Do epiphytic orchids behave as metapopulations? Evidence from colonization, extinction rates and asynchronous population dynamics

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ABSTRACT

Previous in situ studies of orchid population dynamics with conservation relevance have focused on one or a few populations in a limited area. Many species of orchids occur as hyper-dispersed populations in ephemeral habitats (epiphytic, twig epiphytes, short lived or vulnerable host). In this contribution, we show that orchid populations that are patchily distributed and that exist in disturbance-prone environments may act somewhat like a metapopulation with high turnover and low correlation in population dynamics. We tested for evidence of metapopulation dynamics in the riparian orchid *Lepanthes rupestris* by sampling over 1000 sites (250 initially occupied, 750 initially unoccupied) in biannual surveys for 5 years. Extinction and colonization of groups of orchids on a single substrate or patch (either trees or boulders) was common and more or less consistent across different time periods, and asynchronous subpopulation dynamics were evident among the populations. From this we predict non-zero equilibrium values for site occupancy ($\hat{P}$) of *L. rupestris*. Nevertheless, this study species differed from a typical Levins' metapopulation system in that small populations were more likely to go extinct than large populations, and that colonization of previously occupied sites was more common than colonization of initially unoccupied sites suggesting that site quality may influence population persistence and colonization. A major difficulty applying the metapopulation approach to orchid conservation is identifying empty sites suitable for colonization. In spite of this limitation, our study highlights the necessity of following multiple orchid subpopulations (e.g., an entire orchid "metapopulation" in the broad sense) may provide a more accurate basis for predicting persistence in epiphytic orchids.

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1. Introduction

One of the main objectives of conservation biology is to predict the probability of population persistence based on estimates of reproduction and mortality. While many studies have traditionally focused on one, or at most several populations, investigations of groups of interconnected populations—or metapopulations—have rapidly gained prominence, resulting

in a paradigm shift in studies of natural systems and for developing conservation and management strategies for many species (Levins, 1970; Gilpin and Hanski, 1991; Hanski and Gilpin, 1997; Hanski, 1999; Thrall et al., 2000; Hanski and Gaggiotti, 2004a,b). Metapopulations are defined as a set of subpopulations with asynchronous local dynamics occupying discrete patches (Hanski, 1999; Hanski and Gaggiotti, 2004a). In addition, metapopulation persistence requires equilibrium between extinction of populations in occupied patches and colonization of unoccupied patches across the landscape (Levins, 1970). Conservation strategies for species that demonstrate asynchronous local dynamics of extinction and colonization of patches must include the identification of the network of populations in suitable patches—the metapopulation—rather than individual population units.

Studies of metapopulation dynamics in plants are limited (for review see, Eriksson, 1996; Husband and Barrett, 1996; Ouborg and Eriksson, 2004; Freckleton and Watkinson, 2002) but a number of characteristics suggest that a metapopulation approach may be appropriate to understand epiphytic species (Snäll et al., 2003, 2004a,b, 2005a,b) and orchid population dynamics. For example, many orchid species have an epiphytic or lithophytic habit that predisposes them to a discrete or patchy distribution (Ackerman, 1995; Tremblay, 1997a). Epiphytic orchids inhabit ephemeral habitats due to limited life spans of their substrates (i.e., host trees) or the natural process of succession of epiphytic flora on hosts (Catling et al., 1986). Many orchid species also exist as genetically subdivided populations regardless of their habit (Tremblay and Ackerman, 2003; and references therein). In addition, a number of studies have shown that patches of orchids may be prone to extinction (Tamm, 1991; Vanhecke, 1991; Wells and Cox, 1989) because of small population size, skewed life span and stochastic reproductive success (Tremblay, 1997a; Willems, 2002; Tremblay and Hutchings, 2003). In light of these observations, traditional population dynamic models (e.g., life size/stage-based models) may not be the most efficient tools for predicting the long-term population dynamics and equilibrium state of orchids. Most studies on orchid population dynamics have been restricted to terrestrial species (for a review, see Kull, 2002), and few have been carried out on epiphytic and lithophytic species (Zotz and Tyree, 1996; Zotz, 1998, 1999, 2000; Tremblay, 1997c; Tremblay and Hutchings, 2003). Simulations of demographic stochasticity focusing on individual populations predict rapid population extinction for a number of epiphytic species even if mean population growth rates suggest stability (Holsinger, 2000; Tremblay and Hutchings, 2003; Tremblay et al., 2005) perhaps due to short-term occupancy of suitable habitat patches and stochastic recruitment. Gene flow between populations is limited for most species of orchid (Tremblay and Ackerman, 2003) and effective population size as well as observed number of individuals in patch/population is often small (Reddoch and Reddoch, 1997; Tremblay and Ackerman, 2001). Furthermore, many epiphytic orchids have skewed distributions of reproductive success (with considerably low averages) mediated in part by pollinator limitation and short life spans (Calvo and Horvitz, 1990; Calvo, 1993; Tremblay, 2000; Tremblay et al., 2005). Consequently the metapopulation paradigm may prove useful for predict-

ing population dynamics and persistence for some species of orchids.

Recognition of metapopulation structure in orchids would have important implications for the development of management and conservation strategies for genera such as *Lepanthes* within the Caribbean region, an important 'biodiversity hotspot' (Myers et al., 2000) where all except three of the 92 recorded species of *Lepanthes* are limited to one island as well as restricted to small areas within each of the islands.

In this study, we explore the utility of metapopulation theory, specifically the applicability of Levins' model (Levins, 1970) for the study of orchid population dynamics using a species of the genus *Lepanthes* as a model system. *Lepanthes* is a species-rich Neotropical genus of epiphytic and lithophytic orchids (~600 species) that shows high levels of endemicity (Luer, 1986). Within the last decade, a number of studies of Caribbean *Lepanthes* have suggested that populations within this genus may have a metapopulation structure. There are several reasons for this. First, all species are epiphytic or lithophytic, and as a result, many show clumped distribution patterns (Ackerman, 1995; Tremblay, 1997a). Second, population sizes on any particular substrate or rock surface are small and spatially separated, and gene flow between such populations is restricted (Tremblay and Ackerman, 2001). Last, life-stage based models suggest that individual populations may be highly prone to extinction (Tremblay and Hutchings, 2003).

To determine whether or not populations of *Lepanthes* demonstrate the characteristic hallmarks of traditional metapopulations we initiated a long-term study into the dynamics of *L. rupestris* in 1999. In this paper, we summarized the patterns of patch occupancy and estimated patch extinction and colonization probabilities directly from our observations on patch occupancy changes over 5 years of biannual surveys. We then compared the predicted equilibrium patch occupancy according to the classic metapopulation model (Levins, 1969, 1970) with observed patch occupancy. We specifically addressed the following questions: (1) Is there colonization of unoccupied habitats, and if so, at what rate? (2) What is the rate of patch extinction? (3) Are the extinction and colonization dynamics substrate-dependent? (4) Is there asynchrony in these parameters between individual subpopulations? (5) Is the likelihood of patch extinction affected by initial population size? (6) What is the predicted patch occupancy based on the classic metapopulation model?

2. Materials and methods

2.1. Plant species

Lepanthes rupestris Stimson is a miniature orchid endemic to Puerto Rico that is commonly found along the riverbeds of the northwestern slopes of the Luquillo Mountains (Ackerman, 1995; Tremblay, 1997a). Plants are epiphytes and lithophytes (i.e., they anchor roots to the surfaces of trees or rocky boulders) and exhibit a maximum shoot height of 15 cm (Ackerman, 1995). *Lepanthes rupestris* stems are slender and erect with a solitary leaf 1.3–4.3 cm long, and one to few subtending inflorescences capable of producing many

flowers. Flowers are minute (<6 mm) and highly variable in colouration and morphology (Tremblay, 1997b). This species is functionally protandrous and self-incompatible (Tremblay, unpublished). While the pollinator of *L. rupestris* is unknown, elsewhere *Lepanthes* orchids are visited by black-winged fungus gnats (Blanco and Barboza, 2005). Mean plant life span is 3.4 years with a high variance, mainly as a result of differences in survivorship between seedling, juvenile and adult stages (Tremblay, 2000).

2.2.   Study site

The study was carried out along a 1000 m section of Quebrada Sonadora in the Luquillo Experimental Forest (LEF; USDA Forest Service, Fig. 1). Sonadora is a steep first order tributary of the Espíritu Santo River, northeastern Puerto Rico, latitude 18°18′N, longitude 65°47′W. The section studied is between 400 and 500 m in elevation and runs through an area of secondary mature 'Tabonuco' forest dominated by the tree species *Dacryodes excelsa* (Waide and Reagan, 1996). Average annual precipitation is 3600 mm and somewhat seasonal, with the period between May and December generally having greater monthly average rainfall than that between January and April (Brown et al., 1983; McDowell and Estrada Pinto, 1988). Water discharge in these streams is highly variable and closely follows precipitation events (Johnson et al., 1998).

2.3.   Patch selection and sampling

As in other *Lepanthes*, plants are patchily distributed, with small groups of individuals (or populations) scattered among individual boulders and trees (Tremblay, 1997a). Mean population size is 50 individuals but population size distribution is strongly skewed, with a median size of 26 individuals (Tremblay, 1997a). Mean inter-population distance is approximately 5 m (Tremblay, 1997a). Analyses on the population genetic structure of *L. rupestris* at LEF using allozymes suggests that gene flow among groups of individuals is severely limited even at scales of <1 m (Tremblay and Ackerman, 2003). Based on the dispersal behavior of *L. rupestris*, we then defined a patch as substrate area (boulder or tree) available for orchid establishment. All patches (regardless of substrate) where *L. rupestris* occurred along the focal Sonadora section were tagged with permanent metal tags in June and July 1999 (survey 0). A total of 250 occupied patches were identified and their substrate (tree or boulder) was recorded. These occupied patches are here after referred to as "initially occupied" and correspond to sites with orchids found in the initial survey at time zero ("survey 0"). A patch was considered occupied if it had at least one living orchid. For each occupied patch, we selected and tagged patches with no visible seedling, juvenile or adults present (hereafter "initially unoccupied"), and of the same substrate and appearance as the occupied patch. Initially unoccupied patches were tagged at approximately 1,
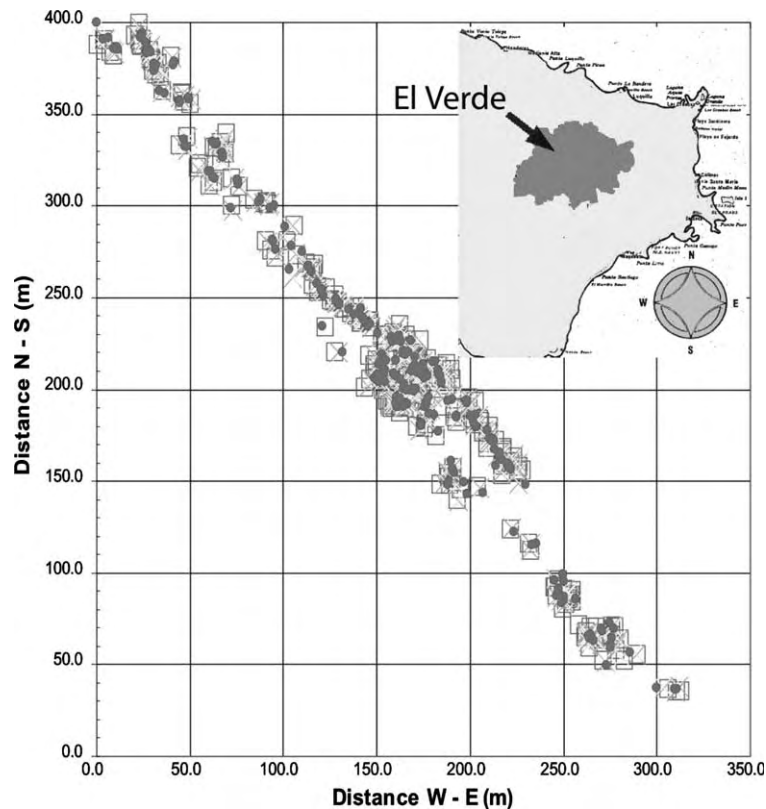

Fig. 1 – Map of distribution of *Lepanthes rupestris* patches along a 500 m section of Quebrada Sonadora. Filled circles are initially occupied patches located on boulders and trees, respectively; fainter crosses and square symbols represent originally unoccupied patches. Inset shows location of El Verde Field Station study area in the Caribbean National Forest (gray) in Puerto Rico.

2 and 5 m (or as close as possible to these distances from their focal occupied patch) for a total of 750 potential colonization sites. The only consideration was distance from an occupied patch. At each pre-selected distance, unoccupied sites were chosen at random within a 360° circumference set with no a priori biases for shape, distance from forest or river or other ecological attributes (i.e., moss cover, or presence of other species of plants). Of the 250 initially occupied patches, 165 were on boulders and 85 were on trees. Initially unoccupied patches followed a similar relative distribution with 495 on boulders and 255 on trees. In the summer of 2003, all patches were mapped to within approximately 10 cm relative (x, y, z position) using rigid metal rules, 0.5° sighting compass and 0.5° clinometers to the centre of all patches (Fig. 1). All 1000 patches were monitored for presence or absence of *L. rupestris* individuals twice a year at 6 months intervals for a total period of 5 years (1999–2003). Surveys two and three were only 2 months apart. Thus, for consistency, survey three was excluded from all analyses. On each census date and for each patch, we recorded its occupancy status (occupied or unoccupied) and the number of individuals in the following life cycle stages: seedlings (n_s, small plants without a developed petiole on any leaf), juveniles (n_j, individuals with at least one lepanthiform sheath on the petiole and no evidence of current or past inflorescence production) and adults (n_a, included non-reproducing adults which were individuals that were not flowering at the time but carried dry inflorescences from a previous flowering event, small reproducing adults which were individuals with one photosynthesizing (green) inflorescence with or without open flowers or fruits and large reproductive adults which constituted individuals with two or more inflorescence). We then added the number of individuals of all life cycle stages to calculate the total population size (N_t) for each patch.

2.4.   Probability of extinction and colonization

We estimated the probability of population extinction and colonization in occupied and unoccupied patches directly from the presence and absence observations. To reduce the likelihood of error in identifying an extinction or colonization event due to errors in data collecting, we inspected the orchid counts for each patch for each survey before reducing the data to "occupied" and "unoccupied" patches. Biologically unfeasible growth rates based on detailed analyses (Tremblay, unpublished) were used to identify populations that were miscoded as absent for the time period (<0.1%). Total population size data were also checked for obvious errors (e.g., particularly high numbers of reproductive adults after complete absence of orchids at the previous survey). In general, we found few populations with unfeasible rates of growth. One potential source of error was the misidentification of *L. rupestris* individuals and other sympatric (but less common) *Lepanthes* species. This source of error could lead to the sudden appearance of reproductive adults or recruits at an otherwise non-*L. rupestris* patch. Careful attention was paid to subpopulations that had more than one species of *Lepanthes*, where misidentification could have lead to erroneous extinction/colonization observations. All these patches were omitted from the analysis. Thus for any given survey, the data on observed

total population size (sum of seedlings, juveniles and three classes of adults) was used to code a site as occupied ("1") if greater than zero, unoccupied ("0") if total population size equaled zero, and "not applicable" ("NA") if the tagged patch was not found for that survey. These survey data were then used to classify patches into one of four possible transition states based on their occupancy status at time $t − 1$ and t. Thus, a previously occupied patch in which a population went extinct was coded as "$1 \rightarrow 0$", a previously unoccupied patch that became colonized was coded as "$0 \rightarrow 1$", an occupied patch that stayed occupied was coded as "$1 \rightarrow 1$", and an unoccupied patch that stayed unoccupied was coded as "$0 \rightarrow 0$". State changes were unknown for patches coded as NA at either $t − 1$ or t (Appendix 1). We then used this information to calculate the probabilities of colonization and extinction between survey dates.

We calculated the probabilities of colonization Pr(C) and extinction Pr(E) as the proportion of those colonization and extinction events occurring from time $t − 1$ to time t divided by the total number of populations available for colonization (i.e., unoccupied) or extinction (i.e., occupied), respectively, at time $t − 1$. Specifically, Pr(C) was calculated as the number of unoccupied patches at time $t − 1$ that contained newly recruited orchids at time t (or "$0 \rightarrow 1$" patches) divided by the number of patches available for colonization (i.e., all unoccupied) at time $t − 1$. Similarly, Pr(E) was calculated as the number of occupied patches at time $t − 1$ that contained no living orchids by time t ("$1 \rightarrow 0$" events) divided by the number of occupied patches at $t − 1$.

2.5.   A test of Levins' model for colonization and extinction probabilities

We tested the assumption of Levins' model of equal colonization and extinction probabilities for *L. rupestris*. We specifically evaluated the effects of patch substrate type (boulders versus trees), initial patch status (initially unoccupied versus initially occupied) and, survey date (9 dates) by modeling the probabilities of extinction and colonization of patches as linear functions of these covariates. For this analysis we carried generalized logistic regressions analyses of the binomial probabilities using an un-weighted linear model with no intercept and a logit link function to evaluate additive effects and interactions in the software Splus-2000 (Lucent Technologies, Inc.). The model included both additive effects and all two-way interactions of each factor but calculation of three way interactions was not feasible due to some missing values (e.g., for Pr(E) there was sufficient observations to calculate only 31 out of a total of 36 levels of the three factors (see Appendix 1). Population size is not independent of initial occupation status since initially unoccupied sites either remain unoccupied (size zero) or have low numbers. Due to this collinearity we performed separate analyses for the population size effects on the probabilities of extinction and colonization. Specifically we asked, if there was an effect of previous population size on future extinction probability and also if there was an effect of being recently colonized on population size. First we divided our occupied patches into two population size categories: small ($N_t \leqslant$ median of 26 individuals) and large ($N_t > 26$ individuals) and then performed a

t-test (Sokal and Rohlf, 1981, 1997) for differences in mean population size at time $t - 1$ between patches that went extinct at time t versus those that did not. In a separate analysis we tested for differences in population size between persistent versus newly colonized populations with a *t*-test on mean population size between patches that were occupied at time t but unoccupied at time $t - 1$ (i.e., all newly colonized patches with transition states equal to "$0 \rightarrow 1$") versus those that were occupied at both times t and time $t - 1$ (i.e., all persistent patches with transition states equal to "$1 \rightarrow 1$").

2.6. Population dynamics (asynchronicity and Levins' Model)

We used our estimates of extinction and colonization probabilities to estimate the number of patches at equilibrium ($\hat{P}$) following Levins' classic metapopulation model (1969; 1970). This model predicts the number of occupied patches at equilibrium assuming that all extant populations have a constant risk of extinction and that all unoccupied sites have a constant probability of colonization. According to the model the equilibrium value of occupied patches ($\hat{P}$) is given by $\hat{P} = 1 - \frac{P(E)}{P(C)}$. This model predicts metapopulation persistence (i.e., positive $\hat{P}$) as long as $\frac{P(E)}{P(C)} < 1$ and applies best to organisms occupying small and patchy microhabitats and, to situations in which local patch dynamics occur at a faster time scale compared to the metapopulation dynamics (Hanski, 1999). To determine if predicted patch occupancy values differed between types of substrate we utilized the two-sample Kolmogorov–Smirnov on repeated samples test assuming binomial data. Values of $\hat{P}_t$ were then compared with observed proportions of occupied patches at time t and time $t + 1$ to assess the stationarity of patch dynamics (and therefore, the predictive power of $\hat{P}_t$) using the Pearson product-moment correlation coefficient on arcsine transformed data.

Population growth within occupied patches was described with the parameter lambda (λ) calculated as N_t/N_{t-1} for each patch at each census date. We plotted the natural log of these values against survey date to assess whether patch population dynamics were asynchronous. Parallel trajectories for λ among different patches are indicative of synchronous population dynamics while intersecting trajectories reflect asynchronous population growth rates among occupied patches. The distribution of λ in each survey was compared with the hypothesis of $\lambda = 1$ (stability) using a *t*-test on the log transformed values to assess whether or not the overall tendency for the population was to increase, decrease or stay the same at any given time period.

3. Results

3.1. Population size distribution

The number of individuals on trees and boulders ranged from 1 to 698 in survey 1. The maximum patch population size was rather variable among subsequent surveys (from the second to tenth survey N_{max} = 568, 486, 522, 604, 592, 465, 496, 423). The overall mean patch population size for all patches combined was 45.3 ± 8.1 (empirical standard error of the mean was calculated as the standard deviation of survey means).

The patch population size distributions were skewed to the right within each substrate type but the median for trees was lower (median 15.5) than that for boulders (median 31; Fig. 2). Medians for each substrate type were smaller than their respective means (overall mean for trees: 36.3, ± 7.4 SE, for boulders: 55.5 ± 11.0 SE). This relative pattern between the median and the mean patch population sizes was consistent for both substrates and in all surveys (data not shown).

3.2. Probability of extinction

The logistic regression analysis showed significant effects on the probability of extinction P(E) related to initial patch status (occupied versus unoccupied $F_{2,29}$ = 152.46, $p < 0.00017$), substrate (boulders versus trees $F_{1,28}$ = 53.34, $p < 0.0019$) and an interaction between initial patch status and substrate ($F_{1,4}$ = 16.53, $p < 0.015$). All other terms in the model were non-significant ($p > 0.15$). Specifically, the extinction probability of a population in a patch (P(E)) was dependent on whether patches were occupied or unoccupied at the beginning of the study (survey zero; Fig. 3). When trees and boulders at survey 10 were pooled, the net probability of extinction in patches that were initially occupied at survey 'zero' (P(E|occupied at t_0 = 0.057) was much lower than in patches that were initially empty at survey 'zero' but were subsequently colonized (P(E|unoccupied at t_0 = 0.55, Table 1). By survey 10, populations on trees and boulder had similar extinction probabilities when recorded as occupied on the first survey (P(E|occupied
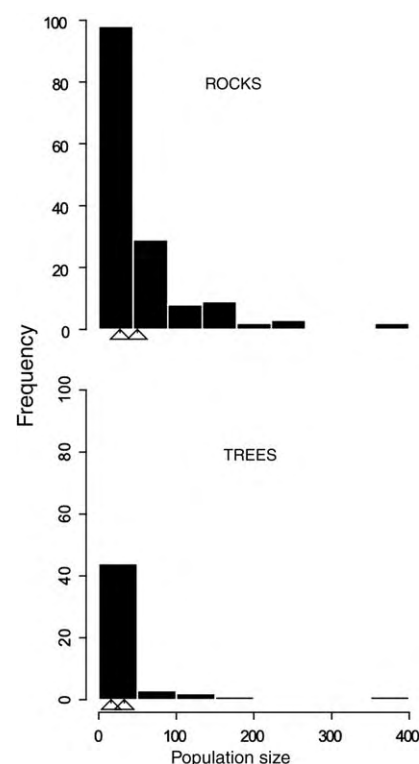

Fig. 2 – Frequency histogram of population size on rock and tree substrates at survey 10. Left and right hand triangles below *x*-axis indicate values for the median and mean population size, respectively.
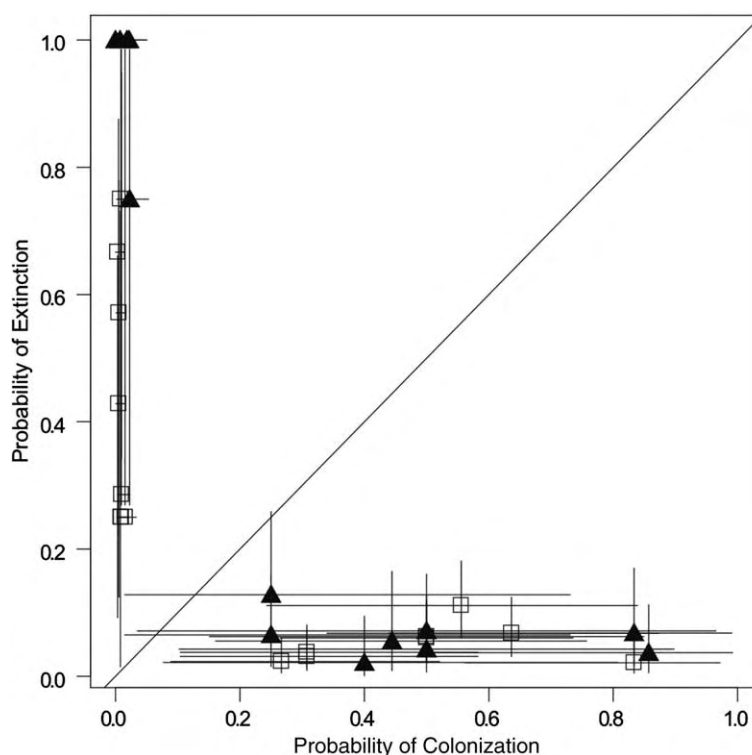
Fig. 3 – Bivariate plot of colonization and extinction probabilities coded by substrate type (boulders, open squares; trees, closed triangles) and original site status (above diagonal originally unoccupied, below diagonal originally occupied) for each survey date (except survey #3; see Section 2). For each combination of substrate type and initial patch status, empirical support limits (~95% confidence intervals of binomial probability estimates) are shown for each census date (Edwards, 1972). Points above the diagonal have zero predicted equilibrium patch occupancy values ($\hat{P}$). Predicted occupancy values for initially occupied patches (below the diagonal can be found in Table 2).

Table 1 – Estimated probabilities of colonization and extinction for different substrate types given their initial patch status (occupied at t_0 or unoccupied at t_0)

Substrate	Probabilities	Survey number									Total
		1	2	4[a]	5	6	7	8	9	10	
Boulders	Pr(C|Occupied t_0)	NA	0.833	0.5	0.636	0.556	0.5	0.267	0.308	0.308	0.473
Boulders	Pr(E|Occupied t_0)	0.077	0.021	0.063	0.068	0.111	0.06	0.023	0.031	0.038	0.053
Boulders	Pr(C|Unoccupied t_0)	0.008	0.009	0.005	0.008	0.015	0.003	0.009	0.005	0.010	0.008
Boulders	Pr(E|Unoccupied t_0)	NA	0.25	0.571	0.75	0.25	0.667	0.25	0.429	0.286	0.456
Trees	Pr(C|Occupied t_0)	NA	0.857	0.5	0.5	0.25	0.444	0.4	0.25	0.833	0.536
Trees	Pr(E|Occupied t_0)	0.108	0.037	0.071	0.042	0.128	0.056	0.022	0.065	0.068	0.066
Trees	Pr(C|Unoccupied t_0)	0	0.004	0.011	0.018	0.007	0	0.015	0.022	0.023	0.011
Trees	Pr(E|Unoccupied t_0)	NA	NA	NA	1	1	1	NA	1	0.75	0.917

a Survey three was excluded from analyses (see Section 2), see Appendix 1 for dates.

at t_0): boulders = 0.053, trees = 0.066, Table 1). However, the probabilities of extinction were different among the types of substrates if the patches were originally unoccupied ($P(E\|$unoccupied at t_0): tree: 0.92; boulders = 0.47, Fig. 3, Table 1).

Patch population size N was also correlated with the likelihood of extinction. Our results indicated that the mean size of populations prior to becoming extinct was significantly smaller than those that persisted at all survey periods except for the second and overall surveys (for example, for survey #10 N extinct: median = 8, mean = 16.75; N persisted: median = 34, mean = 63.51; $t = -3.409$, df = 175, $p < 0.0008$, test on log-transformed data).

3.3. Probability of colonization

The logistic regression analysis for the probability of colonization indicated significant effects of initial patch status ($F_{2,32} = 1433.75$, $p \ll 0.000001$) and survey date ($F_{8,23} = 26.798$, $p < 0.00014$) as well as a significant interaction between substrate

type and survey date ($F_{8,15} = 13.89$, $p < 0.0012$). The substrate effect and the remaining two-way interactions did not have significant effects (all $p > 0.10$). Specifically, the probability of patch colonization (P(C)) was affected by the initial occupancy state (i.e., occupied versus unoccupied) of the patch at the beginning of the survey (Fig. 3; Table 1). The overall probability of re-colonization of an unoccupied patch that was occupied at the beginning of the study was high (P(C|occupied at $t_0 = 0.493$) whereas if the patch was initially unoccupied, the probability of colonization was low (P(C|unoccupied at $t_0 = 0.009$). Different substrates had similar probabilities of colonization and re-colonization. Also, colonization rates for initially empty patches were similarly low for both substrate types (trees = 1.1%, boulders = 0.8%, Table 1) and re-colonization rates of patches that were previously occupied were similarly high for both substrate types (trees: 53.7% and boulders = 47.4%). Newly colonized populations had fewer individuals (overall median: $N = 7$, mean: $N = 14.2$) than populations that persisted between surveys (overall median: $N = 27$, mean $N = 50.9$). This was true for surveys two through ten (for example for survey 10, N extinct: median = 9, mean = 16.22; N persisted: median = 31.5, mean = 58.51; t-test = −2.5261, df = 174, $p = 0.01$, test on log-transformed data).

3.4. Levins' model of patch occupancy and asynchronous dynamics

The equilibrium patch occupancy $\hat{P}$ based on Levins' model for initially occupied patches (regardless of substrate type) was 0.854 while initially unoccupied patches have a $\hat{P}$ of zero. In contrast, when lumping both originally occupied and unoccupied patches the overall $\hat{P}$ is zero demonstrating the necessity of keeping track of these two groups of patches separately (as suggested by the highly significant effect of original status

see above). As expected from the above analysis, $\hat{P}$ is similar for trees and boulders amongst initially occupied patches. A total of 88.9% of available boulders and 83.7% of trees are predicted to be occupied at equilibrium based on overall fitted MLE estimates of subpopulation extinction and colonization rates from the logistic regression analysis. Further supporting the results above, different substrate types did not show statistically significant differences in the distribution of $\hat{P}$ across censuses (two-sample Kolmogorov–Smirnov test, ks = 0.375, p-value = 0.66;). The equilibrium patch occupancy for fitted data from the best fitting models $\hat{P}_t$ (based on transitions observed from $t − 1$ to t) was highly correlated with the observed equilibrium patch occupancy at time t as expected (Pearson product-moment correlation coefficient $r = 0.66$, $t = 3.32$, df = 14, $p = 0.005$ on arcsine square-root transformed data). On the other hand, comparing $\hat{P}_t$ with the observed patch occupancy in the next census $P_{t + 1}$ indicates turnover events occurring between $t − 1$ and t have little predictive power by $t + 1$ ($r = 0.5658$, $t = 0.5905$, df = 2, p = 0.56) suggesting low stationarity.

In general, population growth rates (λ) were asynchronous between patches and surveys (Fig. 4). Comparison of mean λ with the predicted λ for a stationary population ($\lambda = 1$) indicated overall population stability with a significant decrease in λ for surveys 2 ($\lambda = 0.817$, $t = −4.4264$, df = 192, $p \ll .0001$) and 4 ($\lambda = 0.883$, $t = −2.6568$, df = 173, $p = 0.0086$), and increased λ for survey 6 ($\lambda = 1.25$, $t = 3.8838$, df = 131, $p = 0.0002$; all tests and mean λ's were estimated with log-transformed data). Although growth rate was variable at any one time and the mean growth rate differed from one in several time periods, the overall growth rate was not significantly different from one ($\lambda = 0.98$, 95% confidence interval 0.95–1.02), suggesting that over the entire study initially occupied patches are essentially at equilibrium.

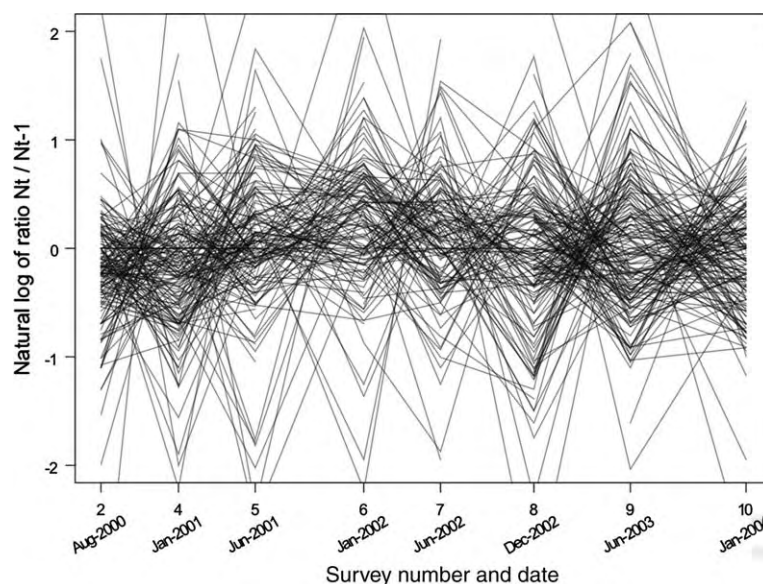Fig. 4 – Population growth rate λ (calculated as the natural log of $N_t/N_{t − 1}$) plotted against survey date. Individual lines connect values for single populations. Values above and below zero represent populations with positive growth and negative growth, respectively. Parallel lines suggest synchronous change in λ between periods, while intersecting lines indicate independent fluctuations in λ.

4. Discussion

4.1. Metapopulation approach

Unlike traditional population dynamics theory, metapopulation theory proposes that populations persist across the landscape as the result of equilibrium between the processes of extinction of local subpopulations and colonization of available (but empty) habitats. We tested whether or not *L. rupestris*, an endemic orchid of Puerto Rico, had a metapopulation structure by examining whether or not the basic conditions describing a metapopulation, according to Hanski (1999), were present. Our results lend some support for the presence of such conditions for *L. rupestris*. First, suitable habitat does occur in discrete patches (trees or boulders) that may or may not be occupied by orchids. Second, even large populations had a measurable probability of extinction. Third, re-colonization of patches that had undergone extinction was possible. Fourth, patch dynamics, measured as the geometric rate of increase (λ), were asynchronous. Direct estimates of these conditions have been scarce in plant population studies (Freckleton and Watkinson, 2002), yet without them one cannot discriminate between a system with true metapopulation structure and one in which the population is merely patchily distributed. These structures, while analogous, will have different implications for the scales at which populations need to be studied and managed for conservation (see Section 4.2).

One of the most difficult tasks in measuring the parameters required for metapopulations studies is being able to define suitable patches available for colonization a priori (Ouborg and Eriksson, 2004). In our selection of empty patches we assumed that our pre-selected patches were suitable and available for colonization. Instead, our results indicated that colonization rates were high for empty patches that had been initially occupied (i.e., recolonized patches) and that they were low for empty patches that were initially unoccupied (i.e., newly colonized patches). While these results do suggest that our original selection of suitable but unoccupied habitats was over-estimated, they also suggest that this system may be at a high equilibrium, where most available patches are observed as occupied. The latter is supported by the relatively constant proportions of occupied patches (especially on boulders) across surveys predicted from Levins model (Table 2).

4.1.1. Departures from Levins model

We used Levins model (Levins, 1970) as a preliminary tool to predict the rate of patch occupancy of the orchid *L. rupestris* in a sector of Quebrada Sonadora. Such application of this model makes two basic assumptions. One is that the ratio between extinction and colonization rates remains constant between surveys. Interestingly, the ratios between extinction and colonization rates (and hence the predicted level of patch occupancy) did remain fairly constant, regardless of substrate type, throughout the survey period, even when there was variation in extinction and colonization rates between surveys (Boulders, range 0.03–0.20; Trees, range 0.04–0.51). Whatever factors leading to temporal changes in extinction and colonization rates of patches affect both rates simultaneously and in the same direction (i.e., when there is more extinction, there is also more colonization and vice versa). One potential source of between-survey differences in extinction and colonization rates may be environmental variation. Water discharge in the stream in the study area is highly variable, and it follows precipitation events closely (Johnson et al., 1998). Flooding may be a potential source of correlated population extinction in this system; large storms have been shown to increase water discharge to levels that are much higher than normal, and capable of moving boulders and large pieces of debris downstream (Johnson et al., 1998). How these flooding phenomena have positive effects on colonization (if any) is unclear. On the other hand, complete loss of substrates could have drastic effects on the metapopulation dynamics and persistence as shown in epiphytic bryophytes and lichens (Snäll et al., 2003, 2005a,b) and would need to be evaluated using a patch-tracking metapopulation approach (Snäll et al., 2003). However, we found few occasions where marked trees (15, occupied and unoccupied) and marked boulders (2, occupied and unoccupied) were moved lower down the study area after such water discharges. These events were always accompanied with the complete loss of orchids from occupied patches.

The other assumption of Levins' classical model is that all patches are equally likely to become colonized if empty, and that populations in all patches are equally likely to become extinct. This assumption was not met. We found a strong effect of initial patch occupancy status on the probability of extinction and colonization. Those substrates that were originally occupied had a high predicted (and observed) probability of

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Table 2 – Equilibrium patch occupancy predicted from Levins' model of initially occupied patches using the fitted parameters given the logistic regression models for Pr(E) and Pr(C) (see Section 2.5)

	Survey number									Total
	1	2	4[a]	5	6	7	8	9	10	
Predicted										
Boulders	NA	0.975	0.875	0.893	0.800	0.880	0.913	0.898	0.877	0.889
Trees	NA	0.957	0.857	0.915	0.487	0.875	0.946	0.739	0.918	0.837
Observed										
Boulders	NA	0.933	0.890	0.914	0.903	0.890	0.800	0.823	0.826	0.872
Trees	NA	0.920	0.868	0.870	0.740	0.849	0.838	0.753	0.919	0.920

a Originally unoccupied patches all have a predicted zero equilibrium patch occupancy. Survey # 3 was excluded; see Appendix 1 for dates.

remaining occupied or being re-colonized if they underwent local extinction, while those that were originally unoccupied had low probabilities of colonization and high probabilities of going extinct if they did become colonized. The higher probability of re-colonization of previously occupied patch could be a result of seed banking, however, we know little about in situ seed viability in orchids (Rasmussen and Whigham, 1993, 1998a,b) and its not clear if orchid seed banking is present in tropical and epiphytic systems. Second, while substrate did not have an effect on the probability of colonization, extinction rates were not equal between substrate types. The probability of extinction of populations occurring on trees that were originally unoccupied but later colonized was twice as high as the corresponding situation on boulders relative to trees (Fig. 3, although this result is based on few data and awaits further surveys for verification). Substrate loss due to flash flood events was larger for trees than for boulders and thus susceptibility to flooding may be partly responsible for this pattern. On the other hand, different substrates could have different mortality dynamics (i.e., higher plant mortality on trees than on boulders) but we did not address this.

The likelihood of extinction was negatively related to population size, an expected pattern based on the effects of population size and demographic stochasticity (Hanski, 1999; Hanski and Gaggiotti, 2004a). Lower extinction rates for larger populations are consistent with the possibility of positive density-dependent regulation (and Allee effects) on local patch dynamics. A separate study on *L. rupestris* at this site indicated weak but significant positive associations between population size and different life cycle stages and positive correlations between the number of reproductive plants and non-reproductive ones (seedlings and juveniles) (Gómez-Rivera et al., in press). These results are consistent with the possibility of positive density-dependent regulation of population size in *L. rupestris*.

Despite differences between the metapopulation structure in *L. rupestris* from the default assumptions of Levins' metapopulation model (Freckleton and Watkinson, 2002; Hanski and Gilpin, 1997), the model appears to be a good first order description of the metapopulation if we recognize that predicted patch occupancy remains consistently high and differs mainly between initially occupied versus empty patches (Table 2). Overall initially occupied patches act as sources while initially unoccupied patches act as sinks. Given that two-thirds of the populations occur on rock substrates along the Sonadora, a one- or two-tiered Levins model accounting for initial occupation status and differences in extinction by substrate may have some utility at predicting general patch occupancy in this system. A further refinement of this analysis would be to include an independent correlate of population size, possibly the size of suitable mossy habitat on a given patch. We are currently measuring the ecological correlates of population size and patch attributes including their spatial structure (Meléndez-Ackerman, Kapan and Tremblay in preparation). When this is complete we will revisit the observed dynamics of colonization and extinction to investigate the effect of patch size (Hanski, 2001; Ovaskainen and Hanski, 2004) and spatial structure (Hanski, 1999; With, 2004) on the metapopulation dynamics utilizing spatially realistic metapopulation theory.

4.2. Conservation implications

Accurate and explicit characterization of the population structure of plants of conservation importance is critical for making effective management decisions (Tilman et al., 1994; Hanski, 1999). When such species have metapopulation structures, conservation efforts need to be directed towards the preservation of suitable habitats for occupation. If metapopulation processes are weak, conservation efforts should be directed at the level of existing populations. Our results may have important implications for the conservation of epiphytic and lithophytic orchids in the Caribbean and elsewhere. Already, one species of *Lepanthes* has been listed as endangered on the island of Puerto Rico (*L. eltoroensis*, Tremblay and Hutchings, 2002). Demographic analyses of *L. eltoroensis* using stage-based models suggest that populations are declining, based on a mean value of λ that is less than one (Tremblay and Hutchings, 2002) and simulations of demographic stochasticity suggest that these populations are highly sensitive to temporal variation in reproductive success (Flecha and Tremblay, unpublished). These analyses assume a more important role for local dynamics relative to regional dynamics, yet the large spatial and temporal variability in demographic parameters in *L. eltoroensis* (Tremblay and Hutchings, 2003) is similar to that found for *L. rupestris* in this study. In view of our findings with *L. rupestris*, it would be appropriate to re-examine the population dynamics of *L. eltoroensis*, to determine the extent of metapopulation structure in that species.

Determining the availability of suitable sites for a species to occupy is important for the conservation of a plant species with a metapopulation structures. To initiate this study, we chose unoccupied trees and boulders that appeared similar and were nearby to occupied sites, but this approach over-estimated available habitats. What factors then would need to be considered in predicting the location of suitable habitats for *L. rupestris*? Finding the answer may be difficult, but some clues are available from the literature. Factors that may affect the likelihood of colonization include the extent of moss cover on the substrate (Tremblay et al., 1998), quality and quantity of light (Fernández et al., 2003), mycorrhizal associations (Bayman et al., 2002) and ambient humidity. On the other hand, persistence of a population at a site may be hindered by limited pollinator attraction, a common problem in orchids (Tremblay et al., 2005) and the Allee effect may precipitate extinction events in small populations. Selecting appropriate sites for colonization for *L rupestris* and other orchids would then require careful experimentation to determine the role of these factors.

Individual λ growth rates for *L. rupestris* subpopulations indicated that on average subpopulations are stable since overall λ is not significantly different from one. Nevertheless, there was considerable temporal variation in λ values at any one time as a substantial fraction of patches show λ's that are suggestive of population decline. Such results underscore the need for studies that take into account the temporal and spatial scale of population dynamics.

Although *L. rupestris* shows some but not all hallmarks of a classic metapopulation, we have shown that the metapopula-

tion approach, if modified to account for differences in substrate quality, may be of great value to this and other epiphytic and lithophytic species. However, its usefulness in studies of terrestrial species remains unknown. While the ultimate conservation value of the metapopulation perspective may depend on our ability to determine what is an appropriate site for colonization a priori, our results do suggest that large-scale studies using multiple populations can be informative at both temporal and spatial scales of population dynamics.

Acknowledgments

Appendix 1

Counts of occupancy state transitions for patches according to their occupancy status (occupied, unoccupied) at time zero (t_0 = survey date "0") and substrate type (boulders, trees). Unoccupied patches were coded as "0" and occupied patches were coded as "1" generating four different transitions. Occupied patches at time zero preclude the possibility of $0 \rightarrow 0$ and $0 \rightarrow 1$ states transitions until after the second full survey and thus were coded as NA for survey date "1". Our observed distributions for each transition class included only those populations for which we could unequivocally assign a transition fate. Survey numbers represent the following survey dates: 0 = Jun-99, 1 = Feb-2000, 2 = Aug-2000, 3 = 07-Oct-2000 (not included in this paper see Section 2), 4 = 27-Jan-2001, 5 = 16-Jun-2001, 6 = 28-Jan-2002, 7 = 03-Jun-2002, 8 = 14-Dec-2002, 9 = 10-Jun-2003, 10 = 24-Jan-2004.

REFERENCES

Ackerman, J.D., 1995. An orchid flora of Puerto Rico and the Virgin islands. Mem. New York Bot. Gard. 73, 1–208.

Bayman, P., González, E., Fumero, J.J., Tremblay, R.L., 2002. Are fungi necessary? How fungicides affect growth and survival of the orchid *Lepanthes rupestris* in the field. J. Ecol. 90, 1002–1007.

Blanco, M., Barboza, G., 2005. Pseudocopulatory pollination in *Lepanthes* (Orchidaceae: Pleurothallidinae) by fungus gnats. Ann. Bot. 95, 763–772.

Brown, S., Lugo, A.E., Silander, S., Liegel, L.H., 1983. Research Opportunities at the Luquillo Experimental Forest. USDA Forest Service Southern Experiment Station General Technical Report SO-44, New Orleans, LA, p. 128..

Calvo, R.N., 1993. Evolutionary demography of orchids: intensity and frequency of pollination and the cost of fruiting. Ecology 74, 1033–1042.

Calvo, R.N., Horvitz, C.C., 1990. Pollinator limitation cost of reproduction, and fitness in plants: a transition matrix demographic approach. Am. Nat. 136, 499–515.

Catling, P.M., Brownell, V.R., Lefkovitch, L.P., 1986. Epiphytic orchids in a Belizean grapefruit orchard: distribution, colonization, and association. Lindleyana 1, 194–202.

Edwards, A.W.F., 1972. Likelihood, second ed. Johns Hopkins University Press, Baltimore, MD.

Eriksson, O., 1996. Regional dynamics of plants: a review of evidence for remnant, source-sink and metapopulations. Oikos 77, 248–258.

Fernández, D.S., Tremblay, R.L., Ackerman, J.D., Rodríguez, E., López, L.-N., 2003. Reproductive potential, growth rate and light environment in *Lepanthes rupestris* Stimson. Lankesteriana 7, 73–76.

Freckleton, R.P., Watkinson, A.R., 2002. Large-scale spatial dynamics of plants: metapopulations, regional ensembles and patchy populations. J. Ecol. 90, 419–434.

Gilpin, M., Hanski, I.A., 1991. Metapopulation Dynamics: Empirical and Theoretical Investigations. Academic Press, London.

Hanski, I.A., 1999. Metapopulation Ecology. Oxford University Press, New York.

Hanski, I.A., 2001. Spatially realistic theory of metapopulation ecology. Naturwissenschaften 88, 372–381.

Original status	Substrate type	State transitions	Survey dates									Total
			1	2	4	5	6	7	8	9	10	
Occupied	Boulders	$0 \rightarrow 0$	NA	2	3	4	4	8	11	9	9	50
Occupied	Boulders	$0 \rightarrow 1$	NA	10	3	7	5	8	4	4	4	45
Occupied	Boulders	$1 \rightarrow 0$	12	3	8	8	12	6	3	4	5	61
Occupied	Boulders	$1 \rightarrow 1$	144	139	120	110	96	94	126	124	127	1080
Occupied	Trees	$0 \rightarrow 0$	NA	1	1	2	3	5	3	3	1	19
Occupied	Trees	$0 \rightarrow 1$	NA	6	1	2	1	4	2	1	5	22
Occupied	Trees	$1 \rightarrow 0$	7	2	4	2	5	2	1	3	3	29
Occupied	Trees	$1 \rightarrow 1$	58	52	52	45	34	34	45	43	41	404
Unoccupied	Boulders	$0 \rightarrow 0$	403	476	451	381	371	320	320	426	409	3557
Unoccupied	Boulders	$0 \rightarrow 1$	4	4	4	2	3	5	1	4	2	29
Unoccupied	Boulders	$1 \rightarrow 0$	2	0	1	4	3	1	6	1	3	21
Unoccupied	Boulders	$1 \rightarrow 1$	5	0	3	3	1	3	3	3	4	25
Unoccupied	Trees	$0 \rightarrow 0$	171	242	225	181	161	135	140	195	181	1631
Unoccupied	Trees	$0 \rightarrow 1$	4	0	1	2	3	1	0	3	4	18
Unoccupied	Trees	$1 \rightarrow 0$	3	0	0	0	2	2	1	0	3	11
Unoccupied	Trees	$1 \rightarrow 1$	1	0	0	0	0	0	0	0	0	1

Hanski, I.A., Gaggiotti, O.E., 2004a. Ecology, Genetics and Evolution of Metapopulations. Elsevier Academic Press, San Diego, California, USA.

Hanski, I.A., Gaggiotti, O.E., 2004b. Metapopulation biology: past, present, and future. In: Hanski, I., Gaggiotti, O.E. (Eds.), Ecology, Genetics and Evolution of Metapopulations. Elsevier Academic Press, San Diego, CA, USA, pp. 3–22.

Hanski, I.A., Gilpin, M.E., 1997. Metapopulation Biology: Ecology, Genetics and Evolution. Academic Press, San Diego, USA.

Holsinger, K.E., 2000. Demography and Extinction in small populations. In: Young, A.G., Clarke, G.M. (Eds.), Genetics, Demography and Viability of Fragmented Populations, Conservation Biology, 4. Cambridge University Press, Cambridge, UK.

Husband, B.C., Barrett, S.C.H., 1996. A metapopulation perspective in plant population biology. J. Ecol. 84, 461–469.

Johnson, S.L., Covich, A.P., Crowl, T., Estrada-Pinto, A., Bithorn, J., Wursbaugh, W.A., 1998. Do seasonality and disturbance influence reproduction in freshwater atyind shrimp in headwater streams, Puerto Rico? Int. Vereinigung Theor. Limnol. Verh. 26, 2076–2081.

Kull, T., 2002. Population dynamics of north temperate orchids. In: Kull, T., Arditti, J. (Eds.), Orchid Biology: Review and Perspectives, vol. VIII. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Levins, R., 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. Bull. Entomol. Soc. Am. 15, 237–240.

Levins, R., 1970. Extinction. In: Gestenhaber, M. (Ed.), Some Mathematical Problems in Biology. American Mathematical Society, Providence, RI, pp. 75–107.

Luer, C.A., 1986. Icones Pleurothallidinarum I. Systematics of the Pleurothallidinea Orchidaceae. Monogr. Syst. Bot. Missouri Botanical Garden, St. Louis..

McDowell, W.H., Estrada Pinto, A., 1988. Rainfall at the El Verde Field Station, 1964–1986. University of Puerto Rico CEER T-228..

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Ouborg, N.J., Eriksson, O., 2004. Towards a metapopulation concept for plants. In: Hanski, I., Gaggiotti, O.E. (Eds.), Ecology, Genetics and Evolution of Metapopulations. Elsevier Academic Press, San Diego, CA, USA, pp. 447–470.

Ovaskainen, O., Hanski, I., 2004. Metapopulation dynamics in highly fragmented landscapes. In: Hanski, I., Gaggiotti, O.E. (Eds.), Ecology, Genetics and Evolution of Metapopulations. Elsevier Academic Press, San Diego, CA, USA, pp. 73–103.

Rasmussen, N.H., Whigham, D.F., 1993. Seed ecology of dust seeds in situ: a new study technique and its application in terrestrial orchids. Am. J. Bot. 80, 1374–1378.

Rasmussen, N.H., Whigham, D.F., 1998a. The underground phase: a special challenge in studies of terrestrial orchid populations. Bot. J. linn. Soc. 126, 49–64.

Rasmussen, N.H., Whigham, D.F., 1998b. Importance of woody debris in seed germination of Tipularia discolor (Orchidaceae). Am. J. Bot. 85, 829–834.

Reddoch, J.M., Reddoch, A.H., 1997. The orchids in the Ottawa district: floristics, phytogeography, population studies and historical review. Canad. Field-Nat. 111, 1–184.

Sokal, R.R., Rohlf, F.J., 1997. Biometry: The Principles and Practice of Statistics in Biological Research, third ed. W.H. Freeman and Company, New York.

Snäll, T., Ribeiro Jr., P.J., Rydin, H., 2003. Spatial occurrence and colonization in patch-tracking metapopulations: local conditions versus dispersal. Oikos 103, 566–578.

Snäll, T., Hagström, A., Rudolphi, J., Rydin, H., 2004a. Distribution pattern of the epiphyte Neckera penneta on three spatial scales—importance of past landscape structure, connectivity and local conditions. Ecography 27, 757–766.

Snäll, T., Fogelquist, J., Ribeiro Jr., J., Lascoux, M., 2004b. Spatial genetic structure in to congeneric epiphytes with different dispersal strategies analysed by three different methods. Mol. Ecol. 13, 2109–2119.

Snäll, T., Ehrlén, J., Rydin, H., 2005a. Colonization-extinction dynamics of an epiphyte metapopulation in a dynamic landscape. Ecology 86, 106–115.

Snäll, T., Pennanen, J., Kivistö, L., Hanski, I., 2005b. Modelling epiphyte metapopulation dynamics in a dynamic forest landscape. Oikos 109, 209–222.

Sokal, R.R., Rohlf, F.J., 1981. Biometry: The Principles and Practice of Statistics in Biological Research, second ed. W.H. Freeman, New York, USA.

Tamm, C.O., 1991. Behaviour of some orchid populations in a changing environment: observations on permanent plots, 1943–1990. In: Wells, T.C.E., Willems, J.H. (Eds.), Population Ecology of Terrestrial Orchids. SPB Academic Publishing, The Hague, pp. 1–13.

Tilman, D., May, R.M., Lehman, C.L., Nowak, M.A., 1994. Habitat destruction and the extinction debt. Nature 371, 65–66.

Thrall, P.H., Burdon, J.J., Murray, B.R., 2000. The metapopulation paradigm: a fragmented view of conservation biology. In: Young, A.G., Clarke, G.M. (Eds.), Genetics, Demography and Viability of Fragmented Populations, Conservation Biology, vol. 4. Cambridge University Press, Cambridge, UK.

Tremblay, R.L., 1997a. Distribution and dispersion patterns of individuals in nine species of Lepanthes (Orchidaceae). Biotropica 29, 38–45.

Tremblay, R.L., 1997b. Morphological variance among populations of three tropical orchids with restricted gene flow. Pl. Sp. Biol. 12, 85–96.

Tremblay, R.L., 1997c. Lepanthes caritensis, an endangered orchid: no sex, no future?. Selbyana 18, 160–166.

Tremblay, R.L., 2000. Plant longevity in four species of Lepanthes (Pleurothallidinae; Orchidaceae). Lindleyana 15, 257–266.

Tremblay, R.L., Ackerman, J.D., 2001. Gene flow and effective population size in Lepanthes (Orchidaceae): a case for genetic drift. Biol. J. Linn. Soc. 72, 47–62.

Tremblay, R.L., Ackerman, J.D., 2003. The genetic structure or orchid populations and its evolutionary importance. Lankesteriana 7, 87–92.

Tremblay, R.L., Hutchings, M.J., 2003. Population dynamics in orchid conservation: a review of analytical methods based on the rare species Lepanthes eltoroensis. In: Dixon, K., Cribb, P., Kell, S., Barrett, R. (Eds.), Orchid Conservation. Kluwer Academic Press, Perth, Australia.

Tremblay, R.L., Ackerman, J.D., Zimmerman, J.K., Calvo, R., 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. Biol. J. Linn. Soc. 84, 1–54.

Tremblay, R.L., Zimmerman, J., Lebrón, L., Bayman, P., Sastre, I., Axelrod, F., Alers-García, J., 1998. Host specificity and low reproductive success in the rare endemic Puerto Rican orchid Lepanthes caritensis (Orchidaceae). Biol. Conserv. 85, 297–304.

Vanhecke, L., 1991. Population dynamics of Dactylorhiza praetermissa in relation to topography and inundation. In: Wells, T.C.E., Willems, J.H. (Eds.), Population Ecology of Terrestrial Orchids. SPB Academic Publishing, The Hague, pp. 1–13.

Waide, R.B., Reagan, D.P., 1996. The rainforest setting. In: Reagan, D.P., Waide, R.B. (Eds.), The Food Web of a Tropical Rainforest. The University of Chicago Press, Chicago, IL, USA, pp. 1–16.

Wells, T.C.E., Cox, R., 1989. Demographic and biological studies of *Ophrys apifera*: some results from a 10 years study. In: Wells, T.C.E., Willems, J.H. (Eds.), Population Ecology of Terrestrial Orchids. SPB Academic Publishing, The Hague, pp. 1–13.

Willems, J.H., 2002. A founder population of *Orchis simia* n the Netherlands: a 30-year struggle for survival. In: Kindlmann, P., Willems, J.H., Whigham, D.F. (Eds.), Trends and Fluctuations and Underlying Mechanisms in Terrestrial Orchid Populations. Backhuys Publishers, Leiden, pp. 23–32.

With, K.A., 2004. Metapopulation dynamics: perspectives from landscape ecology. In: Hanski, I., Gaggiotti, O.E. (Eds.), Ecology, Genetics and Evolution of Metapopulations. Elsevier Academic Press, San Diego, CA, USA, pp. 23–44.

Zotz, G., Tyree, M.T., 1996. Water stress in the epiphytic orchid, *Dimerandra emarginata*. Oecologia 107, 151–159.

Zotz, G., 1998. Demography of the epiphytic orchid *Dimerandra emarginata*. J. Trop. Ecol. 14, 725–741.

Zotz, G., 1999. What are backshoots good for? Seasonal changes in mineral, carbohydrate, and water content of different organs of the epiphytic orchid, *Dimerandra emarginata*. Ann. Bot. 84, 791–798.

Zotz, G., 2000. Size dependence in the reproductive allocation of *Dimerandra emarginata*, an epiphytic orchid. Ecotropica 6, 95–98.