RESEARCH PAPER

The importance of pollinators and autonomous self-fertilisation in the early stages of plant invasions: *Banksia* and *Hakea* (Proteaceae) as case studies

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ABSTRACT

Reproduction is a crucial stage in the naturalisation of introduced plant species. Here, using breeding system experiments and observations of floral visitors, we investigate whether a lack of pollinators or an inability to autonomously self-fertilise limits naturalisation in five Australian *Banksia* species and the co-familial *Hakea salicifolia* in South Africa. *Banksia* species were heavily utilised by native insects and nectar-feeding birds. Although *Banksia* produced fruit when pollinators were excluded, pollinators significantly increased seed set in four of the five species. *H. salicifolia* flowers were visited by 11 insect species; honeybees (*Apis mellifera*) were the main visitors. Flowers in naturalised *H. salicifolia* populations received almost four times the number of visits as flowers in non-naturalised populations; the latter showed both pollen limitation (PLI 0.40) and partial self-incompatibility. This should not prevent invasion, since *H. salicifolia* produces fruits *via* autonomous selfing in the absence of pollinators. The results suggest a limited role of breeding systems in mediating naturalisation of introduced Proteaceae species. Other factors, such as features of the recipient environments, appear to be more important. Spatial variation in rates of reproduction might, however, explain variation in the extent and rate of naturalisation of different populations.

INTRODUCTION

Once an introduced species survives in a novel range, it needs to overcome a series of barriers to become naturalised and invasive (*i.e.,* introduction–naturalisation–invasion continuum; Blackburn *et al.* 2011). One such barrier is reproduction (Barrett 2011; Correia *et al.* 2014). Alien plant species that rely on sexual reproduction must find a mate and a pollinator to reproduce and naturalise (Richardson *et al.* 2000a). Plant species that rely on outcrossing for population growth and spread need to attract floral visitors that transfer pollen. A lack of pollinators, or only partly effective pollinators, will result in pollen limitation, which could prevent or reduce the establishment and spread of introduced species (Parker 1997; Richardson *et al.* 2000a; Le Roux *et al.* 2010; Rodger *et al.* 2010; Bufford & Daehler 2014).

In regions where pollinators are scarce or absent, plants that are capable of autonomous self-fertilisation have a higher chance of naturalising than self-incompatible species, as self-fertilisation offers reproductive assurance (Baker's Law; Baker 1965; van Kleunen *et al.* 2008; Harmon-Threatt *et al.* 2009; Ward *et al.* 2012). Extending Baker's idea, self-incompatible species tend not to become invasive, whereas self-compatible species do (Rambuda & Johnson 2004; van Kleunen & Johnson 2007a; Hao *et al.* 2011). Moreover, self-compatible species

occupy larger novel ranges than self-incompatible species (van Kleunen & Johnson 2007b). However, more work is needed to establish the role of uniparental reproduction across various stages of the invasion process. For instance, does uniparental reproduction provide reproductive assurance during naturalisation (as originally envisaged by Baker? Or is it primarily responsible for increasing spread rates by alleviating pollen limitation in small populations? Testing selfing capabilities and identifying the role of pollinators for alien species placed at different positions along the introduction–naturalisation–invasion (INI) continuum (*sensu* Richardson & Pyšek 2012) will provide a better understanding of the importance of self-fertilisation in determining invasiveness.

Most studies on alien plant species that have assessed the importance of pollination and plant–pollinator interactions have focused on species that are already invasive (*e.g.,* Rambuda & Johnson 2004; van Kleunen & Johnson 2007a; Rodger *et al.* 2010; Ward & Johnson 2012). These species have already overcome the reproduction barrier, which confounds attempts to determine whether reproduction acts as a barrier to invasion (but see Stout *et al.* 2002; Bufford & Daehler 2014). To understand the importance of uniparental reproduction and pollinator limitation as barriers for invasion, the different stages of plant invasions should be considered (Rambuda & Johnson 2004).

To minimise phylogenetic constraints, we use two genera within the family Proteaceae. Proteaceae provides an ideal study group to explore whether breeding system serves as a barrier to invasion. First, Proteaceae in general have low levels of self-compatibility (Horn 1962; Collins & Spice 1986; Whelan & Goldingay 1986; Goldingay *et al.* 1991; Ramsey & Vaughton 1991; Goldingay & Carthew 1998; Offord 2004; Rovere *et al.* 2006). Being largely self-incompatible, Proteaceae are therefore dependent on pollinators for seed production. Second, many Proteaceae are pollinated by only one functional group of pollinators (*e.g.,* see Collins 1983; Collins & Rebelo 1987; Rovere *et al.* 2006; Hanley *et al.* 2009). Third, there are relatively few naturalising and invasive Proteaceae species globally, but a large number of introduced Proteaceae species (Moodley *et al.* 2013). Proteaceae is currently underrepresented on the global list of invasive woody plants (Rejmánek & Richardson 2013), probably because most introductions have been very recent; there is thus the possibility to prevent invasions before they occur. In South Africa, only eight out of 24 introduced species of Proteaceae are invasive, but with the exception of species of *Grevillea* R.Br. ex Knight, *Hakea* Schrad. & J.C.Wendl. and *Macadamia* F. Muell., most species have only been introduced in small numbers (Geerts *et al.* 2013; Moodley *et al.* 2013, 2014).

In addition to comparing different species positioned at different invasion stages, comparisons conducted within one species (*i.e.,* between naturalised and non-naturalised populations) in the introduced range could provide better insights into determinants of invasiveness. Such an approach specifically allows us to determine whether reproduction limits invasion, since there is control over other species-specific traits (*e.g.*, pollination syndrome). *Hakea salicifolia* (Proteaceae) in South Africa presents such an opportunity, with many naturalised and non-naturalised populations scattered across the country (Moodley *et al.* 2014). More importantly, determining reproductive traits that confer invasiveness are crucial for understanding the drivers and limitations of invasions (Rambuda & Johnson 2004).

Here, we aim to assess the importance of pollinators and autonomous selfing at the early stages of plant invasions using Australian Proteaceae species introduced into South Africa as a case study. Specifically, we aimed to determine (i) the ability of autonomous seed production *versus* pollinator contribution for five *Banksia* species along the introduction–naturalisation–invasion continuum; and (ii) whether breeding system and pollinators differ between naturalised and non-naturalised *H. salicifolia* populations.

MATERIAL AND METHODS

Study sites and study species

We selected one population each from two species that have not been recorded as having naturalised (*B. coccinea* and *B. speciosa*); two species that have been recorded as naturalised (*B. serrata* and *B. formosa*); and one species that has become invasive (*B. integrifolia*). We also selected *H. salicifolia* as we could sample both naturalised and non-naturalised populations (Moodley *et al.* 2014). All populations studied were in the Western Cape Province of South Africa (see Table 1).

Banksia is a large genus of trees and shrubs, consisting of 172 species that are widespread in Australia (George 1999; Moodley *et al.* 2013). At least 11 species have been introduced into South Africa (Moodley *et al.* 2013). Most species produce showy inflorescences with copious amounts of nectar that attracts birds (George 1999). A few species are pollinated by insects or mammals (Carpenter 1978; Cunningham 1991). In South Africa, *Banksia* species usually set two seeds per capsule, except for *B. formosa* (R.Br.) A.R. Mast & K.R. Thiele (formerly *Dryandra formosa*), which sets one seed per capsule (personal observation). Winged seeds are stored in woody follicles that open after fire (serotiny), with some species (*e.g., B. formosa*) releasing seeds upon maturity (George 1999; personal observation).

Banksia coccinea R.Br. has only been recorded from three sites in South Africa and has not been recorded as naturalised. The studied population in Elim comprised 48 individuals planted for cut flowers, and ~3070 seeds are stored in the canopy in veld that is 6 years old (Moodley *et al.* 2014). The second species examined that has not naturalised is *B. speciosa* R.Br., which has been recorded at seven sites. The studied population in Elim contained 29 planted trees for cut flower use and comprised a canopy seed bank of ~37,500 seeds in vegetation with a post-fire age of 11 years.

Banksia formosa (four populations in South Africa) and *B. serrata* L.f. (one population in South Africa) are recorded as naturalised. The *B. formosa* population in Elim selected for this study had 1978 individuals, planted for cut flowers. Seeds are produced in abundance (estimated >26 million seeds in the 10-year-old stand) and are released upon maturity (Moodley *et al.* 2014; personal observation). A single population of nine planted *B. serrata* trees in Betty's Bay, planted in natural fynbos, spread after the last fire and has a canopy seed bank of ~180,000 seeds. Although the age of this population could not be determined, the post-fire age of the veld is 14 years.

Lastly, nine *B. integrifolia* L.f. populations have been identified, of which at least one population, at Pringle Bay, is invasive. This population was selected for our pollinator observations and breeding system experiment. From a single tree planted in 1980, the population currently covers ca. 4 ha, with a canopy stored seed bank of around 750,000 seeds.

Hakea salicifolia is a large bushy shrub or small tree (Barker *et al.* 1999) native to southeast Queensland and eastern New South Wales in Australia. It has naturalised in several regions of the world (Moodley *et al.* 2013, 2014), including South Africa, where it was introduced around 1830 and is now widely used as an ornamental or hedge plant (Le Maitre *et al.* 2008). This species has recently been identified as naturalising in some sites, but not in others (Moodley *et al.* 2014). Of the 113 populations recorded in South Africa, 32 populations have naturalised (Moodley *et al.* 2014). For our study, we selected 16 populations comprising eight naturalised and eight non-naturalised populations.

In Australia *H. salicifolia* flowers between August and November (Barker *et al.* 1999), with a similar flowering phenology in South Africa (personal observation). Species belonging to the *Hakea* genus are visited by bees and wasps in their native range (Armstrong 1979), which is a relatively unspecialised pollination syndrome. This is followed by the development of two winged seeds enclosed in woody follicles (Barker *et al.* 1999). The wind-dispersed seeds are released after fire.

Table 1. Locality details and number of self-sown alien Proteaceae in the Western Cape, South Africa, where pollination experiments were conducted.

species	locality	latitude	longitude	no. of seedlings	no. of juveniles	no. of mature plants	area of spreading population (m²)	population statusa
Banksia speciosa	Blomkloof farm	−34.5376	19.8433	1	0	0	1842	casual
B. coccinea	Blomkloof farm	−34.6412	19.7076	0	0	0	NA	casual
B. formosa	Blomkloof farm	−34.5259	19.8124	27,100	6429	9989	19,781	naturalised
B. serrata	Bettys Bay	−34.3506	18.9213	10	34	11	2440	naturalised
B. integrifolia	Pringle Bay	−34.3532	18.8192	68	183	117	39,355	invasive
Hakea salicifolia	Grabouw	−34.2108	19.0433	103	44	16	608	naturalised
H. salicifolia	Tokai	−34.0609	18.4270	1611	29	1	23,842	naturalised
H. salicifolia	Paarl	−33.7331	19.0425	4304	910	38	2844	naturalised
H. salicifolia	Grabouw	−34.1761	19.0758	1693	135	30	2519	naturalised
H. salicifolia	Paarl	−33.7508	19.0376	2439	787	1	2296	naturalised
H. salicifolia	Klapmuts	−33.8336	18.8775	47	47	828	2367	naturalised
H. salicifolia	Theewaterskloof	−34.0171	19.2419	45,786	1886	10	6017	naturalised
H. salicifolia	Grabouw	−34.0749	19.0788	5081	4035	48	13,000	naturalised
H. salicifolia	Theewaterskloof	−34.0183	19.2373	12	12	0	604	non-naturalised
H. salicifolia	Citrusdal	−32.6101	18.9377	1	0	0	13,186	non-naturalised
H. salicifolia	Grabouw	−34.1595	19.0320	66	131	0	1232	non-naturalised
H. salicifolia	Romansrivier (near Wolseley)	−33.4933	19.1905	0	0	0	NA	non-naturalised
H. salicifolia	Paarl	−33.7343	19.0244	0	0	0	NA	non-naturalised
H. salicifolia	Stellenbosch	−33.9939	18.8270	0	0	0	NA	non-naturalised
H. salicifolia	Lynedoch	−33.9653	18.7971	0	0	0	NA	non-naturalised
H. salicifolia	Jonkershoek	−33.9600	18.9158	39	1	0	94	non-naturalised

NA = populations where no seedlings, juveniles or mature plants where found, hence there are no data for area of spread.

Casual species do not form self-replacing populations and rely on repeated introductions for their persistence; naturalised species produce self-sown offspring that reproduce consistently without direct human intervention; invasive species are a subset of naturalised species that produce offspring at a considerable distance from the parent plant (>100 m over <50 years for taxa spreading by propagules; >6 m over 3 years for taxa spreading by roots, rhizomes, stolons or creeping stems).

aPopulation status classified according to Pyšek *et al.* (2004) and Richardson *et al.* (2000b).

Seed viability is generally high in *Hakea*, irrespective of follicle age (Richardson *et al.* 1987). However, seed viability declines in old *H. salicifolia* follicles (Richardson *et al.* 1987).

Floral visitors

Prior to observing visitors, all inflorescences visible from the vantage point (distance of ~5 m) were counted. We observed floral visits and scored visits as potential pollinator visits when there was contact with anthers or stigma. For each observation period, the identity of all pollinator species and the number of inflorescences visited were recorded. Pollinator visitation rates were quantified as the number of visits per inflorescence per hour. To standardise our sampling across sites, all observations were done in the morning, during similar weather conditions and by the same observer.

Starting at 09:30 h, we observed 30 inflorescences for 150 min over 2 days for *B. speciosa* (90 min on 12 July 2011; 60 min on 14 July 2011). A total of 185 *B. integrifolia* inflorescences were observed for 520 min over 2 days (205 min on 20 July 2011; 315 min on 22 July 2011). Observations were only done on two of the five *Banksia* species due to time constraints. Nevertheless, these species comprise a non-naturalised and invasive population status, which should represent visitation rates across the invasion continuum.

For *H. salicifolia*, we conducted floral visitor observations at six populations, 83 min of observations on 1650 inflorescences in two naturalised populations (23 min on 27 September 2012;

60 min on 5 October 2012) and 207 min of observations on 3100 inflorescences in four non-naturalised populations (48 min on 28 September 2012; 60 min on 5 October 2012; 50 min on 5 October 2012; 49 min on 5 October 2012).

Breeding systems

To assess whether pollinators are important for reproduction in the introduced range, pollinator exclusion experiments were conducted to examine autonomous seed production. Pollen addition, hand- self- and hand cross-pollination experiments (described below) were conducted to assess the effectiveness of pollen supplementation and to determine whether pollen limitation restricts invasion.

We randomly assigned *Banksia* inflorescences to a natural or autonomous treatment (for sample sizes see Fig. 2). The autonomous treatment (Fig. 1a) involved bagging inflorescences that were still in bud phase using fine-mesh nylon bags. These bags prevented potential pollinators from accessing the inflorescences but were permeable to air and moisture. The natural treatment (Fig. 1b) served as the control; inflorescences were tagged at the base and left open for pollination. Each pair of autonomous and natural treatments was performed on the same plant.

For *H. salicifolia*, we selected between six and nine individuals at each of the naturalised and non-naturalised populations. We conducted five treatments: open or natural control (where flowers were left open to pollinators); open pollen addition
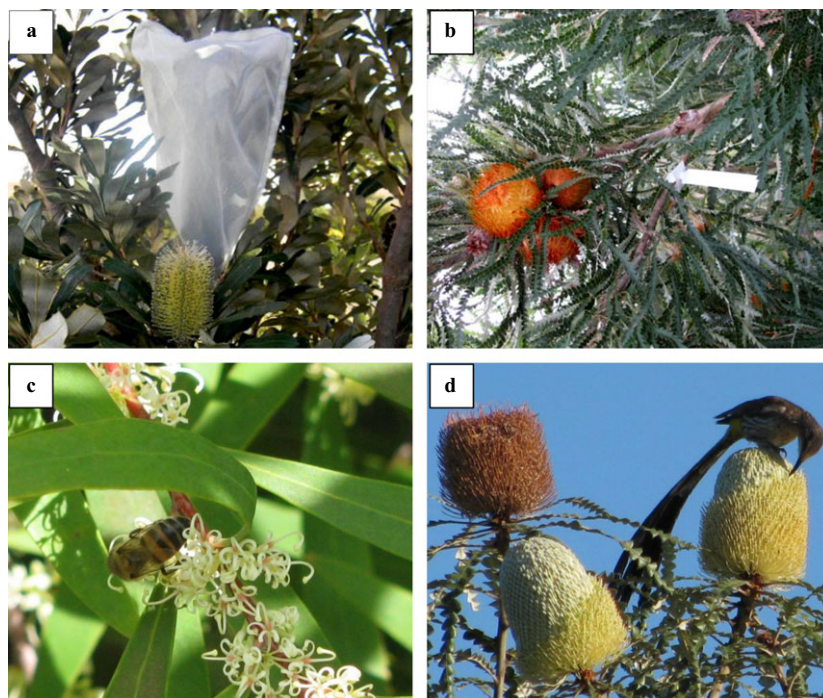
Fig. 1. Aspects of the reproductive biology of *Banksia* and *Hakea* species in South Africa showing: a: Bagged *B. integrifolia* inflorescence; b: Tagged *B. formosa* branch; c: Honeybee (*Apis mellifera* subsp. *capensis*) feeding on a *H. salicifolia* inflorescence; and d: Cape sugarbird (*Promerops cafer*) feeding on a *B. speciosa* inflorescence. Photographs: Sjirk Geerts and Desika Moodley.

(open flowers were augmented with hand-pollination using pollen from donor plants located at least 5 m away); autonomous (flower buds were bagged and left); hand-crossed (flower buds were bagged and once mature were hand-pollinated with pollen from donor plants located at least 5 m away and carefully re-bagged); and hand-selfed (flower buds were bagged, and once receptive, were hand pollinated with pollen from other flowers of the same plant and carefully re-bagged). Prior to blooming, we visited all populations and tagged two branches (for the natural and open pollen addition experiments) and bagged three branches (for the autonomous, hand-crossed, and hand-selfed treatments) on each plant. Branches were bagged with fine-mesh nylon bags to exclude potential pollinators. Hand-pollination treatments involved gently removing anthers from young flowers with clearly visible pollen using tweezers, and rubbing the pollen onto the stigma of the treated flower.

We documented the number of flowers for all treatments in order to measure the percentage fruit set (number of fruits produced/number of flowers × 100%). All branches were harvested 4 weeks later. To identify the number of seeds produced per fruit, we dissected 1896 fruits (at most three fruits per treated plant). Since all dissected fruits contained two seeds, we subsequently only worked with the number of fruits produced.

Data analysis

All statistical analyses were conducted using R (R Development Core Team 2012). Bootstrap means and confidence intervals (bias-corrected and accelerated intervals) were generated by performing 10,000 iterations of sampling with replacement using the 'boot' package.

For each *Banksia* species, we used a generalised linear model (GLM) with Poisson error to determine whether natural and autonomous treatments differed in the number of capsules set per inflorescence.

To test for differences in fruit production between all *H. salicifolia* populations and between the five treatments, we fitted a generalised linear mixed-effect model (GLMM) to allow for random effects caused by repetition of plants and populations. We also used a GLMM to test interaction effect between the treatments and naturalised and non-naturalised populations. Because we treated several flowers on each plant and several plants within each population, we used plant identity nested within population identity as a random effect. We applied the lmer function with a binomial error structure using the 'lme4' package (Bates *et al.* 2012). Proportion fruit set was the response variable and the reproductive treatments (five levels) comprised the predictor variables.

We used two approaches to investigate the influence of breeding system on reproductive success between naturalised and non-naturalised populations. First, we estimated pollen limitation for naturalised and non-naturalised populations using the Pollen Limitation Index (PLI) proposed by Larson & Barret (2000), where PLI = [1 − (natural fruit set/pollen supplementation fruit set)]; a value of zero indicates no pollen limitation. Second, following Lloyd & Schoen (1992) we calculated self-compatibility indices (SCI) for the naturalised and non-naturalised populations, where SCI = seed set after hand-selfing/seed set after hand-crossing. Populations were considered partially self-incompatible if SCI was <0.75 and self-compatible if SCI >0.75. For both indices we calculated the average proportion of fruit set in each treatment per population status.

RESULTS

Visitor observations

Banksia speciosa and *B. integrifolia* were heavily utilised by native nectar-feeding birds and insects (Table 2), with *B. speciosa* receiving more than ten times as many visits per inflores-

Table 2. Visitation rates to *Banksia speciosa* and *B. integrifolia* inflorescences in the Western Cape, South Africa.

species	visits inflorescence$^{-1} \cdot$h^{-1}	
	B. speciosa	*B. integrifolia*
Apis mellifera subsp. *capensis*	0.120	0.0118
Cinnyris chalybeus (lesser double-collared sunbird)	0.0800	0.00311
Nectarinia famosa (malachite sunbird)	0.120	0
Promerops cafer (Cape sugarbird)	0.0267	0.0100
Zosterops pallidus (Cape white-eye)	0	0.00249
total	0.347	0.0274

cence per hour as *B. integrifolia*. *H. salicifolia* flowers were visited by 11 insect species, with similar species present at naturalised and non-naturalised populations (Table 3). Inflorescences were visited by bees, wasps, flies and beetles. Honeybees (*Apis mellifera* subsp. *capensis* Esch.) were the most frequent visitors across all sites and were frequently observed to be collecting nectar and thus foraging between flowers. In total, inflorescences in naturalised populations (0.15 visits inflorescence$^{-1} \cdot$h^{-1}) were visited more frequently than inflorescences in non-naturalised populations (0.04 visits inflorescence$^{-1} \cdot$h^{-1}), but this was not driven by a particular pollinator species (Table 3). In addition, during our extensive pollination experiments, pollinator species and frequencies were noted and found to correspond to the formal floral visitor observations.

Breeding systems

Banksia species produced high levels of autonomously self-pollinated seeds across all casual (*B. coccinea* and *B. speciosa*), naturalised (*B. formosa* and *B. serrata*) and invasive (*B. integrifolia*) species (Fig. 2). In addition, the number of capsules that set seed between the two treatments was significantly different for *B. coccinea* (z = 7.57, *P* < 0.05), *B. serrata* (z = 15.09, *P* < 0.01), *B. formosa* (z = 11.46, *P* < 0.01) and *B. integrifolia* (z = 17.98, *P* < 0.01), with the invasive *B. integrifolia* producing the largest number of capsules in both treatments. However, treatment did not have an effect on

Table 3. Floral visitors and visitation rates at naturalised and non-naturalised *H. salicifolia* populations in the Western Cape, South Africa.

species	visits inflorescence$^{-1} \cdot$h^{-1}	
	naturalised	non-naturalised
Apis mellifera	0.0858	0.0309
Asarkina africana	0.00652	0.00309
Cardiotarsus acuminatus	0.000466	0
Chrysomya albiceps	0.00140	0.00140
Chrysomya marginalis	0.00233	0.00168
Dejeania bombylans	0.00420	0
Eristalinus taeniops	0.00420	0.000281
Musca domestica	0.00326	0.000935
Phytomia incisa	0	0.00112
Polistes fastidiosus	0.00420	0.00159
Scathophaga stercoraria	0.0420	0.00102
total	0.154	0.0420

B. speciosa, since the number of capsules set was similar between the autonomous and natural treatments (z = 0.89, *P* = 0.38). Overall, we only found a significant effect on hand-selfed and hand-crossed treatments between naturalised and non-naturalised *H. salicifolia* populations (Table S1). Furthermore, a comparison between pollination treatments and all populations combined revealed a significant treatment effect (Table S1). *H. salicifolia* produced significantly more fruits naturally than autonomously, but pollen addition significantly enhanced fruit production (Table 4, Fig. 3).

On average, more fruits were produced when pollen was added to flowers (mean for pollen addition: 95% CI, 0.060; range 0.041–0.089; mean for open control: 0.046; range 0.035–0.062). This is especially evident in non-naturalised populations, where the average fruit set increased by more than 70% when pollen was added, as compared to 0% in naturalised populations. The PLI for all populations combined was 0.22, but for non-naturalised populations was 0.40, while naturalised populations were not pollen-limited (PLI of 0). Similarly, the hand-crossed and hand-selfed treatments showed significantly increased fruit set in non-naturalised populations (Table S1).

When we examined population status we also detected variation in self-compatibility between naturalised and non-naturalised populations. Naturalised populations were strongly self-compatible (SCI 0.94), whereas non-naturalised populations were partially self-incompatible (SCI 0.71).

DISCUSSION

Species of Proteaceae introduced to South Africa are able to establish interactions with pollinators in their new range, but they also have the ability to set fruit autonomously without the intervention of pollinators. Pollen vectors increased fruit production in the studied *Banksia* and *Hakea* species. In its native range, *B. coccinea* and *B. formosa* have low levels of self-compatibility (Fuss & Sedgley 1991; Matthews & Sedgley 1998), however, the breeding systems of *B. speciosa*, *B. serrata* and *B. integrifolia* have not been evaluated. In South Africa, a lack of pollinators does not prevent the five *Banksia* species from producing fruits since all species can reproduce *via* autonomous self-pollination to some extent. This suggests that autonomous selfing in *Banksia* species is not related to naturalisation or invasion status. Across all *H. salicifolia* populations, the effect of additional pollen enhanced its reproductive output (Fig. 3), whereas between naturalised and non-naturalised populations self- and cross-pollination treatments significantly increased plant reproductive output (Table S1). This is one of very few detailed studies of the importance of pollinators and breeding systems at the early stages of plant invasion (Stout *et al.* 2002; van Kleunen *et al.* 2008). Here, we established that Australian Proteaceae introduced to South Africa attract similar pollinator guilds as in their native range, and that these bird and insect visitors play an important role in the reproductive performance of Proteaceae. However, whether this increase in reproductive success significantly increases population growth remains to be tested.

In its native range *Banksia* species are pollinated by several nectar-feeding bird species, insects, marsupials and rodents (Collins & Rebelo 1987), and they attract sunbirds, sugarbirds and honeybees in South Africa. Our breeding system experiments and limited pollinator observations show that in South
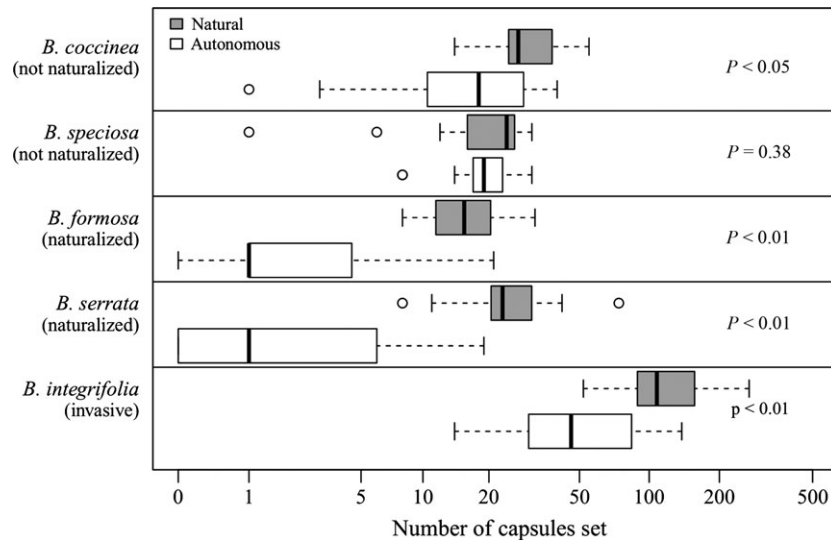
Fig. 2. Natural *versus* autonomous seed production in two casual (*Banksia speciosa* and *B. coccinea*), two naturalised (*B. formosa* and *B. serrata*) and one invasive (*B. integrifolia*) *Banksia* species, in the Western Cape, South Africa. Comparison of the number of capsules (log-transformed) produced between natural pollination (*B. coccinea*, n = 27; *B. speciosa*, n = 18; *B. formosa*, n = 20; *B. serrata*, n = 19; *B. integrifolia*, n = 20) and autonomous treatments (*B. coccinea*, n = 27; *B. speciosa*, n = 19; *B. formosa*, n = 20; *B. serrata*, n = 18; *B. integrifolia*, n = 14) was tested for each species (with significance levels provided for each species). All species produced significantly more capsules in pollinator-visited flowers (natural treatment), except for *B. speciosa*. Boxplots display the median with a solid line, 25th and 75th percentiles in the lower and upper boxes, respectively, and the data range is indicated by the whiskers. Open circles indicate outliers (values >1.5 times interquartile distance below 25th percentile).
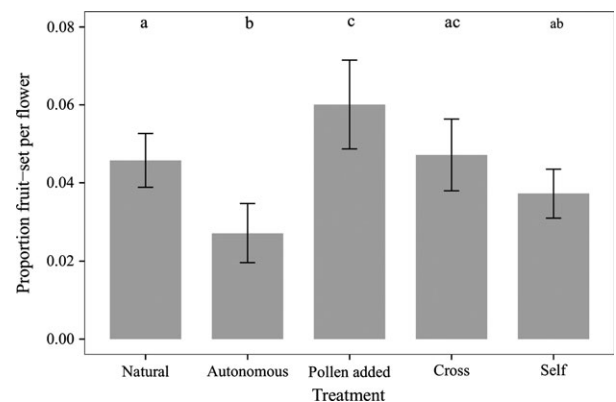
Table 4. Average fruit set (*i.e.*, proportion of flowers producing fruit) in *H. salicifolia* populations in the Western Cape, South Africa, across five pollination treatments. Sample sizes (n) represent number of plants and flowers that were selected for each treatment.

treatment (n)	proportion fruit set per flower mean (range)		fruit set across all sites mean (range)
	naturalised sites	non-naturalised sites	
natural (125/7754)	0.051 (0–0.546)	0.040 (0–0.375)	0.046 (0–0.546)
autonomous (125/6732)	0.023 (0–0.500)	0.030 (0–0.786)	0.027 (0–0.786)
pollen added (122/1857)	0.051 (0–0.600)	0.069 (0–0.800)	0.060 (0–0.800)
cross (125/1309)	0.031 (0–0.272)[a]	0.062 (0–0.750)[b]	0.047 (0–0.750)
self (126/1305)	0.030 (0–0.286)[a]	0.044 (0–0.363)[b]	0.037 (0–0.363)

See Table S1 for the detailed statistical analyses.
[a,b]Indicates a significant difference between naturalised and non-naturalised populations.

Fig. 3. The effects of five pollination treatments (natural, autonomous, pollen added, hand-crossed and hand-selfed) on the average fruit to flower ratio in all *Hakea salicifolia* populations (*i.e.*, naturalised and non-naturalised populations combined). Error bars depict SE of means.

Africa these species set more seeds in the presence of pollinators. All *Banksia* species (except *B. speciosa*) showed a higher reliance on pollinators, but the invasive species demonstrated higher selfing capabilities. This provides support for Baker's Law (Baker 1955), which states that establishment is more likely for self-fertilising species, and also supports studies showing that pollinators can improve seed production in self-fertilising invasive species (Geerts & Pauw 2009; Rodger *et al.* 2010). Similarly, the only known invasive *B. ericifolia* population in

South Africa, although self-compatible, relies on native nectar-feeding birds and honeybees to maintain its population (Geerts *et al.* 2013). Many widely recognised invasive plants are well integrated into pollination networks in their introduced range (Olesen *et al.* 2002; Morales & Aizen 2006). However, if these *Banksia* species are introduced to regions without nectar-feeding birds, we would expect seed production to be lower and the chance of invasion to be significantly lower (see Ollerton *et al.* 2012 for regions with no specialised nectar feeders). Nevertheless, the invasive *Banksia* was still the most successful species, even in the absence of pollinators.

The ability of plants to attract suitable pollinators that are efficient in transferring pollen determines reproductive success

(Harder & Barrett 2006; Mitchell *et al.* 2009). Honeybees were the most frequent visitors to *H. salicifolia* inflorescences (*i.e.,* 59% of visits were made by honeybees). Moreover, reproduction will be maintained in regions where this generalist pollinator occurs (Rodger *et al.* 2010). The PLI revealed that non-naturalised populations were pollen-limited and appear to be partially self-incompatible (*i.e.,* SCI), thereby requiring some degree of cross-pollination to significantly enhance set seed (Table S1). Pollen quantity may be limiting when plants compete for the services of pollinators (*i.e.,* Allee effects; Groom 1998; Cappuccino 2004; Davis *et al.* 2004). Pollinator visitation rates can influence whether populations naturalise or not (Razanajatovo *et al.* 2015). We found that visitation rates are more than three times higher in naturalised *H. salicifolia* sites than non-naturalised populations. Lower fruit production in non-naturalised populations can therefore be explained by pollen limitation. Two naturalised populations were surrounded with vineyards and orchards, whereas the four non-naturalised populations were planted close to a vineyard, along a road, in pastoral land, and along an old orchard (Tables 1 and 4). The presence of many fruit trees increases the number of insect visitors and this probably explains why populations surrounded by fruit trees have more visitors. Pollen limitation and self-incompatibility may not restrict non-naturalised *H. salicifolia* populations from spreading, but could partly explain why some populations have not yet naturalised. For example, the rate of spread in *Cytisus scoparius* along the west coast of North America was reduced due to pollen limitation, but the species still became a widespread invader (Parker 1997).

Studies on other invasive plant species have also demonstrated that self-compatibility and suitable pollinators in the introduced range are important for successful invasions (Rambuda & Johnson 2004; van Kleunen & Johnson 2005, 2007a; van Kleunen *et al.* 2008; Rodger *et al.* 2010; Pyšek *et al.* 2011; Ward & Johnson 2012). Our study adds to the sparse literature on self-compatibility in invasive woody plants. Self-compatibility is clearly not a prerequisite for invasion, and self-incompatible species can become successful invaders. Pollinator limitation and self-incompatibility will lower seed production and perhaps slow down population expansion, but it will not prevent it (Richardson *et al.* 2000a; Traveset & Richardson 2006). As such, except for the advantage of legitimate self- and cross-pollen transfer, our results do not show a strong role for reproduction in explaining plant invasions. Rather, characteristics related to species traits (*e.g.,* serotiny and native range size; Moodley *et al.* 2013) and invasibility (*e.g.,* phylogenetic novelty; Burns *et al.* 2011) and the reason for introduction appear to be the key drivers of these invasions.

Studies that focus on the mechanisms that limit invasions have the potential to provide practical guidelines for risk assessment and management (*e.g.,* Bufford & Daehler 2014). In the cases examined here, a lack of floral visitors could result in a lag phase and might reduce rates of spread, but are unlikely to completely prevent invasions. We suspect that research comparing the breeding systems of populations of introduced woody species at different stages of the invasion process will continue to provide important practical insights. With an increasing number of such empirical studies, it should also become possible to demonstrate when and where Baker's Law applies.

SUPPORTING INFORMATION

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

Table S1. Results of GLMM, testing the effect of five treatments on *H. salicifolia* fruit set (*i.e.,* between all populations combined) and the interaction between the treatments and population status (*i.e.,* between naturalised and non-naturalised populations).

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1 **Table S1.** Results of generalized linear mixed-effect models, testing the effect of five
2 treatments on *Hakea salicifolia* fruit set (i.e. between all populations combined) and the
3 interaction between the treatments and population status (i.e. between naturalized and non-
4 naturalized populations). Plant identity nested within population identity was considered as a
5 random factor. We used the relevel function to test the effect between all treatments.

Fixed effects / response variables	Estimate	Std. Error	z-value	Pr(>|z|)
Natural				
Natural x Autonomous	-0.392	0.097	-4.057	P<0.0001***
Natural x Pollen added	0.350	0.126	2.775	0.00552***
Natural x Cross	0.096	0.146	0.655	0.512
Natural x Self	-0.086	0.157	-0.550	0.582
Natural x Status	0.080	0.154	0.521	0.603
Autonomous				
Autonomous x Pollen added	0.741	0.132	5.631	P<0.0001 ***
Autonomous x Cross	0.488	0.152	3.219	0.00129 **
Autonomous x Self	0.306	0.163	1.879	0.060
Autonomous x Status	0.276	0.184	1.502	0.133
Pollen added				
Pollen added x Cross	-0.254	0.170	-1.494	0.135
Pollen added x Self	-0.436	0.180	-2.426	0.0152*
Pollen added x Status	0.402	0.237	1.701	0.089
Cross				
Cross x Self	-0.182	0.195	-0.935	0.350
Cross x Status	0.917	0.289	3.170	0.00152**
Self				
Self x Status	0.730	0.310	2.352	0.0187*

*P < 0.01; **P < 0.001; ***P < 0.0001

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7

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*$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$