



Technical note

Getting around: Effects of fragmentation on a bird-pollinated *Erica* speciesSiow Yan Jennifer Angoh^{a,*}, Jeremy Midgley^a, Mark Brown^b^a Department of Biological Sciences, University of Cape Town, Rondebosch 7701, South Africa^b School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg 3209, South Africa

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ABSTRACT

Small or isolated plant populations might be expected to provide limited nectar resources to attract or sustain pollinators. We determined whether the plant-pollinator mutualism between the obligate out-crossing ornithophilous species *Erica discolor*, and its pollinators was resilient to habitat fragmentation. To do this we recorded pollinator visitation rates (using % flowers with at least one visit as a proxy) and seed set in patches of different sizes and degree of isolation in the easternmost section of the Cape Floristic Region, South Africa. For *E. discolor*, there was no negative effect of patch size and isolation on visitation rates. Plants in small patches (< 100 ha) did not have lower proportions of viable seeds compared to plants in large patches (> 240 ha). Our findings suggest that conserving small isolated fragments is important in maintaining ornithophilous plant populations.

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1. Introduction

Habitat fragmentation can potentially affect pollination of animal-pollinated plants and thereby lead to population declines in both animal and plant species (Pauw, 2007). There are numerous ways in which animal-mediated pollination mutualisms can be disrupted by fragmentation (Harris and Johnson, 2004). In fragmented habitats, declines in the availability of food resources, nesting sites, or larval host plants may eventually decrease flower visitations by pollinators (Rathcke and Jules, 1993; Winfree et al., 2011). Pollinators may also respond negatively to the alteration in abiotic and biotic factors in small isolated fragments and their surrounding matrix (e.g., change in fire frequency and increased alien invasion) and thus avoid these areas (Montero-Castaño and Vilà, 2012). In the case of a severe decline in pollinator abundance and richness, significant pollination deficiency within a plant community would be expected (Aguilar et al., 2006; Pauw, 2007).

Large parts of the distribution range of many Cape *Erica* species have been subjected to a multitude of anthropogenic and ecological perturbations, including habitat fragmentation, alien plant invasion and disturbed fire regimes (Rebelo, 1992; Cowling et al., 2009). In the eastern coastal part of the Cape Floristic Region, the natural habitat of wild pollinator and many plant communities has been severely transformed and fragmented by agriculture, plantations of alien trees (e.g., *Pinus pinaster* and *Pinus radiata*), natural spread of

alien trees (e.g., *Acacia mearnsii*), and the urbanization of the southern Cape lowlands (Rouget et al., 2003; Cowling et al., 2009; Kraaij et al., 2011). As a result, many of the remaining fynbos species populations occur in small isolated remnants of natural and transformed vegetation (Rouget et al., 2003). We hypothesized that in the widespread bird-pollinated species, *Erica discolor*, (1) pollinator visitation rates would increase with increasing patch size and decreasing distance to mainland (isolation) and (2) seed set would be lower in smaller patches of fragmented natural habitat, compared to larger mainland patches.

2. Methods

2.1. Study site

Twenty patches composed of montane mesic proteoid fynbos habitat were identified and their boundaries delineated using remote sensing data in both Google Earth Pro-V7.1.4.1529, ArcGIS 10.2.2 (ESRI, 2014), and on-site ground-truthing (Fig. S1 in supplementary material). The study area encompassed a matrix of natural fynbos, stands of invasive alien vegetation, agriculture, farmsteads, *P. pinaster* and *P. radiata* plantations, indigenous forests, natural features, and rural and urban settlements (Table S1 in supplementary material).

Of the 20 patches, three were large (mainland patches > 240 ha) and 17 were patches smaller than 100 hectares (Fig. S1; Table S1). The 20 patches ranged in size from two hectares to 337 hectares (Fig. S1; Table S1). We measured the minimum distance of every small patch from its closest large mainland patch. The distances of these 17

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islands from their respective nearest mainland ranged between 0.18 km and 8.29 km (Fig. S1; Table S1). Fynbos habitat fragmentation in the study area, through afforestation and cultivation, has been recorded since the early 1900s (Kraaij et al., 2011). Although exact dates were not available, the 17 fragments used in our study would have most likely been isolated from the large mainland patches for over a century (Kraaij et al., 2011). All the patches were composed of mature fynbos vegetation with either no alien species or very few alien species (some patches were recently cleared of *P. pinaster* and *Hakea sericea*; Table S1).

2.2. 'Snapshot' of pollinator visitation

Erica discolor, which is particularly common in the study area, was used to investigate impacts of habitat fragmentation on pollination systems. Since *E. discolor* occurs in all 20 of the fynbos patches that were visited, it was the ideal study species for assessing the effect of habitat fragmentation on pollination by birds. To quantify visitation rate, a proxy for visitation (i.e., disturbed anther ring technique instead of direct observations) (Geerts and Pauw, 2010) was used in this study. Previously, the probing beak of a visiting sunbird has been shown to disturb the anther ring of two bird-pollinated *Erica* species (i.e., *E. versicolor* and *E. perspicua*) (Geerts and Pauw, 2010). Similar to the latter two *Erica* species, the anther thecae of mature *E. discolor* flowers are connected laterally at their pores into a perfect ring (i.e., un-tripped anther ring). Through the anther ring, the style is exerted (Fig. S2A in supplementary material). No pollen is released until the anther ring is ruptured by a pollinator.

In December 2015, populations of *E. discolor* were scored for tripped anther rings in 20 fynbos patches of varying sizes and different degrees of isolation from mainland patches. In each patch, a minimum of 10 plants were selected at random and for each plant, one apical inflorescence (with three mature flowers), was scored for tripped anther rings (Fig. S2B). We only chose inflorescences with mature open flowers and standardized for flower age by verifying that the stigmas were receptive (shiny and sticky) and exerted out of the corolla by at least three millimeters (Fig. S2C).

We analyzed the pollinator visitation data (i.e., proportion of visited flowers with increasing patch size) using the 'lme4' package in R 3.2.3 (Bates et al., 2015; R Core Team, 2020). We fitted a generalized linear mixed model (GLMM) with a binomial error structure using the 'glmer' function (Bates et al., 2015). The latter regression model was based on a Laplace approximation (logit scale). Patch size was added as a categorical predictor, and patch ID added as a random intercept to account for pseudo-replication at the patch level (10 plants sampled per patch). We also fitted the above GLMM with proportion of visited flower as a function of patch isolation and with patch ID as a random intercept to determine if patch isolation had a significant effect on the proportion of visited flowers per patch.

2.3. Seed set success

Seed set samples of *E. discolor* per patch were also collected in order to further assess pollination success. In October 2015, 10 fruits (one fruit each from 10 different randomly chosen plants) were collected in each of the 20 patches. Seed set across patches was standardized for maturity by only picking ripe uneaten fruits (red colored fruits; Fig. S2D) and to negate seed predation as a factor. The seed set obtained only provided an approximate measure of the effectiveness of natural pollination as we did not follow the fate of individual flowers, nor use supplementary pollen to test for resource limited seed set. The main aim of this study was to determine whether fragmented populations of a bird-pollinated self-sterile plant produce reasonable seed set.

The fruits produced were dissected and the viable and nonviable seed numbers were recorded. *Erica discolor* is a self-sterile species in

which most self-fertilized ovules are subsequently aborted. That is, *E. discolor* has late-acting self-incompatibility or early inbreeding depression (Angoh et al., 2017; Arendse et al., 2021). Given that *E. discolor* flowers that are selfed produce small fruits with few seeds (Angoh et al., 2017), we only selected large mature fruits (implying a pollinator visit). We assumed that most of the nonviable seeds in these fruits resulted from selfing or heterospecific pollen rather than pollen limitation due to a lack of pollinator visitation (Angoh et al., 2017; Coetzee et al., 2020). Viable seeds were distinguishable from nonviable ones by their larger size and darker, deeper color (Turner et al., 2012). Viable and nonviable seeds per fruit collected was scored using an image processing and analyzing software package (Image-J; Rasband, 2015). Photographs of every seed set were taken and analyzed in Image-J (Abramoff et al., 2004; Rasband, 2015). Size and/or color traits pertaining to the appearance of viable and unviable seeds in each seed set were measured. In order to test the accuracy of the Image-J results, a direct comparison (*t*-test) between automated and manual seed set counts was done for 30 fruits of *E. discolor*.

Our patch sizes were bimodal: smaller than 100 hectares and larger than 240 hectares. Therefore, the seed set data was split into the two groups and analyzed separately (17 small and three large patches). We considered three large patches (i.e., mainland/large island) to be more similar to the natural environment for seed set and therefore compared seed set in many small islands with seed set in the large ones. A non-parametric Wilcoxon rank sum test with continuity correction was used to compare between the averaged proportion of viable seed set of the samples belonging to the < 100 ha group ($n = 17$) and the > 240 ha group ($n = 3$).

3. Results

There was no significant relationship (GLMM, z -value = -0.76 , $SE = 0.002$, $P > 0.05$) in the proportion of flowers visited per plant as patch size increased (Fig. 1A). Across all patches, the proportion of flowers visited per plant was high on average ($\geq 0.67 \pm 0.15$). There was also no correlation between flower visitation success and isolation (i.e., distance from closest mainland patch) (GLMM, z -value = -0.491 , $SE = 0.085$, $P > 0.05$; Fig. 1B).

The mean for the averaged proportion of viable seed set of the 17 patches measuring < 100 ha ($\bar{x} = 0.74$) was significantly higher ($W = 3118$, $P < 0.01$) than the mean of the three larger patches (> 240 ha group; $\bar{x} = 0.61$, Fig. S3 in supplementary material).

4. Discussion

Our results suggest that the pollination mutualism between the ornithophilous plant species, *E. discolor*, and its nectar-feeding bird-pollinators could be resilient to fragmentation in the Cape Floristic Region. Visitation rates were high and did not differ across patches of varying size and degree of isolation for this *Erica* species (Fig. 1). Neuschulz et al. (2013) suggest that in KwaZulu Natal, some bird-pollinators are able to use small, isolated forest fragments as stepping-stones to move across and forage throughout transformed landscapes. We cannot comment on this for our study site. No indigenous trees are bird pollinated and only a few understory shrubs are (e.g., *Halleria lucida*, *Burchellia bubalina*). Moreover, relying on common fynbos generalist sunbird species (e.g., *Cinnyris chalybea* and *Anthobaphes violacea*; Geerts and Pauw, 2009), some populations of specialized bird-pollinated *Erica* species seem unlikely to lack pollinators as has been found elsewhere (Bascompte et al., 2006). The short-billed sunbird species, *Cinnyris chalybea*, can be found throughout matrices of transformed urbanized landscapes and presumably find enough nectar resources in fragmented habitats (Pauw and Louw, 2012). Hence, such bird-pollinators may be crucial in maintaining populations of plant species with short nectar tubes, such as

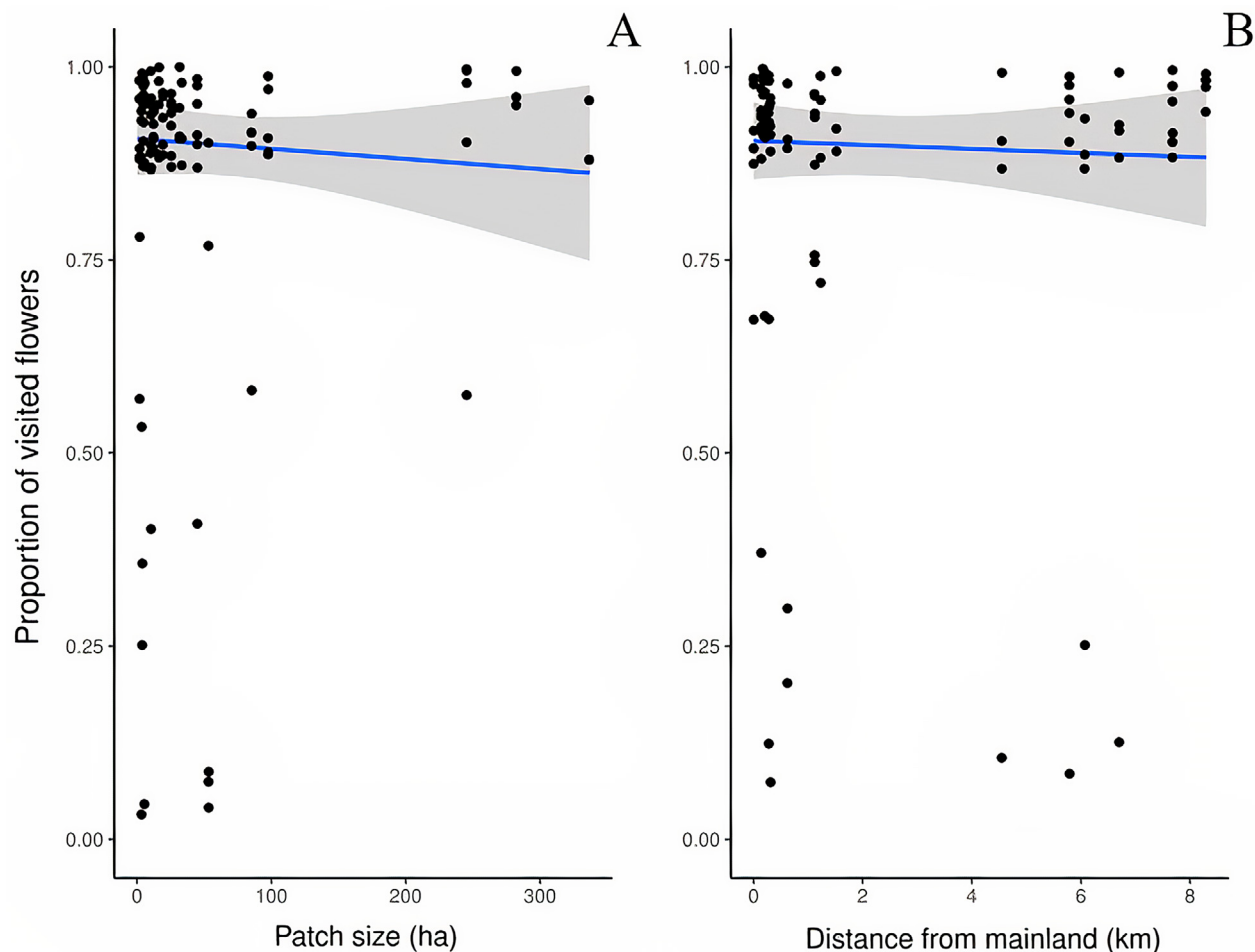


Fig. 1. Relationship between proportion of visited flowers per plant and (A) patch size, and (B) isolation (distance from closest large mainland patch). 95% confidence intervals (gray shaded area). ≥ 10 *Erica discolor* plants per patch (plant $n = 203$; patch $n = 20$).

E. discolor, in small and isolated fragments of natural habitats. On the other hand, the long-billed sunbird, *Nectarinia famosa*, was found to only venture from mainland habitats by at most 1 km (Pauw and Louw, 2012). This could possibly make specialized plant species with long nectar tubes more vulnerable to pollinator loss and less resilient to fragmentation.

Average proportions of viable seed set of *E. discolor* collected in smaller patches (< 100 ha) were significantly higher than in patches larger than 240 hectares. We interpret this to indicate more zenogamous visits per flower, as is suggested by the above high visitation rates, in smaller patches. In larger patches, the sheer number of available nectar resources may saturate the available pollinator pool (Johnson et al., 2012). This could explain the lower seed set obtained in the larger patches (Engel and Irwin, 2003; Waites and Agren, 2004; Johnson et al., 2012). Limited seed set in larger patches could also be due to fewer resources in these sites. For example, additional pollen occasionally did not alleviate low seed set for *Erica multiflora* (Santandreu and Lloret, 2011). In our study however, large *E. discolor* populations exist on the mainland patches and this suggests adequate resources availability there. Hence, resource limitation is unlikely to explain lower seed set on the larger patches in our case. Also, *E. discolor* seeds require low resource allocation because they are microscopic in this genus. Nevertheless, even if resource limitation is higher on the mainland patches, this does not detract from the ability of this bird-pollinated out-crossed plant species to be demographically viable on small isolated islands. As we only sampled seed set in ripe fruits, rather than in marked flowers, another factor that

could explain lower seed set on the mainland patches is greater abortion of unvisited flowers on small islands. However, we observed high visitation rates on small patches.

The habitat of many plant and pollinator species is being lost at an alarming rate in the lowland fynbos (Cowling et al., 2009; Kraaij et al., 2011). Our results highlight the resilience of a bird-pollinated obligate out-crossed *Erica* species to the impacts of fragmentation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2021.05.012.

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