately predict species responses to climate change. Secondly, in quantifying direct links between different environmental drivers and demography we not only identified environmental drivers throughout the life cycle and at species level, but also show the strength and direction of effects. This provides hypotheses for transplant experiments, with the notable advantage to provide clearer inference about the effects of environmental drivers on plant performance, which are important inputs for the demographic modelling of species niches and range dynamics (Pagel and Schurr 2012; Schurr *et al.* 2012).

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Figures and Tables

Table 1. Environmental variables (population density, fire interval, climate and soil) used to analyse range-wide demographic data of 26 serotinous Proteaceae species, with explanations and data sources

Environmental variables		Description	Source
Density	Population density	Adult density; calculated as density per m^2	Field observations
Fire	Fire return interval	*Fecundity*: Time since fire (years) *Recruitment*: Length of the previous fire interval (years)	Field observations (node counts on adult plants) and/or personal communication with landowners or conservation staff; and/or historical fire database (De Klerk 2008) or satellite observations (MODIS; Roy et al. 2008)
Climate	Summer SMD (summer soil moisture days)	Soil water stress in January (% days with soil moisture stress)	Schulze (2007)
	Heat units (summer)	Sum of daily temperatures exceeding 10°C (October – March)	Schulze (2007)
	Frost days	Average number of days per year with frost	Schulze (2007)
	Altitude anomaly	Difference between altitude of field sites and mean altitude of the grid cell (Schulze 2007)	Field observations and Schulze (2007)
Soil	Soil fertility	Soil fertility score	Schulze (2007)

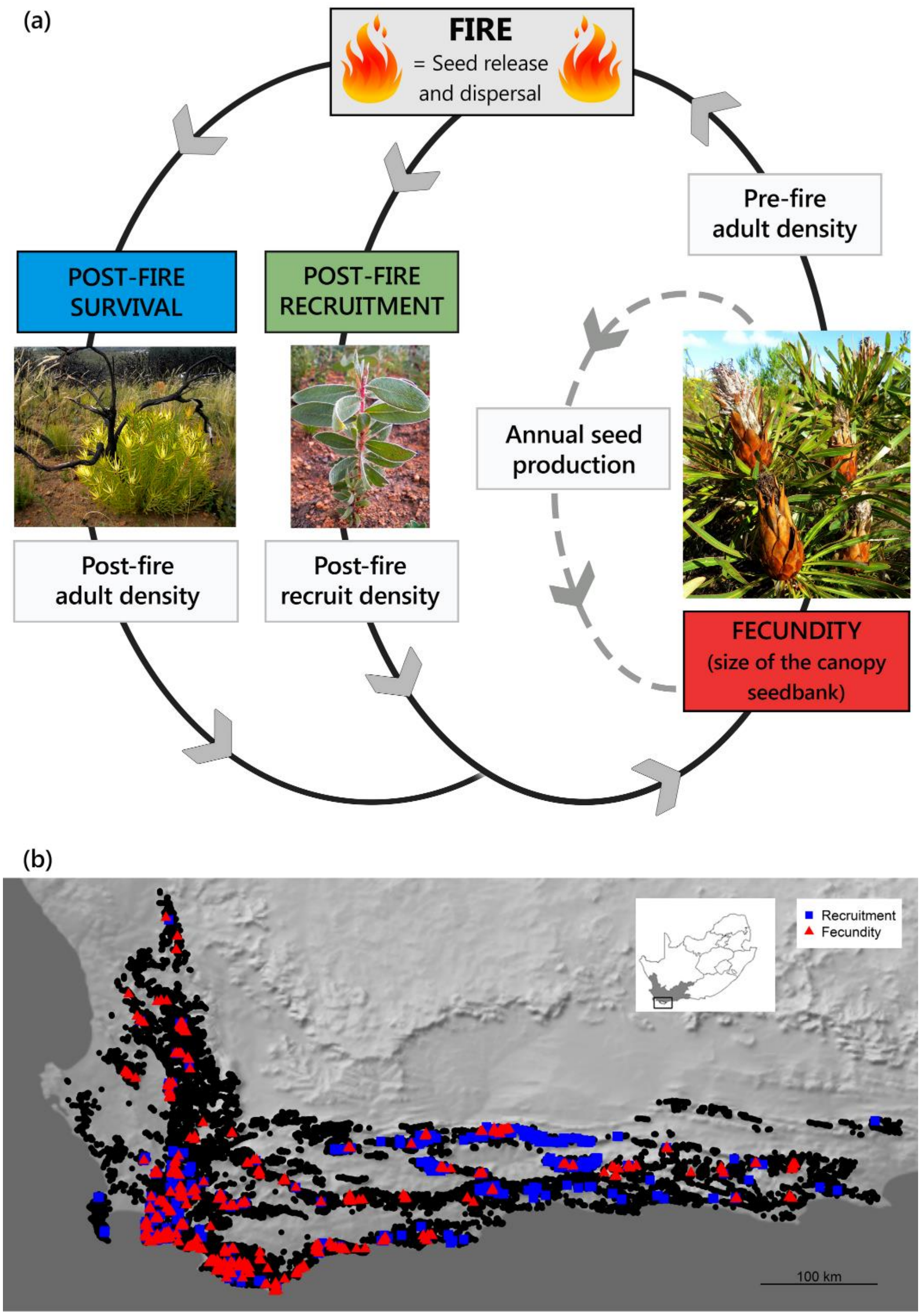


Fig.1. (a) The fire-driven life cycle of serotinous Proteaceae in the South African Cape Floristic Region (CFR) showing key demographic rates measured in this study (fecundity, post-fire recruitment and adult fire survival), (b) Map of study sites for recruitment (blue squares) and fecundity (red triangles) surveys. Black dots depict the geographical distribution of the Proteaceae family (presence records from the Protea Atlas Project; Rebelo, 2001) which largely covers the fynbos biome, a major constituent of the CFR.

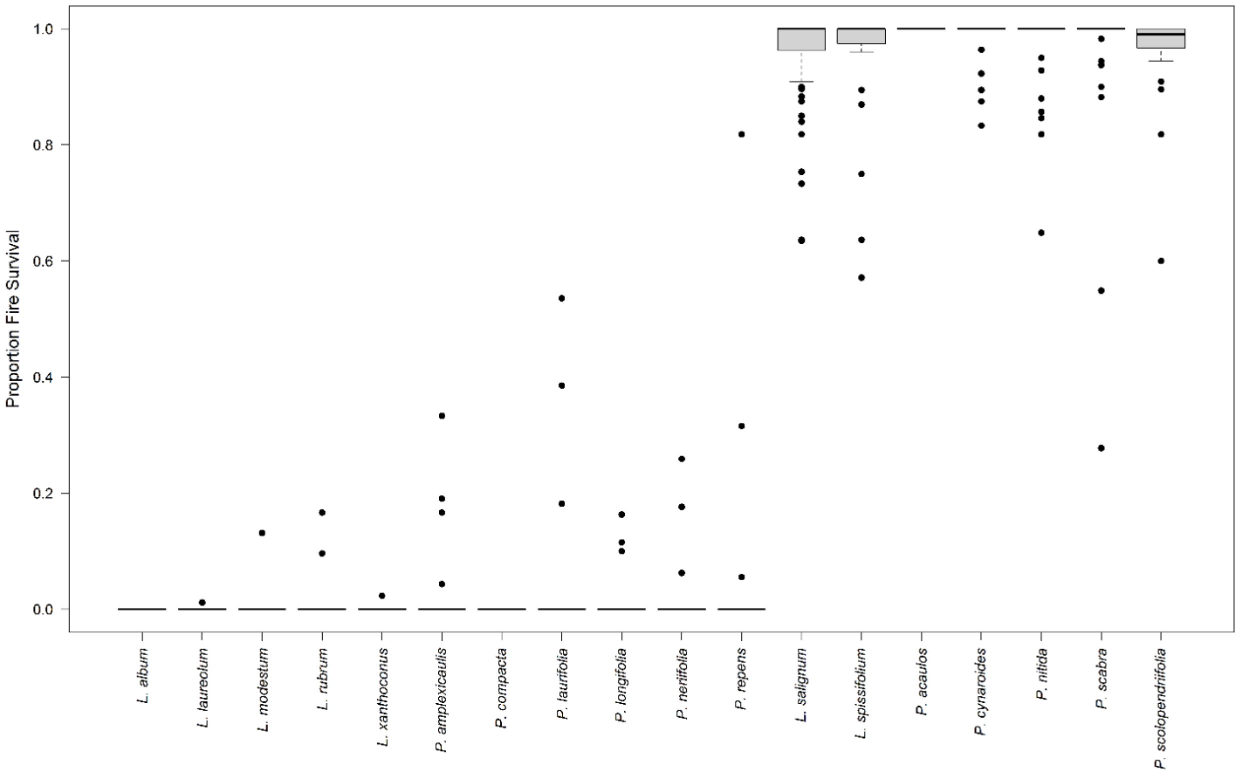


Fig. 2. Variation in adult fire survival in 11 nonsprouting (arranged left) and 7 resprouting (arranged right) serotinous Proteaceae species (*Leucadendron* = L.; *Protea* = P.). We only show species with fire survival records > 10 populations.

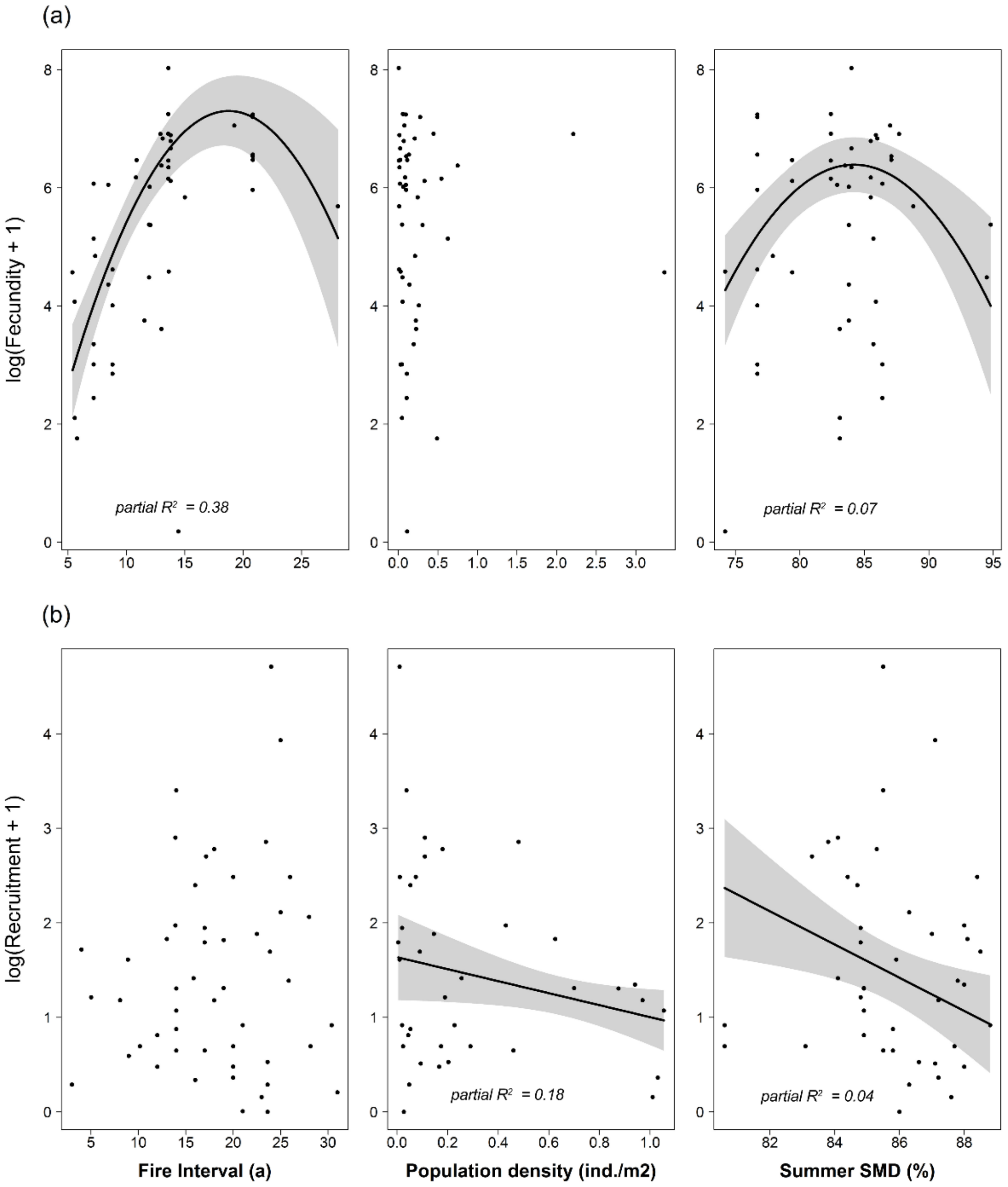


Fig. 3. Range wide variation in (a) fecundity and (b) recruitment of *Protea punctata* in response to fire interval (time since fire), adult population density and summer soil moisture days (SMD; % days with soil moisture stress). Response variables are plotted on the log(x+1) scale; lines indicate mean predictions of the best model and grey areas show 95% confidence intervals. Partial R^2 values are given for variables retained in the best models.

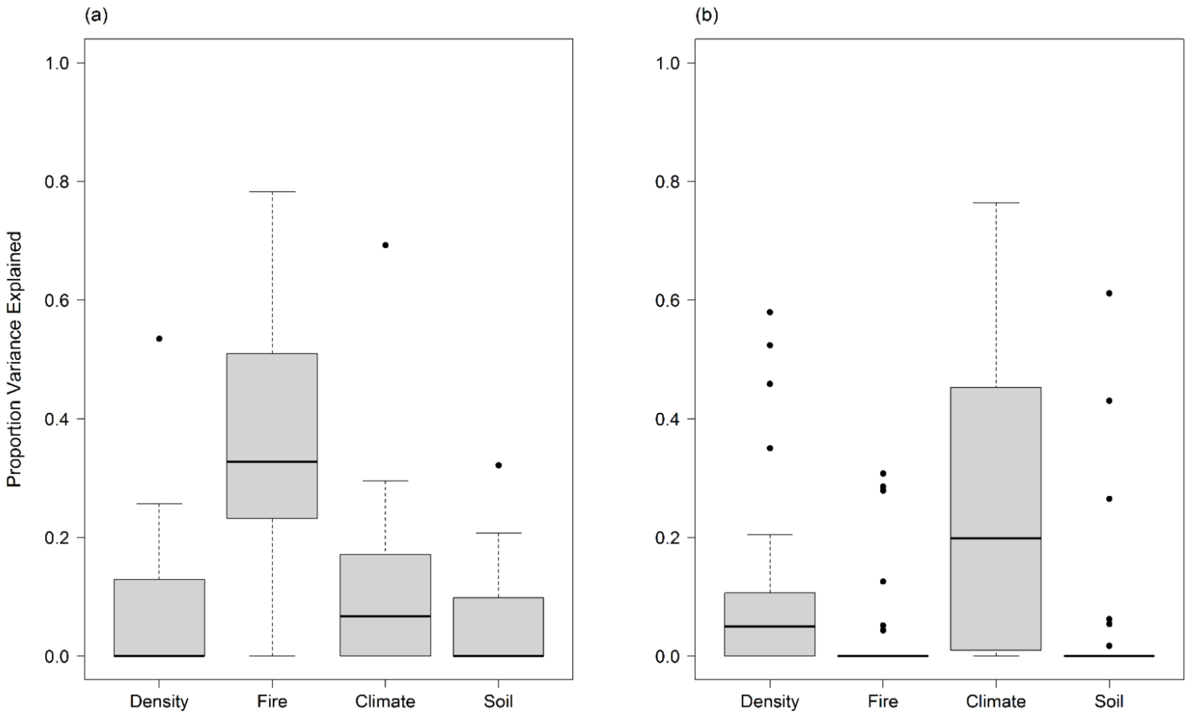


Fig. 4. Variance in (a) fecundity and (b) recruitment of 26 serotinous Proteaceae species that is explained by density (adult population density), fire (fire interval), climate (summer soil moisture days, summer heat units, frost days, altitude anomaly) and soil (soil fertility). Variance explained is the partial R^2 of the respective variable(s) in the best model for each species.

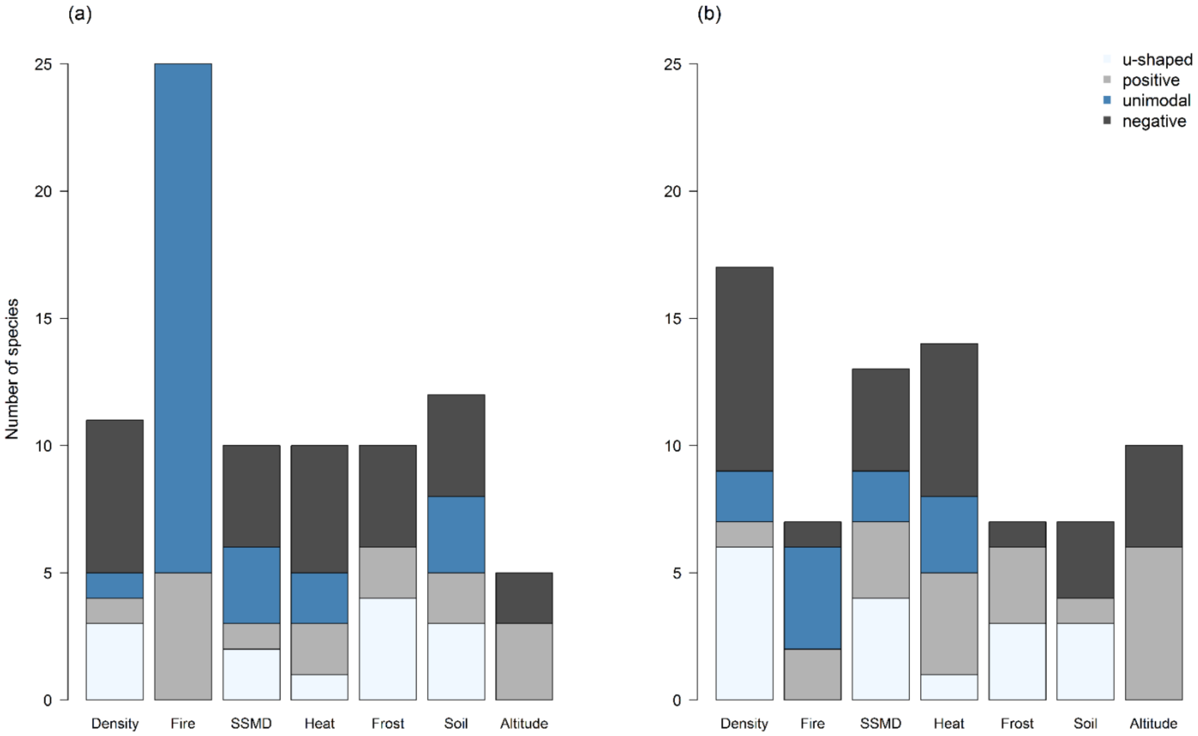


Fig. 5. Shape of environmental effects on (a) fecundity and (b) recruitment of 26 serotinous Proteaceae species. The barplots show the number of species for which the best model predicts a given environmental response (u-shaped (◡); negative (-); unimodal (⌒) or positive (+)). See Table 1 for descriptions on environmental variables.

SUPPLEMENTARY INFORMATION (Appendix II)

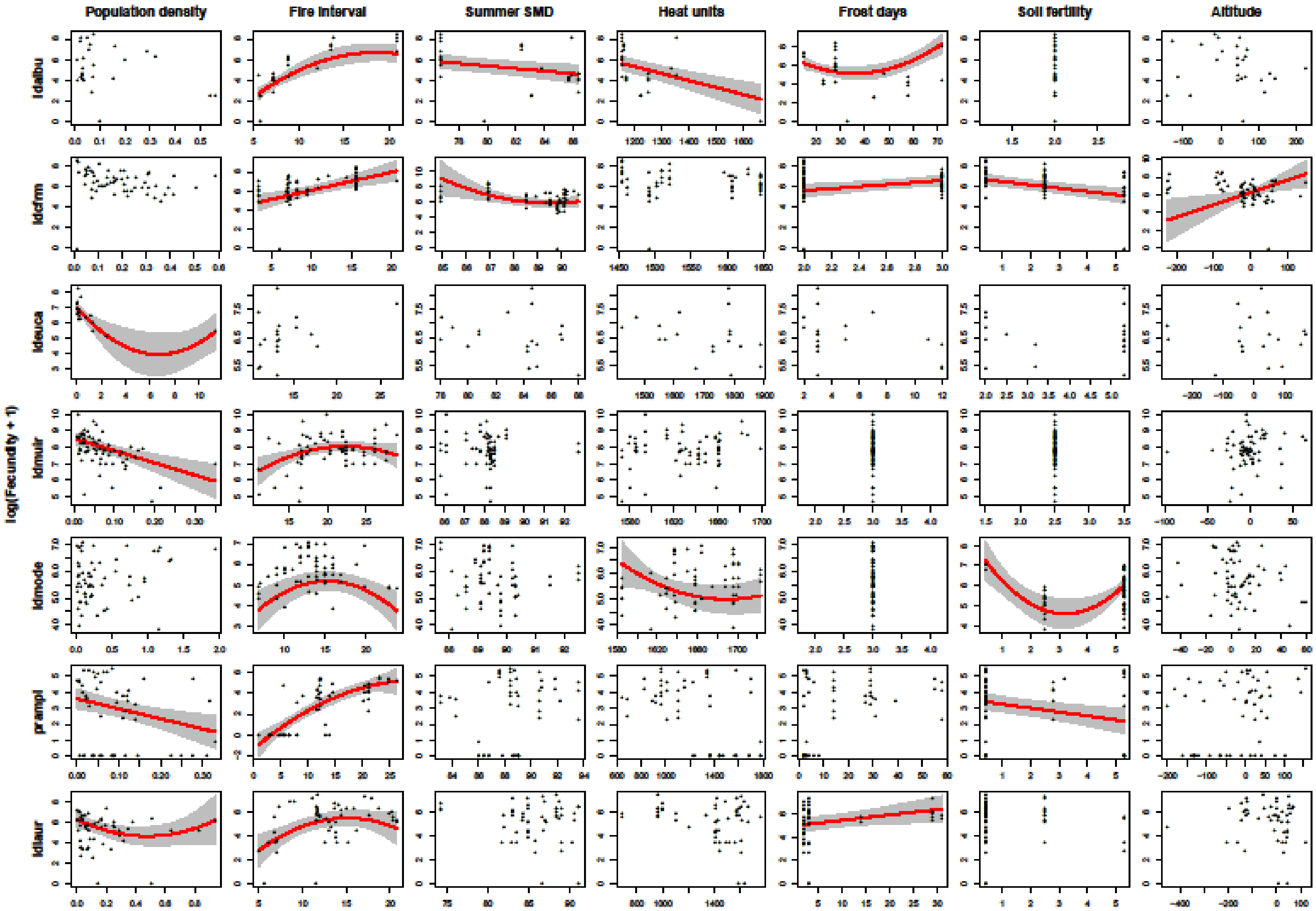
Figure S1. Response of fecundity (plotted on the log(x+1) scale) to adult population density (population density), fire interval, climate variables (summer soil moisture days (Summer SMD); Heat units; Frost days; Altitude anomaly (Altitude)) and soil fertility from 26 serotinous Proteaceae study species (see Table S1 for sampled (n) populations per species). Table 1 in the main text describe environmental variables and data sources. The plotted bold red lines show the mean prediction of the best model for each species and the grey areas indicate the 95% confidence interval.

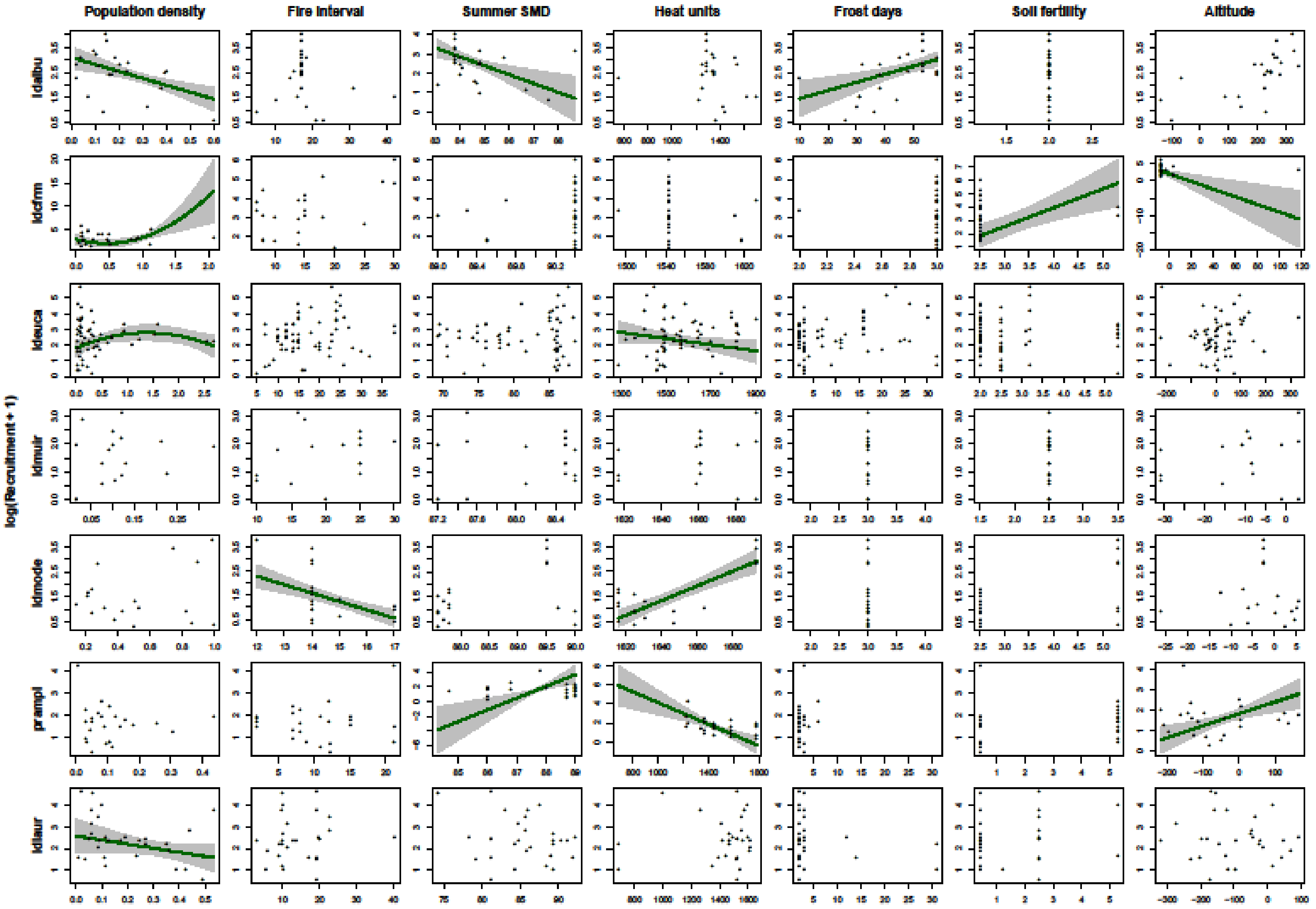
Figure S2. Response of recruitment (plotted on the log(x+1) scale) to adult population density (population density), fire interval, climate variables (summer soil moisture days (Summer SMD); Heat units; Frost days; Altitude anomaly (Altitude)) and soil fertility from 26 serotinous Proteaceae study species (see Table S1 for sampled (n) populations per species). Table 1 in the main text describe environmental variables and data sources. The plotted bold green lines show the mean prediction of the best model for each species and the grey areas indicate the 95% confidence interval.

Table S1. List of study species with abbreviations (used in Fig. S1 and Fig. S2), range size and fire persistence type (Rebelo, 2001); and number of populations sampled (n) to estimate demographic variation in fecundity, recruitment and adult fire survival. Range size was calculated based on occurrence records from the Protea Atlas Project (Rebelo 2001) aggregated to a spatial resolution of 1’ × 1’.

Species name	Abbreviation	Range size (1’ grid cells)	Fire persistence type	Fecundity (n)	Recruitment (n)	Fire survival (n)
Leucadendron album	ldalbu	213	Nonsprouter	26	24	15
Leucadendron coniferum	ldcfrm	287	Nonsprouter	59	22	0
Leucadendron eucalyptifolium	ldeuca	1407	Nonsprouter	19	55	0
Leucadendron laureolum	ldlaur	458	Nonsprouter	51	29	22
Leucadendron modestum	ldmode	223	Nonsprouter	62	18	14
Leucadendron muirri	ldmuir	203	Nonsprouter	64	15	6
Leucadendron rubrum	ldrubr	1538	Nonsprouter	55	80	14
Leucadendron salignum	ldsgnm	6007	Resprouter	100	75	85
Leucadendron spissifolium	ldspis	1338	Resprouter	80	33	38
Leucadendron xanthoconus	ldxant	891	Nonsprouter	50	39	16
Protea acaulos	pracau	891	Resprouter	80	51	48
Protea amplexicaulis	prampl	377	Nonsprouter	54	24	24
Protea compacta	prcpct	391	Nonsprouter	55	30	22
Protea cynaroides	prcyna	1719	Resprouter	83	27	24
Protea eximia	prexim	840	Nonsprouter	51	50	2
Protea laurifolia	prlaur	2752	Nonsprouter	60	38	18
Protea longifolia	prlong	453	Nonsprouter	51	34	28

Species name	Abbreviation	Range size (1’ grid cells)	Fire persistence type	Fecundity (n)	Recruitment (n)	Fire survival (n)
Protea lorifolia	prlori	1469	Nonsprouter	51	103	4
Protea neriifolia	prneri	1811	Nonsprouter	56	106	15
Protea nitida	prniti	2727	Resprouter	78	31	35
Protea obtusifolia	probtu	470	Nonsprouter	64	24	7
Protea punctata	prpunc	707	Nonsprouter	48	41	2
Protea repens	prrepe	4070	Nonsprouter	69	231	35
Protea scabra	prscbr	476	Resprouter	85	66	66
Protea scolopendriifolia	prsrfl	484	Resprouter	76	30	30
Protea susannae	prsusa	359	Nonsprouter	48	32	1


Population density
Fire interval
Summer SMD
Heat units
Frost days
Soil fertility
Altitude
ldalbu
ldcfm
ldeuca
ldmuir
ldmode
prampl
ldlaur
log(Fecundity + 1)


Population density
Fire interval
Summer SMD
Heat units
Frost days
Soil fertility
Altitude
ldalbu
ldcfrm
ldeuca
ldmuir
ldmode
prampl
ldlaur
log(Recruitment + 1)