

Variation in seed set amongst populations of a rodent pollinated geophyte, *Colchicum coloratum*

C. Kleizen^{a,*}, J.J. Midgley^a, S.D. Johnson^b

*University of Cape Town, South Africa
The University of Kwazulu Natal, South Africa*

Received 5 February 2009; received in revised form 25 July 2009; accepted 27 July 2009

Abstract

Agricultural activities around the rural village of Nieuwoudtville in the Succulent Karoo region of South Africa has led to the confinement of many plant and animal species to fragmented patches of relatively untransformed habitat. The geophyte *Colchicum coloratum* subsp. *coloratum* (Colchicaceae) was studied in five patches of variable size in and around Nieuwoudtville. This species is dependent on rodent visitation for seed production. The influence of variation in population size and plant size on seed set was investigated, as well as whether there is pollen limitation in this species. A pollen-supplementation experiment indicates that there is pollen limitation in *C. coloratum*, and that much of the natural seed set could be the result of pollinator-mediated selfing. The five populations appeared to have different rodent abundances, however, neither population size nor the abundance of rodents in the area have an effect on seed set. This suggests that the mutualism between *C. colchicum* and its rodent pollinators is robust, and that habitat fragmentation in Nieuwoudtville has not yet affected the seed production of this geophyte.

© 2009 SAAB. Published by Elsevier B.V. All rights reserved.

Keywords: Colchicaceae; *Colchicum coloratum* subsp. *coloratum*; Habitat fragmentation; Pollen limitation; Rodent pollination; Southern Africa; Succulent Karoo

1. Introduction

The pervasive nature of habitat fragmentation makes it one of the most important factors threatening the survival of plant and animal species (Saunders et al., 1991). To predict the effects of habitat fragmentation on particular plant species it is necessary to determine the factors that affect the seed set in the plants. A variety of factors may limit seed production in natural populations, including the availability of suitable pollinators and pollen (pollen limitation), the availability of resources (resource limitation), herbivory or predation of plant structures, and unfavourable environmental conditions (Vaughton and Ramsey, 1995).

Habitat fragmentation can have disruptive effects on plant-pollinator mutualisms (Lamont et al., 1993; Aizen and Feinsinger, 1994a,b; Ågren, 1996; Murren, 2002). Small plant populations are expected to be less attractive to pollinators than large populations (Sih and Baltus, 1987), which could result in certain plant populations not being visited by pollinators. There are

however, some plant-pollinator mutualisms that appear robust against habitat fragmentation, with pollinators mediating gene flow between fragments (Dick, 2001; White et al., 2002). Flowers are distributed within populations at a number of levels: flowers may be clustered into inflorescences, inflorescences may be clustered on a single plant, and individual plants may be clustered or scattered. Potential pollinators are required to make decisions concerning how best to exploit the plants and the associated rewards (Goulson, 2000). Several studies have shown that large floral displays are “optimal” (Lack, 1982; Bell, 1985; Queller, 1985). Plants with many flowers have been shown to attract more pollinators, which also visit flowers for longer (Klinkhamer et al., 1989; Klinkhamer and De Jong, 1990).

The isolation and reduction in the size of plant populations are also common consequences of habitat fragmentation, which in turn can reduce the likelihood of pollination (Ågren, 1996; Morgan, 1999). Many studies have shown that foragers spend shorter time and visit fewer flowers in small patches (Klinkhamer et al., 1989; Klinkhamer and De Jong, 1990; Brody and Mitchell, 1997). This may result in lower seed set as a consequence of a reduction in the quantity and quality of available pollen, which

* Corresponding author.

E-mail address: ckleizen@gmail.com (C. Kleizen).

constitutes respectively an ecological Allee and a genetic Allee effect respectively (Ellstrand and Elam, 1993).

Colchicum coloratum subsp. *coloratum* from the Succulent Karoo region of South Africa grows in variable sized populations, and has been shown to be dependent on rodents for seed set (Kleizen et al., 2008). In order to determine the effect of habitat fragmentation, variation in seed set amongst different populations of this species was investigated to assess the following. 1) Is there pollen limitation in *C. coloratum* populations with different rodent abundances? and 2) Is there any correlation between the rodent abundance in a plant population and the size of the population, density of the plants, or the plant size?

2. Materials and methods

2.1. Study species and study sites

Colchicum coloratum J.C. Manning and Vinnersten (Colchicaceae) is a deciduous geophyte (Membrives et al., 2002) that flowers in winter, producing a robust, geoflorous inflorescence with red bracts (Membrives et al., 2002). Membrives et al. (2002) performed breeding system experiments on cultivated plants of *C. coloratum* that had been grown from seed and concluded that the species was “preferentially self-incompatible”. This breeding system was confirmed by field experiments conducted by Kleizen et al. (2008), who demonstrated that self-fertilization occurs only through pollinator-mediated movement of pollen between the flowers of an inflorescence (Kleizen et al., 2008). Therefore, this species does depend on its rodent pollinators for seed production.

This study was carried out in the semiarid Succulent Karoo region of South Africa, during August and September of 2007. Five populations were chosen in the vicinity of the town of Nieuwoudtville, situated at the following sites: a slope on Glen Lyon Farm (31°23'S, 019°09'E, elev. 742 m), alongside a river on Glen Lyon Farm (31°22'S, 019°15'E, elev. 703 m), the Nieuwoudtville Flower Reserve (31°21'S, 019°08'E, elev. 747 m), Matjiesfontein Farm (31°28'S, 019°04'E, elev. 700 m) and Hotbergfontein Farm (31°22'S, 019°12'E, elev. 758 m). Voucher specimens of the study species are deposited in the Bews Herbarium, Pietermaritzburg.

2.2. Natural seed set and pollen limitation

To determine if natural seed production is the result of pollination or by lack of visitation, a pollen-supplementation experiment was conducted. At each population, ten pairs of plants, the members of each pair not more than 4 m apart, were randomly selected. In each pair, one plant received supplemental pollen and the other was marked as a control, and was left untreated. To accomplish pollen supplementation, pollen-laden anthers were collected from plants growing 4–6 m from the experimental plant, and were brushed over the stigmas of the fully opened experimental plants, so to saturate them with pollen. At the end of the flowering season, seed set was assessed by counting the number of seeds per flower from all of the 20 inflorescences.

An earlier study (Kleizen et al., 2008) indicated the approximate numbers of seeds produced per flower when flowers were

selfed, outcrossed or left unmanipulated. This experiment was conducted in one of the same study sites and at the same time as the current study. In that study all the stigmas were saturated with pollen in order to ensure that the seed set was the result of pollen quality and not pollen quantity. The mean number of seeds per flower that resulted from the unmanipulated, self-pollinated, and cross-pollinated treatments was rounded off, and used as an indication of whether the plants in the present study were selfed, crossed or whether neither had occurred. Based on this, in the present study we assume that if there are less than five seeds per flower, there has been no pollination activity. If there are more than five seeds and less than 50 seeds per flower, we assume self-pollination has occurred; and if there are more than 50 seeds per flower, we assume that cross-pollination has taken place.

2.3. Rodent trapping

The total number of rodent individuals captured at each of the *C. coloratum* populations was taken as a measure of rodent abundance at each population. To test whether the rodent abundance varied between different populations of *C. coloratum*, live trapping was conducted. A total of 90 Sherman traps were set every evening for three consecutive evenings at each of the five *C. coloratum* populations. Traps were set at dusk and laid out in lines of 15 traps, with four metres between each trap; and all traps were baited with rolled peanut butter and oats (as in Kleizen et al., 2008). Traps were checked in the early morning (between 0600 and 0700) and the species of captured rodents identified. The weather conditions during the trapping at each population were very similar.

2.4. Differences between populations

In order to establish whether the population size and/or plant size affect the rodent abundance at a site, the length and breadth of ten randomly chosen inflorescences were measured (in order to calculate inflorescence area) as well as the number of flowers per inflorescence at each of the five sites. The density of *C. coloratum* plants was also measured by counting the number of plants growing in four 5 × 5 m quadrats per population. The area of each of the five populations of *C. coloratum* plants was also measured (a measure of population size). The statistical analyses conducted on the data were simple (R^2 coefficients) due to the fact that only five populations were included in this study.

3. Results

3.1. Pollen limitation in *Colchicum coloratum* populations

Supplemental hand pollination increased mean seed set by 57%. Seed set was significantly higher in the inflorescences that received supplemental pollen than the control inflorescences in all of the five populations (Fig. 1) ($n = 10$ plants per site, $p < 0.05$).

Based on the assumptions made from the earlier breeding system experiment (Kleizen et al., 2008), it appears that in the

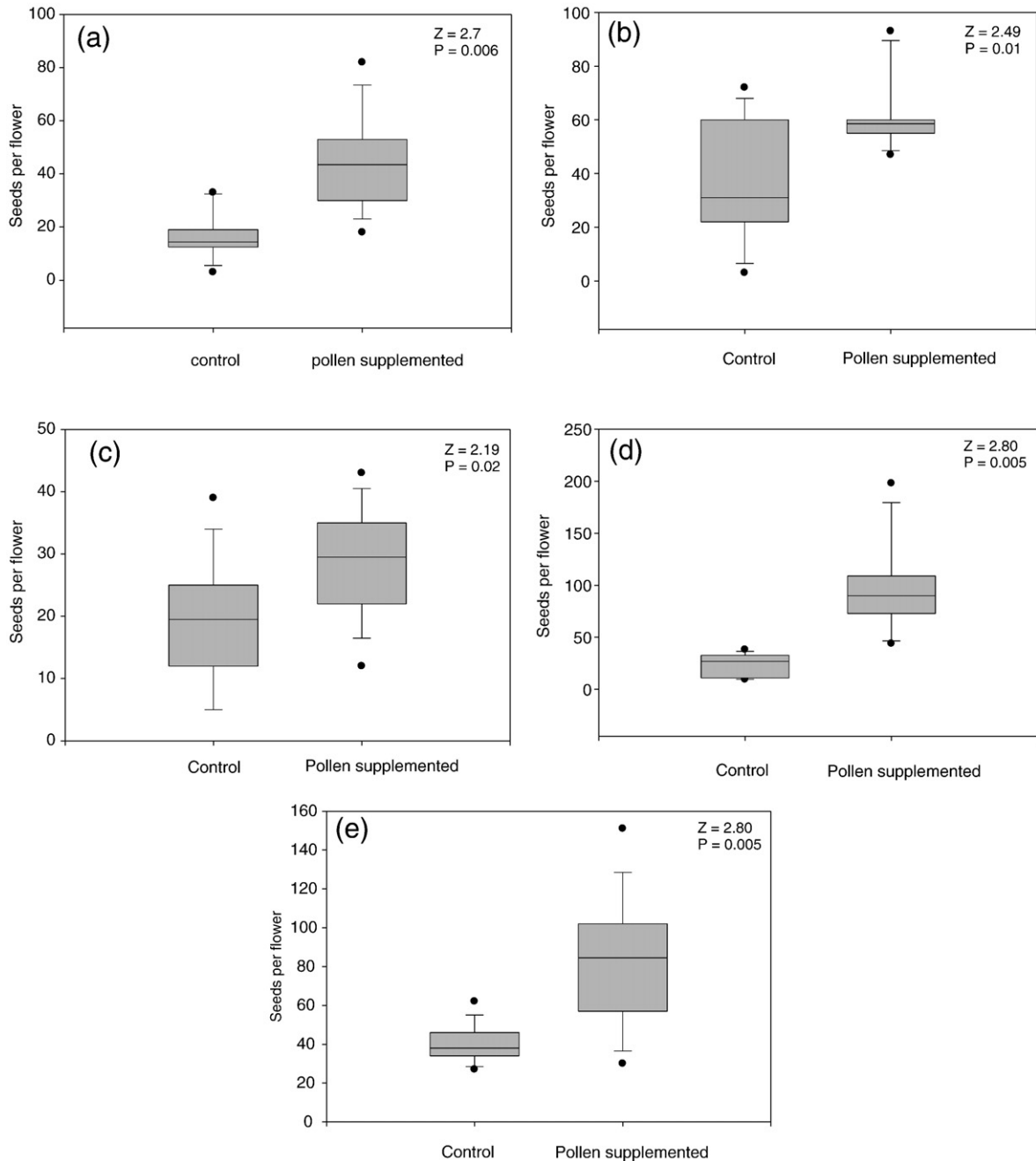


Fig. 1. The median number of seeds produced by the flowers supplemented with pollen and the control flowers in the *Colchicum coloratum* population on the slope at Glen Lyon farm (a), alongside a river at Glen Lyon farm (b), the Nieuwoudtville Flower Reserve (c), Matjiesfontein Farm (d) and Hotbergfontein farm (e). Z values were obtained from the Wilcoxon test for paired samples. Sample sizes were 10 plants per treatment group, with a p -level < 0.05 considered significant.

unmanipulated inflorescences at our five sites, 80% were self-pollinated (< 50 seeds), 12% were cross-pollinated (> 50 seeds) and only 8% were not visited (fewer than 5 seeds).

3.2. Rodent abundance and *Colchicum coloratum* characteristics

A total of 19 animals were captured at all five populations, representing four rodent species: *Aethomys namaquensis* (A. Smith), *Mus minutoides* (A. Smith) *Rhabdomys pumilio* (Thomas) (all Muridae: Murinae); and *Gerbillurus paeba* (A. Smith) (Muridae: Gerbillinae). Rodents were most abundant at the population

on Hotbergfontein farm with the capture of 6 individuals over the three trapping nights; whereas the least number of rodents were captured at the *C. coloratum* population on a slope on Glen Lyon (1 individual) (Table 1).

There was no relationship between population size and mean seed set (Pearson's $r = 0.0095$, $N = 6$, $p = 0.9858$). Rodent abundance was however, positively correlated with the mean inflorescence size (area) (Fig. 2a) and the mean number of flowers per inflorescence (Fig. 2b) at each of the *C. coloratum* population (Pearson's $r = 0.913$, $N = 5$, $p = 0.021$; and, Pearson's $r = 0.951$, $N = 5$, $p = 0.012$ respectively). Rodent abundance was

Table 1
Numbers and species of rodents captured at each of the *Colchicum coloratum* populations.

Site	Total no. rodents captured	Rodent species
Slope, Glen Lyon	1	<i>Gerbillurus paeba</i> (1)
Alongside river, Glen Lyon	3	<i>Mus minutoides</i> (2) <i>Rhabdomys pumilio</i> (1)
Flower Reserve	4	<i>Aethomys namaquensis</i> (3) <i>Mus minutoides</i> (1)
Matjiesfontein	5	<i>Aethomys namaquensis</i> (3) <i>Rhabdomys pumilio</i> (2)
Hotbergfontein	6	<i>Rhabdomys pumilio</i> (2) <i>Gerbillurus paeba</i> (4)

not affected by either the size of the *C. coloratum* population (Pearson's $r=0.671$, $N=5$, $p=0.214$) (Fig. 3a), nor the plant density (Pearson's $r=0.226$, $N=5$, $p=0.714$) (Fig. 3b).

4. Discussion

4.1. Pollen limitation

A previous study on *C. coloratum* demonstrated that autonomous self-pollination is limited and that seed production depends strongly on pollinators (Kleizen et al., 2008). In all of

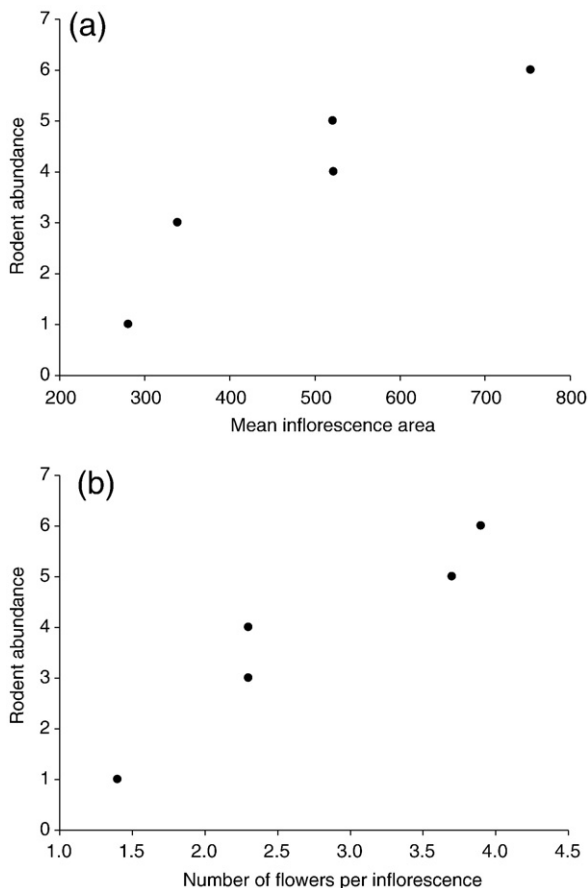


Fig. 2. Relationship between the rodent abundance and the corresponding mean inflorescence area (cm²) (a) and number of flowers per inflorescence (b) at each *Colchicum coloratum* population.

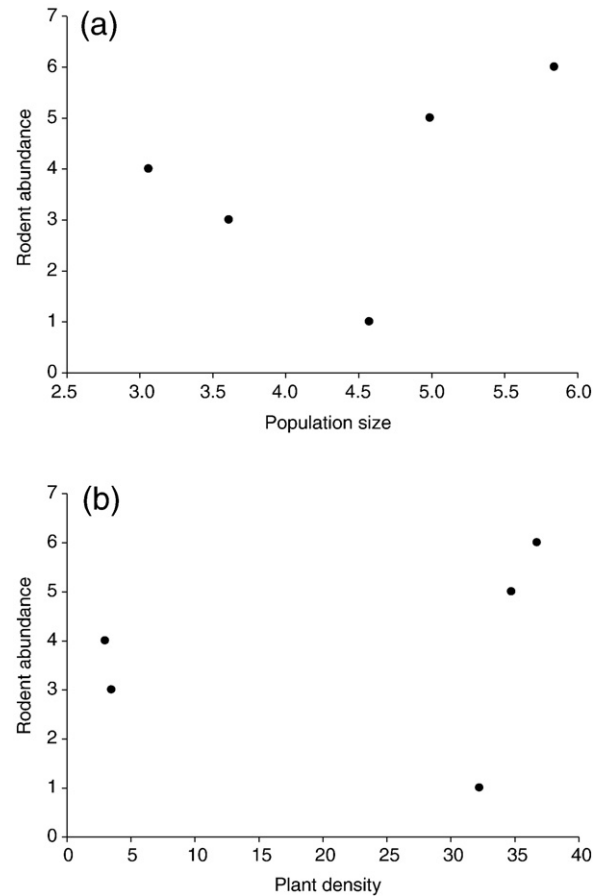


Fig. 3. Relationship between the rodent abundance and the corresponding population size (m² and log-transformed) (a) and plant density (number of plants in four 5 × 5 m quadrats per population) (b) at each *Colchicum coloratum* population.

the five populations included in this study there was a significantly lower mean seed set in the control inflorescences than in the inflorescences that received supplemental pollen, indicating that pollen limitation is a factor in these populations. Pollen limitation results either from flowers receiving too few visits by pollinators, or from pollinator behaviour that results in insufficient amounts of compatible pollen being deposited on stigmas (Vaughton and Ramsey, 1995). Although seed set was lower in unmanipulated flowers, relatively few were unvisited (i.e. had fewer than 5 seeds per flower). This suggests that floral visitation rates are high within all 5 populations but that geitonogamy dominates.

4.2. Effects of different population parameters

The Allee effect is broadly defined as a low per capita rate of increase of small populations relative to larger ones (Stephens et al., 1999). The lack of any relationship between population size and seed set in *C. coloratum* suggests that there are no Allee effects in the studied populations, where populations with low rodent abundance produced a similar mean number of seeds as those with greater rodent abundances (Fig. 1). This suggests that even small rodent populations are sufficient to pollinate the plants in an area.

Rodent abundance was positively correlated with inflorescence size and the number of flowers per inflorescence. If it is assumed that the amount of nectar in an inflorescence is positively correlated with inflorescence size; then a likely explanation for this is that the larger, more conspicuous, and more rewarding an inflorescence is, the more attractive the inflorescence is to rodents and the likelihood of rodent visitation increases. Our study is apparently the first to investigate the consequences of variation in population size and density on a plant-small mammal pollination mutualism. We detected that pollen limitation is evident in these populations of *C. coloratum*, but that it is probably the result of pollinator-mediated selfing rather than non-visitiation. These apparently small rodent populations appear to provide relatively efficient pollination, such that despite the *C. coloratum* occurring in patches separated by farming activities, the mutualism between *C. coloratum* and its rodent pollinators appears to be resilient to this habitat fragmentation.

Acknowledgements

We thank the Northern Cape Department of Nature and Environmental Conservation for providing us with the appropriate permits and Neil McGregor for his friendly facilitation and discussions regarding Namaqualand flora. The fieldwork was made possible by grants from the South African National Research Foundation.

References

- Ågren, J., 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* 77, 1779–1790.
- Aizen, M.A., Feinsinger, P., 1994a. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology* 75, 330–351.
- Aizen, M.A., Feinsinger, P., 1994b. Habitat fragmentation, native insect pollinators, and feral honeybees in Argentine “Chaco Serrano”. *Ecological Applications* 4, 378–392.
- Bell, G., 1985. On the Function of Flowers. *Proceedings of the Royal Society of London* 224, 223–265.
- Brody, A.K., Mitchell, R.J., 1997. Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis*. *Oecologia* 110, 86–93.
- Dick, C.W., 2001. Genetic rescue of remnant tropical trees by an alien pollinator. *Proceedings of the Royal Society London B* 268, 2391–2396.
- Ellstrand, N.C., Elam, D.R., 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* 24, 217–243.
- Goulson, D., 2000. Why do pollinators visit proportionally fewer flowers in large patches? *Oikos* 91, 485–492.
- Kleizen, C., Midgley, J.J., Johnson, S.D., 2008. Pollination systems of *Colchicum* (Colchicaceae) in southern Africa: evidence for rodent pollination. *Annals of Botany* 102, 747–755.
- Klinkhamer, P.G.L., De Jong, T.J., 1990. Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). *Oikos* 57, 399–405.
- Klinkhamer, P.G.L., De Jong, T.J., De Bruyn, G., 1989. Plant size and pollinator visitation in *Cynoglossum officinale*. *Oikos* 54, 201–204.
- Lack, A.J., 1982. Composition for pollinators in the ecology of *Centaurea scabiosa* and *Centaurea nigra*. III. Insects visits and the number of successful pollinations. *New Phytologist* 91, 321–339.
- Lamont, B.B., Klinkhamer, P.G.L., Witkowski, E.T.F., 1993. Population fragmentation may reduce reproduction to zero in *Banksia woodii*. *Oecologia* 94, 446–450.
- Membrives, N., Caujape-Castells, J., Pedrola-Monfort, J., 2002. Reproductive biology of the genus *Androcymbium* (Colchicaceae) in western southern Africa. *Orsis* 17, 37–59.
- Morgan, J.W., 1999. Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. *Conservation Biology* 13, 266–273.
- Murren, C.J., 2002. Effects of habitat fragmentation on pollination: pollinators, pollinia viability and reproductive success. *Journal of Ecology* 90, 100–107.
- Queller, D.C., 1985. Proximate and ultimate causes of low fruit production in *Asclepias exaltata*. *Oikos* 44, 373–381.
- Saunders, D.A., Hobbs, R.J., Margules, C.R., 1991. Biological consequences of ecosystem fragmentation: a review. *Conservation Biology* 5, 18–32.
- Sih, A., Baltus, M., 1987. Patch size, pollinator behavior, and pollination limitation in catnip. *Ecology* 68, 1679–1690.
- Stephens, P.A., Sutherland, W.J., Freckleton, R.P., 1999. What is the Allee effect? *Oikos* 87, 185–190.
- Vaughton, G., Ramsey, M.W., 1995. Pollinators and seed production. In: Kigel, J., Galili, G. (Eds.), *Seed Development and Germination*, pp. 475–490.
- White, G.M., Boshier, D.H., Powell, W., 2002. Increased pollen flow counteracts fragmentation in a tropical dry forest: an example from *Swietenia humilis* Zuccarini. *Proceedings of the National Academy of Sciences, USA* 99, 2038–2042.