Effects of habitat fragmentation on pollination: pollinators, pollinia viability and reproductive success

COURTNEY J. MURREN*

Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269, USA

Summary

1 For 3 years, I examined the effects of fragmentation on pollination of an endemic, euglossine bee-pollinated, epiphytic orchid, *Catasetum viridiflavum*, on 10 islands created during the construction of the Panamá Canal and at five sites in nearby large tracts of mainland forest.

2 I investigated the viability of pollinia over time, availability of pollinators and male and female reproductive success (measured as removal of pollinaria and fruit set, respectively) at different sites.

3 Viability of pollinia declined dramatically over time, although some remained at least partially viable for up to 60 days. Bees moved between trapping stations at different sites within a day, and viable pollen therefore could have been transferred between populations.

4 Although euglossine bees were equally frequent in fragments and continuous forest, those recorded on islands were visitors from mainland sites, and not island residents.

5 Neither male nor female reproductive success were significantly different between island and mainland forest types in 1997. The higher reproductive success in certain sites within both forest types throughout the flowering season could not be explained by variation in overall population floral display. Larger inflorescences had higher male and female reproductive success.

6 In 2 of 3 years, plants on islands had significantly lower fruit set than plants on mainland sites. Female reproductive success differed dramatically across years and sites.

7 The low fruit set in 1996 and 1998 on islands in comparison with mainlands, and the observation that bees are not island residents, conform with expectations of physical isolation of forest patches. In spite of the evidence of fragmentation in this system, patterns of pollinator behaviour and of reproductive success suggest significant connectedness among sites. This 3-year study shows that a single year study may not have highlighted the complexity of the system.

Key-words: Barro Colorado Island, epiphytic orchid, euglossine bee, forest fragmentation, plant reproduction

Journal of Ecology (2002) **90**, 100–107

Introduction

When large tracts of forest are dissected, organisms remaining in these smaller patches are exposed to many abiotic and biotic changes (Saunders *et al*. 1991). Selection pressures associated with fragmentation can cause local plant populations to: (i) decline in size (Colas *et al*. 1997); (ii) go extinct (McGarrahan 1997); (iii) become locally adapted after isolation (Young *et al*. 1996); (iv) increase in size in concert with increased edge habitat availability (Cunningham 2000); or (v) maintain interconnections with subpopulations through gene flow (Asquith *et al*. 1997; Nason & Hamrick 1997). The status of a particular population will depend on the life history and ecological characteristics of the species concerned and of its mutualists (Jennersten 1988; Rathcke & Jules 1993; Aizen & Feinsinger 1994). Few empirical data are available on these issues for plants in habitat fragments (Laurence & Bierregaard 1997; Storfer 1998).

Several components of the life history of tropical epiphytic orchids suggest that their populations in forest fragments are at risk of local extinction. Foremost, fragmentation reduces the available habitat (tree trunks and branches) upon which seedlings may germinate. Tropical orchids often rely on a single or few

*Present address and correspondence: Courtney J. Murren, Department of Biology, University of Maryland, College Park, MO 20742, USA (e-mail cmurren@wam.umd.edu).

pollinators for successful fruit set (Ackerman 1986) and fragmentation may exacerbate pollinator limitation, which is common among epiphytes across a variety of landscapes (e.g. Calvo & Horovitz 1990; Murren & Ellison 1996) if it is accompanied by local extinction of the pollinator. Together these factors put epiphytic orchids at risk of reduced reproductive success, and subsequently at an increased risk of local extinction.

On the other hand, tropical plants which naturally occur at low densities may have features that contribute to the maintenance of reproductive success following fragmentation. These include characteristics such as long distance pollinator movement and obligately outcrossing mating systems (Ellstrand & Elam 1993; Kearns *et al.* 1998; shown in the fig/fig wasp mutualism by Nason *et al.* 1998). Orchid species share these characteristics of their natural history (Dressler 1981; Janzen *et al.* 1982). Continued reproductive success requires species-specific pollinators to travel across the intervening non-forest matrix. If floral attraction mechanisms are sufficiently strong, and if orchid pollinia remain viable for days or even weeks, then reproductive success may be similar in continuous forest and forest fragments. In such cases, fragmentation may result in little change or may increase pollen movement among fragments (Young *et al.* 1996; Nason & Hamrick 1997; Aldrich & Hamrick 1998). Therefore, while physical isolation may be an indicator of extinction risk, the probability of persistence is more likely to be a complex set of interactions than a single causal link between fragmentation and extinction.

The epiphytic orchid, *Catasetum viridiflavum*, Hook, is pollinated by a single species of euglossine bee and has a functionally dioecious mating system and is a representative of obligately outcrossing plants with a species-specific pollination system. I hypothesized that if the mutualism is disrupted by landscape level changes, reproductive success will be lower in small fragments compared with continuous forest sites. Although euglossine bees may travel long distances between plants within continuous forest, they may only infrequently cross the intervening matrix to visit plants in forest fragments (Powell & Powell 1987), increasing pollinator limitation (Zimmerman 1991).

I examined several components of the reproductive ecology of *C. viridiflavum* to determine how local populations have been affected by fragmentation. During construction of the Panama Canal (1910–14) the flooding of the Chagres River caused the expansion of Lake Gatun (Croat 1978) and created numerous islands from previously continuously forested regions (Leigh *et al.* 1993). In this context, I studied 15 populations of *C. viridiflavum* on islands from 1 ha to 10 ha in size, and in several nearby continuously forested regions. I addressed the following questions:
• Do pollinators visit fragmented as well as continuously forested sites?
• How long are pollinia viable?

• How does floral display size (at the plant and population level) affect pollinarium removal and fruit set?
• Are there differences in fruit set or pollinarium removal between fragmented and continuously forested sites?
• Are there differences in fruit set among years?

To my knowledge this is the first study to specifically examine the influence of fragmentation on a tropical orchid. The answers to these questions will shed light on several components that affect reproductive success and population interconnection.

Materials and methods

STUDY SPECIES

Catasetum viridiflavum, Hook. is an epiphytic orchid endemic to central-lowland Panamá. It flowers from April to December, producing one to nine inflorescences per year. The inflorescences develop sequentially, sometimes several months apart. Plants are nominally monoecious but rarely produce both male and female flowers in the same season and are therefore functionally dioecious (a.k.a. sex switching) (Zimmerman *et al.* 1989). Male racemes have up to 17 flowers that remain open for about 5 days, whereas female inflorescences have fewer flowers and remain open for up to 30 days. Sex ratio of inflorescences in this species is strongly male biased (C. Murren, personal observation, Gregg 1975, 1978). *Catasetum viridiflavum* grows on trees at 1–35 m above the ground and, when necessary, rope climbing techniques were used to access the canopy (Dial & Tobin 1994).

Each male flower has a pollinarium with pollen packed into two pollinia (Dressler 1981). The entire pollinarium is removed by a single successful visit by a pollinator. Its removal can be easily scored and used as a measure of male reproductive success. *Eulaema cingulata* (Euglossini, Apidae) is the single pollinator (see Zimmerman *et al.* 1989; Zimmerman 1991 for further details). The male bees are attracted by the fragrance produced by the flowers (Dressler 1981, 1993; Ackerman 1989).

STUDY SITES

This study was executed in the Barro Colorado National Monument (Fig. 1). Ten island sites (forest fragments) within reasonable boating distance from the Smithsonian Tropical Research Institute field station on Barro Colorado Island were chosen such that the census population size was at least 30 (except site N, with only 10 individuals), and the sites were at least 100–500 m apart. The area of these islands ranged from 1 ha to 10 ha and they were isolated (by water) from nearby forest for 80 years.

The island and five mainland sites (large tracts of forest > 1500 ha, Fig. 1) were initially surveyed between June and August 1996. The area on Barro Colorado Island (BCI) was included as a mainland site because several previous studies showed that resident population densities of the euglossine bee pollinator and
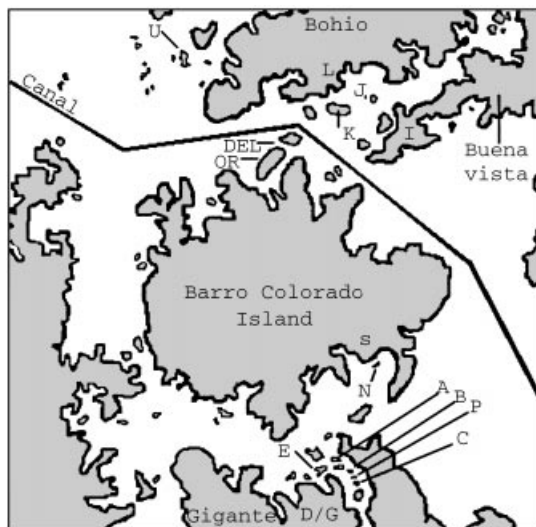
Fig. 1 Region of the Barro Colorado National Monument. The 15 study sites are labelled with letters. The Panama Canal is delineated north and east of Barro Colorado Island.

population sizes of *C. viridiflavum* were similar to nearby mainland forest sites (Parqué National de Soberania) (e.g. Ackerman 1983, 1989; C. Murren, personal observation). Mainland sites were forests of mixed ages and areas of secondary growth included remnant trees left when the forest was cut.

VARIATION IN POLLINATOR ABUNDANCE AMONG SITES AND BETWEEN FOREST TYPES

Euglossine bee pollinators were baited by providing commercially available scent melted onto paperboard (Zimmerman *et al.* 1989). I used skatole (3-methylindole; Sigma M-2127) as my scent bait as this has been shown to attract the greatest number of *Eulaema cingulata* (Ackerman 1983). I randomly sampled each of five island and five mainland sites on three separate non-consecutive days, between September and December 1997. I baited between 08.00 and 12.00, the time of peak abundance (C. Murren, personal observation; Zimmerman *et al.* 1989). No bees were ever observed at scent baits during rain, thus I only baited when no rain had occurred since 07.30. Each bee was marked with a dot of paint on its thorax to identify the site if the bee was recaptured. I used nested ANOVA (PROC GLM, SAS 1989) to assess whether bee abundance (number of bees trapped/day) differed between forest types (islands and mainlands) or among sites. Data were log-transformed to conform more closely to the assumption of normality.

MARK AND RECAPTURE AT A SINGLE PAIR OF SITES

An additional experiment was run for 12 days (from 08.00 to 12.30, for four consecutive days a month in May, June and July) concurrently at one island site and one mainland site 500 m apart. Trapped bees were

marked with paint unique to the location and observation date. Abundance, direction of arrival and recapture frequency were recorded.

POLLINIA VIABILITY

I collected pollinia from newly opened flowers from populations at an additional mainland site. Pollinia were kept in sterile Eppendorf vials until tested for viability with fresh triphenyl tetrazolium chloride (TTC) in pH 7.5 Tris-HCl buffer solution (Kearns & Inouye 1993). At testing, a pollinium was scored for viability (rank of 0–4) depending on the percentage colour change relative to the pollinia of a newly opened flower (scored as 4). Pollinia were sampled approximately every 7 days for up to 75 days. Insufficient female flowers of *C. viridiflavum* were available across study sites to allow for this question to be addressed by hand pollination experiments (Murren 1999). Data were analysed using a Kruskal–Wallis analysis (PROC GLM on ranked data, SAS 1989) to determine if pollinium viability decreased over time, and Spearman's rank correlation to determine the strength and sign of the relationship.

MALE REPRODUCTIVE SUCCESS

Throughout the 1997 flowering season (15 April to 22 December), I assessed the number of flowers produced and reproductive success of all plants in the 15 study sites. Every 3 days, I visited all sites to assess maturity of inflorescences, flower opening and pollinia removal (a measure of male reproductive success, Murren & Ellison 1996). Absence of the anther (which covers the pollinia) and pollinarium were scored by examination of the flower through binoculars, or through rope access to the canopy.

FEMALE REPRODUCTIVE SUCCESS

In 1997 I examined season-long female reproductive output, noting date of flower opening, the number of flowers per inflorescence and successful pollination. Within a day of successful pollination, the flower changed from upright to pendant, and the ovary walls began to swell. Hand pollination resulted in 95% (Zimmerman 1991) to 100% fruit set (C. Murren, unpublished data), suggesting that individuals that were able to produce female flowers could also bear the cost of fruit production. In 1997 population floral display (number of other flowers open within the population at the same time as the focal inflorescence) was included as a covariate in the analysis.

Fruit are maintained on the plant until the beginning of the dry season (late December or January), when they dehisce and the wind-dispersed seeds are released. In December of 1996 and 1997, and early January of 1999, I assessed fruit set at all 15 sites and counted total floral output per plant. Total floral output was assessed

by counting racemes that remain attached to the pseudobulb for many months and counting flower pedicel scars, which are sexually dimorphic.

DATA ANALYSIS OF POLLINARIUM REMOVAL AND FRUIT SET

I used a non-parametric nested analysis of variance (PROC GLM on ranked data, a Kruskal–Wallis test, SAS 1989) to examine the effects of forest type, site and plant on pollinaria removed or fruit set per inflorescence. Because neither measure of reproductive success could be transformed to meet the assumption of normality of the error, the analysis of variance was performed on ranked data. Number of flowers per inflorescence and population floral display were used as covariates in the model. Forest type (island or mainland) was a fixed effect. Site nested within forest type and plant nested within site by forest type were random effects. F-tests for random effects in an unbalanced design use a linear combination of the mean squares of the fixed effects, thus the F-tests use Satterthwaite approximations (SAS 1989; see Tables).

DATA ANALYSIS OF THE 3-YEAR PATTERNS OF FRUIT SET

Year, forest type and site were used as main effects in this nested mixed model non-parametric analysis (PROC GLM on rank of fruit number) with forest type by year and year by site nested within forest type included as interaction terms. Each inflorescence was a replicate, with number of fruit as the response variable. Number of flowers on the inflorescence was used as a covariate. Fixed and random effects were as above.

Results

VARIATION IN POLLINATOR AVAILABILITY

The number of bees trapped at island sites (mean = 13.1, SD ± 1.16) was only slightly lower than the number attracted to mainland sites (mean = 15.33, SD ± 2.21). This difference was not significant ($F = 0.4$; d.f. = 1; $P = 0.55$), nor were variations in bee abundance among sites nested within forest type ($F = 0.60$; d.f. = 8; $P = 0.76$). All bees observed at island baits came across the water, suggesting that these small islands do not support resident populations of *E. cingulata* but are visited by bees. All bees trapped in mainland sites arrived from within the surrounding forest. Two mainland bees were recaptured in the following month at their marking station.

MARK AND RECAPTURE AT A SINGLE PAIR OF SITES

Of 301 bees observed in the mark–recapture study, 12 (4%) were recaptured. Five of these bees were recap-
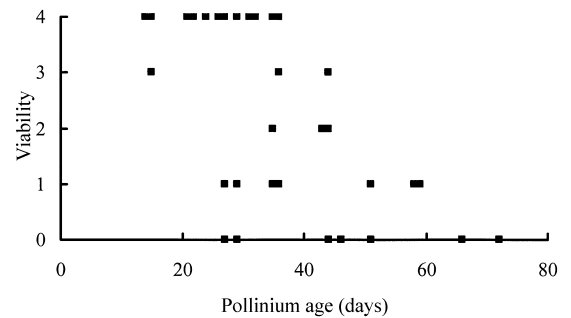

Fig. 2 Relationship between age of a pollinium and its estimated viability. Viability was estimated on a scale of 0–4, based on relative percentage colour change compared with a fresh pollinium in a TTC assay.

tured on the island and all were marked at the mainland site, whereas six of the seven bees recaptured on the mainland were observed at the same site where they had been marked. During one paired trapping episode, a bee arrived at an island from the mainland, was marked, and later recaptured at the mainland trapping station. All bees arriving on the island came in a direction opposite to the wind, presumably following a positive odour concentration gradient.

POLLINIUM LONGEVITY

Age of a pollinium was a significant predictor of viability ($F = 36.7$; d.f. = 1; $P = 0.0001$). Viability decreased with age ($r_S = -0.67$; $P = 0.05$) (Fig. 2). Few pollinia were viable after 45 days.

POLLINARIUM REMOVAL

There was no significant difference between island and mainland sites in the number of pollinaria removed. The number of pollinaria removed was strongly site-dependent and positively correlated with the number of flowers on the inflorescence (Table 1). The number of conspecific flowers that were open at the same time in that site was not a significant predictor of pollinarium removal (Table 1).

FEMALE REPRODUCTIVE SUCCESS IN 1997

There was no difference between the two forest types in the number of fruit set per inflorescence, but there were significant differences among sites (Table 2). Number of flowers per inflorescence, which explained the largest variation for male reproductive success, was also a significant predictor for females. A significant effect of plant suggested that certain plants were successfully pollinated more often than others.

FEMALE REPRODUCTIVE SUCCESS: 3-YEAR SURVEY

Absolute number of fruit set was greater in 1997 than in the other 2 years for both forest types (Fig. 3). In 1996

Table 1 Nested Kruskal–Wallis results (an ANOVA on ranked data) for number of pollinaria removed per inflorescence (a measure of male reproductive success). Data were taken over the entire 1997 flowering season. Forest type (island or mainland) was a fixed effect. Site was a random effect. Flowers per inflorescence and flowers open on the same site during the same days the inflorescence of interest was open, were covariates in the model. Overall model $R^2 = 0.73$

Source of variation	Type III MS	Numerator d.f.	Denominator d.f.	F	P
Forest type*	4985.3	1	12	0.1	0.73
Site (forest type)†	42938.8	11	497	2.4	0.007
Plant (forest × site)	18662.8	335	210	1.1	0.169
Flowers per inflorescence	523031.7	1	210	32.0	0.0001
Flowers per site	2865.6	1	210	0.2	0.68
Model	26424.6		349	1.6	0.0001
Error	16535.1		210		

*Forest type denominator MS: $0.8811 \times$ MS (site (forest type)) $+ 0.0972 \times$ MS (plant (forest type × site)) $+ 0.0217 \times$ MS (error).
†Site (forest type) denominator MS: $0.7431 \times$ MS (plant (forest type × site)) $+ 0.2569 \times$ MS (error).
Plant (forest × site), flowers per inflorescence and flowers per site all use the model MS (error) as denominator MS.

Table 2 Kruskal–Wallis results examining the effect of forest fragmentation and floral display on fruit set (number of fruit: a measure of female reproductive success on a per inflorescence basis). Data were taken in 1997 over the entire flowering season. Factors are as in Table 1. Overall model $R^2 = 0.85$

Source of variation	Type III MS	Numerator d.f.	Denominator d.f.	F	p
Forest type*	362.5	1	17	0.2	0.69
Site (forest type)†	3266.6	11	124	2.0	0.04
Plant (forest × site)	1841.6	105	52	2.2	0.001
Flowers per inflorescence	372	1	52	10.5	0.002
Flowers per site	8756	1	52	0.5	0.51
Model	1967.6		119	2.4	0.0004
Error	832.5		52		

*Forest denominator MS: $0.5627 \times$ MS (Site (forest)) $+ 0.132 \times$ MS (plant (forest × site)) $+ 0.3053 \times$ MS (error).
†Site (forest) denominator MS: $0.8271 \times$ MS (plant (forest × site)) $+ 0.1729 \times$ MS (error).
Plant, flowers per inflorescence and flowers per site all use the MS (error) as the denominator MS.
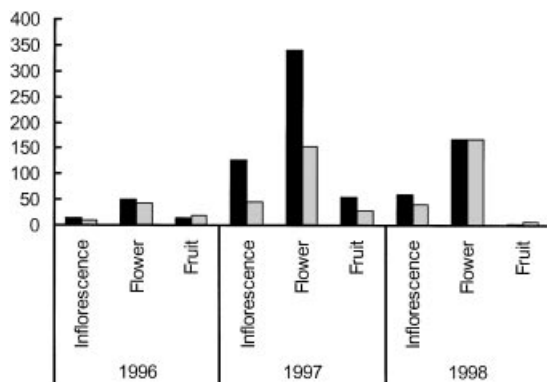

Fig. 3 Yearly differences in number of inflorescences, number of flowers and number of fruit for the two forest types: islands are in grey and mainlands are in black.

and 1998, overall the proportion of number of fruit to number of inflorescences on mainland sites was significantly greater than on island sites (Fig. 3). In 1997 the proportion of fruit set to inflorescences was similar between islands and mainlands, as inflorescence production on islands was extremely high. Proportion of fruit set per inflorescence at island sites was much higher in 1997 than in either 1996 or 1998 (Fig. 3). Flower number also varied among years, with 1997 having the highest flower production on islands and 1998 having the highest flower production on mainland sites. Forest type, site, year and the interaction of forest type and year (marginally significant) explained a large proportion of the variation in fruit set (Table 3; Fig. 3). However, in contrast to the 1997 study, the number of flowers per inflorescence was not a good predictor of fruit set.

Discussion

Presence of pollinators is essential for plant population persistence in obligately outcrossing, animal-pollinated species (Rathcke & Jules 1993; Murcia & Feinsinger 1996; Murcia 1996). Although it is known that most tropical plant populations are dependent upon pollinators for successful pollen removal and fruit set (Leigh 1999), there are very few ecological data

Table 3 Analysis of female reproductive success over 3 years (1996–98) for all sites. Year was a fixed effect. Other factors are as in **Table 1**. Overall model R^2 value is 0.33

Source of variation	Female reproductive success 1996–98				
	Type III M.S.	Numerator d.f.	Denominator d.f.	F	P
Forest type*	28972.6	1	25	8.73	0.003
Site (forest type)†	8006	13	26	2.41	0.004
Flowers per inflorescence	8493.8	1	265	2.56	0.11
Year‡	76503.7	2	26	23.05	< 0.0001
Site (forest) Year	4841.2	16	265	1.46	0.12
Year × forest§	8209.7	2	25	2.47	0.09
Model	12484.9	35		3.8	< 0.0001
Error	3318.8	265			

*Forest type error: 0.518 MS (site (forest)) – 0.00 × 5 × MS (year × site (forest)) + 0.482 × MS (error).
†Site (forest) error: 0.719 × MS (year × site (forest)) + 0.2814 × MS (error).
‡Year error: 0.719 × MS (year × site (forest)) + 0.2809 × MS (error).
§Year × forest error: 0.723 × MS (year × site (forest)) + 0.28 × MS (error).
Flowers per inflorescence and site (forest) × year and year × (forest) used only MS (error).

available on plant–pollinator mutualisms to predict whether local populations for any given species will persist (Kearns & Inouye 1997). Ecological and life history characters that maintain reproductive success in low density populations of *C. viridiflavum* may also contribute to their reproductive success and population persistence across a fragmented landscape.

Predictions that female reproductive success would be low in forest fragments (Aizen & Feinsinger 1994; Young *et al*. 1996) are borne out for 2 years of this study, but during 1997 (an El Niño year) absolute fruit set was much higher on islands than mainland sites and was greater than other years. Variations among sites in both forest types were significant, indicating that individual sites may strongly differ from the overall landscape level pattern. The combined data on pollinator abundance, observation of pollinator flight patterns and reproductive success of male and female orchids indicate that pollinator visitation to islands is needed for reproductive success to be similar to mainland sites. Reproductive success must therefore be examined across a number of sites and multiple years to identify the factors contributing to population persistence in forest patches.

POLLINATOR BEHAVIOUR

Males of *Eulaema cingulata* – the sole pollinator species of *C. viridiflavum* – are present on islands, the first step for continued reproduction within fragments. Interestingly, *E. cingulata* trapped on islands were always observed to fly across the lake to reach the bait traps, suggesting that individuals are not island residents, but rather transient visitors from larger continuously forested areas. Overall, these data suggest that island populations of *C. viridiflavum* rely on mainland populations of *E. cingulata* for pollination, and thus for fruit set.

Studies in both Brazil and Panamá, however, have shown that many euglossine bee species do not cross the intervening matrix between forest fragments (Powell & Powell 1987; Becker *et al*. 1991; C. Murren and S. Gotsch, unpublished data) leading to unequal abundances. Certain species appear to be less likely to be trapped in forest fragments than in larger tracts of forest (Powell & Powell 1987), suggesting with this study that not all species of euglossine bees respond similarly to open areas (see also Roubik 1993). The fact that *E. cingulata* visit bait traps on both islands and mainland sites indicates that their behaviour is an important component of continued population persistence in fragments for plant species that depend on this bee.

POLLINIA VIABILITY

In many plant species, pollen remains viable for only a few hours (Stanley & Linskens 1974), making pollinator behaviour within a single day a strong predictor of whether successful pollination will occur within or among spatially isolated populations, and thus making length of pollen viability critical for many plants in fragments (Aizen & Feinsinger 1994). Several pollinia were viable for up to 2 months and bees travelled between sites within a given day, suggesting that long distance movements occur and that pollinia may be transferred successfully between individuals in separate forest patches.

MALE AND FEMALE REPRODUCTIVE SUCCESS

Although there were highly significant differences among sites for both pollinia removal and fruit set during 1997, the two forest types did not differ in reproductive success per inflorescence. This contrasts with theoretical predictions, and with most empirical studies

that have found a decrease in fruit set in fragments relative to continuous habitats (e.g. Oostermeijer *et al.* 1992; Groom 1998; Wolf *et al.* 1999). Increased fruit production in fragments has been shown by Cunningham (2000) in *Senna artemisioides*, a Caesalpinoid herb common in the mallee wood of New South Wales, Australia, and Aizen and Feinsinger (1994) showed similar results for *Tillandsia ixioides*, a self-incompatible, hummingbird-pollinated bromeliad of the dry forest in Argentina.

Among-site variation in reproductive success is critical for conservation decisions. For example, two fragments had strongly male-biased sex ratios of the inflorescences and only one or two plants produced female flowers during the course of the study, yet the census population sizes on these islands were comparable with other fragments. If conservation decisions in this circumstance were made through census population size alone these populations with little or no female reproductive success would be among those considered for conservation. It is unlikely that such limited fruit set would be sufficient to maintain these populations over the long-term, persistence would depend on plants switching sex or seed influx from other sites. Therefore, populations at all sites are not equivalent in their potential contributions to male and female reproductive success, and many populations are highly dependent upon neighbouring sites for persistence.

The El Niño season was very different from other years, suggesting that such events may be extremely important for local population persistence. Light has been shown to be the proximate cue for both flowering and gender determination in *C. viridiflavum* (Zimmerman 1991) and much greater light availability and less rainfall was recorded in 1997 than in a typical Panamanian rainy season (S. Paton, personal communication). In that year, season-long light availability was positively correlated with an increase in the number of female inflorescences (C. Murren, unpublished data), as well as with greater absolute fruit production, greater percentage fruit set in both forest types, and increased seedling recruitment compared with 1996 or 1998.

Female reproductive success in 1997 may have been further enhanced by effects of pollinator behaviour. Euglossine bees only flew between island and mainland forest patches on sunny days (C. Murren, personal observation) and the more favourable weather may have ameliorated a perhaps more common pattern of pollinator limitation at these sites (Ågren 1996; see also Zimmerman *et al.* 1989; Zimmerman 1991). A survey of pollinia removal over 3 months in 1996 at two mainland sites and five island sites (a total of 325 plants) showed that pollinia removal was 37% on mainland sites but only 23% on island sites, vs. 45% in both forest types for 1997. Male reproductive success and fruit set across several years were reduced in forest fragments, which followed theoretical predictions. However, the infrequent El Niño events may significantly influence local population persistence.

Understanding the interaction between mutualistic relationships and forest fragmentation is critical to gauging the impact of landscape level changes on population persistence. My results suggest that populations of *C. viridiflavum* remain interconnected and highlight several ecological factors responsible for these links. Pollinator behaviour is an integral component, as fruit set on islands depends on pollinator visits from mainlands. Additionally, increased fruit set associated with El Niño may be an important factor in long-term population maintenance. Further studies comparing the reproductive ecology of plants in continuous and fragmented systems are required to allow effective prediction of the response of a broader range of species to habitat alteration. Such detailed data are necessary for the establishment of management practices that limit the loss of species of conservation interest. Data for many growth forms (such as trees, epiphytes and herbs), as well as for different mating and breeding systems, will be needed to provide a foundation for understanding the range of plant responses to habitat disturbance. Understanding the patterns of variation in reproductive success among sites and among years, as well as the interconnection among sites and between forest types, is crucial for making sound management decisions.

References

Ackerman, J.D. (1983) Diversity and seasonality of male euglossine bees (Hymenoptera, Apidae) in central Panama. *Ecology*, **64**, 274–283.

Ackerman, J.D. (1986) Coping with the epiphytic existence, pollination strategies. *Selbyana*, **9**, 52–60.

Ackerman, J.D. (1989) Geographic and seasonal variation in fragrance choices for preferences of male euglossine bees. *Biotropica*, **21**, 340–347.

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. (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology*, **75**, 330–351.

Aldrich, P.R. & Hamrick, J.L. (1998) Reproductive dominance of pasture trees in a fragmented forest mosaic. *Science*, **281**, 103–105.

Asquith, N.M., Wright, S.J. & Clauss, M.J. (1997) Does mammal community composition control recruitment in neotropical forests? Evidence from Panama. *Ecology*, **78**, 941–946.

Becker, P., Moure, J.S. & Peralta, F.J.A. (1991) More about euglossine bees in Amazonian forest fragments. *Biotropica*, **23**, 586–591.

Calvo, R.N. & Horovitz, C.C. (1990) Pollinator limitation, cost of reproduction, and fitness in plants: a transition-matrix demographic approach. *American Naturalist*, **136**, 499–516.

Colas, B., Olivieri, I. & Riba, M. (1997) *Centaurea corymbosa*, a cliff-dwelling species tottering on the brink of extinction, a demographic and genetic study. *Proceedings of the National Academy of Science*, **94**, 3471–3476.

Croat, T.B. (1978) *Flora of Barro Colorado Island*. Stanford University Press, Stanford.

Cunningham, S. (2000) Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. *Conservation Biology*, **14**, 758–768.

Dial, R. & Tobin, S.C. (1994) Description of arborist methods for forest canopy access and movement. *Selbyana*, **15**, 24–37.

Dressler, R.L. (1981) *The Orchids, Natural History and Classification*. Harvard University Press, Cambridge.

Dressler, R.L. (1993) *Phylogeny and Classification of the Orchid Family*. Dioscorides Press, Portland.

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**, 217–242.

Gregg, K.B. (1975) The effects of light intensity on sex expression in species of *Cynoches* and *Catasetum* (Orchidaceae). *Selbyana*, **1**, 101–113.

Gregg, K.B. (1978) The interaction of light intensity, plant size, and nutrition on sex expression in *Cynoches* (Orchidaceae). *Selbyana*, **2**, 212–223.

Groom, M.J. (1998) Allee effects limit population viability of an annual plant. *American Naturalist*, **151**, 487–496.

Janzen, D.H., DeVries, P.J., Higgins, M.L. & Kimsey, L.S. (1982) Seasonal and site variation in Costa Rica euglossine bees at chemical baits in lowland deciduous and evergreen forests. *Ecology*, **63**, 66–74.

Jennersten, O. (1988) Pollination of *Dianthus deltoides* (Caryophyllaceae), effects of habitat fragmentation on visitation and seed set. *Conservation Biology*, **2**, 359–366.

Kearns, C.A. & Inouye, D.W. (1993) *Techniques for Pollination Biologists*. University of Colorado, Niwot.

Kearns, C.A. & Inouye, D.W. (1997) Pollinators, flowering plants, and conservation biology, much remains to be learned about pollinators and plants. *Bioscience*, **47**, 297–366.

Kearns, C.A., Inouye, D.W. & Waser, N.M. (1998) Endangered mutualisms, the conservation of plant–pollinator interactions. *Annual Review of Ecology and Systematics*, **29**, 83–112.

Laurence, W.E. & Bierregaard, R.O. (1997) *Tropical Forest Remnants, Ecology, Management and Conservation in Fragmented Communities*. University of Chicago Press, Chicago.

Leigh, E.G. (1999) *Tropical Forest Ecology, a View from Barro Colorado Island*. Oxford University Press, Oxford.

Leigh, E.G.J., Wright, S.J., Herre, E.A. & Putz, F.E. (1993) The decline of tree diversity on newly isolated tropical islands, a test of a null hypothesis and some implications. *Evolutionary Ecology*, **7**, 76–102.

McGarrahan, E. (1997) Much-studied butterfly winks out on Stanford preserve. *Science*, **275**, 479–480.

Murcia, C. (1996) Forest fragmentation and the pollination of Neotropical plants. *Forest Patches in Tropical Landscapes* (eds J. Schelhas & R. Greenberg), 19–36. Island Press, Washington.

Murcia, C. & Feinsinger, P. (1996) Interspecific pollen loss by hummingbirds visiting flower mixtures, effects of floral architecture. *Ecology*, **77**, 550–560.

Murren, C.J. (1999) *Ecological and genetic examinations of reproduction in a tropical epiphytic orchid across a fragmented forest habitat*. PhD dissertation, University of Connecticut, Storrs.

Murren, C.J. & Ellison, A.M. (1996) Effects of habitat, plant size, and floral display on male and female reproductive succes of the Neotropical orchid *Brassavola nodosa*. *Biotropica*, **28**, 30–41.

Nason, J.D. & Hamrick, J.L. (1997) Reproductive and genetic consequences of forest fragmentation - two case studies of neotropical canopy trees. *Journal of Heredity*, **88**, 264–276.

Nason, J.D., Herre, E.A. & Hamrick, J.L. (1998) The breeding structure of a tropical keystone plant resource. *Nature*, **391**, 685–687.

Oostermeijer, J.G.B., den Nijs, J.C.M., Raijmann, L.E.L. & Menken, S.B.L. (1992) Population biology and management of the marsh gentian (*Gentiana pneumonanthe* L.), a rare species in The Netherlands. *Botanical Journal of the Linnean Society*, **108**, 117–130.

Powell, A.H. & Powell, V.N. (1987) Population dynamics of male euglossine bees in Amazonian forests. *Biotropica*, **19**, 176–179.

Rathcke, B.J. & Jules, E.S. (1993) Habitat fragmentation and plant-pollinator interactions. *Current Science*, **65**, 273–277.

Roubik, D.W. (1993) Tropical pollinators in the canopy and understory: field data and theory for stratum 'preferences'. *Journal of Insect Behavior*, **6**, 659–673.

SAS (1989) *SAS/STAT User's guide, Version 6*, 4th edn. SAS institute, Cary.

Saunders, D.A., Hobbs, R.J. & Margules, C.R. (1991) Biological consequences of ecosystem fragmentation, a review. *Conservation Biology*, **5**, 18–32.

Stanley, R.G. & Linskens, H.F. (1974) *Pollen*. Springer-Verlag, New York.

Storfer, A. (1998) Gene flow and endangered species translocations, a topic revisited. *Biological Conservation*, **87**, 173–180.

Wolf, A., Brodmann, P.A. & Harrison, S. (1999) Distribution of the rare serpentine sunflower, *Helianthus exilis* (Asteraceae), the roles of habitat availability, dispersal limitation and species interactions. *Oikos*, **84**, 69–76.

Young, A., Boyle, T. & Brown, T. (1996) The population genetic consequences of habitat fragmentation in plants. *Trends in Ecology and Evolution*, **11**, 413–418.

Zimmerman, J.K. (1991) Ecological correlates of labile sex expression in the orchid *Catasetum viridiflavum*. *Ecology*, **72**, 597–608.

Zimmerman, J.K., Roubik, D.W. & Ackerman, J.D. (1989) Asynchronous phenologies of a neotropical orchid and its euglossine bee pollinator. *Ecology*, **70**, 1192–1195.