Pollination in *Pelargonium dolomiticum* Knuth (Geraniaceae)

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Anthesis in the self-compatible, dichogamous flower of *Pelargonium dolomiticum* Knuth can be divided into three different phases. The male phase precedes the female phase, indicating protandry. Dichogamy is complete and asynchronous. The activity pattern of *Apis mellifera*, the pollinator of this species, does not correlate with the rewards offered by the flower but is intrinsic to the behaviour of the pollinators. In *P. dolomiticum* cross-pollination is favoured by the behavioural pattern of the honeybees.

Drie verskillende fases kan tydens antese van die selfverenigbare, protandriese, digogame blom van *Pelargonium dolomiticum* Knuth onderskei word. Digogamie is volledig en ongesinkroniseer. Die blomme word *deur Apis mellifera* bestuif. Bestuiwersaktiwiteit hang nie van die beskikbaarheid van belonings af nie maar is ’n resultaat van die gedragspatroon van die bye. Hierdie gedragspatroon bevorder kruisbestuiwing by *P. dolomiticum*.

**Keywords:** Dichogamy, Geraniaceae, mellitophily, *Pelargonium*, self-compatibility.

**Introduction**

The only information collected in South Africa on pollination syndromes in the Geraniaceae is that of Vogel (1954) who concentrated mainly on *Pelargonium*. Since then Van der Walt et al. (1990) have published observations on aspects of the pollination biology of *P. ocellatum* J.J.A. van der Walt. In their study they place this species within the syndrome of 'rhinomyiophily' (Rebelo et al. 1985; Whitehead et al. 1987). The authors also mention that bees visit and pollinate *P. ocellatum* in a generalist fashion (Van der Walt et al. 1990).

One of the few studies relating to the breeding system and pollination of any of the indigenous *Pelargonium* species is that of McDonald & Van der Walt (1992). They investigated the pollination of *P. tricolor* Curt. and based on their findings made certain predictions of insect pollinator response to the flowers of *P. capillare* (Cav.) Willd., *P. ocellatum* and *P. incarnatum* (L’Hérit.) Moench. These four species of the section Campyjia (Sweet) DC. exhibit embossed areas on their posterior petals. From their observations on *P. tricolor* they concluded that these embossed areas are false nectaries which are effective in attracting dipteran pollinators. These findings support the untested hypothesis of Hugo (1978) who speculated that the 'shiny-wet' surface of the embossed areas probably leads pollinators to the closely-associated nectar tube in the hypanthium, thus promoting pollination.

This study was conducted on *P. dolomiticum* (voucher: P.C. Zietsman 300, NMB), a herbaceous perennial which dies back during the winter months. In the study area this much-branched subshrub reaches a height of 0.60 m. It has a rounded habit and varies on average between 0.48 and 0.54 m in diameter. Depending on the rainfall, it flowers between September and April in the study area. In this study the pollination biology and breeding system of this species was investigated.

**Material and Methods**

**Study area**

The research was conducted in natural veld on an undisturbed part of the Orange Free State National Botanic Garden (2926AA). *P. dolomiticum* is abundant in the gardens and occurs on a dolorite outcrop. The area forms part of the Dry *Cymbopogon – Themeda* veld type (Acocks 1988).

**Plant habit and floral behaviour**

Ten different plants were used to monitor the behaviour of individual flowers. This was done between 07:00 and 19:00 for three consecutive days. Movement of the various floral parts of different individual flowers (N = 100) was closely recorded from the onset of anthesis at 15-min intervals. Three hours and onwards after the onset of anthesis, flowers were monitored hourly. The number of individual flowers in the different phases was noted. The number of flowers per phase during the mornings (07:00 – 12:00) and afternoons (12:15 – 19:00) was compared.

**Pollen viability and potential germinability**

To get an indication of the viability of pollen grains in this species Alexander’s viability stain was used for the differential staining of aborted and non-aborted pollen grains (Alexander 1969). Pollen grains were mounted in a drop of the medium and viewed under high light intensity using light microscopy. To assess potential germinability of the non-aborted pollen grains, the fluorochromatic test procedure (FCR) was used (Heslop-Harrison et al. 1984). Mature, ripe pollen was used during both tests. For all epifluorescence microscopy, a Zeiss Axioskop microscope equipped with a 100-W high pressure Hg lamp and filter set for UV light excitation was used. Kodak Ektachrome 100 ASA colour slide film was used throughout.

**Pollination experiments**

Pollination experiments (N = 50) were conducted to test for self-compatibility, self-pollination, cross-pollination, geitonogamy and apomixis (Zietsman 1991). Care was taken to ensure that stigmatic surfaces were covered with pollen. The flowers were collected after 48 h and fixed in Carnoy’s fluid (Samaha et al. 1989). The number of pollen grains adhering
to the stigmatic surfaces of flowers that were a) self-pollinated and b) cross-pollinated were counted to determine the difference in pollen load between the crosses (Samaha et al. 1989). Pollen germination and pollen tube growth were studied using epifluorescence microscopy as described. Seed set in hand and naturally-pollinated flowers (control) was determined.

Pollen-ovule ratio and pollination mechanism
Pollen counts to determine the pollen/ovule ratio were conducted as described by Zietsman (1991). Insects visiting the flowers of *P. dolomiticum* were collected and killed in killing jars containing 100% ethyl acetate. The presence and placement of pollen on each insect was noted. As *P. dolomiticum* is the only species with orange-coloured pollen at any stage in that area, foreign pollen could easily have been identified, thus obtaining an indication of flower constancy.

Pollinator behaviour
Visitors to *P. dolomiticum* flowers were observed for a total period of 8 days, from 07:00 to 19:00, on 10 randomly chosen plants. Every hour, 5 min were spent at each of these plants observing pollinators. Insect visits were expressed as number of insects per hour per plant. The duration of visits to individual flowers was timed and the number of flowers visited consecutively was noted. Flight distances between two successively visited flowers were obtained.

Results
Plant habit and floral behaviour
Individual flowers last for approximately two days and during this period three different phases can be distinguished (Figure 1). The number of flowers in the different phases during the morning and afternoon does not differ markedly (Figure 2).

1. Presexual phase: *P. dolomiticum* exhibits diurnal anthesis and the flowers on the same plant are not in synchrony. Anthesis starts when the closed stigma protrudes through the sepals. About 2–3 h later the tips of the petals start to appear between the sepals and are followed by the anthers. At this stage the sepals start to diverge. Anthesis ends when the petals and anthers are fully exposed. At this stage the anthers are undehisced.

2. Male phase: The first sexual phase follows shortly after the short presexual phase. *P. dolomiticum* flowers have seven fertile stamens (four long, one of medium length and two short). The first of the anthers to dehisce are the two on the short stamens closest to the base of the flower. The pollen is quickly removed by pollinators after which the anthers drop off. Next to dehisce are the anthers on two of the long stamens together with the anther in the middle. The two remaining anthers (on long stamens) are the last to dehisce. The anthers drop off after the pollen has been removed. During the male phase the style elongates. The closed stigma is never in touch with the dehiscing anthers. The duration of the male phase is from 5–8 h.

3. Female phase: This phase starts with the opening of the stigma and exposure of the receptive surfaces. After 2–3 h these areas are fully exposed. These surfaces are on the
interior part of the stigmatic lobes and are papillate. The papillae are multicellular and arranged in multiseriate order. On receiving compatible pollen, the stigma closes after a few hours.

Pollen-ovule (P/O) ratio, pollen viability and pollination mechanism

The P/O ratio for this species is 1484:1. Staining with Alexander's stain resulted in all pollen grains staining dark red, and with the FCR procedure all the grains fluoresced bright green. This indicates that all the pollen grains in this species are viable.

Of all the insect visitors that were collected and scrutinized, *P. dolomiticum* pollen was found only on *Apis mellifera*. Although pollen grains are deposited on the underside of the body and on the feet, the highest concentration of pollen was found on a bunch of hair on the vertex of the head. None of the bees examined carried foreign pollen.

Pollination experiments

Seed set in the flowers that were marked and left untouched to serve as a control for cross-pollination (N = 50) was 70%. None of the flowers emasculated and bagged to test for apomixis, set seed. Only one of the flowers bagged to test for selfing had a single pollen grain on a stigma. In 75% of the flowers that were used to determine self-compatibility, pollen grains adhered to the receptive surfaces of the fixed stigmatic material. The average number of pollen grains per stigma in the latter flowers was 1.75 (Figure 3a). Pollen tube growth in these flowers was not arrested.

Germinated grains were found on all the geitonogamous-pollinated flowers and pollen tubes penetrated the length of the style. An average of 19 pollen grains per stigma were found in these crosses. The results obtained from cross-pollinated flowers were virtually the same except that an average of 33 pollen grains were found on these stigmas (Figure 3b).

![Figure 3](image-url) Fluorescent micrograph of germinated pollen grains on the stigma of *Pelargonium dolomiticum* after (a) self-pollination and (b) cross-pollination.
Pollination

Attractants and rewards

Neither an odour nor osmophores were detected during any of the stages following anthesis. Pollen is available on an individual flower during approximately the first 24 h. Although the nectar quantity appears to vary between the different phases following anthesis, nectar is present during both sexual phases.

Pollinator behaviour

Behaviour on individual flower: Pollinators visit flowers irrespective of the floral phase. They approach the flower from the front and alight on the anthers. Although active pollen collecting was not observed, pollen is deposited on their legs and various parts of their bodies during a visit. The pollinators gain access to the nectar either by hanging upside down from the two posterior petals or by sitting on the anthers and probing the nectar tube.

Patterns of insect visits: Visits to P. dolomiticum flowers start early, at approximately 07:30 – 08:00. It increases steadily during the morning and peaks during midday with as many as 132 pollinators/plant/hour (Figure 4). The number of visits then decreases sharply until 18:00 after which very few pollinators are active. No pollinators have been observed at or later than 19:00.

The frequency distribution of the duration of visits to individual flowers (Figure 5) shows that most pollinators (43%) visit flowers for no longer than 2 sec. The duration of only 15% of all visits is between 4 and 6 sec.

In the majority of observations (48%) pollinators visit one flower per plant before moving on (Figure 6). These observations represent visits to individual plants and/or periods during which the pollinators moved out of sight. The latter situation was therefore interpreted as a single-flower visit to an individual plant. In those instances where more than one flower was visited consecutively, it was on the same individual plant. Windy conditions during the study period, as well as the quick and erratic movements of the pollinators, made the observation of interplant flights very difficult. In 62% of the observations (Figure 7) pollinators moved to flowers closer than 10 cm from the previously-visited individual.

Discussion

The temporal separation of the anthers and stigmas in P. dolomiticum indicates dichogamy. Dehiscence of anthers before stigma receptivity is characteristic of a protandrous
flower. Intrafloral dichogamy is achieved by the temporal separation of pollen and a receptive stigma in an individual flower. The lack of overlap between pollen presentation and stigma receptivity in *P. dolomiticum* indicates that dichogamy is complete (Lloyd & Webb 1986). In this species it results in the almost complete absence of self-pollen on the stigmatic surfaces, thus preventing self-pollination and clogging of the stigmatic surfaces. Asynchronous dichogamy is illustrated in *P. dolomiticum* by the random onset of anthesis in individual plants throughout the day.

The figures that Cruden (1977) presents for the pollen/ovule ratio of some of the angiosperms suggest that *P. dolomiticum* is a facultative xenogamous species. The relatively high P/O ratio of 1481:1 indicates relatively inefficient pollen transfer. This is contradictory to the actual seed set of 70%. Pollen transfer in this species should therefore be regarded as effective.

*P. dolomiticum* is self-compatible and geitonogamous. As cross-pollination is not a prerequisite for successful seed set, geitonogamy may explain in part, the high percentage seed set. Pollination experiments on *P. dolomiticum* indicate that more grains from cross-pollinations adhere to stigmatic surfaces than from self-pollinations. Samaha *et al.* (1989) found, in their study on the self-incompatibility of *Zinnia angustifolia* HBK, that compatible crosses resulted in much higher pollen loads as opposed to incompatible crosses. They attributed this phenomenon to the inhibition of pollen germination and the interference with the binding of pollen grains and pollen tubes to the stigmatic papillae (Ferrari *et al.* 1981). This results in the differential anchorage of compatible and incompatible pollen, and can be seen following the fixation and staining of pollinated pistils (Samaha *et al.* 1989). Although *P. dolomiticum* is self-compatible, the binding of cross-pollinated grains is stronger than that of self-pollinated grains.

The average visitation rate of 66 bees/plant/hour indicates the importance of bees as actual pollinators and is in line with the hypothesis of Wyatt (1983) in that bee- and/or fly-pollinated plants are predominantly protandrous. The flowers differ in certain aspects from the typical mellitophilic flower in providing facilities for landing and a foothold for pollinators. Although the flowers are not strong, the most important difference is the absence of a detectable odour during any stage of anthesis (Faegri & Van der Pijl 1979; Wyatt 1983). Even though honeybees may not be the most effective pollinators of the majority of *Pelargonium* species (Van der Walt *et al.* 1990), it is apparent that *A. mellifera* is the most important pollinator of *P. dolomiticum* in the study area. The role that colour and nectar guides play in pollinator behaviour is not yet clear. The total absence of foreign pollen on visiting honeybees indicates the constancy of these opportunists to *P. dolomiticum* flowers in a low reward area. Bees have a very keen sense of time which is integrated with information on food value and site location (Waddington 1983). The availability of flowers in the different phases following anthesis results in pollinators being able to visit the flowers at any time and utilize the maximum quantity of rewards.

The short duration of the majority of floral visits (2 sec) results in the continuous movement of pollinators, thus enhancing the possibility of cross-pollination. The closest neighbouring flower is visited which results in the leptocurtic distribution of flight distances (Waddington 1979; 1980; 1981; Zimmerman 1979) thus minimizing flight cost (Waddington 1983). This increases the possibility of geitonogamous flight cost (Waddington 1983) which increases the possibility of geitonogamous pollinations. However, in the majority of observations (48%) only a single flower is visited per plant before the pollinator moves away. Cross-pollination in *P. dolomiticum* is therefore favoured by the behavioural pattern of the honeybees. This pattern may be explained by the hypothesis of Waddington (1983) who found that bees adjust their flight distance in response to the volume of recently received nectar rewards. When rewards are encountered, they minimize their flight distance. When non-rewarding flowers are encountered, nearby flowers are passed.

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**References**


