The Reproductive Biology of *Erica pudens*

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Abstract

Erica is the largest genus in the Cape Florisitic Region (CFR) boasting a diverse range of floral morphology and pollination systems. Even though it is such a diverse genus, there is minimal research examining the pollination biology of specific species. This research inspects the pollination biology of *Erica pudens*. To do this we carried out pollinator exclusions, hand pollination experiments, rodent trapping, camera observations and pollen/ovule counts. This research also establishes whether *E. pudens* is another example of convergent evolution in *Erica* by establishing its phylogenetic position. *E. pudens* possesses floral characteristics that are consistent with the rodent-pollination syndrome. These characteristics include tightly-packed, pendulous inflorescences with a prostrate habit, found close to the floor, with a dull flower colour and winter flowering times. This research also found that *E. pudens* offers a high volume of nectar per floral head (up to 20.9µl) with a comparably high sugar concentration (23.7%). Even though these characteristics suggested rodent-pollination, there was no other evidence that conclusively demonstrated this. Only three rodents were captured, and few pollen tetrads were found in the faeces of the two *Rhabdomys pumilio* individuals (average of 13 and 1 respectively). There was very little footage captured of rodent activity around *E. pudens* flowers and none to demonstrate the foraging activities of a potential pollinator. The exclusion of pollinators showed no significant difference in swollen ovule dimensions between bagged flowers and caged flowers. Breeding experiments showed no significant difference between self-pollinated flowers and cross-pollinated flowers. These results suggested no need for a pollinator and the ability of *E. pudens* to undergo self-pollination. This could be an example of pollinator failure (due to small rodent populations) and the consequent evolution of self-pollination. The phylogenetic studies showed that *E. pudens* was another example of convergent evolution within *Erica*.

Introduction

The Cape Floristic Region (CFR) of South Africa is well-known for its high levels of species richness and endemism (Schnitzler *et al.*, 2011, Pirie *et al.*, 2011, Johnson, 2004). The Ericaceae is one of the many families in this region and is the largest genus in the area with 680 described species (Rebelo *et al.*, 1985, Rebelo & Siegfried, 1985, Barnes *et al.*, 1995, Mcguire & Kron, 2005, Pirie *et al.*, 2011, Turner *et al.*, 2011). *Erica* boasts a wide range of floral structure and morphology which is matched by a diverse range of pollination systems (Turner *et al.*, 2011, Rebelo *et al.*, 1985). These pollination systems include bird, wind and insect and have been described extensively in Rebelo *et al.* (1985).

There are few studies pertaining to *Erica* pollination. However, those that have been carried out, include some of the first examinations of groups of *Ericas*, their floral characteristics and their respective pollinator guilds. Barnes *et al.*, (1995) examined the nectar sugar compositions for 50 *Erica* species, and related this to their pollinators, with a specific focus on ornithophilous species. This study was based on the idea that there is a relationship between the sugar proportions in a flower's nectar and its type of pollinator (Barnes *et al.*, 1995). They found that flowers pollinated by birds showed a much higher incidence of sucrose-dominated nectar and therefore there appeared to be a relationship between the nectar characteristics and the pollinator (Barnes *et al.*, 1995). Colour and size of *Erica* flowers have also been examined and related to specific pollinator guilds for 341 species (Rebelo and Siegfried, 1985). More recently, studies have examined the pollinators for specific *Erica* species, namely *E. halicababa* which is pollinated by birds (Turner *et al.*, 2012) and *E. hanekmoii* which is rodent-pollinated (Turner *et al.*, 2011). There is certainly a deficit of studies examining the reproductive biology and pollination of specific *Erica* species.

Another aspect of research that has taken place regarding the *Erica* genus is that of phylogenetic studies based on molecular data (Pirie *et al.*, 2011; McGuire and Kron, 2005). McGuire and Kron (2005) studied *Erica* species from both Africa and Europe to establish the origin of this genus. The phylogenetic studies revealed that the African taxa are most likely descendent from a European common ancestor (McGuire and Kron, 2005). Another phylogenetic study investigated the relationship between morphological characters and

pollination syndromes (Pirie *et al.*, 2011). A significant finding from Pirie *et al.* (2011) showed that there has been convergent evolution of floral morphology in un-related *Erica* species (Pirie *et al.*, 2011). This implies that there has been the evolution of similar floral characteristics in independent lineages (Pirie *et al.*, 2011).

A central idea of pollination biology is that of pollination syndromes. Since its first mention by Vogel (1954-cited in Johnson, 2004), it has become a key concept in determining specific pollinators (Johnson, 2004; Turner *et al.*, 2012). Pollination syndromes are a collection of floral traits that reflect convergent evolution, and are usually associated with the attraction of a specific group of pollinators (Turner, 2012; Ollerton *et al.*, 2009; Fenster *et al.*, 2004). The application of pollination syndromes has been seen as restrictive, because it imposes too much dependence of flowers on specific pollinators (Ollerton *et al.*, 2009, Merxem *et al.*, 2009, Waser *et al.*, 1996). However, applying the pollination syndromes concept can assist in creating testable hypotheses for assessing which pollinator guild applies to certain plants (Turner *et al.*, 2012).

Take *Erica pudens* H.A Baker for example, it has floral traits that include tightly-packed, pendulous inflorescences with a prostrate habit (Fig. 1 A and B), dull flower colour (Fig. 1) and winter flowering times (peak flowering time between July and September). The initial examination of *E. pudens* floral traits suggests that it conforms to the rodent-pollination syndrome which is indicated in Wiens *et al.* (1983). *Erica pudens* also exhibits similar traits to another rodent-pollinated *Erica* species, *E. hanekomii* (Turner *et al.*, 2011). Therefore it is hypothesised that *E. pudens* is rodent-pollinated.

Another aspect of this study entails a molecular analysis of *E. pudens* to establish its position within the greater *Erica* phylogeny. This will assist in determining if the floral characteristics observed in *E.* pudens evolved only once or more than once in unrelated lineages. This will further determine if there is convergent evolution within Erica. Consequently the second hypothesis is that *E. pudens* is another example of convergent evolution in *Erica*.

To test the first hypothesis and further understand the reproductive biology of *E.pudens*, this study carried out pollinator exclusions, hand pollination experiments, rodent trapping, camera observations and pollen/ovule counts. This was done to address four questions: 1) What are the characteristics of the floral rewards? 2) What is the pollinator? 3) Which

breeding system does *E. pudens* utilise? 4) What is the relationship between the breeding system and the pollinator? To test the second hypothesis molecular analysis was carried out to answer the question; is *E. pudens* another example of convergent evolution in *Erica*?

Methods

Study site

The field studies took place at one of the known locations of *Erica pudens*; a rocky plateau on a farm, above the small town of Aurora, near the Piketburg Mountains, South Africa (-32.718S 18.575E, 970m). Two isolated sites were found containing *E. pudens*. The one site contained approximately 100 plants, whilst the other site had a total of 50 plants. On inspection of the sites, there were very few other species of *Erica* and none that were rodent pollinated. This knowledge would prevent the confusion of the pollen tetrads of *E. pudens*. Observation of the plants took place for three days from 12 to the 14 of July 2013.

Nectar Properties

The nectar properties of 30 florets were examined from separate plants. This was done by extracting nectar from the florets using calibrated micro-syringes, accurate to 0.05 µl. The nectar sugar concentrations were measured using an Eclipse handheld refractometer that could take sugar measurements up to 50% and 80%. The nectar was then placed onto Whatman filter paper and dried for the determination of the constituent sugars later.

Rodent trapping and observation

To determine the state of the rodent population in the area, trapping was done over 3 nights and two days. Fourteen metal traps were laid out on the evening of the 25 July 2013 in both sites. Ten more traps were set up on the evenings of the 9th and 10th of August 2013. Day trapping was done on the 10th and 11th of August 2013. These traps were set up in the morning and checked every two to three hours during the day. All traps were baited with balls of peanut butter and oats. If a rodent was caught, the species would be identified before they were released. Any faeces left in the traps was collected and stored in separate Eppendorf tubes, in 70% alcohol for pollen analysis later.

All the faecal material was transferred to new eppendorfs with 500 microlitres of 70% alcohol. The faecal material was then broken up using a toothpick, after which each eppendorf was placed onto a vortex for ten seconds. If the material was not separated enough, it was broken up again with a toothpick and placed on the vortex for a further ten seconds. Two microlitres of the sample was placed onto a piece of dried fuschin dye on a microscope slide. The dye was melted with a lighter held under the slide for a few seconds and a coverslip (±22mm^2) was placed over the sample. On each microscope slide there were two sub samples made, and for each sample of faecal material (from each rodent species) four slides were mounted. This gave a total of 8 subsamples from each sample of faecal material. The pollen tetrads were counted under 100x magnification by moving across the entire area of the slide so that all the pollen grains were counted.

Rodent behaviour towards *E. pudens* was captured using five Ranger camera traps which were set up for three nights of filming between 12 and 14 July 2013 and for one night on the 25 July. They were also left on from the 29 July until 9 August 2013 for night and day observation of the rodents. The cameras were mounted on tripods or attached to wooden poles with elastic bands and placed approximately 2 metres away from the plants, and about 30cm off the ground. Once mounted, any plant material that might obscure the view of the camera was removed to allow a clear view of the plants. The cameras are triggered by motion in front of the lens, so any rodent activity was captured by the cameras.

Selective exclusion experiments

Selective exclusion experiments were carried out on the 13 July 2013, to determine the importance of pollinators for *E. pudens*. Eleven individual plants were chosen for the exclusion experiments from different localities within the population. The flowers were too small and tightly packed within the floral head to allow for individual exclusions. Instead, one undisturbed flower was selected from the floral head and all other flowers were cut off removing all the anthers. This was done to prevent the spread of pollen from other flowers within the floral head, but would not prevent the movement of pollen within a floret. Rodent exclusion was achieved by covering flower heads with chicken-wire exclosures that had an aperture of approximately 15 x 17mm. The exclusion of all pollinators was achieved by covering the flowers with veil exclosures (small gift bags) that were tied at the base.

Control flowers were left uncovered and marked with a piece of string. The plants were removed seven weeks later for dissection. When dissection of the flowers took place, it was discovered that they had not fully developed; however some flowers had swollen ovules while others did not. It was assumed that the swollen ovules indicated that the flower had been pollinated, and subsequently the length of the ovules was measured to distinguish between those that were pollinated successfully, and those that were not. This assumption was checked by examining gynoecia for pollen tubes, which is described later. To assess swollen ovules for the breeding experiment, an individual flower was chosen (the oldest one of the floral head) from each treatment, and dissected. A subset of five ovules from each floret was mounted onto a slide and the length was measured in graticule units at 40x magnification.

Breeding system

The breeding system was determined by performing pollen transfers for 11 individual florets from different plants. Eleven floral heads, in bud, were bagged 4 weeks prior to treatments to ensure no disturbance by any potential pollinators. The four treatments were carried out on the 13th of July 2013. The treatments were control, cross-pollinated, self-pollinated and autogamous. The control florets were left open and marked with string. The crossed florets received pollen from another plant that was 10 metres away. The self-pollinated florets received pollen from their own flower, and the autogamous florets were left covered to see if the plants could pollinate themselves. All pollen transfers were carried out by removing the pollen from the anthers on a toothpick and placing the pollen onto the stigma. All other florets on the floral head were cut away, removing the anthers, to ensure no further pollen transfer from these other florets. The plants were all covered after manipulations to eliminate any further interference.

Seven weeks later, the flowers were collected for dissection in the lab. When dissection of the flowers took place, the same discovery was made concerning the exclosure treatments; the flowers had not fully developed. The same protocol was carried out where a subset of five ovules from each floret was mounted onto a slide and the length was measured in graticule units at 40x magnification.

Pollen:Ovule Ratio

Single flowers were separated into individual eppendorfs for pollen and ovule counts. Ten flowers were used for the pollen counts and ten for the ovule counts. Ovules were counted by dissecting the flowers and releasing all the ovules from the ovary and counting them. For the pollen count, 3ml of 95% glacial acetic acid was added to each eppendorf, to soften the tissue, and was centrifuged for 15 minutes at 1850 rpm. All liquid was then decanted from the eppendorfs. A solution was made containing 27ml acetic anhydride and 3ml of concentrated sulphuric acid. 1ml of this solution was added to each of the eppendorfs and placed in a 100 °C bath for 15 minutes, this broke down the tissues. The eppendorfs were them removed from the bath and centrifuged for another 10 minutes at the same settings as above. 20 µl of this solution was placed onto a haemocytometer. The pollen grains were counted at 40x magnification.

Pollen tubes

To check the assumption that swollen ovules indicated a successful pollination and non-swollen ovules indicated no pollination, flowers were prepared so that pollen tubes could be examined. If pollen and pollen tubes were seen on flowers with swollen ovules and no pollen or pollen tubes were found on non-swollen ovules, then this would confirm the assumption. The presence of pollen tubes infiltrating the ovule indicated successful pollination.

To prepare the flowers, gynoecia were removed and submerged in Carnoy's solution (1 glacial acetic: 3 95%ethanol) for two hours to arrest metabolic processes. They were then placed in 70% ethanol for preservation until they could be stained. The stigma, style and ovary were rinsed twice in distilled water for one hour to remove all excess ethanol. This was followed by treatment with 8M NaOH (sodium hydroxide) for three hours to soften the tissue. The tissue was then rinsed twice for one hour, after which 20% H_2O_2 (hydrogen peroxide) was applied for 2 hours. After sufficient time had lapsed, the tissues were rinsed again, twice for an hour in distilled water. Staining of the styles and ovaries enables better visualization of the pollen tubes. To prepare the stain, 21ml 1% aniline blue (0,2g Gurr aniline blue), 7ml K_3PO_4 (potassium phosphate) (1,4g grains or pellets in 7ml) and 182ml distilled H_2O were added together to make 200ml 1% aniline blue stain. The stain was left to

decolour in the fridge for 12 hours before applying to the tissues. The tissues were mounted in glycerine with a drop of the stain, and examined using a Diaphot-tmd Nikon Inverted fluorescent microscope, model Diaphot-tmd. Images were taken with the Zeiss axiocam camara, attached to the microscope. When analysing the pollen tubes, pollen was identified on the end of the stigma, then any tubes from the pollen grains were followed down the style and to penetration of an ovule.

Molecular analysis

Plant material was collected on the 24 June 2013 for DNA analysis. DNA was collected for analysis by Dr Mike Pirie (University of Stellenbosch). Internal Transcribed Spacer (ITS) data was used to create a phylogeny, with the use of maximum likelihood and boostrapping. The phylogeny was restricted to the North West clade of *Erica*, which are the *Erica* species found in the North West part of the Cape Fold Mountains, including the Cederberg, Ceres and Hex River Mountains.

Statistical analysis

The assumptions of a parametric test were met, therefore a one-way ANOVA was used to determine the differences between the treatments for the exclosure experiments and for the breeding system experiments. Where a significant difference was found, a Post-Hoc HSD test was carried out to determine which treatments were different. All statistical analyses were carried out in STATISTICA version 11.

Results

Nectar Properties

The mean (±SD) nectar volume of *E. pudens* florets was 1.9µl (±0.52), with a range of 1µl to 2.9µl. The mean (±SD) nectar sugar concentration was 23.7% (±6.6) with a range of 12.5% to 42.5%. The average number of florets per floral head was 11. Although nectar samples were taken for constituent analysis, laboratory problems prevented the analysis of the constituents consequently there are no results for this.

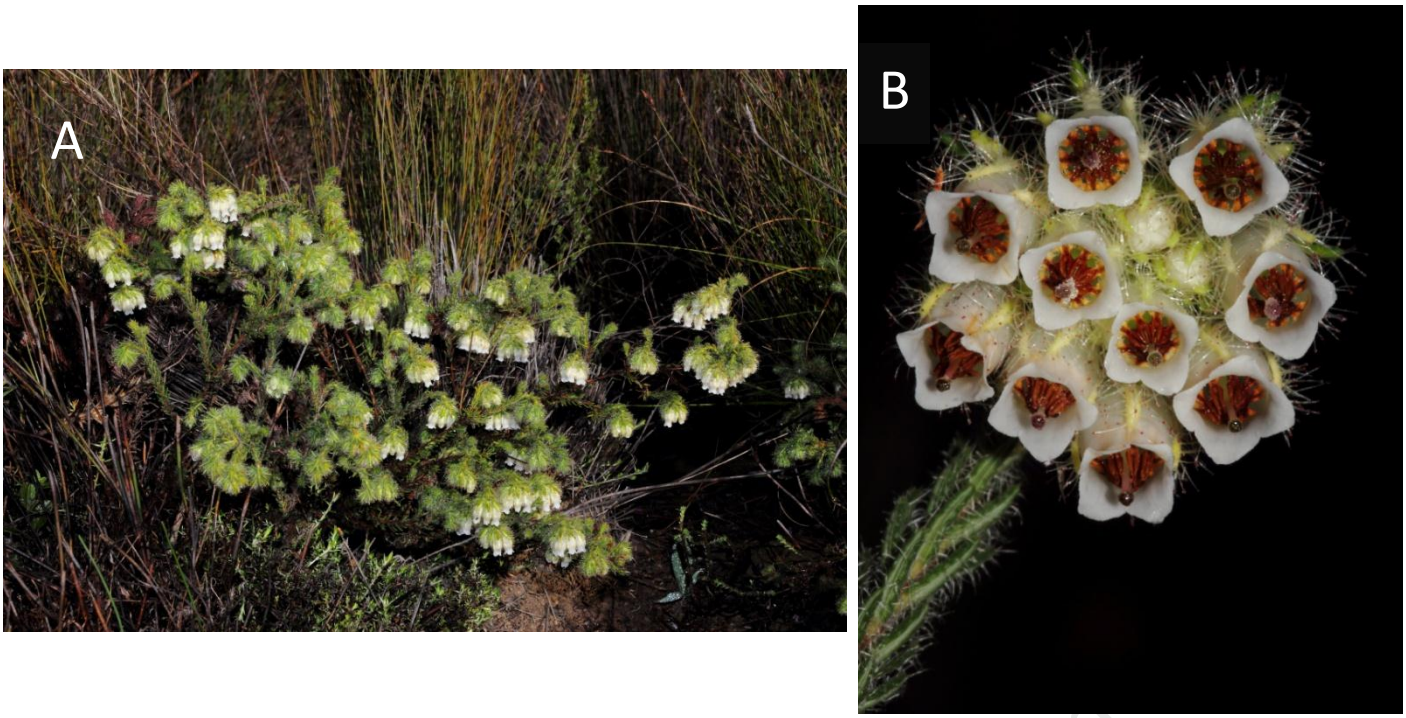

Figure 1: A, *Erica pudens* growth habit showing pendulous flowers, and habitat. B, The tightly packed inflorescence of *E. pudens*.

Exclusion experiments

There was no significant difference between the ovule dimensions of the three treatments applied for the pollinator exclusion (F-value= 0.66, p-value=0.52).

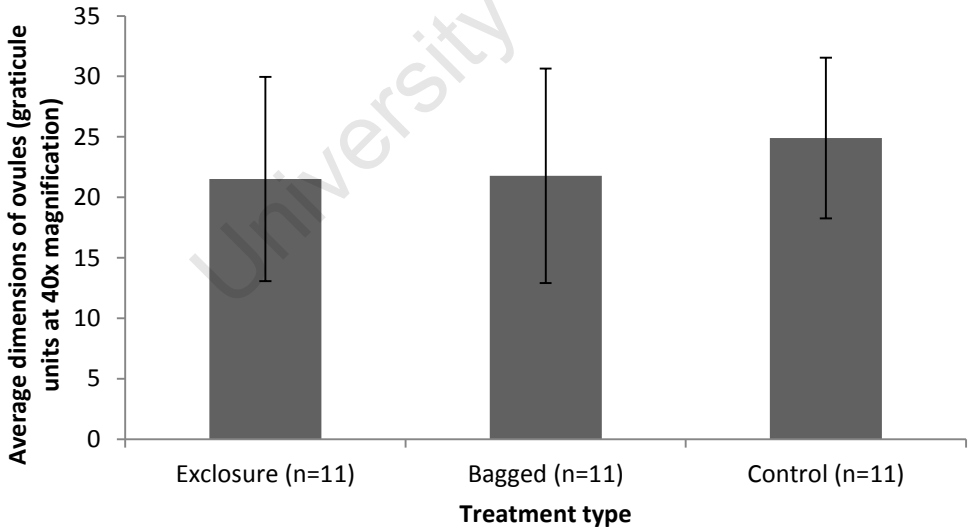

Figure 2: The average dimensions of ovules for three different treatments (exclosure, bagged and control) each with a sample size of 11, with ±1 Standard deviation.

During observations of *E. pudens* no insects were seen approaching or surrounding the flowers. The Malachite Sunbird (*Nectarinia famosa*), the Cape Sugarbird (*Promerops cafer*) and the Orange-Breasted Sunbird (*Anthobaphes violacea*) were all seen in the general area; however, none were observed approaching *E. pudens* flowers.

Rodent trapping and observation

On the first trapping night during July 2013, no rodents were captured and often the bait ball was found still sitting in the traps. On the second trapping night during August 2013, three rodents were captured: Two *Rhabdomys pumilio* (Striped mouse) and one *Acomys subspinosus* (Cape Spiny mouse). Faecal material was collected from all three of the rodents. No pollen grains were found in the faecal material of *A. subspinosus*. The average number of pollen tetrads was 13 (range : 7-25) for the one *R. pumilio* individual and 1 (range: 0-3) for the other *R. pumilio* individual. The pollen grains in the droppings are the results of preening by the rodents while caught in the traps.

Between the 12 July and 14 July, still photographs were taken on the Ranger cameras; there were a total of 47 still images captured. Two of these images showed a rodent moving near the *E.* pudens flowers; however there was no clear evidence of any pollinating activity with the flowers and it was difficult to identify the species. Video footage taken from 29 July to 9 August 2013 recorded a total of 32 hours and 45 minutes of video footage. One video showed a rodent moving past the flowers however it did not make any attempt to retrieve nectar from them.

Breeding System

There was a significant difference between the four treatments (F-value=4.13, p-value=0.012, p-value<0.05). A post-hoc Tukey HSD test showed that there was a significant difference between the self-pollinated and the bagged treatment (p-value=0.03) as well as the crossed and the bagged treatment (p-value=0.019).

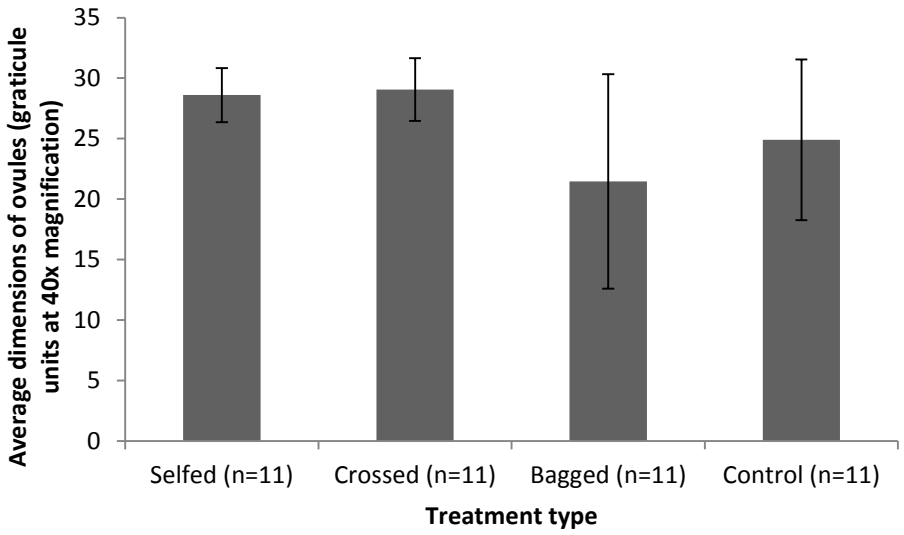

Figure 3: The average dimensions of ovules for 4 different treatments (Selfed, crossed, bagged and control) each with a sample size of 11, with ±1 standard deviation.

Pollen/Ovule Ratio

The average number of pollen grains per *E. pudens* flower was 7200 (±4859.24) and the average number of ovules per flower was 24.5 (±4.08). The pollen-ovule ratio was 294.

Pollen tubes

Flowers that did not have swollen ovules showed no evidence of pollen grains or pollen tubes. The flowers with swollen ovules showed evidence of pollen grains on the stigma, pollen tubes developed from the pollen grains, and penetration of the pollen tube into the ovule (Fig. 4).

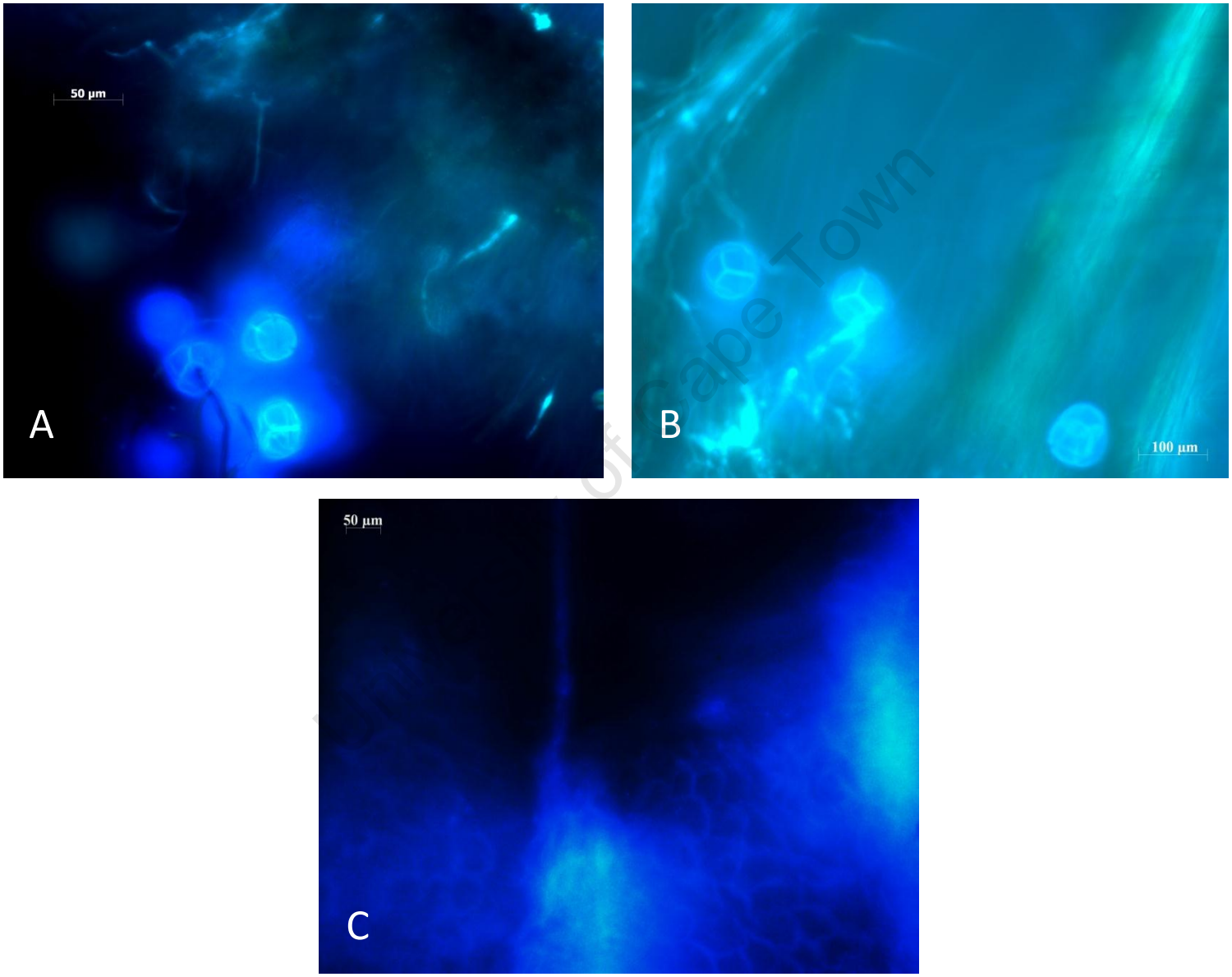

Figure 4: Images taken on a Diaphot-tmd Nikon Inverted fluorescent microscope to show the presence of pollen grains on the stigma (A), pollen tubes being released from pollen grains (B) and the infiltration of pollen tubes into the ovule (C). Scale bars, 50µm (A); 100µm (B); 50µ (C).

Molecular analysis

E. hanekomii and *E. pudens* are not related according to the phylogenetic position of the two species (Fig.5)

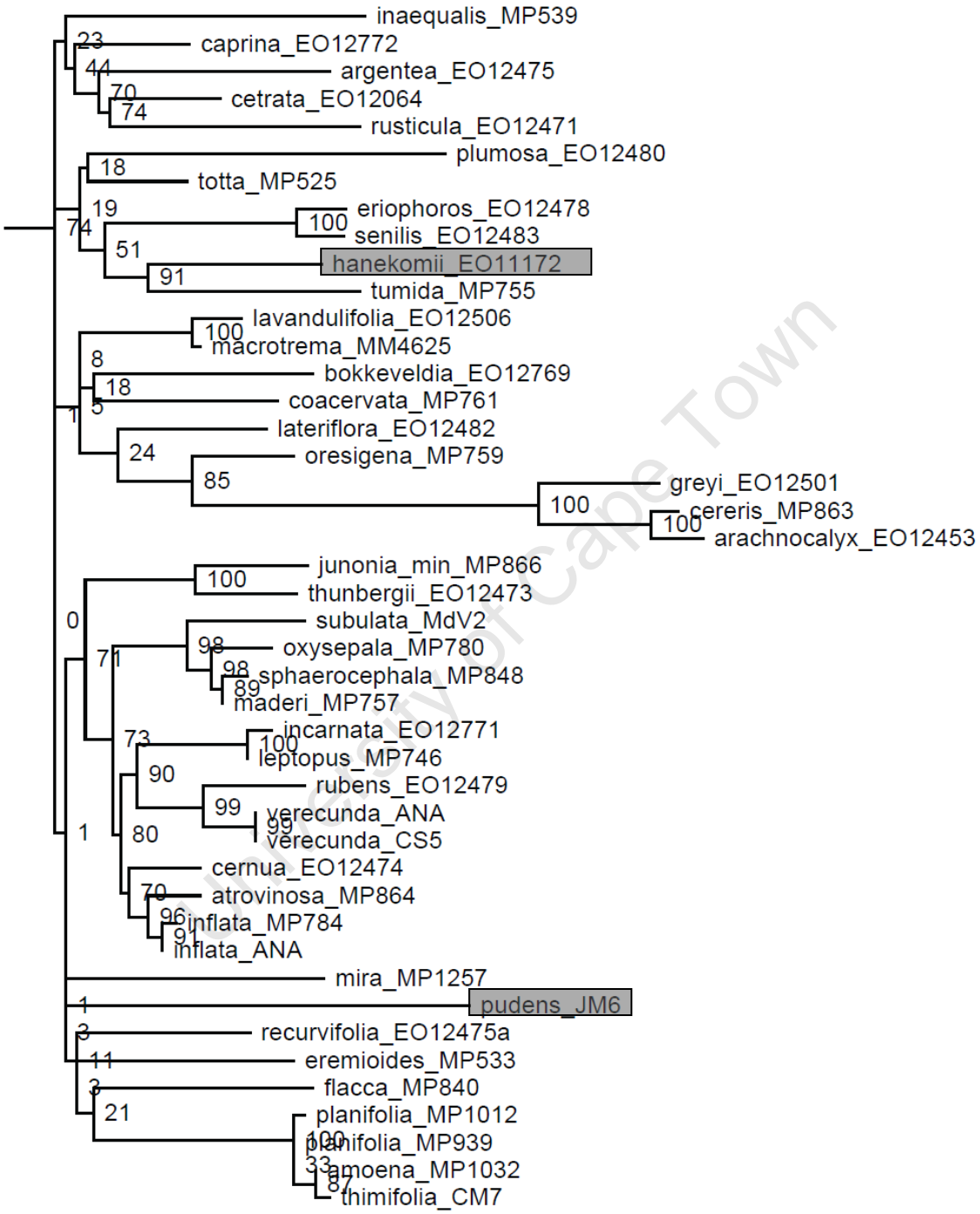

Figure 5: A phylogeny, produced by Dr Mike Pirie (University of Stellenbosch), of the North-West clade of Cape *Ericas*, with the bootstrapping units adjacent to the nodes. *E. hanekomii* (the known rodent-pollinated species) and *E. pudens* (this study) are highlighted.

Discussion

What are the characteristics of the floral rewards?

Erica pudens flowers have a lot less nectar than *E. hanekomii* flowers, which produce 9µl per flower (Turner *et al.*, 2011). However, it must be considered that on average there are 11 florets per floral head for *E. pudens* and so for the entire floral head there is approximately 20.9µl of nectar which falls in the range of *E. hanekomii* (Turner *et al.*, 2011). The sugar concentration of 23.7% is comparable to *E. hanekomii* as well as other rodent-pollinated species (Turner *et al.*, 2011; Letten and Midgley, 2009; Johnson *et al.*, 2001). Wiens *et al.*, (1983) describes copious amounts of sucrose-rich nectar as one of the characteristics of rodent-pollinated Proteas. Even though nectar properties may play a role in attracting rodents, research has not found distinctive nectar traits that specifically classify certain plants as being rodent-pollinated (Turner *et al.*, 2011). However, one can still compare *E. pudens* to other known rodent-pollinated species and assess if its nectar properties fall in the range of these known rodent-pollinated species, which it does.

Another aspect to consider when examining the floral reward is how and when the reward is presented to potential pollinators. *E. pudens* flowers are geoflorous and have a very distinctive prostrate habit (Fig. 1).This offers the nectar to pollinators close to the ground (Wiens *et al.*, 1983; Turner *et al.*, 2011). The peak flowering time for *E. pudens* is between July and September during the winter months, which means that nectar, is offered when other food sources are low and when insects are generally inactive (Johnson *et al.*, 2001; Letten and Midgley, 2009). These characteristics are consistent with the rodent-pollination syndromes, however further experimental evidence needs to confirm that *E. pudens* is rodent-pollinated.

What is the pollinator?

Two species of rodent were captured; *Acomys subspinosus* and *Rhabdomys pumilio*. No pollen was found in the faeces of *A. subspinosus* which suggests that it is not taking part in foraging activities on *E. pudens*. This is contrary to other results since *A. subspinosus* has been found as a principle pollinator for many other species (*E. hanekomii*-Turner *et al.*, 2011; *Liparia parva*- Letten & Midgely, 2009 and *Protea nana*-Biccard & Midgley, 2009).

In contrast to this, the pollen found in the faeces of *Rhabdomys pumilio* might suggest that it takes part in foraging activities on *E.pudens* flowers. However, this species is usually associated with destroying floral heads rather than foraging for nectar and the pollen might have been picked up during this destruction (Letten & Midgley, 2009, Turner *et al.*, 2011). There was also evidence of destructive behaviour found around the *E.* pudens plants, where parts of the white corolla had been removed from the flowers (N Dignon, RC Turner and JJ Midgley, pers. observ., this study). This evidence for destructive behaviour suggests that R. *pumilio* might not be the pollinator of *E. pudens* because legitimate pollinators are associated with non-destructive behaviour toward floral heads (Turner *et al.*, 2011; Letten & Midgley, 2009; Biccard and Midgley, 2009). Although the results suggest that *R. pumilio* is coming into contact with *Erica* pollen, nothing conclusive can be said about whether or not it is actually pollinating the flowers because the capture rate was so low.

This low capture rate might suggest that the population of rodents at this site is much depleted. This could be explained by the altitude of the site or persistent human presence and disruption. The altitude of the site (980m) does not seem like a valid reason because *E. hanekomii* plants were found at an even higher altitude (1170m) and a number of rodents were captured at that site (Turner *et al.*, 2011). The consistent presence of humans on the site and near to the *E. pudens* plants could explain the depleted rodent population. The land is used as a guest farm, however there are also privately owned houses, which are frequented by owners and guests. There are roads located near to the sites of *E. pudens* and disturbance caused by cars and human traffic could disturb the rodent populations causing them to seek better, less disturbed localities. Human disturbance has been reported to disturb pollinators and consequently disturb the pollination of certain plant species, which could be the case at this site (Geerts and Pauw., 2011).

With the exclusion experiments, no significant difference between the treatments indicates that the exclusion of all pollinators does not make a difference to the flowers pollination success. This suggests that *E. pudens* does not necessarily require a vector, or pollinator to move the pollen from the anthers to the stigma. The lack of research into pollination biology of *Ericas* makes it difficult to assess whether or not this is a trend in other *Erica* populations. However the fact that *E. pudens* does not require a pollinator can have implications on the breeding system which was also considered in this study.

The direct evidence, using cameras to capture footage of any rodent activity around *E. pudens*, gave very little support to the idea of rodent-pollination. Conversely, the numerous sightings of known bird pollinators taking no interest in *E. pudens* and the lack of insects around *E.pudens* flowers does not assist in establishing another potential pollinator. The lack of footage showing rodent movements was surprising considering the extensive visual evidence of rodent trails, holes and activity around the *E. pudens* flowers (N Dignon, RC Turner and JJ Midgley, pers. observ., this study). The footage did not show that the rodents had an interest in the flowers, and certainly did not show their foraging activities with the flowers. This could further support the speculation that the rodent population is very small, or it could show that *E. pudens* is in fact not rodent-pollinated. The consistency of the floral traits of *E. pudens* with rodent-pollination syndromes seems to nullify the second reason, and the low capture rate in traps and on cameras supports the idea that the rodent population is small.

Which breeding system does E. pudens utilise?

The results for the hand-pollination experiments suggest that *E. pudens* is self-compatible, because swollen ovules were detected in the ovary, and there was no significant difference between the ovule dimensions for the cross-pollinated flowers and the ovule dimensions for the self-pollinated flowers. This suggests that *E. pudens* can undergo self-pollination. This is also consistent with what was seen in the exclusion experiments; that *E. pudens* does not require a pollinator for movement of pollen. Once again, the lack of literature on *Erica* breeding systems does not assist in establishing if this is a trend amongst small *Erica* populations. Studies looking at other plant species have found that the isolation of a population or the lack of pollinators can cause the rapid evolution of autonomous self-pollination (Kalisz *et al.*, 2004, Moeller & Geber, 2005). The evolution of this self-pollination is seen to be advantageous because it can offer reproductive assurance where pollinator visitation fluctuates or is unreliable (Kalisz *et al.*, 2004). This may be the case with *E. pudens* where the lack of pollinators, in the form of rodents, has allowed for the evolution of autonomous self-pollination. This, however, is speculation and makes the assumption that these plants are in fact pollinated by rodents.

The pollen/ovule ratio found for *E. pudens* falls within the 'facultative autogamy' category (Cruden, 1977). Cruden (1977) showed that the pollen/ovule ratio is integrally connected to the breeding system of a plant, as well as other aspects like plant habitat and successional stage. If a plant is autogamous (can self-pollinate), the number of pollen grains it produces is less than a plant that is xenogamous (cross-pollinates) (Cruden, 1976, 1977). A plant that self-pollinates has reproductive assurance and so does not need to produce as many pollen grains as one that relies on a pollinator (Cruden, 1977; Kalisz *et al.*, 2004). This implies that it produces enough pollen to be able to self-pollinate, however it does so facultatively (Cruden, 1977). This appears to be the case with *E. pudens*.

What is the relationship between breeding system and pollinator?

The results of this experiment do not conclusively show that rodents are the primary pollinators of *E. pudens*, however they do demonstrate two ideas. Firstly, *E. pudens* does not necessarily require a pollinator for the movement of pollen and successful pollination of flowers. Secondly, the breeding system appears to be autogamous (self-pollinating), however only when there is a need for self-pollination (facultative). When these two ideas are considered, in conjunction with the low rodent population, there appears to be a case of pollinator failure with a consequent need to evolve a system that deals with this failure. This system is what is observed as autogamous self-pollination (Kalisz *et al.*, 2004, Goodwillie, 2001). If one assumes that *E. pudens* can be pollinated by rodents, as the floral traits suggest, then it would have relied on out crossing, however the low rodent population may have caused the evolution of self-pollination. This concept has been termed as the "reproductive assurance hypothesis" and explains the idea that self-pollination might evolve out of cross-pollination when pollen delivery is not sufficient (Goodwillie, 2001; Baker, 1955; Stebbins, 1957). This scenario may be the case in this population if *E. pudens* when all the direct and indirect evidence is considered. This is however, speculation, due to the lack of convincing conclusions regarding the pollinator of *E. pudens*.

Is Erica pudens another example of convergence in Erica?

The phylogeny of the NW clade of *Ericas* demonstrates that *E. pudens* is another example of convergence in *Erica.* This is due to the fact that it is not a sister species to another known rodent-pollinated species, *E. hanekomii*. *E. hanekomii* exhibits floral characteristics such as

tightly packed inflorescences, dull flower colour, geoflorous flowers, copious amounts of sucrose-rich nectar, and winter-flowering time (Turner *et al.*, 2011). Although this study did not demonstrate conclusively that *E. pudens* is rodent-pollinated, the similarity in floral characteristics to *E. hanekomii* and the unrelatedness of the two species, suggests that convergent evolution has taken place. This is consistent with the findings of Pirie *et al.*, (2011) who demonstrated that similar adaptations in floral morphology and pollination syndromes have occurred in independent lineages within *Erica*.

Limitations

Site selection and modifications to the methods described in this study, could potentially improve the chances of establishing the pollinator for *E. pudens* or allow for a better understanding of the breeding system. The site we selected for this study appeared to have a very low rodent population, which made it difficult to make any clear conclusions regarding the rodent-pollination hypothesis for *E. pudens*. In this study we removed the flowers too early and so the flowers were not fully developed. Improvements on the method would entail the removal of breeding and exclosure experiment flowers at a later stage, to ensure seed set. This would give a better view of how well the flowers set seed under certain treatments.

The direction of our study was too rodent focused which resulted in our methods specifically addressing the rodent-pollination hypothesis. The study showed that *E. pudens* is not conclusively rodent-pollinated and it seems to be undergoing self-pollination. However, our conclusions regarding self-pollination are largely speculative and our methods did not address a further understanding of the mechanism of self-pollination. Even so, this study has indicated a potential new direction of study on *E. pudens* where the focus is not on whether or not it is rodent-pollinated but rather on the breeding system and how this isolated population of *E. pudens* uses its breeding system to maintain the population.

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