Evolution of floral morphology in *Brunsvigia* and *Crossyne* (Amaryllidaceae)

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Front cover: Painted Lady Butterfly, *Vanessa cardui* on *Crossyne flava* flower at midday.

Rear cover: Noctuid moth on *Brunsvigia bosmanniae* flower during early evening in Nieuwoudville.

Abstract:

Floral morphology and its relationship to pollination syndromes is examined for the genera *Brunsvigia* and *Crossyne* (Amaryllidaceae). These two genera have similar vegetative morphologies and share the same mode of seed dispersal (anemogeochory). They differ ~~is~~ in their floral and inflorescence structures. The species *Brunsvigia bosmaniae* and *Crossyne flava* are chosen as representative species of the two genera *Brunsvigia* and *Crossyne*. Floral morphology is studied in relationship to an outgroup species *Nerine humilis.* Pollination syndrome, ability to self-pollinate, levels of natural seed set and patterns of seed dispersal are studied so that reproductive strategies of the two species can be compared. *Crossyne flava* is pollinated by a suite of small diurnal insects and can be considered to have a generalist pollination syndrome. The first observation of pollination by moths in *Brunsvigia bosmaniae* is reported. I show that neither species is able to self, hence pollination events are important. Experimental manipulation reveals that *Brunsvigia bosmaniae* is pollinator limited. Although this experimental manipulation was not possible for *Crossyne flava,* high seed set levels in *Crossyne flava* suggests that levels of pollination are high in *Crossyne* and low for *Brunsvigia.* The amount of pollination that takes place is shown to be closely related to floral morphology. Floral divergence of the two genera is thus proposed to be the result of adaptation to a pollinator driven selective regime.

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Introduction:

The tribe Amaryllideae belongs to the family of perennial bulbs, the Amaryllidaceae. The Amaryllideae consists of 11 genera, which occur throughout sub-Saharan Africa (Snijman, 1992). Species in this tribe occur in a range of different habitats including grassland, savannah and tropical forests, they are however, most speciose in the semi-arid winter rainfall areas of Southern Africa (Snijman and Linder, 1996). The Amaryllideae are an unusual group of perennial geophytes, as they flower during autumn in a leafless stage.

Flowering in early autumn, after a dry summer in an arid area, from reserves stored during the previous winter's growth, is presumably a costly strategy. Members of the Amaryllideae tribe flower in autumn because they have non-dormant seeds which need to be released prior to the growing season. Snijman and Linder (1996), hypothesise that the early evolution of this group took place during the Tertiary when conditions were moist. Subsequent aridification of Southern Africa lead to the radiation of the subtribe Amaryllidinae during the Pliocene. The key innovation which allowed for this radiation is hypothesised to be the evolution of a seed with a thick green integument and stomatose testa (Snijman and Linder, 1996). This seed type reduces the carbon cost of flowering and setting seed in arid conditions before the vegetative growing season has begun.

There are two adaptive advantages to flowering in Autumn: The first is the immediate germination of seedlings at the start of the rainy period, which prevents risk of long seed exposure to environmental hazards. The second is improved exploitation of insect pollinators during autumn when few other plants are in flower (Dafni et al. 1981 b). The severity and length of the dry season, and the mass spring floral displays and species richness of the flora of the winter rainfall semi-arid regions of South Africa, have probably favoured autumn flowering plants.

The Amaryllideae, while being remarkably uniform in vegetative morphology, exhibit a diverse range of inflorescence, and to a lesser extent, floral characters (Snijman and

Linder, 1996). Snijman and Linder (1996) have shown that the evolution of different modes of azoochorous seed dispersal have driven some of the changes in inflorescence structure in this tribe. Floral divergence and how this relates to pollinator interactions has however not been studied. What is of particular interest is how these plants, which flower at such an unusual time of the year, are pollinated. Presumably pollinator abundance and diversity would be affected by long arid summers. How members of the Amaryllideae have adapted to pollinator pressures has as yet been unquestioned.

In order to test whether a pollination selective pressure is important in this group floral divergence is studied. As a starting point the floral morphologies of the two sister genera, *Brunsvigia* and *Crossyne,* are examined. The reasons for choosing these two genera are included in the discussion that follows.

Brunsvigia and *Crossyne* have very different floral morphologies. The genus *Brunsvigia* consists of 19 species, the genus *Crossyne* has 2 species. The floral and morphological characters of the taxa within these genera are uniform enough for one specie to be representative of the rest of that genus. This research project will look at differences floral traits of the species, *Brunsvigia bosmaniae* (*Brunsvigia b.)* and *Crossyne flava (Crossyne f.).*

Brunsvigia and *Crossyne* share the same mode of seed dispersal, anemogeochory: the dispersal of a tumbling infructescence by wind (Snijman and Linder, 1996). Floral and inflorescence structures are, however very different. There are two possible explanations for this: either the two genera have diverged due to a selective pressure for increased pollination or for increased efficiency of seed dispersal. In a study done by Johnson and Bond (1996), the flora of the South Western Cape was found to be severely pollinator limited. If this is the case it would make sense that the divergence of these to genera, may be pollinator related. Both genera however have evolved wind dispersal of seeds by having a tumbling infructescence (anemogeochorous dispersal). Anemogeochorous dispersal is basal to the *Brunsvigia/Crossyne* clade (Snijman and Linder, 1996). The more complex inflorescence structure of *Crossyne* may be a specialisation of this seed dispersal mechanism. The number of times that anemogeochory has evolved in the tribe Amaryllideae: twice in the subtribe Crininae, and potentially three times in the tribe Amaryllidinae (See Snijman and Linder, 1996) suggests that fruiting characters associated with seed dispersal are plastic.

Brunsvigia and *Crossyne* are sympatric in the Nieuwoudville area with the species *Brunsvigia b.* and *Crossyne f.*. Studying these two species in an area where they flower and grow at the same time, means that both share the same set of selective pressures including the same suite of potential pollinators.

This research project aims to answer the following questions:

1)How do *Brunsvigia b.* and *Crossyne f.* differ in floral morphology?

In order to establish which of the floral morphologies are plesiomorphic the two species are compared to *Nerine humilis* (*Nerine h.)*. This species will act as a representative of the genus *Nerine*, which is the outgroup genus to the *Brunsvigia/ Crossyne* clade (Snijman 1996).

2)Do the *Crossyne f.* and *Brunsvigia b.* have different pollination syndromes? Is the difference in floral morphology related to the pollination syndrome of the species.

3)How dependent is fruit set of the two study species on pollination, and how effectively are they being pollinated?

4)Both *Brunsvigia b* and *Crossyne f.* have anemogeochorous seed dispersal, their inflorescence structures are however very different. Are these differences an adaptation by either for more efficient method of seed dispersal, or is the difference in inflorescence structure related to flowering strategy?

Methods:

Study species

Brunsvigia bosmaniae Leight, and *Crossyne flava* W.F. Baker ex Snijman are bulbous herbs (Snijman and Linder, 1996). Both species have leaves which are hysteranthous and grow during the winter months from Late April to September. The inflorescence heads, emerge after the first rains received at the end of summer in March, or April (MacGregor, pers. comm.), and consist of a naked scape which is terminated by an umbel like cluster of flowers (Snijman and Linder, 1996). The scape of both species detaches at ground level before seeds are released (Snijman and Linder, 1996). The Infructescence is dispersed as a single unit for both species (Snijman and Linder, 1996).

Brunsvigia bosmaniae has bulbs ranging from 2 to 20 cm in size depending on age. The species has 6 broad recumbent leaves. The inflorescence of *Brunsvigia bosmaniae* consists of 20 - 40 zygomorphic flowers (Snijman and Linder, 1996), on pedicels which during flowering are about 10 cm long (Manning and Goldblatt, 1997). Flowers are densely packed on an inflorescence and all flowers open within 3 days of one another. On an inflorescence of 20 flowers, 15 flowers may be flowering at one time (Dyer, 1950). The perianth is shell-pink with variation in perianth colour among populations and individuals ranging form deep pink to white (pers.. obs.). Floral lobes are about 4cm long (Dyer, 1950). Stamens are dimorphic, and exerted from the perianth. The style is up to 6 cm long (Dyer, 1950). The Perianth is sweetly scented an unusual character for the genus *Brunsvigia* (Dyer, 1950). The ovary is ovate and acutely angled, when ripe it develops into a triangular almost winged capsule (Dyer, 1950). *Brunsvigia b.* occurs from Springbok in the North Western Cape to Tygerberg near Cape Town (Figure 1).

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Figure 1. *Brunsvigia bosmanniae* inflorescence in typical habitat at Nieuwoudtville.

Figure 2. *Crossyne flava* in typical habitat at Nieuwoudville.

Figure 2b. Close up of *Crossyne flava* infructescence showing typical high seed set

Figure 3. *Nerine humilis*, the outgroup species used in this study.

Crossyne flava, has a large subterranean bulb and 4-6 prostrate, fairly narrow leaves (Manning and Goldblatt, 1997). The leaves of *Crossyne flava* of differ from *Brunsvigia b.* in having long bristle cilia around the leaf margins (Snijman and Linder 1996). Leaves are abaxially speckled in red, this character is unique to the genus *Crossyne* in the tribe Amaryllideae (Snijman and Linder, 1996). The inflorescence emerges at the same time, or slightly earlier than *Brunsvigia bosmaniae.* The inflorescence has many flowers ranging from around 70 to 400 flowers depending on the size of the inflorescence (pers. obs.). Flowers are small (less than 2cm), slightly zygomorphic (Snijman and Linder, 1996) and pale yellow. Inflorescence scape and pedicel colour varies from pale yellow to deep red. Tepals curve back during flowering (Manning and Goldblatt, 1997). Only a small percentage: less than 30% of flowers are open at one time. Flower period extends to 2 weeks (pers. obs.). Flowers are lightly scented. Ripe capsules are rounded, and may split open if seed number is high (pers. obs.) (Figure 2a and Figure 2b). *Crossyne f.* ranges in it's distribution from Springbok to the Biedow valley.

Study sites

Populations of the study species, *Brunsvigia b.* and *Crossyne f.* were sampled in and around the town Nieuwoudville in the South Western Cape (31°22′S, 19° 8′E), in March 1998. Rainfall in the region is low, 350 mm per annum, most of which falls in winter (Manning and Goldblatt, 1997).

The two genera grow together, in small fragments of Renosterveld which remain between fodder pastures. Where possible, as many different populations of the two genera were sampled. Two populations of *Crossyne f.* were sampled for seed set within a distance of 10 km's of one another. The first from Glen Lyon Renosterveld Reserve, the other from the Nieuwoudville Wildflower Reserve. Both these sites are situated on Dwyka Tillite soils.

Seed set sampling for *Brunsvigia b.* was performed on one population of about 2000 plants, on a Dolerite outcrop on the farm Glen Lyon. Pollinator observations were recorded at five different populations where both study genera were flowering together: Glen Lyon Renosterveld reserve, Nieuwoudville Wild Flower Reserve, and three different Dolerite soil populations on the farm Glen Lyon.

Nerine humilis was sampled in April 1998 on the Bainskloof mountain range, behind Wellington (33° 38′ S, 19° 0′ E). One population of 150 plants growing in an area of 15m2 was sampled. The population grows on Sandstone nutrient poor soils and receives high rainfall.

Floral morphology

The floral morphology of *Brunsvigia* and *Crossyne* was studied and compared to the morphology of the outgroup specie *Nerine* so that any changes in floral structure could be phylogenetically traced. Buds from flowering plants of all three genera were picked in the field at different stages of floral development, from at least five different individuals of each of the three study species. These were fixed in FAA and analysed in the laboratory.

Buds for each species were divided into size classes, ranging from the smallest bud to open flowers. An ontogenetic sequence for flower development was decided on by the size of the bud. Four different bud sizes, varying from the smallest to the largest bud were allocated the time stage 1,2,3 and 4. The fully developed flowers were divided into the male flower stage (Anthers dehisced, but stigma not ripe- stage 5), and the female flower stage (Anthers dehisced and stigma ripe), stage 6. Three buds for each time stage, for all three species, were dissected and the length of the various floral structures were measured. The orientation, arrangement and angle of different floral parts were also recorded for each bud. Exact measurements were:

i) Petal orientation: measured as the angle of the tepals in relation to the inferior ovary.

ii)Perianth length: The distance measured from the base of the inferior ovary to the edge of the tepals.

iii) Filament ratio: All three species have 6 filaments, which are dimorphic.

Filament length was measured and the ratio, between the whorl of long filaments and the whorl of short filaments, was calculated.

iv) Style to filament ratio: This was calculated by measuring the length of the style in relation to the length of the whorl of long filaments.

v)Anther orientation: Four different anther orientation states were identified for the three study species. State 1: The double levelled packing of anthers into the bud. State 2: Filament whorls at different lengths with anthers declinate. State 3: Filaments almost the same length and orientated around the style. State 4: anthers wilted and declinate in comparison to the style.

vi) Style angle: Three different states were identified for the way in which the stigma was situated compared to the rest of the style. State 1: Stigma and style strait, (stigma not mature). State 2: End of style with stigma slightly curled up at an angle of 45° from the rest of the style (stigma mature). State 3: End of the style and stigma curved at an angel of 90° to the rest of the style.

The three measurements for each bud stage were averaged so as to obtain a relatively accurate measure of the floral feature being studied.

Pollinator observations

Populations of flowering *Brunsvigia b.* and *Crossyne f.* were observed at peak flowering times. Observations were made at different times of the day starting at 7:30 in the morning with four to six hour intervals. This was done to ensure that both nocturnal and diurnal pollinators were observed. Pollinator activity was also observed at five different sites on different days so that a full range of pollinators were observed. Pollinator activity was closely studied to ensure that the recorded animals were touching the reproductive parts of the flower and hence effecting pollination. Where possible, pollinators were caught. These animals were observed under dissecting microscopes in the lab, to determine whether they had pollen attached to their bodies, or not. If pollen was found on the insect, the insect was viewed under the scanning electron microscope to determine whether the pollen present was Amaryllideae pollen. Amaryllideae pollen is bisulcate (Snijman, 1992; Meerow, 1995), and large in comparison to other Monocot pollen grains (Erdtman 1966). The surface of the pollen is uniform: gemmate with large spinulae (Meerow, 1995). These features makes it easy to identify. Unfortunately it is not possible to identify pollen of different genera within the tribe Amaryllideae as the pollen characters are conservative (Meerow, 1995).

Seed set sampling

In order to test what contribution pollinators make to the number of seeds *Brunsvigia b.* and *Crossyne f.* set, the ability of these two species to self was tested. Fifteen emerging inflorescence heads of each species were bagged. Owing to the unknown early flowering of *Crossyne f.*, plants were bagged at the early stage of flowering, rather than at the emerging bud stage. To ensure that no pollination had already taken place, open flowers were cut, leaving only closed buds on the inflorescence. One week after bagging *Crossyne f.* and *Brunsvigia b.* inflorescence heads were manipulated. For each species:

i) 10 flowers on each of the five inflorescence heads were self-pollinated. Pollen from the anthers of a neighbouring flower on the same inflorescence, was used. This treatment simulated a pollinator depositing pollen of one flower, on the stigma of another flower, on the same inflorescence.

ii) 10 flowers on each of a further five plants were cross-pollinated using pollen from stamens of neighbouring unbagged plants. This treatment imitated the action of a pollinator depositing pollen from one inflorescence to the flower of a different inflorescence.

iii)10 flowers on the five remaining bagged plants were tagged and left unmanipulated , to determine if the plants are capable of self pollinating in the absence of pollinators.

iv) 5 control plants were randomly chosen from the remaining population of unbagged plants. 10 flowers on each plant were tagged and left unmanipulated. The control plants served to represent the amount of natural seed set that occurred for *Crossyne f.* and *Brunsvigia b.*

The number of seeds that developed in each manipulated and tagged flower capsule, were counted two weeks after manipulation. Ten plants of *Brunsvigia b.* were completely cross-pollinated by hand. The seed set of these plants was compared to ten naturally pollinated plants. This was done to determine whether the plants are setting seed close to their optimum seed set, and whether they are pollinator limited. Unfortunately a similar treatment could not be carried out for *Crossyne f.* due to the nature of the flowering of this species. Unlike *Brunsvigia b.*, which has an inflorescence where all the flowers open within a minimum period of two days, *Crossyne f.* flowers over a 2 to 3 week period. Therefore on any particular inflorescence, seeds from early flowers have already developed while late flowers are in bud. As this was the case no plants were cross-pollinated but ten plants were still randomly chosen and seed set recorded. When determining seed set per inflorescence, every fruit capsule for *Brunsvigia b.* was counted.*Crossyne f.*, has an average of 1000 capsule per inflorescence, counting each capsule for this species was thus too time consuming. A subset sample of capsules for each inflorescence was therefore chosen and the seeds present in these capsules were counted. To work out total seed set per inflorescence, the total number of seeds in the subset of counted capsules was multiplied up by the ratio of: the number of counted capsules to the total number of capsule in an inflorescence.

Seed dispersal

To test the hypothesis that there is a difference~~s~~ in seed dispersal patterns of *Crossyne f.* and *Brunsvigia b.*, due to specialisation of either species to the mode of anemogeochory seed dispersal:

i) 10 inflorescence heads, with good seed set, were chosen for each species.

ii) These were collected from the field before they had broken away form the subterranean bulb and dried in a sheltered room for 1 week.

iii) The number of capsules with seeds in them for each inflorescence was counted.

iv) Each inflorescence was then allowed to roll over a 100m stretch of flat, stony vegetation free stretch of ground, on an afternoon when the wind was blowing at $15 m.s^{-1}$ as measured with an anemometer.

v) The number of empty seed capsules on each inflorescence was counted after the roll.

vi) The above process was repeated up to six times, so that each dried inflorescence rolled up to a distance of 600m.

Analyses:

Seed set

To determine how dependent the two study species were on pollinators, their ability to self was statistically tested. An analysis of variance was used to test whether there was a significant difference for the number of seeds set per capsule, between the four treatments: selfed, outcrossed, unmanipulated (bagged) and unmanipulated control plants. As the distribution of the seed per capsule values for the two treatments: selfed, and unmanipulated were not normal the non-parametric equivalent of an one way ANOVA, the Kruskal-Wallis ANOVA test, was performed on this data. In order to determine exactly which treatments were significantly different from one another, a multiple range comparisons test, Dunn's test was used. The median values of seed set per capsule were also graphed for each of the four treatments and two species.

The comparison of total seed set per inflorescence was calculated, and the median of 10, sampled inflorescence heads for each species was plotted.

In order to determine whether *Brunsvigia b.* was pollinator limited, the number of seeds in each capsule of the ten completely outcrossed inflorescence heads was compared to the number of seeds, in each of the capsules, of the ten control inflorescence heads. As the number of seeds per capsule for both treatments were not normally distributed and the sample sizes for treatments were different, the non-parametric Mann-Whitney U test for comparing two groups was performed. The mean number of seeds per capsule for both treatments was plotted.

Results:

Floral morphology

Petal orientation in *Nerine h.*, *Brunsvigia b.* and *Crossyne f.* differ during flowering (stage 5 and 6, Figure 4.). *Nerine h.* and *Brunsvigia b.* have slightly reflexed tepals, at an angle of 45° form the ovary. *Crossyne f.* has more reflexed tepals which during the male flowering stage, are orientated at right angles to the ovary and in the female stage are completely reflexed at a 180° angle to the original orientation of the tepals in the bud stage.

Key to floral development stage:	
1	**Smallest, bud on inflorescence**
2	**2nd smallest bud on inflorescence**
3	**Medium bud stage**
4	**Mature bud stage**
5	**Open flower, male flowering stage**
6	**Open flower, female flowering stage**

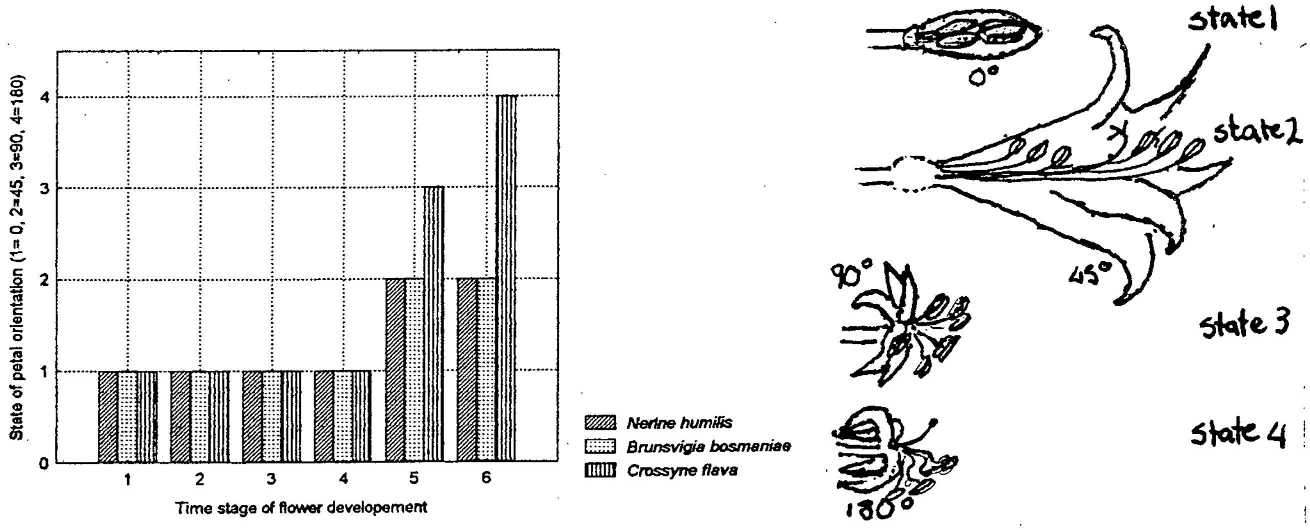

Figure 4: The differences in tepal orientation during flowering for *Crossyne f.*, *Brunsvigia b.* and *Nerine h.*

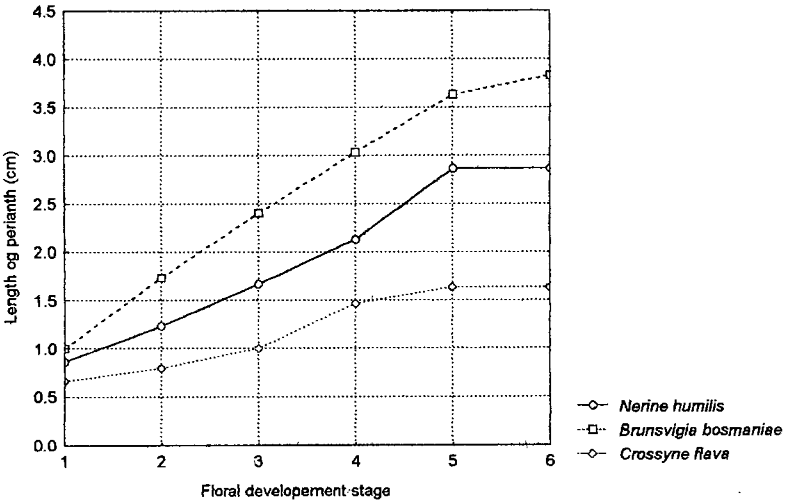

Figure 5: The change in perianth length during bud development for *Nerine h.*, *Brunsvigia b.* and *Crossyne f.*

Flower size is an important measure of the difference in floral morphology of the three species. The development of the perianth during ontogeny shows that all three species start of with buds of similar size (Figure 5, stage 1). *Nerine humilis* shows continual perianth enlargement until the flower opens. *Brunsvigia b.* when compared to *Nerine h.* shows a faster rate of perianth development. The perianth of this species continues growing even once the flower has opened (Figure 6 stage 5 and 6). *Crossyne f.* has a far lower rate of perianth development, when compared it's two outgroup taxa. The perianth also stops developing at the mature bud stage (stage 4). During flower opening, the male flowering stage, and the female flowering stage only the angle of the tepals change (see Figure 4), there is no change in the perianth length. Figure 6 shows not only the difference in relative growth of the perianth during development but also the difference in absolute perianth size during flowering. The final perianth size of *Crossyne f.* is the same as the perianth size of the second smallest bud for *Brunsvigia b.*

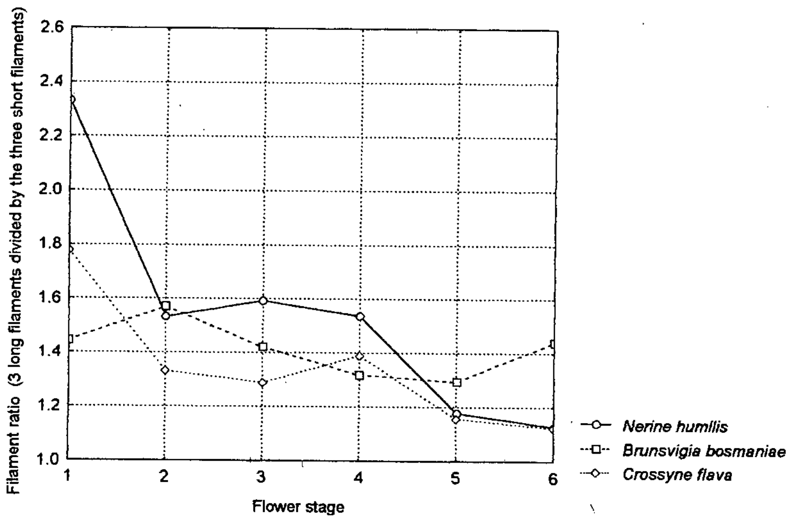

Figure 6: The change in the ratio between the long filament whorl and the short filament whorl during floral ontogeny of *Nerine h.*, *Brunsvigia b.* and *Crossyne f.*

The change in length and orientation of reproductive structures during the ontogeny of the species *Nerine h.*, *Brunsvigia b.* and *Crossyne f*, may reflect important adaptations related to pollination.

All three species have dimorphic filaments (Figure 6.) During different stages of ontogeny, the ratio between the long and short filament whorls changes. Each species shows a varying amount of filament whorl separation at different stages of bud development. For example, the long filament whorl is twice the length of the short filament whorl, in the smallest bud stage of *Nerine h.* This large difference decreases during bud development so that during flowering (Stage 5 and 6), the filaments are almost of equal length. *Crossyne f.* shows a pattern of filament development similar to that of *Nerine h.* (Figure 6). *Brunsvigia b.* also shows a decrease in filament ratio during bud development (stage 1 to stage 6), however during the female stage of flowering (stage 6) the ratio between filament whorls increase.

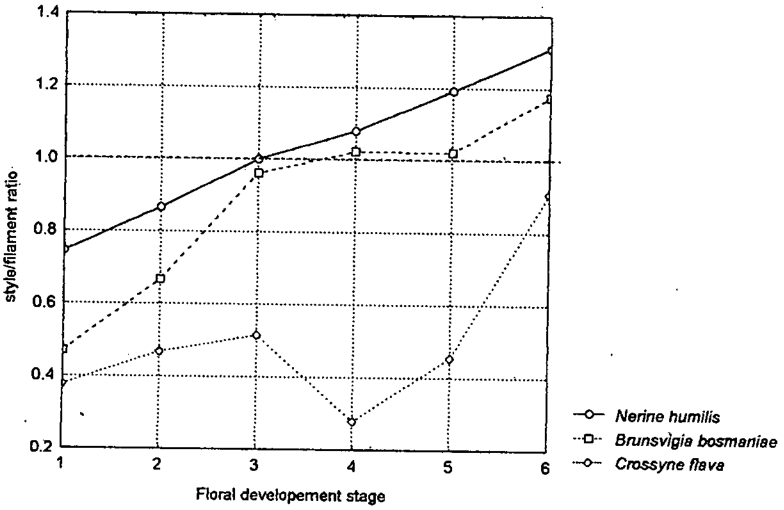

Figure 7: The relative length of the style in comparison to filament length for the species *Nerine h., Brunsvigia b.,* and *Crossyne f.* A ratio of above 1, indicates that the style is longer than the filaments.

Style development varies between the three study species. All three species have short styles in relation to filament length during the early ontogenetic stages. Style elongation for *Nerine h.* and *Brunsvigia b.* occurs almost continuously and during the female flower stage (Figure7. stage 6) the length of the style exceeds that of the filaments. *Crossyne f.*, shows style elongation in the three early stages of bud development (stage 1, 2 and 3). There is then a decline in the style to filament ratio caused by rapid elongation of the filaments (stage 4). The style elongates during the male phase (stage 5) but even during the female stage does not become longer than the filaments.

The orientation of anthers by the filaments is very different in *Crossyne f.* when compared to the two outgroup species. During the flowering stages (stage 5 and 6), both *Brunsvigia b.* and *Nerine h.* have declinate anthers. The anthers of *Crossyne f.* are centrally orientated during the male flowering stage of the flower, and in the female stage the anthers wilt, and drop below the style which elongates up (Figure 8).

The style of *Brunsvigia b.* becomes increasingly curved during flowering (Figure 9.) The style of *Nerine h.* is only strongly curved during the female flowering stage.

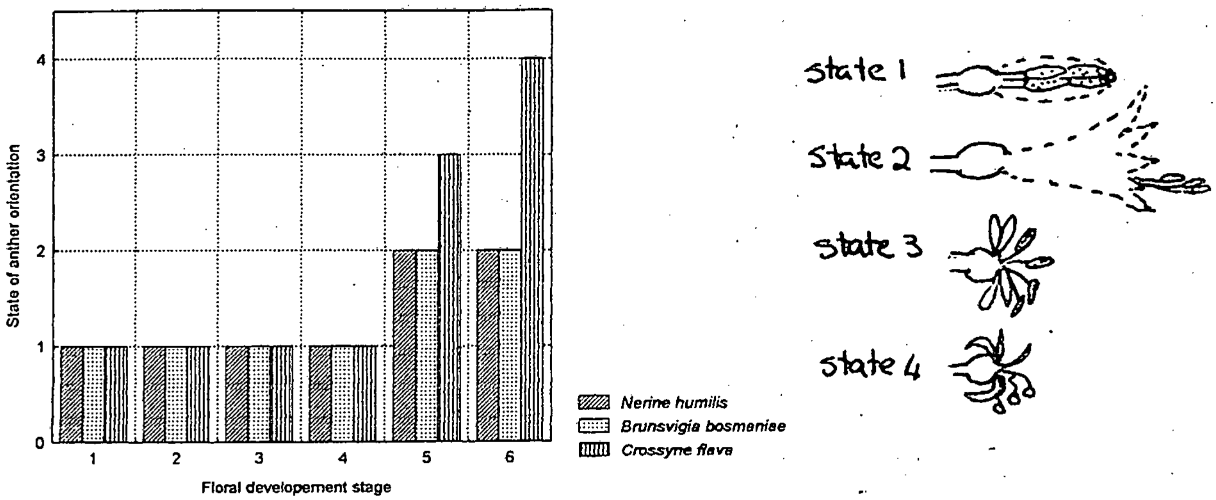

Figure 8: The four different states of anther orientation for the species *Nerine h.*, *Brunsvigia b.* and *Crossyne f.*

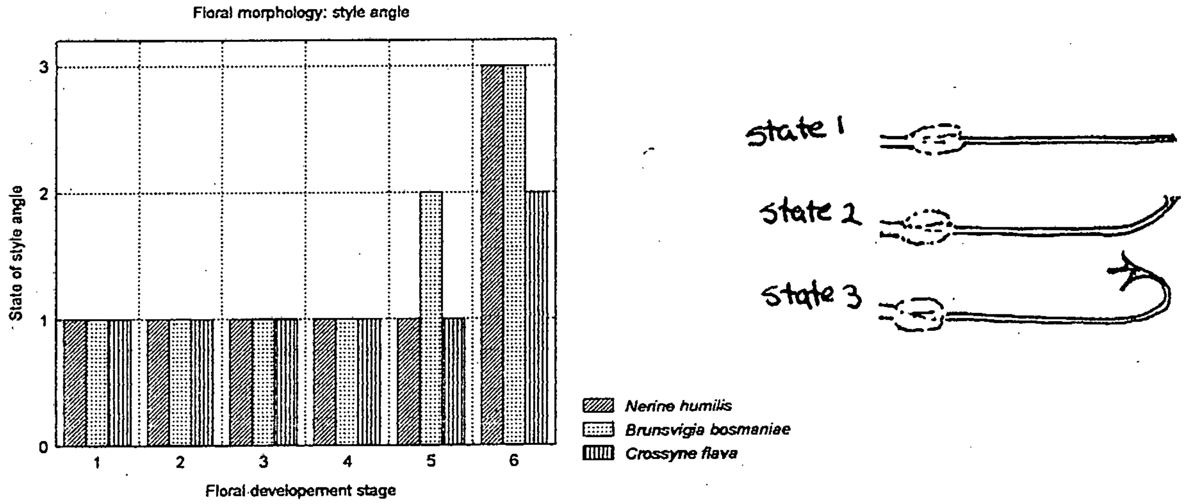

Figure 9: The angle of the stigma and tip of the style in comparison to the rest of the style, at the different stages of floral development for the species, *Nerine h.*, *Brunsvigia b.* and *Crossyne f.*

Pollination results

The results of pollinator observations are shown in Table 1. Both *Crossyne f.* and *Brunsvigia b.* are visited by a number of different pollinators. Flower visitors for *Crossyne f.* are two short tongued Bombelid fly species, *Australecous hypoleucus,* and a species from the genus *Heterolinia,* a Nymphalid butterly, *Vanessa cardui* and the honeybee *Apis mellifera* . All these four visitors were observed on a number of different occasions. The foraging pattern for all four visitors was to perch on the flower while feeding on nectar. The underside of all four insect visitors came into contact with both the anthers and stigma of the *Crossyne* flowers, during the period that they were feeding on nectar. Each insect systematically visited a number of flowers on one inflorescence before moving to the next inflorescence.

The insects that were caught while visiting *Crossyne f.*, were observed under a dissecting microscope in the lab. Pollen was found on all caught insects. Samples of the insects viewed under the scanning electron microscope all contained pollen of the tribe Amaryllideae (Figure ~~9~~ 10 A, E, F).

Diurnal floral visitors to *Brunsvigia b.* are the Honeybee *Apis mellifera* and a Bombelid fly *Australeucous hypeoleucous* (Table 1.) . Both these two insects are small and while foraging, land on the tepals of the Brunsvigia flowers and crawl into the tube. Due to the small size of the insects, and the fact that one whorl anthers and the stigma of Brunsvigia are exerted from the tepals, these insects hardly touched the reproductive structures of the flowers of *Brunsvigia b.* Their behaviour suggests that they do not effect pollination of *Brunsvigia* to any large extent.

The Bombelid fly, *Australeucous hypeoleucous,* does not systematically visit a large number of flowers on each inflorescence. Table 1, shows that the number of Bombelid visits observed were low and the maximum number of flowers visited by one Bombelid is one. This insect was observed to visit a single flower on an inflorescence and then rest on the ground for a substantial period of time. Honeybees

Table 1: Insect pollinators of the species Brunsvigia bosmaniae and Crossyne flava.

Date	Plant	Population	Estimated plant popualtion size	Insect	Time of observation (min)	Number of Flowers visits	Number of visits per hour	Estimate of number of plants observed	Number of flowers visited per inflorescence per hour	Max. number of visits by one insect	Insect in contact with stigma (0 = no contact with stigma;1= contact with stigma)
10.03.98	*Crossyne f.*	Glen Lynn Tillite Reserve	100	Autraleucous hypeoleucous	10	32	192	40	4.8	9	1
16.03.98	*Crossyne f.*	Glen Lynn Tillite Reserve	120	Autraleucous hypeoleucous	60	16	16	50	0.32	7	1
10.03.98	*Crossyne f.*	Glen Lynn Tillite Reserve	100	Apis mellifera	10	3	18	40	0.45	3	0
16.03.98	*Crossyne f.*	Glen Lynn Tillite Reserve	120	Apis mellifera	60	40	40	50	0.8	32	1
10.03.98	*Crossyne f.*	Glen Lynn Tillite Reserve	100	Vanessa cardui	10	1	6	40	0.15	1	1
13.04.98	*Crossyne f.*	Glen Lynn Tillite Reserve	40	Vanessa cardui	15	10	40	8	5	2	1
16.03.98	*Crossyne f.*	Glen Lynn Tillite Reserve	120	*Vanessa cardui*	60	4	4	50	0.08	1	1
10.03.98	*Crossyne f.*	Glen Lynn Tillite Reserve	100	*Heterolinia sp.*	10	10	60	40	1.5	6	1
13.04.98	*Crossyne f.*	Glen Lynn Tillite Reserve	40	*Heterolinia sp.*	15	2	8	8	1	2	1
16.03.98	*Crossyne f.*	Glen Lynn Tillite Reserve	120	*Heterolinia sp.*	60	50	50	50	1	10	1
17.03.98	*Brunsvigia b.*	Glen Lyan Dolerite Patch	50	*Autraleucous hypeoleucous*	30	10	20	15	1.333333333	1	0
17.03.98	*Brunsvigia b.*	Nieudville Flower Reserve	100	*Autraleucous hypeoleucous*	25	11	26.4	48	0.55	1	0
29.03.98	*Brunsvigia b.*	Glen Lyan Dolerite Patch	50	*Herse convolvuli* and *Hipppotion clerio*	35	40	68.57142857	35	1.959183673	6	1
17.03.98	*Brunsvigia b.*	Glen Lyan Dolerite Patch	50	*Apis mellifera*	30	35	70	15	4.666666667	10	0
17.03.98	*Brunsvigia b.*	Nieudville Flower Reserve	100	*Apis mellifera*	25	27	64.8	48	1.35	0	0
17.03.98	*Brunsvigia b.*	Nieudville Flower Reserve	1200	*Apis mellifera*	20	92	276	350	0.788571429	4	0
14.04.98	*Brunsvigia b.*	Charlles Hoek	1000	Species in the family Noctuideaeae	20	29	116	7	16.57142857	4	1
29.03.98	*Brunsvigia b.*	Glen Lyan Dolerite Patch	50	Species in the family Noctuideaeae	35	60	102.8571429	35	2.93877551	2	1

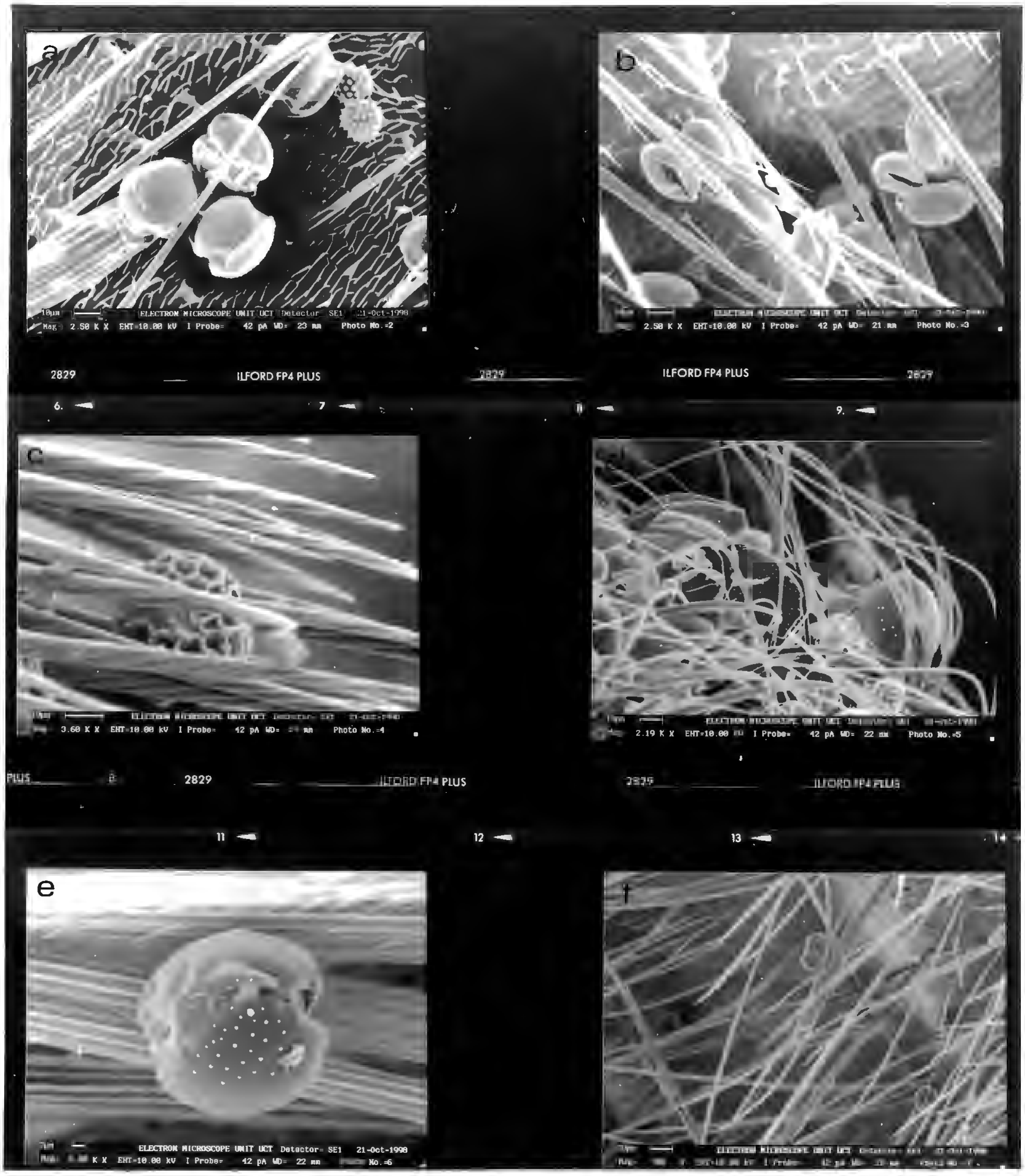

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Figure: Scanning electron micrographs of: a) Four Amaryllideae pollen grains found on a *Helliophylla* fly caught on *Crossyne flava*. Asteraceae pollen grains are also present . b) and c)Pollen grains found on the surface of a bee caught on *Brunsvigia b.* belonging to other species of flowering plants. d)The only Amaryllideae pollen grains found on the surface of a bee caught on *Brunsvigia b* These are very scarce in comparison to the heavy load of pollen of other flowering species present on the bee's body. Pollen grains were found on the leg of the bee. e) An Amaryllid pollen grain found on the fly *Austroleucous hyperleucus* caught on *Crossyne f.* f) Two Amaryllid grains caught on the hairs of *Austroleucus hyperleucus.*

were one of the most common visitors to *Brunsvigia b.* flowers (Table 1.). Figure 11. shows that although Honeybees are frequent visitors to *Brunsvigia* the chance of them effecting pollination is extremely low. Out of 87 observations, Honeybees only touched the stigma of a *Brunsvigia* flower on 5 occasions.

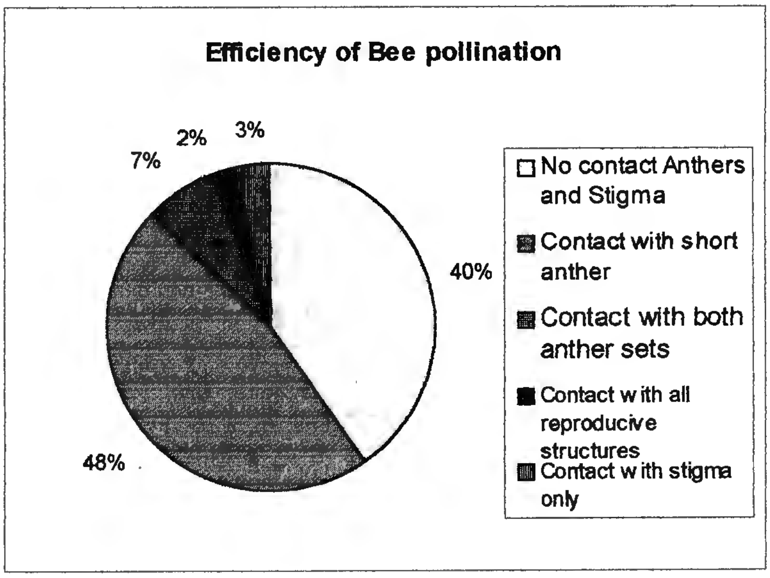

Figure ~~10~~ 11: Shows how often Honey bee flower visitors touch the various reproductive structures of *Brunsvigia b.* N= 87 observations.

When looking at a bee specimen caught on *Brunsvigia bosmaniae* under the electron microscope, only three grains of Amaryllideae pollen (probably *Brunsvigia bosmaniae* pollen) were found on this insect (Figure ~~4~~ 10D). The rest of the surface of the animal was thickly coated in the pollen of other flowering species. Although the sample number of insects observed under the scanning electron microscope was very low (n=1), the low pollen grain number found on the bee may suggest inefficient pollinator behaviour.

Nocturnal visitors to *Brunsvigia b.* are two species of hawkmoth: *Herse convolvuli,* and *Hippotion celerio.* Moths in the family Noctuideae, possibly a number of different species, were also very common visitors to *Brunsvigia b.* Both Noctuids and Hawkmoths touched the stigmas and anthers of the *Brunsvigia b.* flowers while visiting. Hawkmoths feeding on nectar, hover in front of the flower. By so doing they come into contact with the reproductive flower parts (stigma and anthers of the long filament whorl) which are exerted from the tepals. Noctuid moths do not hover outside of the flower but land on one of the tepals, and walk into the flower tube. By so doing the insects come into contact with the short whorl of anthers. When leaving the flowers, noctuids often brush the stigma with their bodies. Noctuid moths are the most common visitors of *Brunsvigia b* .flowers (Table 1.) Both nocturnal visitors visit a large number of flowers per inflorescence (Table 1.). Hence nocturnal floral visitors to *Brunsvigia b.* are more efficient pollinators than diurnal visitors.

Seed set

The results of the Kruskal Wallis test performed on the four treatments for *Crossyne f.* are: H=119.70065 this value is highly significant p=0.000. Hence the null hypothesis that there is no difference in the number of seeds set per capsule for the four different treatments may be rejected. Figure 12(i) shows that there is no significant difference between the number of seed set per capsule for the treatments unmanipulated and selfed. There is a significant difference between these two treatments and both other treatments, crossed and control. A significant difference of number of seed set per capsule exists between the treatments control and outcrossed.

The results for of the Kruskal-Wallis ANOVA and Dunn's for *Brunsvigia b.* are similar to those of *Crossyne f.* With H=141.8648 at a significance level of p=0.000. Again showing that there is a highly significant difference between the number of seeds set in each of the four treatments. Dunn's test, seen in Figure 12 (ii) shows that a significant difference exist between all groups except the treatments selfed and unmanipulated.

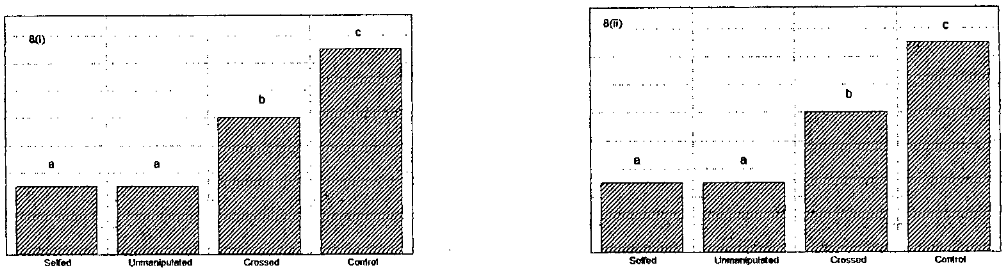

Figure 12(i) The results of the Dunn's test for the four treatments performed on *Crossyne f.*, showing which groups are significantly different from one another p<0.05. A change in the letter represents a significant difference between treatments. Figure 12(ii) The results of the Dunn's test for the four treatments performed on *Brunsvigia b.*, showing which groups are significantly different from one another p<0.05. A change in the letter represents a significant difference between treatments.

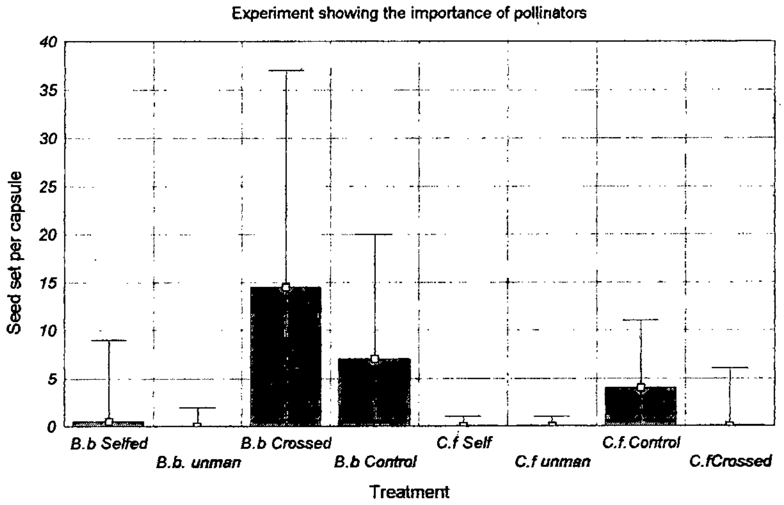

Figure 13: The median number of seeds per capsule for the four treatments performed on *Brunsvigia b.(B.b)* and *Crossyne f. (C.f)*. Bar lines show the range for the minimum and maximum values for each treatment. N for each treatment for both species =50, except for *Crossyne f.* selfed treatment where N=37 .

Bagging and manipulating plants of each species, showed that neither species is able to self pollinate. Figure 13 shows that both species are unable to self- pollinate without the presence of a pollinators (treatment unmanipulated Figure 13). The treatment of unmanipulated plants that were bagged, have median seed set per capsule of 0 and very low deviation from this number. The maximum number of seed set per capsule for both these treatments was 1. *Crossyne f.* is unable to self pollinate with the help of a pollinator, the treatment selfed in Figure 13, shows this. The seed set per capsule for *Crossyne f.* flowers that were crossed is also extremely low. This result was probably due to a resource problem. Some flower heads were chopped of the inflorescence before manipulation took place. This resulted in loss of water form cut pedicels which probably caused resource problems for the remaining flowers. The range bar in Figure 13, shows that there were flowers that set as many as 7 seeds per capsule when cross pollinated, this shows that *Crossyne f.* does respond to cross pollination if resources are not limited. The seed set in capsules of the control unbagged plants for *Crossyne f.* were the highest. Suggesting that a high level of pollination is taking place for Crossyne flowers.

Self pollinating flowers of *Brunsvigia b.* did result in a few capsules setting seed (Figure 13). The majority of capsules however had a seed set of 0 thus the conclusion can be made that *Brunsvigia b.* is unable to self pollinate. The high level of seed set per capsule for both the control (unbagged) plants and cross pollinated plants (Figure 13), along with the results of the Kruskal -Wallis ANOVA, are evidence that *Brunsvigia b.* needs to have outcrossed pollen. An interesting result shown in Figure 13 and Figure 12 (ii) is that the median seed set per capsule for the crossed treatment is significantly higher than the seed set per capsule in unbagged control plants. This suggests that *Brunsvigia b.* has the potential to produce more seeds than it is producing in capsules of unmanipulated naturally pollinated inflorescence heads.

The above observation was further tested using a Mann-Whitney U test on the number of seeds per capsule of ten inflorescence heads that had been outcrossed by hand and ten inflorescence heads that had been naturally pollinated. The sample size of the above two treatments were $N=390$ capsules outcrossed by hand, and $N=422$ capsules naturally pollinated. The result of this test was $U=53393$, $Z=-8.654$ and a significance of $p=0.000000$. This result shows that the Null hypothesis: *Brunsvigia b.* is not pollinator limited, can be rejected.

not so

Should be done per plant not capsule

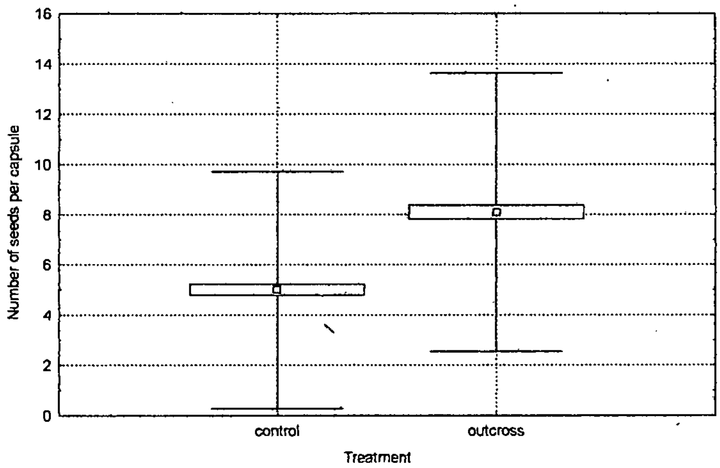

Figure 14: The mean number of seed per capsule for *Brunsvigia b.* in the control and outcross treatments. outcross N=390 , control N=422.

The above results compared the ability of the two species to set seed. One other factor that still needs to be accounted for is the absolute number of seeds produced on an inflorescence of each of the two study species. Figure 15 shows the median of the total number of seeds set per inflorescence for ten inflorescence heads of *Crossyne f.* and *Brunsvigia b.* Quite clearly the number of seeds set on a *Crossyne f.* inflorescence heads is far higher than the number of seeds set on a *Brunsvigia b.* inflorescence. With a maximum seed number being as high as 1800 seeds.

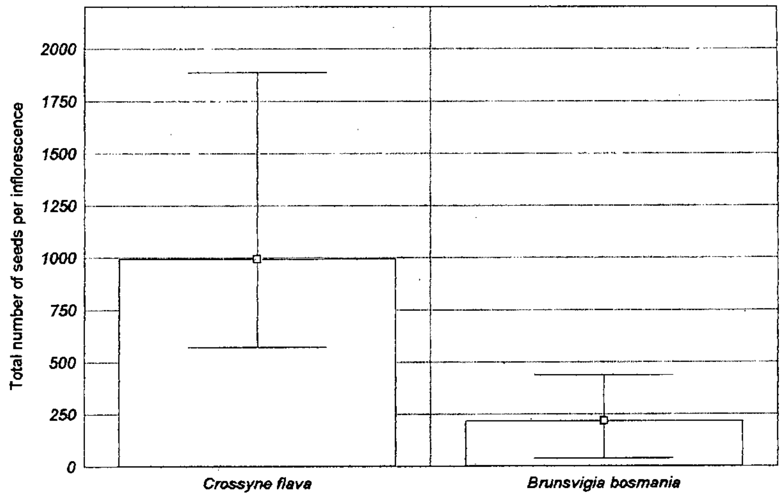

Figure 15: Shows the median and minimum, maximum range for the total seed set per inflorescence for the species *Crossyne f.* and *Brunsvigia b.*. N=10 *Crossyne f.*, N=10 *Brunsvigia bosmaniae.*

Seed dispersal

The rate of seed loss during tumbling for *Brunsvigia b.* and *Crossyne f.* differs markedly. *Crossyne* has many more capsules per inflorescence than *Brunsvigia* (Figure 16, distance 0m). Assuming that the number of emptied capsules can be used a as a representative of the amount of seeds released while tumbling. *Crossyne f.* releases over 80% of it's seed during the first 100 meters of rolling (Figure 16.).

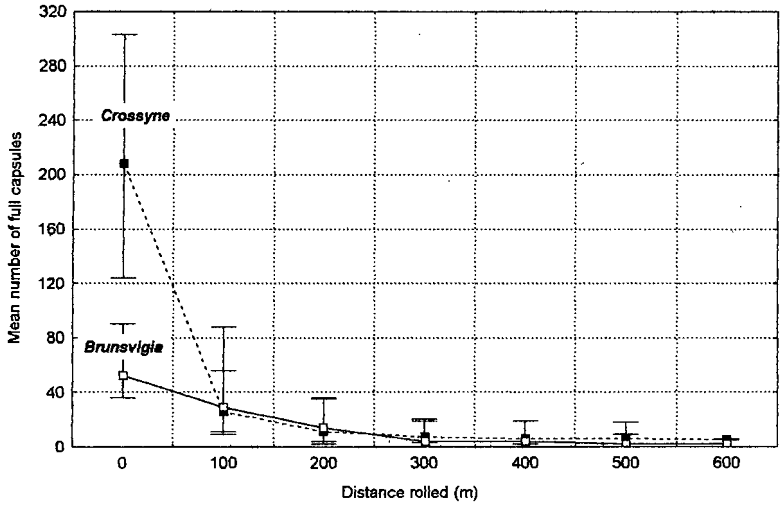

Figure 16: Number of seeds retained by *Brunsvigia b.* and *Crossyne f.*, at 100m intervals over a distance of 600m. N= 10 inflorescence heads for species. Seed number is represented by the number of full capsules present on each inflorescence

Brunsvigia b. on average releases only 40% of its seeds in the first 100m. Only 80% of *Brunsvigia b.* seeds are released after a rolling distance of 300m (Figure 17.). Using the number of full capsules as a representative of the number of seeds remaining on an inflorescence, Figure 16 shows that although Crossyne starts off with a larger number of seeds than *Brunsvigia*, after rolling 100m, the two plants have a similar number of capsules containing seed. These remaining full capsules seem to empty at similar rates with both species retaining a small number of full seed capsules for over 500m (Figure 16). In general *Brunsvigia* releases proportionally fewer seeds than *Crossyne* for the first 100m of rolling but then releases proportionally more seeds over the next 200m. After rolling 300m both species having released the majority of their seeds, but retain a few seeds for well over 300m.

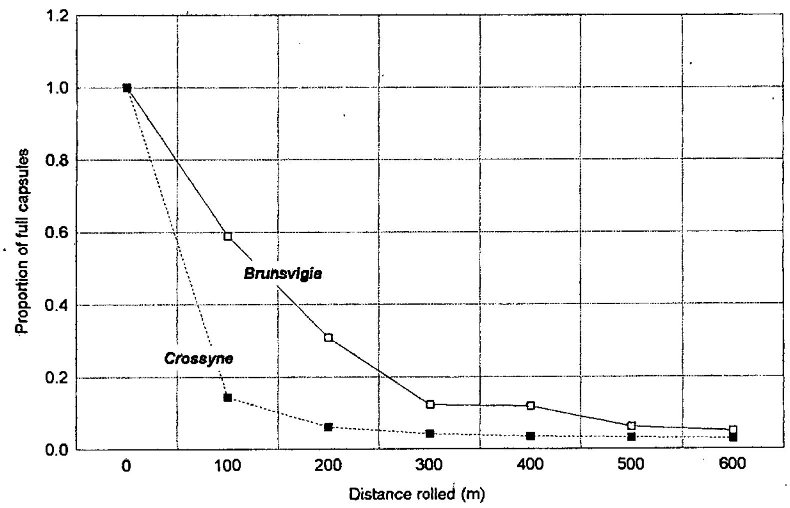

Figure 17: Shows the mean proportion of full capsules remaining per inflorescence for *Brunsvigia b.* and *Crossyne f.* after rolling intervals of 100 metres for 600m . N=10 inflorescence heads per species.

Discussion:

The evolution of floral morphologies

It is possible to speculate on the mode of evolutionary change in the floral developmental processes if the phylogenetic tools of a cladistic analysis are available (Raff and Wray, 1989). A cladistic analysis is necessary so that the ancestral condition of the species of interest can be determined. For the species *Brunsvigia b.* and *Crossyne f.* the cladistic analysis of the tribe Amaryllideae (Snijman and Linder, 1996) shows that *Nerine* is the outgroup genus to these two genera. Hence using the floral structures of *Nerine h.* as the ancestral condition and comparing change in floral ontogeny of the two study species it is possible to speculate on the mechanism of floral divergence for these two species.

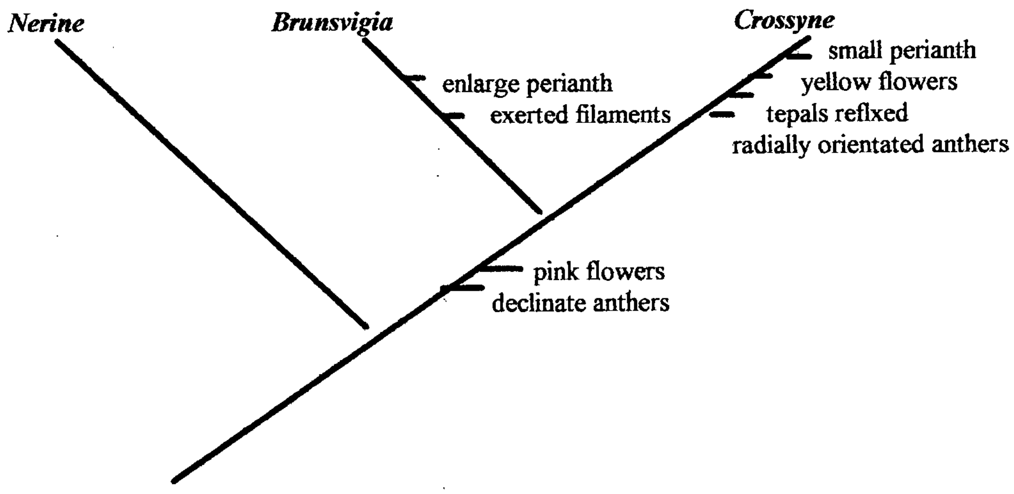

Figure 19: The *Brunsvigia/Crossyne* clade of the strict consensue tree for the Amaryllideae (Snijman and Linder). Floral characters are mapped onto the cladogram.

The main difference between *Brunsvigia b.* and *Nerine h.* is the relative size of the two flowers. *Brunsvigia b.* is far larger than *Nerine h.* Other floral features such as petal, anther and style orientations do not differ largely. The obvious heterochronic change that has taken place in the evolution of the flower of *Brunsvigia b.* is the relative increase in the rate of growth of all floral structures. Such a phenomena is known as *proportioned gigantism* (Gould, 1977 in Alberch et al. 1979) and results in the descendent species being the same shape but larger than the ancestral species.

Crossyne f. has a very different floral morphology to *Brunsvigia b.* and *Nerine h.* However the length of the tepals of *Crossyne f.* during flowering, are the same length as the tepals of the second smallest bud stage of *Brunsvigia b.* The largest bud of *Crossyne f.* also closely resembles that of the 2nd smallest bud stage of *Brunsvigia b.* and *Nerine h.* Negative permutations in the rate of change in shape during ontogenetic development leads to a descendant which resembles a juvenile state of the ancestor (Albrech et al, 1979 , Raff and Wray, 1989). This phenomena is known as neotony. Neotony as mode for evolutionary change in floral morphology makes sense in the case of *Crossyne f*, especially when the relative size of all the floral features of this specie are compared to the two outgroup species. The style, filaments and tepals are very much smaller. Tepals of the two outgroup species form a "pseudo-floral tube" these however are not fused tepals, so the separation and reflexation of tepals in *Crossyne f.* is not an unrealistic adaptation.

The mapping of floral characters on to the phylogeny produced by Snijman and Linder (1996) shows that *Crossyne* has more autapomorphic floral characters than *Brunsvigia* when compared to the outgroup genus *Nerine.* This may suggest that the genus *Crossyne* is a more derived than the genus *Brunsvigia.* A possible problem with the above postulation is that only one outgroup species was used to compare floral divergence of *Brunsvigia* and *Crossyne.* Ideally, these two genera should be compared to a number of basal genera in the tribe Amaryllideae. Instead of using an outgroup species character polarity may be determined by looking only at ontogeny (Wheeler, 1990). In light of the fact that ontogenetic changes were considered in the analysis of floral morphology, it is unlikely that an incorrect pattern of floral divergence was postulated.

Floral morphology in relation to pollination

The small size of the *Crossyne* flower allows for pollinators to rest on the flower during nectar feeding. The central arrangement of the anthers, and the style that curves up at an angle of 45° result in the contact of reproductive parts with the body of the pollinators at all times. Filament and style length are in proportion to the rest of the flower and are hence relatively small. Because these structures are short they do not prohibit access of pollinators to nectar and thus pollinators do not directly try to avoid these structures. *Crossyne f.* also has yellow flowers, an unusual colour for a s/ specie in the tribe Amaryllideae. The above floral features are adaptations in the genus *Crossyne* (Figure 17) which are related to pollination.

Floral morphology of *Brunsvigia b.* and *Nerine h* are similar in the following ways. Both species have tepals which, during flowering, are only slightly reflexed at the edges. The almost horizontal angle of the rest of the tepals which are closely packed together result in a pseudo-floral tube being present in both species. Both also have reproductive structures, (filaments and style) that are exerted from the tepals, and styles that are strongly curved during the female stage of flowering. These characters suggest that both these species may be adapted to pollinators that hover in front of the flower while feeding on nectar. The dimorphic filaments of *Brunsvigia b.* were ly. original thought to be part of a specialised pollination syndrome. On studying this feature for all three species, it was discovered that dimorphic filaments are present in all three species. The difference between the length of the two filament whorls is most extreme in the earliest bud stage. As pollen and anthers are one of the first floral part to be produced during ontogeny, the two different whorls of a filament appear to be a function of packing already developed relatively large anthers in the small early bud. As the flower buds develop, the difference in length between the filament whorls, gets smaller. Only in the female flowering stage of *Brunsvigia b.* is the difference in length increased. While observing pollinators on *Brunsvigia b.* it was noted that the shorter filament whorl, which rests inside the tepals edge, touched visiting non-hovering pollinators, who landed on the tepals and crawled down the "floral tube". These insects appear to make an effort to avoid the stigma and longer filament whorl which prohibit easy entrance to the flower. Thus increased anther dimorphism during flowering in *Brunsvigia b.* appear to be a bet hedging feature which may help which utilises not only hovering flower visitors.

The pollinators that were observed visiting *Brunsvigia b.* and *Crossyne f.* are typically animals one would expect when looking at the floral morphology. The small yellow flowers of *Crossyne* are pollinated by a number of small insects. These insects don't all belong to one insect order. The relatively loose association of plants to particular orders of insects for pollination may indicate a prevailing low degree of floral specialisation to particular pollinators (Herrera, 1996). The open structure of the *Crossyne* flower is typical of that of a generalist plant. The long flowering period of *Crossyne f.* 2 to 3 weeks and the fact that only about 20 flowers are open at one time on the inflorescence, result in the inflorescence having a large window of time in which pollination can take place. Given the fact that Amaryllids flower in autumn when few insects are around, this long flowering period could be a reason for the high seed set found in this genus. Having only a few flowers open on one inflorescence may also force flower visitors to move between inflorescence heads and hence improve the chance of a flowers being outcrossed.

The pollinators observed on *Brunsvigia b.* point to this plant being moth pollinated. Undoubtedly bees and small Bombelid flies that visit the *Brunsvigia b.* flowers affect pollination on some occasions. However the behaviour of these two animals and their small size in comparison to the flower point to them being extremely inefficient pollinators. Moths on the other hand visit the flowers regularly. They have large bodies, which brush against the reproductive floral parts while they visit the flowers. As mentioned above, the floral structure of *Brunsvigia b.* seems best adapted to pollinators that hover. The large bodied Hawkmoths would appear to be the perfect pollinators. Unfortunately these animals were only observed visiting the flowers on two occasions and at only one of the populations of *Brunsvigia b.* These animals are difficult to see, because they fly at high speeds and spend ~~milliseconds~~ seconds on each flower they visit. This could perhaps be the reason for them not being observed more often. However it is more likely that they occur in low number and are not the

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most important pollinator of *Brunsvigia b.* The smaller Noctuid moths, which were observed in large abundance on many *Brunsvigia* inflorescence heads, are the most important pollinators of *Brunsvigia b.* What can definitely be concluded is that this species is moth pollinated.

Differences in flowering strategies

Brunsvigia b. has more flowers open at the same time. Numerous studies have shown that the more flowers open on an individual plant, the more likely the removal or receipt of pollen is. (Fritz and Nilsson, 1996). Thus the *Brunsvigia b.* is advantaged by having all it's flowers open at one time. Having most flowers on an inflorescence open at one time is especially important when considering that this plant is mainly pollinated by nocturnal visitors. The two disadvantages of flowering all at one time is firstly the window of opportunity in which pollination can take place is shortened. The second is that a large number of open flowers may lead to geitonogamy (loss of self pollen to stigmas on the same inflorescence) (Klinkhamer and de Jong, 1993).

Crossyne f. flowers for a longer period. Slow extended flowering may be an advantage when there are limited pollinators around (Dafni et al, 1981a) The few flowers open at one time on *Crossyne f.* would presumably lower the attractiveness of the inflorescence as a whole to a pollinator (Fritz and Nilsson, 1996). This however is unlikely to be the case for *Crossyne f.* as the actual inflorescence structures (pedicels and scape) may act as insect attractants. The centre of the inflorescence, where the pedicels attach to the top of the scape, are often a bright pale yellow. The rest of the pedicels and scape are a deep red. This coloration of inflorescence structures may help attract pollinators, and decrease the costs of flowering all at once.

Pollen limitation

In the above discussion, three potential reasons why Brunsvigia b. is pollinator limited have been mentioned. Firstly geitonogamy may lower the amount of outcrossed pollen received by flowers on the plant. Secondly flowering simultaneously for short periods may decrease the chance of a flower being visited by a pollinator. The most likely reason for pollen limitation of *Brunsvigia b.* is the inefficient transfer of pollen by floral visitors. Small insects like Honey bees, Bombelids and even Noctuid moths don't effect pollination as efficiently as would a larger insect for example a hawkmoth.

The influence of pollen availability on the evolution of floral characters depends on the degree to which pollen limits life time fitness (Johnson, 1997). Whether the pollen limitation shown in *Brunsvigia b.* is present in other members of this genus and whether this pollen limitation is effecting life time fitness so as to drive the evolution of floral divergence away from specialised pollination is a question which remains to be researched.

Seed dispersal

The differences in seed dispersal rates for the two study species can best be explained by looking at the structures of their respective fruiting bodies. Both genera have infructescences that are very well adapted to tumbling. *Brunsvigia* has broad kitelike capsule surfaces at the base of a few strengthened pedicels (Snijman and Linder, 1996). *Crossyne* has an infructescence which is uniformly spherical because there are a large number of pedicels all of which equal the scape in length. When released the capsule of each *Crossyne f.* pedicel does not have much contact with the ground and this ball-like inflorescence tumbles easily (Snijman and Linder, 1996).

The most obvious reason why *Brunsvigia b.* takes longer to release seeds than *Crossyne f.* is because the fruit capsules in this genus are large, indehiscent, and strongly ribbed. When the infructescence is released each capsule has to be worn down by rolling before the release of seeds can take place (Snijman and Linder, 1996). *Crossyne f.* on the other hand has relatively small capsule with no obvious reinforcement. The outgroup species for this study, *Nerine h.* also has small seed capsules with no reinforcement. The reinforced capsules of *Brunsvigia* are likely an adaptation to increased efficiency of seed dispersal. The small capsule of *Crossyne* often dehisce before the release of the infructescence from the subterranean bulb. This occurs due to a lack of space for the large seeds, when seed set levels are high. Scarabid beetles were also observed chewing away the outer layer of the capsules while the infructescence was still attached to the subterranean bulb (pers. obs.). Thus a large proportion of *Crossyne's* capsules are already opened before the infructescence breaks loose. Those that are not open have a very thin capsule covering. This breaks open rapidly once the infructescence begins to tumble. For the above reasons it is not surprising that *Crossyne f.* drops the majority of it's seeds as soon as the infructescence starts rolling.

The small proportion of seeds remaining in the infructescence of both species has to do with both infructescence structure and capsule shape. Both species have late developing pedicels which generally do not grow as long as earlier pedicels. The capsules of these short pedicels make no contact with the ground while tumbling and hence little seed is released. Some seeds of both species are firmly wedged into the base of the capsules of the infructescence. These seeds may also take a long period of tumbling before they are released, capsules may need to be completely worn away before the seeds will drop out.

To determine whether either of two study genera have evolved a more efficient manner of dispersal, two angels of approach should be looked at. The first is whether one tumbles more easily than the other. The second is whether the one disperses seeds in a manner which would optimise potential seedling germination success. The above discussion of infructescences arrangement shows that both species have infructescences that are well adapted to rolling. While carrying out seed dispersal experiments in the field it was noted that both study species tumbled extremely fast and at very similar rates. *Brunsvigia b.* however releases seeds evenly over a distance of 300m and as the infructescence empties seed release is slowed down. *Crossyne* drops most of it's seeds near the parent plant in the 1st 100 m but still gets the number of propagules dispersed to a far distance.

The evolutionary advantages of the two different methods are as follows. Firstly there is a definite advantage to dispersing propagules far from the parent plant if plant populations are dense (Peart, 1985). There is also an advantage to having long distance dispersal to a plant growing in an environment which varies both temporally, and spatially (Venable and Brown, 1988). The genera *Brunsvigia* and *Crossyne* are hypothesised to have evolved in the South Western Cape, (Snijman, 1992) an area where vegetation is either spatially variable through subjection to fires or temporally variable because of the severe summer drought. The centre of diversity for both genera is in both the Fynbos system of the South Western Cape and the Succulent Karoo of the West coast. These two areas are both extremely rich in species of vascular plants (Cowling and Hilton-Taylor, 1994), and especially rich in bulbous species. Geophytes may comprise up to 40% of the of the regional flora (Esler et al. 1998). In the study area the density of bulbous species is especially dense with up to 1000 bulbs per meter squared (MacGregor, pers. comm.). Thus plant populations are dense and competition for space is important.

In the light of the above discussion the fact that *Crossyne* drops most of it's seeds around the parent plant and within the first 100 meters does not appear to advantages. The only situation in which this method of seed dispersal is advantageous is when conditions around the parent plant are more favourable than surrounding areas (Venable and Brown, 1988). There is an advantage of not dropping seeds before infructescence release and dropping seeds slowly over a far distance as *Brunsvigia b.* does. The advantage is that propagules fall far from the parent plant, this reduces the chance of reproducing with the parent plant and also increases the probability of a propagule falling in an area where plant competition is low(Peart, 1985 and Venable and Brown, 1988.) In view of the dense populations of plants which grow in the study area and the fact that the environment is subject to temporal and spatial variation, the general method of dispersal of *Brunsvigia b.* is probably more effective for each propagule dispersed. However the fact that both species keep a few propagules in the infructescence for a rolling distance of over 600m means that that they both disperse a few propagules for a long distance and neither plant can be said to be a significantly more efficient dispersal of seed over long distances.

Resource limitations

One major factor, that relates to the flowering strategy and ability to set seed in all plants, especially geophytes, is resource availability. For hysteranthous bulbs an accumulation of storage materials is a prerequisite for flowering (Burt 1970 in Dafni et al. 1981 a). Flowering in hysteranthous geophytes may also only take place once a critical amount of storage reserves have been accumulated. Once these reserves are accumulated there should be very little change in the flower abundance from year to year (Dafni et al. 1981 a).

As yet few studies have been done on how the different members of the Amaryllideae differ in resource allocations. Resource allocation patterns of this tribe are important because geophytes growing in seasonal climate with restricted growth periods must have complicated use of reserves in order to reduce risks(Dafni et al. 1981 a). Snijman 1992 showed that a large bulb is basal to the tribe and bulb size decreases in the more derived genera of *Strumaria, Hessea, Namaquanula, Kamiesbergia,* and *Carpolyza.* Other than actual size of the storage organs, the time taken to accumulate enough resources to flower and the allocation of resources to reproduction are not known. The way in which the *Brunsvigia* and *Crossyne* utilise storage material is an important feature which most certainly affects both floral morphology: allocation to floral structures, and seed set. Looking at resource problems was unfortunately beyond the scope of this project. However during the collection of seed set data, a difference in allocation to seeds was observed. The late developing flowers of *Brunsvigia b.* had very low seed numbers typically 1 or no seeds per capsule. *Crossyne f.* with four times as many pedicels had high levels of seed set in the late developing flowers. This difference and the high absolute numbers of seeds set in *Crossyne f.* inflorescence heads point to a more efficient use of resources than

Brunsvigia b. Looking at resource allocation differences between these and other species in the Amaryllidinae remain to be studied and may prove interesting in light of the unusual flowering strategies of these plants.

Conclusion:

An adaptive shift in pollination system is the most probable explanation for floral divergence in the genera *Brunsvigia b.* and *Crossyne f.*. Pollination driven speciation has been underrated in the Cape Flora (Johnson, 1996b). The diversity of floral and inflorescence form in the tribe Amaryllideae suggests that there are two factors that have played an important role in the evolution of this group. The first undoubtedly important factor is the seed dispersal mechanism. Snijman and Linder (1996) have shown that different methods of abiotic seed dispersal have arisen a number of times in this tribe. The second factor is adaptation to different pollinator modes. We hypotheses that the shift from a specialised pollination to a more generalised pollination syndrome, seen in the in the two genera studied here, may be common in other parts of this tribe.

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