Self-pollination in the genus *Erica*

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Abstract

The ability of flowers to self-pollinate was investigated in eight species of the diverse *Erica* genus. Self-pollination was found to occur in five out of the eight species, with mainly bird pollinated species having a high degree of selfing. The use of a broken anther ring as an indication of visitation and pollination of the flowers was also investigated and it was found that two species are potentially useful in this regard. An inverse relationship between the degree of selfing and near neighbour distance was found across the species. The resprouting *Erica cerinthoides* was found to have a UV signal, it also had the most dispersed population, highest nectar sugar concentration and a high degree of self pollination. *Erica paludicola*, which is an endemic that occurs in only one other population on the Cape Peninsula, had the highest degree of self pollination. I speculate that the high degree of selfing in *Erica* is one of the reasons the genus is so diverse, and that the ability to self will preserve this diversity in the face of increasing habitat destruction and fragmentation, at least temporarily.

Introduction

The *Erica* genus comprises ±860 species, 660 of which occur in the fynbos biome (Manning 2007). *Erica* is the largest genus in the fynbos biome which is famed for its high levels of diversity (Goldblatt and Manning 2002a). The cause of the diversity of the fynbos region is as yet unconfirmed, different theories attribute it to the prolonged geological history of the area (Cowling 2008) and the low nutrient status and highly variable soil characteristics of the area (Goldblatt and Manning 2002a). Johnson (1996) suggests that while the rampant speciation in the Cape flora has been attributed to the heterogenous environment, the fact that most of the variation occurs in floral rather than vegetative characteristics suggests that speciation is pollinator driven.

In the genus *Erica*, the vast range in floral features may be attributed to specialisation for specific pollinators, explaining why several species can grow in such close proximity to each other. The other option is that plants self pollinate, causing high diversity in a landscape where populations may so easily become geographically separated, especially where many flowers and therefore pollinators are small and aren't able to travel long

for growing together? I don't see the connection —

how does selfing prevent hybridization?

distances, causing populations to become reproductively isolated and diverge. Johnson and Bond (1997) found that a scarcity of pollinators led to pollen limitation of fruit set in Orchidaceae and Amaryllidaceae in the Cape flora. They hypothesise that plants in a fire prone environment such as the fynbos would be prone to pollen limitation because fire would deplete pollinator populations. In the absence of pollinators self pollination may evolve as a method of reproductive assurance. The reproductive assurance hypothesis predicts that population variation in mating system should covary with the long-term abundance of effective pollinators (Moeller 2006), so in areas of low pollinator availability plants may evolve self pollination to supplement the production of seeds. The drawback of self pollination is the occurance of inbreeding depression, the reduced fitness of inbred offspring (Moeller 2006). In the fynbos, other genera avoid the lack of pollinator problem by being wind pollinated and prevent selfing by being dioecious (*Leucadendron, Restio*) (Bond 1994), while flowers such as those in the genus *Protea* have a structure that keeps the pollen away from the stigma, avoiding the chances of pollination within a flower.

In this study I investigate whether species from the genus *Erica* have the ability to self pollinate. The relationship between degree of selfing and characteristics such as population density and anther-stigma distance is explored. I predict that *Erica* species do in fact self-pollinate, perhaps explaining the ability of so many small, isolated populations to survive. The degree of self-pollination should decrease with an increasing degree of attractiveness to pollinators, as self-pollination is a form of reproductive assurance, not necessary with high rates of pollinator visitation. The ability of a species ability to self-pollinate should also be related to the distance between the anther and the stigma within the flower (herkogamy). A shorter distance should increase the chance of autogamy (pollination within a single flower) occurring, while the other form of self-pollination, geitonogamy, requires pollen to be carried between flowers on the same plant by some agent of pollination, and isn't related to the anther-stigma distance (Herlihy and Eckert 2004).

In a separate investigation, I look at the potential of using the characteristically tight anther rings of the *Erica* genus as indicators of pollinator visitation. Orchid species such as *Disa* are valuable as an indicator species in ecological studies because it can clearly be seen whether they have been visited by a pollinator or not, by observing whether the pollinia have been removed (Johnson and Bond 1992). This makes them a popular species for pollination studies. *Erica* flowers have a very tight anther ring which may be broken when pollinators force their way into the flower in search of nectar. If this can be proven to be a measure of successful visitation and pollination we can show whether pollinators are present and therefore whether seed set is pollen or pollinator limited.

The aims of this study are first to determine whether *Erica* species can self-pollinate and second to investigate the possibility of using observations of broken anther rings as a measure of pollination.

Methods

Study Site

The study site was situated on the slopes of Chapman's Peak, below Noordhoek peak on the Cape Peninsula, Western Cape, South Africa. The vegetation in the area is peninsula sandstone fynbos which consists of proteoid and ericoid-leaved shrubland growing on acidic lithosol soils (Mucina and Rutherford 2006). This area contains many endemics including *Erica abietina* and *E. paludicola.*

Field work took place from August to October 2009. Eight *Erica* species (Table 1) were selected for this study. The fire recovery strategy of all but *E. cerinthoides* is to reseed, making the production of seed an important trait. *E. cerinthoides* is the sole resprouter, and the assumption is that species which resprout after fire are less reliant on seedlings and therefore would invest less energy in attracting pollinators and producing seed (Bond 1994).

Table 1: Species used in this study (Oliver and Oliver 2002; Oliver and Oliver 2000).

Species	**Flowering time**	**Pollination syndrome**	**Environment description**
E. abietina subsp. constantiana	July-October	Suspected insect	Stony slopes
E. baccans	August-November	Insect (bee)	Stony slopes
E. cerinthoides	Summer and after fire	Bird (sunbird)	Plateau
E. curviflora	September-March	Bird (sunbird)	Wet seep
E. hirtiflora	Year round	Insect	Along river
E. paludicola	September- February	Suspected insect	Wet seep
E. plukenetii.	Year round	Bird (sunbird)	Stony slopes
E. viscaria subsp. viscaria	January -September	Suspected insect	Stony slopes

Self pollination:

In August and September, at the beginning of the flowering season for most of the *Erica* species, flowering branches with unopened buds on a minimum of five individuals of each species were bagged using bags made of fine mosquito netting to exclude pollinators. A control branch, containing unopened buds at the same stage as the bagged flowers was marked for each bagged branch using a twist of wire. Control flowers were left open to be pollinated. Flowers were collected a maximum of eight weeks later and examined for evidence of pollination using a hand lens and/or dissecting microscope. A maximum of five flowers from each plant were examined for each treatment (control and bagged). Flowers were considered to be pollinated if the ovary was swollen and contained healthy looking seeds. If possible the anther ring was examined to see if it had been broken by a visiting pollinator or not, and this was analysed as a predictor for fertilised ovaries in control flowers.

Floral characteristics:

In order to determine the relationship between floral characteristics and the degree of self pollination, several floral attributes relating to the species attractiveness and adaptation to pollinators were measured. Width and length of flower and distance between stigma and anther ring were measured for several flowers from each species using callipers. Width was taken at the widest point of the corolla. The distance between the anther and stigma should predict the occurance of self-pollination, while flower shape (width and length) helps determine the pollinator syndrome for species in which the pollinator has not yet been confirmed (Table 1). Nectar samples were extracted from unvisited flowers of the bird pollinated species early in the morning using a syringe and sugar concentration was determined using an Eclipse handheld refractometer (Bellingham and Stanley Ltd.) (Morrant *et. al* 2009). Colour was analysed by measuring the spectral reflectance over the UV-visible range (300-700 nm) of the corolla of 3 flowers per species using an Ocean Optics S2000 spectrophotometer and fibre optic reflection probe (UV/VIS 400 microm). The distance between nearest neighbours of 10 individuals of each *Erica* species was also determined as an estimate of population density.

Results

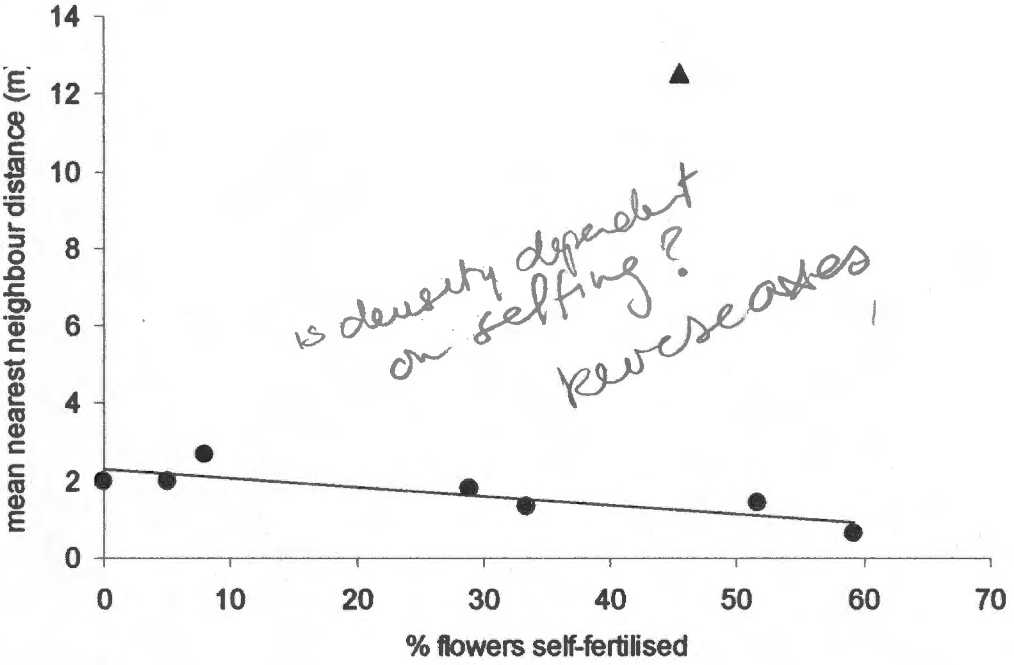
Table 2: Results of self fertilisation experiment. Bold type indicates bird pollinated species. Self pollination was considered to occur where more than 10% of bagged flowers were pollinated (indicated by *).

Species	% fertilised (control)	n	% self-fertilization (bagged)	n	Nectar conc. (% sucrose)	Std dev	n
E. paludicola	90.0	20	59.3*	27			
E. plukenetii	**68.4**	**19**	**51.6***	**31**	**16.1**	**2.7**	**5**
E. cerinthoides	**53.3**	**15**	**45.5***	**11**	**38.37**	**1.4**	**4**
E. curviflora	**68.4**	**19**	**33.3***	**30**	**14.92**	**2.9**	**6**
E. baccans	100.0	19	28.9*	38			
E. hirtiflora	94.1	17	8.0	25			
E. abietina subsp. constantiana	85.0	20	5.0	20			
E. viscaria subsp. viscaria	66.7	24	0.0	30			

why?

Species with a self fertilisation percentage greater than ten were considered to be capable of self fertilisation. The number of flowers fertilised in the control is likely to be an overestimation as many species drop flowers that have not been fertilised and these were lost for the control plants although they were retained on the bagged branches. The selfing measured here is not necessarily autogamy, as there were several flowers contained within each bag and it was possible that pollen was transferred between adjacent flowers, ? wouldn't this be outcrossing? within the bags as well as from outside, by wind, resulting in geitonogamy. The ovaries of many of the samples from *E. plukenetii* were not yet fully ripened so estimates of self fertilisation for this species may not be accurate.

The endemic *E. paludicola* had the highest percentage of self pollinated flowers while *E. viscaria subsp. viscaria* had no evidence of self pollination (Table 2). Apart from *E. paludicola*, which is insect pollinated (bees were observed to visit the flowers in the field) the three bird pollinated species (*E. plukenetii, E. cerinthoides* and *E. curviflora*) had the highest percentage selfing. *E. abietina subsp. constantiana,* which is an endemic to the area, had only five percent selfing and is considered not to self-pollinate. There was no correlation found between nectar concentration and percentage selfing in the bird pollinated species (Table 2), although a sample size of only three species is too small to make assumptions. ?



is density dependent on selfing?
Reverse axes!

Figure 1: Relationship between nearest neighbour distance and percent flowers self-fertilised (bagged flowers). ● = the seven reseeding species, ▲ = the resprouting species, *E. cerinthoides*.

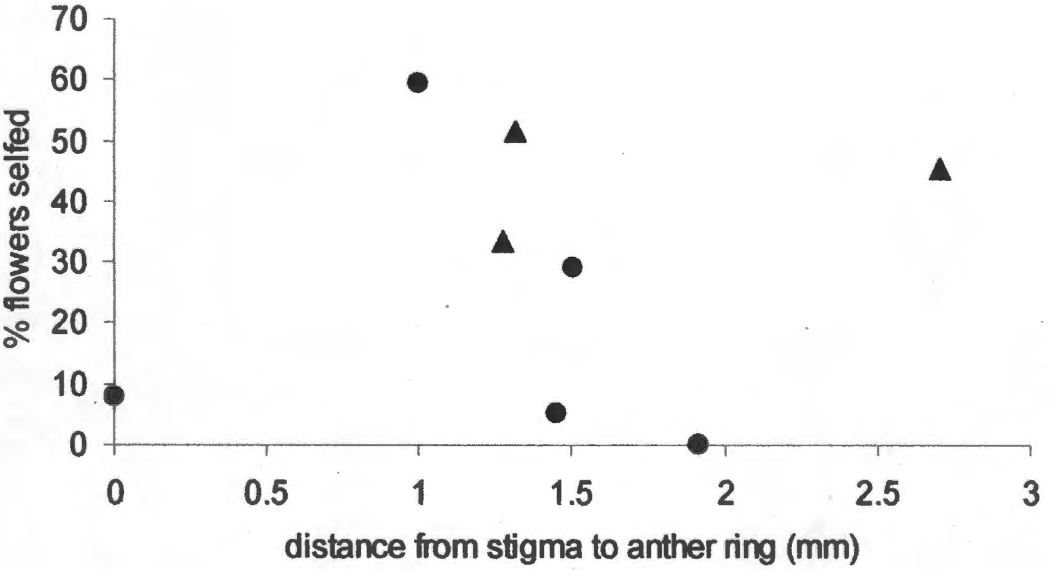


Figure 2: The relationship between percentage self fertilisation and herkogamy (distance from stigma to anther). ▲ = bird pollinated species; ● = insect pollinated species.

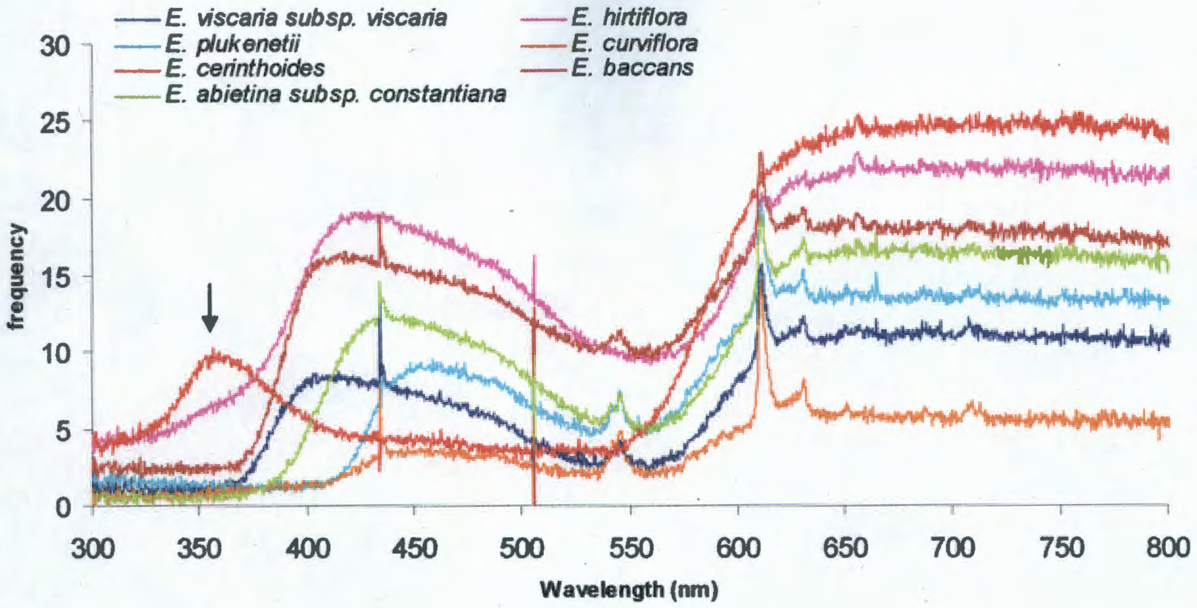


Figure 3: Colour analysis of the corolla of the *Erica* species (excluding *E. paludicola*). The arrow shows the peak of Erica cerinthoides in the near ultraviolet light range (300-400nm).

p = ?

There is a negative trend between nearest neighbour distances and percentage selfing in reseeding species, with an R^2 value of 0.72 (Fig. 1). There was no clear relationship between the anther to stigma distance of each species and the degree of selfing (Fig. 2). Colour analysis of the corolla of each species (Fig. 3) shows that the colour reflectance of most species is similar, apart from the bird pollinated *E. cerinthoides* which has a UV signal, seen as a spike at 350nm (near ultra violet light has a wavelength range of 300-400nm).

Observations: Anther ring breakage

It was observed that the anther ring of *E. curviflora* was broken after being visited by sunbirds, however, as the flowers aged and the ovary developed the anthers were damaged, presumably by the high winds that occur in this area, so that it was impossible to tell whether the anther rings of this species had been broken by a pollinator or other forces in the matured flower. In other species such as *E. baccans*, *E. paludicola* and *E. hirtiflora*, the anthers are so short that when the ovary develops it pushes them apart. Younger flowers of *E. baccans* were not often seen to have broken anther rings, and in one observation a visiting bee failed to break the anther ring, this may also be due to the compact and sturdy nature of the flower. Bees visiting *E. paludicola* also failed to break the anther ring, presumably because of the open, cup shape of the flower, they do not have to force their way in. In contrast, the sturdy, tubular form of *E. cerinthoides* preserves the anther ring and it was possible to see in mature flowers whether the anther ring had been broken or not. Eight out of twelve broken anther rings in the control flowers of *E. cerinthoides* led to a fertilised ovary (Table 3), while *E. viscaria subsp. viscaria was* also useful as an indicator, with eight out of nine broken anthers leading to fertilised ovaries (Table 3). None of the bagged *E. viscaria subsp. viscaria* flowers had fertilised ovaries (seeds were present but shrivelled and brown as opposed to the larger, plump, golden seeds of the control flowers) so the 13 fertilised control flowers are presumably the result of a pollution event/insect that was not forceful enough to break the anther ring. Bagged flowers of *E. cerinthoides* and *E. viscaria subsp. viscaria* did not have broken anthers.

Table 3: Number of broken rings and fertilised ovaries for control flowers of *E. cerinthoides* and *E. viscaria subsp. viscaria.*

species	**Anther ring**	**# fertilised**	**# unfertilised**
E. viscaria	Broken	8	1
	Unbroken	13	7
	Total	**21**	**8**
E. cerinthoides	Broken	8	5
	Unbroken	0	2
	Total	**8**	**7**

E. cerinthoides was the only resprouting species sampled and its characteristics were different to the other seven reseeding species in several respects: *E. cerinthoides* had the highest nectar concentration of the bird pollinated species (Table 2); the population was most scattered, with the greatest nearest neighbour distances (Fig. 1) and colour analysis shows that the flower corolla had an ultraviolet signal (Fig 3).

Discussion

Contrary to expectations, selfing was not correlated with stigma to anther distance (Fig. 2), as found in Moeller (2006) for *Clarkia* species. Allison (1990) found that there was a negative relationship between nearest neighbour distance and ovule production for an evergreen wind pollinated coniferous shrub, which leads to the speculation that some of the selfing occurring in the bagged flowers was due to wind pollination, as the fabric used for the exclusion bags had a mesh that excluded most insects, but would allow pollen to blow in. High wind velocities are a frequent occurance in this region and although the

dont erica's
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pollen tetrads?

unlikely to
be effectively
moved by
wind

stigma of these species is not adapted for wind pollination, it is possible that pollen blown from adjacent flowers could land on the stigma of some flowers, especially the more open flowers such as *E. plukenetii, E. abietina subsp. constantiana* and *E. paludicola.*

It was also expected that species growing in more scattered populations would have a higher degree of selfing, as they are less assured of a pollinator reaching them. This was true for *E. cerinthoides*, which had the greatest nearest neighbour distances between individual plants, but not for the other species with scattered plants such as *E. hirtiflora, E. viscaria subsp. viscaria* and *E. abietina subsp. constantiana.* It is however difficult to generalise about patterns in the genus, and more realistic to discuss each species separately.

Scatterd
NN ± 2

E. cerinthoides is bird pollinated species which resprouts after fire when it flowers most prolifically. The corolla of *E. cerinthoides* has a near UV signal (Fig. 3) which is visible to both birds and insects, which may explain why plants can afford to be so far apart. By flowering in the post-fire environment the species has less competition for pollinators, but Johnson and Bond (1997) suggest that pollinator populations may be depleted after fire. Nectivorous bird species may not return in great numbers until there is sufficient food available, so plants which flower after fire such as *E. cerinthoides* may have very low pollinator visitation rates, therefore having to evolve a degree of self fertilisation for reproductive assurance (Bond 1994). In the case of *E. viscaria subsp viscaria*, plants are relatively scattered over the mountain slopes, with an average nearest neighbour distance of two metres between plants (max: 7.8m, min: 30cm), yet the species doesn't appear to self at all. It does however have a very strong, syrupy sweet fragrance which would attract pollinators, decreasing the need for reproductive assurance by self-pollination.

E. paludicola, which had the highest degree of selfing of all the species in this study, is a narrow endemic confined to only two populations on the Cape Peninsula. The high degree of selfing may explain why these two populations can survive, separated by a distance to great for their presumably small, insect pollinators to travel. *Erica* seeds are not adapted for wide dispersal, most will just drop straight to the ground below their mother plant and remain in the seed bank until the next fire comes round. A combination of short dispersal distances and lack of pollen transport between the two populations because of small insect pollinators is most likely the reason that the two *E. paludicola* populations are diverging in terms of flower shape: the Chapman's Peak population is more open and bowl-shaped while the Silvermine population, a few kilometres away, has a narrower corolla. Is this difference pollinator driven or could it be caused by genetic drift due to isolated populations and a high degree of selfing? In the future it would be interesting to compare the degree of selfing in endemic *Erica* species to those which are widespread

Is it possible that *Erica* species have evolved self-pollination strategies because of lack of pollinators in a frequently burnt environment? Inbreeding depression, the decline in fitness of inbred plants, causes self fertilization to be selected against (Harder and Barrett 2006), so self-fertilization shouldn't occur unless there are advantages to being able to self. Studies on fynbos genera such as *Ixia* (Goldblatt *et. al* 2000) and *Gladiolus* (Goldblatt and Manning 2002b) find that these genera are mostly self-incompatible and rarely self pollinate, other fynbos genera such as *Leucadendron* and *Restio* avoid self pollination by being dioecious and escape the problem of a lack of pollinators by being wind pollinated. If other fynbos genera actively avoid self-pollination, then the occurance of self-pollination in *Erica* raises more questions than it answers.

The use of anther rings as a method of estimating pollinator visitation is still unclear. It would be very useful in pollination studies if we were certain that a broken anther ring in an *Erica* flower was a sign of the flower having been visited and successfully pollinated. Although it is possible to determine whether the anther rings of *E. cerinthoides* and *E. viscaria subsp. viscaria* have been disturbed or not, this is not a reliable predictor for fertilisation of the flower (Table 3). Further investigation with a larger sample size and pollinator observations may clear up this issue. In certain species such as *E. curviflora*, the broken anther ring can be seen directly after the pollinator has visited, but with the ripening of the ovary and the shrivelling of the corolla, the anthers are broken or disturbed. With species such as this the visited flowers would have to be marked while they are still fresh, and revisited later to see if the visit resulted in a fertilised ovary.

The fynbos biome is becoming increasingly fragmented by development, especially in the greater Cape Town area where reserves are mostly confined to mountainous areas surrounded by urban areas. If the already pollinator scarce fynbos environment is impacted by this development, losing more pollinators due to lack of habitat and, in the case of birds, lack of pollination corridors. The equilibrium theory of island biogeography predicts that as habitat fragments or 'islands' decrease in size, the number of species occurring on that island will also decrease (Bond *et. al* 1988). Bond et. al's study on fynbos island in the Knysna forest found that small islands have up to 80% less species than similar sized areas in bigger fragments. One cause of a decrease in diversity in fragments such as these is the decrease in pollinator visits. Self-pollination in *Erica* may preserve the species diversity in the face of increasing fragmentation of habitats. But for how long? Little is known of the pollination biology of species within the genus *Erica* in the fynbos. Rebelo and Siegfried (1985) predicted pollination syndromes of 341 of the south-western Cape's *Erica* species based on the colour and size of the flowers. The nectar sugar composition of bird pollinated *Erica* species has been studied (Barnes *et. al* 1995), and recently Geerts and Pauw (2009) investigated the adaptation of *Erica* species to pollination by malachite sunbirds, but the pollinators of many *Erica* species are still unknown (Oliver and Oliver 2000). This study did not include *Erica* species adapted to specialised pollinators, and it is the specialised species that are under the greatest threat from human development. If their pollinators disappear and they do not have a reproductive assurance strategy such as self-pollination, the specialised species, many of which have not yet been studied, may disappear.

Opportunities for further research within the *Erica* genus are boundless. It would be particularly interesting to compare the degree of selfing in Cape *Erica* species to those growing elsewhere, especially where the genus is not as diverse, for example, in Europe where they have only a few, widespread species. *Erica* may be useful as a model from which to investigate the reasons behind the diversity of the fynbos, as well as the implications for its survival in the future.

Conclusion

The high degree of self pollination in some of the *Erica* species in this study could be due to the fact that fynbos is regularly burnt, banishing pollinator populations from the area until sufficient food is available for their return. Self pollination could have evolved as a reproductive assurance mechanism in frequently burnt areas, and then acted as an agent of speciation when populations were isolated, causing them to diverge and create the diverse genus we know today.

In a world increasingly affected by human activity important ecological services such as pollination may be affected and in turn this may lead to a decrease in diversity in many ecosystems. These results show that much of the diversity of the genus *Erica* may be preserved in the absence of pollinators as they have reproductive assurance in the form of self-pollination.

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