Spatial and demographic population genetic structure in *Catasetum viridiflavum* across a human-disturbed habitat

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Abstract

Spatial and temporal genetic structures were examined across sites on islands and mainland (continuous forest) populations of an epiphytic orchid, *Catasetum viridiflavum*, using 17 polymorphic allozyme loci. I tested whether patches on islands or at mainland sites comprised small local populations or a large population. Low among population differentiation was observed across the landscape suggesting that the species-specific pollinator and tiny wind-dispersed seeds maintain interconnections among distant patches. Temporal genetic structure among stage classes, and among breeding individuals are important components of the maintenance of genetic variation in this orchid. The natural history of this species including small breeding populations, probable high frequency of mating among relatives, and the high rates of seed movement among sites contribute to the high F_{IS}. These data show that physically isolated patches in this epiphytic orchid comprise a single larger genetic population, which is independent of the physical distances among sites. Although quite different in ecological and life history characteristics, the genetic structure of this orchid demonstrates a pattern similar to temperate and tropical trees in fragmented landscapes.

Introduction

Physical isolation among patches of plants often reflects genetic isolation as well. This pattern has been observed for many temperate herbs particularly following anthropogenic fragmentation of habitats (e.g. Young *et al*., 1999). However, data from both temperate and tropical trees growing in spatially isolated forested areas indicate that large distances among patches may not translate into comparable high genetic differentiation (Fore *et al*., 1992; Nason & Hamrick, 1997; Aldrich & Hamrick, 1998; Aldrich *et al*., 1998; White, 2002). The ecological and life history characters of a particular species will influence whether physically separate patches are completely genetically isolated, have infrequent gene flow among them, or experience regional panmixia. Whether genetically isolated or completely interconnected, the placement of a species along this continuum is likely to be fluid in time (Knutsen *et al*., 2000), and influenced by the behaviour of pollinators and seed dispersers (e.g. Asquith *et al*., 1997; Nason & Hamrick, 1997; Groom, 1998; Nason *et al*., 1998; Murren, 2002). Investigations of species with distinct ecological and life history strategies, such as tropical trees and epiphytes, are useful to examine whether generalities exist across for multiple members of a forest community.

Most research of isolated patches of plants has focused on patterns of spatial genetic structure. However, temporal differences in genetic structure among age classes or temporally discrete breeding populations of long-lived perennial species have been found to be integral to the maintenance of genetic diversity within native populations (e.g. *Delphinium*, Epling & Lewis, 1952; *Verbascum*, Wells & Wells, 1980; *Trillium*, Kalisz *et al*., 2001). Contrasts of genetic structure of canopy trees, hypothesized to be remnants from prefragmentation populations, to juvenile/seedling populations, presumed to represent

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examples of present day pollen / seed flow has illuminated the influence of landscape level disturbance during this time interval on current genetic structure (Fore *et al*., 1992; Young *et al*., 1993; Aldrich *et al*., 1998). General results from studies of temporal genetic structure indicate that tree species with generalist pollination systems and long-distance seed dispersal have little differentiation among areas in spite of landscape fragmentation via human disturbance. Therefore in these tree species with long-distance dispersal probabilities, physical separation of groups of individuals does not necessarily translate into genetic isolation.

The association between physical and genetic isolation is influenced by the relative roles of gene flow and drift. Hutchinson & Templeton (1999) formulated a method that assesses the relative contributions of gene flow and drift on regional genetic structure. This method examines the relationship between pairwise F_{ST} among patches of individuals and their pairwise geographical distances. Hutchinson & Templeton (1999) present several general cases, which range from regional equilibrium between gene flow and drift, to an increase in importance of one or the other of these evolutionary mechanisms. This approach does not rely on an *a priori* assumption of regional equilibrium, and therefore is particularly appropriate for species in disturbed landscapes where drift is hypothesized to play an important role, as well as being a circumstance where the assumption of equilibrium may not be met.

Here, I examine the spatial and temporal population genetic structure of an epiphytic orchid, *Catasetum viridiflavum*, with a species-specific pollination system. Broadly I ask the question, does physical isolation reflect genetic isolation? Several characteristics of this species and location make it an appropriate system to address this question. *C. viridiflavum* occurs abundantly in the lowland forest surrounding the Panama Canal and like many epiphytic orchids has a single pollinator species, occurs in low densities throughout tropical forests, is pollinator-limited, and has infrequent seedling recruitment and establishment (Schemske, 1980; Ackerman, 1985; Zimmerman & Aide, 1989; Zimmerman, 1991; Murren, 2002). Additionally it has a growth form that allows individuals to be classified into stage classes, an asset for studying temporal genetic structure. Patches of varying numbers of plants occur on a large set of equal aged islands which were created during the construction of the Panama Canal, and in areas of continuous forest (mainlands) surrounding the Canal. These characteristics serve as a comparison for patterns of correlation between physical and genetic isolation documented for tree species with strikingly different life history characters.

I use several lines of evidence to examine the temporal and spatial genetic structure of *C. viridiflavum*, and to determine if patterns are consistent across the landscape. (1) Are island populations less genetically diverse than mainland populations? This pattern may result if individuals from islands comprise local populations where drift plays an important role and if physical isolation results in genetic isolation. This is consistent with some components of the biology of this orchid as number of individuals per island is relatively few, dispersal opportunities are limited (fruit set is uncommon, Zimmerman, 1991; Murren, 2002), and founder events are likely. Alternatively, equivalent genetic diversity values between island and mainland sites would suggest that gene flow maintains allelic richness across sites. (2) Are there differences in population genetic structure between mainland and island sites? Greater differentiation among island sites than among sites within mainland forest would suggest that island sites are either newly founded or relictual. This could be because different islands may be composed of few individuals of distinct genotypes, either as a result of migration into the island, or by the reduction of an existing larger population on each island. Comparable population genetic structures indicate that similar processes are acting in both forest types, and that physical isolation is not sufficiently great to be reflected in the genetic structure of this particular species. (3) Is there a reduction in differentiation among younger stage classes across island and mainland sites? This would be consistent with the hypothesis that gene flow among sites has increased during recent history. Alternatively, patterns of differentiation among stage classes may vary by stage class because of unique sets of parents contribute to the subsequent generations. (4) Using the Hutchinson & Templeton (1999) model, I ask whether gene flow (through pollen and seed) or drift has relatively greater importance in this system. By answering these questions, I will show whether groups of individuals of a long-lived perennial orchid on islands (forest patches) are independent isolated populations or part of a larger population encompassing individuals across the island and mainland sites.

Methods

Study species

Catasetum viridiflavum grows on tree branches and trunks 1–35 m above the ground, and is endemic to central lowland Panama. *C. viridiflavum* is functionally dioecious: each individual can produce either male or female flowers. Individuals may change the sex of the flowers they produce either between sequential inflorescences in the same year or between years (Gregg, 1975; Gregg, 1978; Zimmerman, 1991). A single pseudobulb (a modified stem from which the leaves and inflorescences develop) is produced each year, and the number of pseudobulbs is directly related to the age of each plant (although it is an underestimate of the exact age for the oldest plants, whose older back bulbs may rot and fall off the plant). It flowers from April to December, producing one to nine inflorescences per year. Male racemes have one to 17 flowers that remain open for about 5 days; female inflorescences have fewer flowers (one to six) that

remain open longer (up to 30 days). Individual racemes are maintained on the plants throughout the flowering season, and floral scars are dimorphic, allowing both the gender of each plant and the number of flowers it produced to be assessed at the end of the flowering season (personal observation; Zimmerman, 1991).

Catasetum viridiflavum has a single pollinator, *Eulaema cingulata* (Euglossini, Apidae). Male bees are attracted by the fragrance produced by the flowers, and use these chemicals as precursors to sex pheromones (Dressler, 1981). *E. cingulata* individuals can fly to islands and their abundance at bait traps is as great on islands as it is on mainlands (Murren, 1999). *C. viridiflavum* has tiny wind-dispersed seeds that have been observed to travel distances as great as that between populations especially if plants are high in the canopy (personal observation; and following ballistic seed dispersal models in Murren & Ellison, 1998).

Study sites

During the construction of the Panamá Canal (1910–1914), the flooding of the Chagres River caused the expansion of Lake Gatun (Croat, 1978; Leigh *et al.*, 1993). At this time, numerous islands were created from previously continuously forested regions. In subsequent discussion of the methods, site refers to the sampling unit and is hypothesized *a priori* to be a population. Island sites (from 1.8 to 10 ha in size) were chosen in close proximity to Barro Colorado Island (BCI, Fig. 1). Ten sites had census sizes >20 individuals. Additional islands with <20 individuals (islands F, N, P, De Lessups and T) that were in the sampling area surrounding BCI, were included in the analyses to examine among site differentiation. All islands in this study have been isolated (by water) from nearby forest for 80 years. All sampled *C. viridiflavum* sites were separated from one another by at least 100 m and the majority were separated by >500 m.

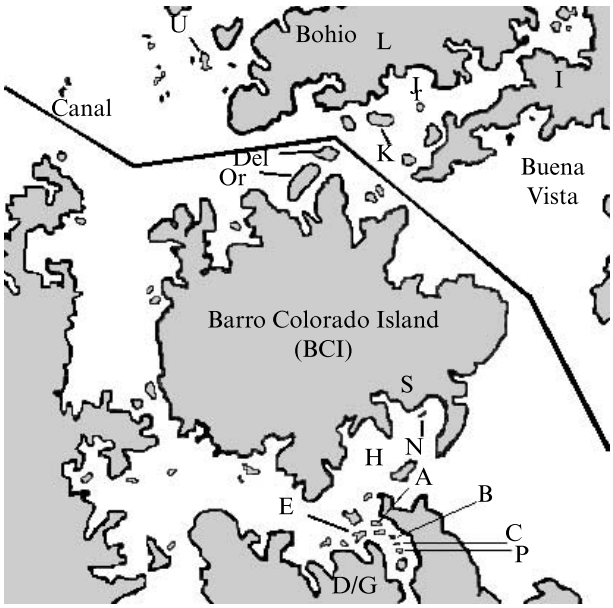

Fig. 1 Map of Barro Colorado National Monument in Panama. Study sites are indicated by letters. The Panama Canal is indicated to the north and east of Barro Colorado Island. Redrawn with permission from Journal of Ecology.

Forested areas surrounding the Canal are protected and act as a buffer zone for erosion control. Six mainland sites were included in the analysis. Bohio (mainland site letter L – Fig. 1) and Buena Vista (mainland site letter I) are probably only 100 years old (J. Denslow pers. comm. 1997), whereas the two sites sampled on Barro Colorado are within older forest (*c.* 350 years age Croat, 1978). Site S is along the southern border of BCI and the other BCI site is near the radio tower at the centre of the island. Two sites were sampled on Gigante Peninsula (site D, with 35 individuals on a single tree, is considered a subpopulation of site G). BCI is grouped with the mainland sites because several previous studies showed that population densities of the euglossine bee pollinator are similar on BCI and nearby mainland forest sites (Parqué National de Soberania, Ackerman, 1983, 1985, 1989), and BCI maintains resident populations of *E. cingulata*. Island sites receive only transient visits by the pollinator (Murren, 1999), thus the distinction between large areas of forest and island sites is equivalent to sites with and without resident bees. I compared the amount and partitioning of genetic variation between island and mainland sites to examine the differences in genetic structure associated with these two forest types.

Between June and August 1996, each of these sites (except Island U, added in early 1997) were chosen and surveyed, and plants were marked. All plants were surveyed for reproductive output in 1996, 1997 and 1998 (see below). Flowering at Island U in 1996 was estimated by counting racemes that were present in the 1997 census on the pseudobulb produced in 1996.

Few, if any, individuals are 80 years old. Most plants had between five and seven pseudobulbs (approximately 5–7 years old). The largest plants across all sites had 17–20 pseudobulbs. The largest and oldest individuals produced the largest number of flowers throughout the flowering season. Female flowering plants are generally larger than those flowering male, and those with the largest number of flowers per inflorescence are most likely to set fruit (Zimmerman, 1991; Murren, 2002). There is a significant relationship between inflorescence size and probability of male reproductive success for plants flowering male. Very few fruit are produced across the entire geographical area in any flowering season although large females may set multiple fruit each year (see Murren, 2002). Therefore, the likelihood of sib mating is high.

Sampling method and analysis

I collected a small leaf sample (approx. 16 cm^2) from all individuals located in each of the study sites. A total of 1442 individuals were sampled, including 300 seedlings collected at nine of the sites (two mainlands and seven islands). I collected samples using arborist climbing techniques (Dial & Tobin, 1994) and pole pruners. Mean sample sizes for the two forest types were similar. However, islands were exhaustively sampled whereas mainlands were subsampled (only one area was exhaustively sampled), thus there is a positive relationship between number of individuals and habitat size.

Samples were kept on ice in the field, and tissues were refrigerated until transported to the STRI Naos Marine / Molecular labs in Panamá City, Panamá, where they were used fresh or flash frozen in liquid nitrogen and stored in an ultracold freezer at −75 °C. A grinding buffer of ethylenediaminetetraacetic acid (EDTA, 0.04 g), sodium metabisulphate (0.08 g), borax (0.2 g), bovine albumin (0.25 g), Dithiothreitol (DTT, 0.025 g), Diethylcarbamazine (DIECA, 0.11 g), nicotinamide adenine dinucleotide phosphate (NADP, 0.0005 g) and nicotinamide adenine dinucleotide trihydrate (NAD 0.001 g) mixed in 25 mL of stock solution of sucrose (0.25 g) and polyvinyl-pyrrolidone [PVP 40 000 (1.75 g) and 360 000 (0.25 g)] (Waycott, 1995) was freshly prepared and adjusted to pH 6.8. Samples were ground either the morning of the gel run or the evening before and wicks (Whatman filter paper no. 1) were stored at −75 °C until loading. The samples were subjected to protein electrophoresis in 11% starch gels using four buffer systems to resolve 23 loci. Genetic interpretations were based on the known cytological distribution and subunit properties of the encoded enzymes (Wendel & Weeden, 1989; Kephart, 1990). EST (Esterase), ACO-1 and ACO-2 (Aconitase), HEX (Hexokinase), IDH-1 and IDH-2 (Isocitrate dehydrogenase) were all monomorphic during the initial pilot study (of over 300 individuals), and were not included in subsequent data analyses, leaving 17 polymorphic putative loci. Buffer systems and sources for each enzyme system are listed in Appendix S1 (see Supplementary material section). Citrate morpholine gels were run at constant 25 mA, and LIB and TC gels were run at 55 mA. All gels were run for 9 h.

Gels were photographed using the Stratagene Eagle-Eye system and electronically stored images of the banding patterns among run dates were compared in ADOBE PHOTOSHOP (Adobe Systems Inc., San Jose, CA, USA). Approximately 10% of individuals were run on multiple gels for crosschecking and comparison among putative alleles.

Genetic structure analyses were performed with the program Genetic Data Analysis (Lewis & Zakin, 1999). This program uses the Weir & Cockerham (1984) method for examining spatial genetic structure, which considers populations as a random sample of all possible populations. The resulting statistics are: f, a measure of the correlation of genes within individuals within populations, corresponding to Wright's F_{IS}; F, the inbreeding coefficient for the correlation of genes across all populations, corresponding to Wright's F_{IT}, and θ_p, the differentiation among populations, corresponding to F_{ST} (Weir & Cockerham, 1984). Following directly from Wright's framework additional hierarchical levels can be defined to examine differentiation among subpopulations expressed as θ_s (Hartl & Clark, 1989). In GDA, θ_s is equal to $(1-F_{sp})(1-F_{pt})$ (P. Lewis, personal communication). Bootstrapping across loci was used to estimate 95% confidence intervals for all parameter estimates.

I use the Hutchinson & Templeton (1999) model to examine the relative importance of gene flow and drift in this system. Pairwise θ_p's were calculated using FSTAT version 2.9 (Goudet, 1995; Goudet, 1999). Pairwise geographical distances were measured for all sites from topographical maps and rounded to the nearest 10 m. Matrixes of genetic and geographical distance were compared using a Mantel test (NTSYSpc version 2.0).

Because most previous measures of genetic structure have reported Nei's parameter G_{ST}, I also calculated G_{ST} (GENESTAT for PC- version 3.31, Lewis & Whitkus, 1989) for all 20 sites. G_{ST} should approach the value of θ_p as the number of sites sampled increases (see Weir, 1996, for a detailed discussion of the differences).

Ecological data

In the field, all individuals were categorized into one of five stage classes. A single pseudobulb is produced each year, thus I used the number of pseudobulbs as a proxy for age: stage class 1, 1997 seedlings; stage class 2, two to four pseudobulbs / years old; stage class 3, five to seven pseudobulbs / years old; stage class 4, eight to10 pseudobulbs / years old, and stage class 5, >10 pseudobulbs / years old. I consider individuals in stage classes 2–5 to be adults. All individual plants were censused in 1996, 1997 and 1998, and incidence and frequency of flowering, and gender of inflorescences were recorded over the entire flowering season.

Comparing island and mainland genetic variation and genetic structure

I used P (the proportion of polymorphic loci), A_p (the mean number of alleles for a polymorphic locus), H_e (the expected average heterozygosity based on Hardy–Weinberg equilibrium), and H_o (the actual observed heterozygosity) as measures of genetic diversity. Island and mainland sites were contrasted. Separate analyses of genetic structure were examined for mainland sites alone and island sites alone. 95% confidence intervals were compared for the measures of genetic differentiation among sites within a forest type in order to determine whether the measures of θ_p were statistically different. A final test to examine temporal variation in

genetic structure was to construct a model that examined one stage class at a time; all sites were included in this analysis. Again, 95% bootstrapped confidence intervals were compared between each pair of stage classes to determine if measures of θ_p were statistically different.

Testing for wahlund effects

A Wahlund effect refers to the deficiency of heterozygotes (relative to Hardy–Weinberg expectations) produced by including individuals from two or more subpopulations in what is assumed to be a sample from a single population (Hartl & Clark, 1989). A Wahlund effect can result from combining either spatially isolated subpopulations within which random mating occurs, or temporally isolated breeding populations. If a species is unable to self-fertilize and has a large inbreeding coefficient within populations (F_{IS}), it is natural to ask whether the excess of homozygotes might be the result of combining samples from spatially or temporally isolated subpopulations with different allele frequencies (found in plants Ingvarsson & Giles, 1999; invertebrates Wilson *et al*., 1999).

There are two expectations for the data presented here if a temporal Wahlund effect contributes significantly to F_{IS}. First, I expect the within population inbreeding coefficient (f) to be smaller in young age classes than in older ones, because fewer age classes are included in earlier stages than in late stages. Therefore, in the seedling stage class fewer subpopulations (here temporal populations) are considered part of a single population. To address this prediction, I conducted analyses of differentiation (θ_p) by stage class and examined the correlation of genes within individuals within them (f). A decrease in f from oldest to youngest stage class was tested with a Wilcoxon signed-rank test. Secondly, there may be detectable differences among the allele frequencies of individuals that are reproductive in different years, leading to a temporal Wahlund effect. A deficiency of heterozygotes could result from statistically lumping subpopulations that were reproductive in different years into a single population. To address this possibility, I conducted an analysis that considered both temporal differences in allele frequencies in the breeding populations and spatial differentiation, and examined if these could account for the observed pattern. A hierarchical model can account for additional differences among subpopulations within a population (θ_s) or among subsub-populations within a subpopulation (θ_{ss}). Through a hierarchical analysis of genetic differentiation, flowering and nonflowering individuals were contrasted, and these contrasts were examined across 3 years, with the spatial differentiation among sites taken into account. This model is analogous to a nested analysis of variance. If temporal differences in allele frequency contribute significantly to inbreeding within populations, extracting the effects of those differences should lead to substantially reduced estimates of f.

Results

Allozyme diversity

Overall, high levels of allelic diversity were obtained (Appendix S2, see Supplementary material section), with a total of 94 alleles detected for the 17 polymorphic loci. Only four alleles were found exclusively at one site. All sites had uniformly high levels of polymorphism ($P = 0.71$–1.00) averaged across polymorphic loci (Table 1). This is in part because samples included in this study were prescreened only for loci that had already been identified as polymorphic in a pilot survey. These data demonstrate that islands or mainland sites with *C. viridiflavum* were polymorphic for roughly the same set of loci. One may expect *a priori* that mainland sites would have more genetic variation than islands if islands were remnant populations. However, island and mainland sites did not differ significantly in levels of polymorphism (Table 1). Individual locus polymorphisms are presented in Appendix B.

Spatial genetic structure

Site differentiation

In a model treating each of the sites separately, there was very little differentiation among adults (for sites where number of individuals censused were >20; Table 2), as indicated by the low θ_p. In spite of the small magnitude of θ_p, its 95% confidence interval (derived from bootstrapping) did not overlap zero, indicating that there were detectable allele frequency differences among sites. This pattern held for analyses that included sites with few individuals – field census size <20 where $\theta_p = 0.06$, that included seedlings where $\theta_p = 0.07$, or that included stage classes nested within sites in a hierarchical analysis where $\theta_p = 0.08$. Moreover, the values from these alternative analyses were statistically indistinguishable from each other, and mean values of f did not differ when stage classes or sites with few individuals were included in more complex models. f was high (0.58) and was significantly different from zero.

There was little differentiation among mainland sites ($\theta_p = 0.08$; Table 2). Excluding the two mainland sites with secondary forest (L and I) did not alter the mean values for any of the parameter estimates. Genetic structure was similar for island and mainland forest types: differentiation among island sites ($\theta_p = 0.06$) was not significantly different than among mainland sites, nor did inbreeding coefficients differ (based on 95% confidence intervals, Table 2). In a test of the association between the degree of differentiation among populations and their geographical distance, I found no relationship

Table 1 Genetic variation in island and mainland sites.

Population	Forest type	*n*	*P*	A_p	H_e	H_o
A	Island	33.7	0.88	3.26	0.30	0.15
B	Island	48.7	0.88	3.73	0.27	0.11
C	Island	67.4	1.00	3.88	0.35	0.15
DEL	Island	12.9	0.63	3.00	0.29	0.19
E	Island	35.8	0.76	3.77	0.33	0.13
H	Island	79.2	1.00	3.82	0.32	0.13
J	Island	37.8	0.88	3.53	0.28	0.11
K	Island	94.0	1.00	4.35	0.32	0.13
OR	Island	41.0	0.94	4.00	0.35	0.15
U	Island	168.5	0.88	5.00	0.35	0.14
Mean	Island	61.9	0.88	3.83	0.32	0.14
(±SD)		(44.5)	(0.12)	(0.56)	(0.03)	(0.02)
BCI – radio tower	Mainland	12.9	0.71	2.91	0.24	0.12
S – BCI	Mainland	56.9	1.00	3.94	0.34	0.16
D – Gigante	Mainland	26.1	0.76	3.08	0.27	0.17
G – Gigante	Mainland	15.2	0.65	2.73	0.25	0.11
I – Bohio	Mainland	38.7	0.82	3.71	0.28	0.09
L – Buena Vista	Mainland	69.8	0.88	4.33	0.34	0.13
Mean	Mainland	36.6	0.80	3.45	0.29	0.13
(±SD)		(47.1)	(0.26)	(1.27)	(0.10)	(0.04)
Grand Mean		52.4	0.86	3.69	0.31	0.14
(±SD)		(39.0)	(0.12)	(0.59)	(0.04)	(0.03)

Populations follow Fig. 1. *n* is the average number of samples per locus. *P* is the proportion of polymorphic loci. A_p is the mean number of alleles for a polymorphic locus. H_e is the expected average heterozygosity based on Hardy–Weinberg equilibrium, and H_o is the actual observed heterozygosity.

Table 2 Adult populations: genetic structure using all adult individuals (stage classes 2–5) from all island and mainland sites. There was very little differentiation among sites, with a high level of inbreeding within sites. Number of individuals examined was 1122. Islands and Mainlands: comparison of population genetic structure of island (post-fragmentation) and mainland (prefragmentation) sites. Only island sites that had >20 individuals were included in this analysis. There were no apparent effects of fragmentation.

	f	*F*	θ_p
Adult populations	0.58	0.61	0.06
	(0.42–0.75)	(0.45–0.77)	(0.04–0.09)
Islands	0.60	0.62	0.06
	(0.42–0.76)	(0.46–0.77)	(0.03–0.09)
Mainlands	0.57	0.61	0.08
	(0.41–0.75)	(0.44–0.77)	(0.03–0.13)

95% CI are in parentheses.

between pairwise F_{st} (θ_p) and physical distance between sites (Fig. 2; Mantel $r^2 = -0.035$; $P = 0.39$). This result held whether mainland and island sites were analysed separately or together.

Stage class differentiation

There were two objectives for examination of stage-class differentiation: (1) to examine if there was significant variation in genetic structure among stage classes, and (2) to examine whether the relationship between θ_p and *f* changes in a systematic way among age classes (indicative of a temporal Wahlund). Values of θ_p decreased monotonically from seedlings / stage class one (0.098) to stage class five (0.04; Table 3). Although stage classes 1 and 5 had overlapping 95% confidence intervals, this is a very conservative test (Weir & Cockerham, 1984), and the monotonic decrease suggests a trend towards differences between these age classes (Wilcoxon signed-rank test $Z = -2.02$; $P = 0.04$). There was no similar trend for *f* (inbreeding within populations): *f* ranged from 0.50 in seedlings to 0.63 in stage class 2 individuals. *f*-values were all significantly different from zero and 95% confidence intervals of all stage classes overlapped. Patterns of genetic structure were similar whether all data were included or only the sites which had representatives from all of the five stage classes.

Temporal changes in genetic structure

Individuals in this study were of varied reproductive status. If allele frequencies among reproductive individuals differ substantially among years, the inclusion of all individuals in a single sample could result in a large apparent deficiency of heterozygotes as a result of a temporal Wahlund effect. To test for a temporal Wahlund effect, I used a hierarchical *F*-statistic approach with three levels: reproductive vs. nonreproductive individuals, year, and site. The results from this analysis indicated that overall inbreeding coefficient was large ($f = 0.589$, 95% CI = 0.44–0.72). Both reproductive status

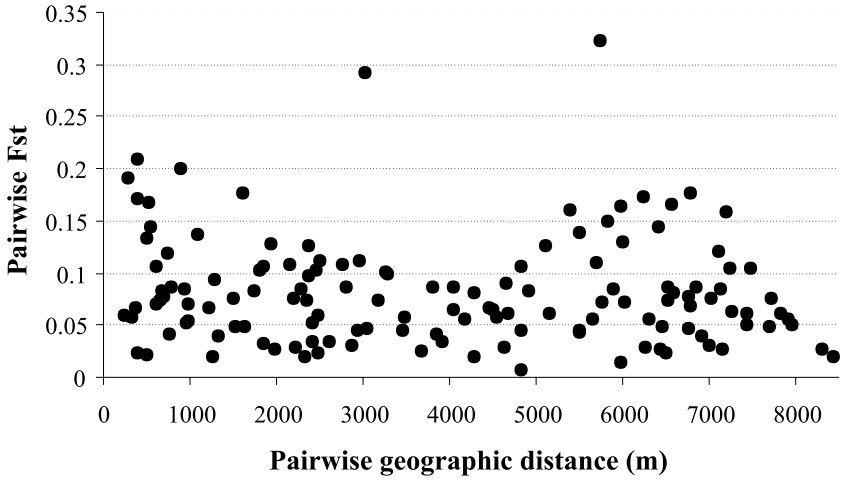

Fig. 2 Relationship between geographical distance (in metres) and pairwise F_{st} among local populations. There was no relationship between distance and pairwise F_{st} suggesting that gene flow and drift were equally important in this system.

Table 3 Temporal population genetic structure: data for the five stage classes present in existing populations. Mean and 95% confidence intervals are presented. Members of stage class 1 are 1997 seedlings. Stage class 2 has two to four pseudobulbs. Stage class 3 has five to seven pseudobulbs. Stage class 4 has eight to 10 pseudobulbs. Stage class 5 has >10 pseudobulbs. One pseudobulb is produced per year (see text of methods for details).

Stage Class	*f*	*F*	θ_p
1 (Seedlings)	0.44	0.50	0.10
	(0.26–0.68)	(0.29–0.74)	(0.05–0.17)
2	0.60	0.63	0.09
	(0.44–0.76)	(0.47–0.79)	(0.04–0.15)
3	0.59	0.62	0.08
	(0.42–0.76)	(0.46–0.79)	(0.06–0.12)
4	0.55	0.58	0.06
	(0.39–0.73)	(0.41–0.75)	(0.04–0.09)
5	0.60	0.61	0.04
	(0.43–0.78)	(0.46–0.79)	(0.001–0.10)

($\theta_{ss} = 0.064$, 95% CI = 0.06–0.11) and year of reproduction ($\theta_s = 0.058$, 95% CI = 0.04–0.08) had significant effects on genetic structure. The effects of both were of magnitude similar to the among-site differences ($\theta_p = 0.077$, 95% C.I. = 0.05–0.09).

Discussion

Combined evidence from measures of genetic diversity, similarities of genetic structure between island and mainland sites, and stage class analyses suggest that the sites examined in the Barro Colorado National Monument comprise a single large population of the orchid, *C. viridiflavum*. Island and mainland sites shared similar patterns of within and among site genetic diversity regardless of physical distance. The overall pattern of genetic structure (differentiation among sites on the order of 6–8%) is consistent with the life history characteristics of this orchid.

The low value of θ_p (0.06) or G_{st} (0.10) indicated there was very little genetic differentiation among the sites with *C. viridiflavum*. This level of differentiation is within the range reported for wind dispersed, long-lived perennial, and endemic species (G_{ST} range: 0.09–0.26: Hamrick & Godt, 1996), and is consistent with the mean reported for 16 other species of Orchidaceae ($G_{ST} = 0.087$: Hamrick & Godt, 1996) and the only published measures for epiphytic orchid species (Ackerman, 1998; Ackerman & Ward, 1999). Hamrick & Godt (1996) attribute the generally low values found to the species-specific relationship of orchids with their pollinators. The low level of differentiation among *Catasetum* sites is likely influenced by the joint effects of long distance pollinator / pollen movement and the potentially long dispersal distances of the tiny wind dispersed seeds of orchids (Murren & Ellison, 1998; Murren, 1999; Murren, 2002). The tiny seeds likely traverse distances of 100 m to >1 km depending on the height of seed release and local wind speed (estimates are based on a ballistic seed dispersal model; see Results in Murren & Ellison, 1998). The small number of reproductively successful individuals per year, and the greatest reproductive success in the largest / oldest stage class translates into a likelihood that multiple individuals within a stage class are half or full sibs (Murren, 2002). In turn, this results in potential mating among relatives, even if mates are physically distant from one another. Among year genetic differences in sets of reproductive individuals corroborate these observations, as well as parallel evidence published for other long-lived perennial species (Epling & Lewis, 1952; Wells & Wells, 1980).

Across a landscape of physically isolated patches of orchids, population genetic structure of this species-specific pollinated orchid resembles the pattern of interconnection among individuals in forest fragments of temperate and tropical trees with generalist or specific pollination systems (e.g. Young *et al.*, 1993; Nason & Hamrick, 1997), rather than the high differentiation

among populations in isolated sites of temperate herbs with generalist pollination syndromes (e.g. Oostermeijer *et al*., 1995; Young *et al*., 1999). For *C. viridiflavum*, temporal genetic structure corroborates the spatial patterns. Genetic structure is statistically indistinguishable across stage classes. However, there is an apparent trend that the largest / oldest stage class was less differentiated than seedling classes, suggesting temporal variation in patterns of gene flow. Other studies with limited number of years since landscape disturbance have ascribed such differences between stage classes to fragmentation [e.g. Fore (1991) who suggested that seed dispersal of *Acer saccharum* into sites is associated with edge affects and changes of wind patterns following fragmentation]. However, in *C. viridiflavum* I consider the pattern to be attributed to natural variation in reproductive success of a limited number of adult individuals among years. In a single year, a large number of seedlings across sites may be offspring of an extremely limited subset of individuals, whereas older larger stage classes are comprised of multiple reproductive bouts.

I found a high level of inbreeding (f) combined with the low among site differentiation. A plausible explanation for a pattern of low among site differentiation and high inbreeding is a Wahlund effect – where two subpopulations are statistically grouped as a single population resulting in reduced observed heterozygosity compared with expectations (e.g. Falniowski *et al*., 1999; Wilson *et al*., 1999). I examined whether breeding groups across years represented temporally distinct populations. Allele frequency differences between reproductive and nonreproductive individuals, were statistically detectable, however, were not large enough in magnitude to support the hypothesis of a temporal Wahlund effect defined in this way. A second test for a temporal Wahlund effect examined if there was a detectable decrease in the f from oldest to youngest stage class. I found variable values of f or θ_p across stage classes, lending no support to a temporal Wahlund effect when analysed in this manner.

There are both spatial and temporal factors that contribute to the high f in *C. viridiflavum*. Extremely localized populations can contribute to a spatial Wahlund effect as found by Ingvarsson & Giles (1999). An analysis of localized structure in *C. virdiflavum,* examining data from two islands for which detailed spatial information was available, lent some support for the presence of substructure within islands, although this factor did not entirely account for the high f. Spatial substructure in this species may in fact reflect patches established by a few families across the geographical boundaries of island or mainland sites. This would give rise to subpopulations that occur in portions of two or more island or mainland sites. This view of the substructure is consistent with the long-distance seed movement probability of the tiny seeds, and the low number of females that produce seed in a given year. In addition to the spatial substructure, temporal breeding groups comprising of relatives that may be physically distant from one another also may influence the patterns of genetic structure. Together these spatial and temporal factors contribute to the patterns observed here (described in general terms in Kaj & Lascoux, 1999). Additional detailed spatial data within the numerous island and mainland sites and encompassing multiple years of flowering and paternity data are beyond the scope of the present study, but are warranted to pinpoint in detail the relative contributions of these factors to the high f.

Taking a regional view, both drift and gene flow play important roles in defining population genetic structure. Hutchinson & Templeton (1999) constructed a method of detecting the relative importance of gene flow and drift based on the relationship between pairwise F_{st} and geographical distance. Their expectation is that a positive linear relationship between pairwise F_{st} and geographical distance is a result of regional equilibrium of gene flow and drift. In the orchid system, I did not find a linear relationship between pairwise F_{st} and geographical distance (Fig. 2). The lack of positive relationship could be a result of the spatial scale examined in relation to the dispersal distance of pollen and seed of this orchid. Therefore, mean per generation dispersal distances may be sufficiently great that isolation by distance could be detected at a geographical scale different from that of this study. Alternatively, the sites in this study may have been recently founded as random samples from a single, large source population, implicating the joint importance of gene flow and drift in this system. The relationship between pairwise F_{st} and physical distance is consistent with the hierarchical F statistics.

General conclusions

I found that there is little genetic differentiation among physically distant patches of *C. viridiflavum*. Additionally, the high inbreeding coefficient is consistent with the life history of this orchid, with frequent sibling matings and long-distance seed movement, and an understanding of this pattern warrants further spatial data collection from the field. Genetic data in other species have also been interpreted as indicating an interdependence of individuals located in physically distinct patches, connection of isolated trees with nearby patches, and connection of individuals in forest fragments to others in continuous forested regions (e.g. Nason *et al*., 1996; Young *et al*., 1996; Nason & Hamrick, 1997; Aldrich & Hamrick, 1998; Aldrich *et al*., 1998; White, 2002). The species that have been studied so far may, in fact, be those which are capable of maintaining populations in human altered landscapes. Their persistence may be due primarily to their ecological and life history characters that allow for continued reproduction and seed dispersal across a wide geographical scale. Species that have gone locally extinct, or occur in very small populations are less likely to have been the object of study. The species at risk likely have ecological

and life history attributes distinct from this orchid, or following more extreme forms of fragmentation.

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Supplementary material

The following material is available from http://www.backwellpublishing.com/products/journals/suppmat/JEB/JEB517/JEB517sm.htm

Appendix S1

Appendix S2

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