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LEPANTHES CARITENSIS, AN ENDANGERED ORCHID: NO SEX, NO FUTURE?

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ABSTRACT. Persistence of any population depends on the ability of its members to reproduce, asexually or sexually. When reproductive success and/or survivorship are low, this may lead to population decline or in extreme cases extinction. Estimating population growth rate by matrix analysis is effective in determining population stability, although rarely used in orchids. The newly described epiphytic orchid *Lepanthes caritensis,* is limited to the Charco Azul area in the Carite Forest Reserve, Puerto Rico and occurs only on one species of tree, *Micropholis guyanensis* (Sapotaceae). Reproductive success, growth and survivorship of individuals of *L. caritensis* were recorded monthly for two years and a matrix analysis was applied to estimate the asymptotic growth rate of two populations. Reproductive effort was infrequent, few pollinaria were removed and fruit set was nil in both populations. Recruitment and mortality during the sampled years was 8.2% and 22.0% respectively. Mortality was significantly higher in juveniles as compared to adults (28.9% and 10.6% respectively). Recruitment of seedlings must have resulted from the presence of a seed bank or long distance seed dispersal. Intrinsic population growth rate was estimated at 0.995 and 0.999 for population one and two, respectively. Elasticity analysis suggests that non-reproductive adult stage in both populations is the most sensitive to change and would have the largest effect on the population growth rate as compared to other stages and reproductive success. The estimated half-life of *Lepanthes caritensis* populations is approximately 9.5 and 47 years for population one and two, respectively. Population persistance will depend on the consistency of the mortality rate and stochastic reproductive success.

Conservation of rare and endangered species requires integration of many facets of the life history of the species including its ecology, genetics and demography. A recent special feature of Ecology focused on the importance of basic ecological life-history traits for conservation biology (Kareiva 1994, Schemske *et al.* 1994, Mangel and Tier 1994, Doak and Mills 1994). Reproductive success clearly affects population growth rate although many ecological factors may result in substantial bottlenecks for growth rate (Schemske *et al.* 1994). Orchids are without a doubt endowed with many constraints and bottlenecks that potentially limit population growth rate (Ackerman and Zimmerman 1994), including obligate mycorrhizal associations for seed germination, seedling growth that require (Hadley 1982) specialized substrates (Migenis and Ackerman 1990, Tremblay *et al.* in press), and pollinator and resource limitations (Ackerman and Montalvo 1990). However, orchids have the potential to produce astonishing numbers of offsprings: each capsule may contain from a hundred to a million seeds (Dressler 1981). It is thus possible that population size can be maintained or even increased from low fruit set if seed establishment and survivorship is sufficient.

Stage projection matrix models in orchids have been to used to estimate population growth rate in *Cleistes divaricata* (Gregg 1991) and *Laelia speciosa* (Hernandez Apolinar 1992), to test for pollinator limitation and cost of reproduction in *Tolumnia variegata* (Calvo and Horvitz 1990), and to evaluate the effects of different management regimes on *Ophrys sphegodes* (Waite and Hutchings 1991).

Recently a new orchid species, *Lepanthes caritensis* Tremblay and Ackerman was discovered in the Carite State Forest of Puerto Rico (Tremblay and Ackerman 1993). Typical of many *Lepanthes*, it is restricted to a small area (Tremblay 1997). *Lepanthes caritensis* is a small epiphytic perennial plant limited to a single host, the tree *Micropholis guyanensis* (Sapotaceae). The presence of the orchid is positively associated with the size of the host tree, with high moss cover, and reduced mycorrhizal fungal abundance (Tremblay *et al.* in press). Plants usually have 2 to 4 leaves with one (rarely two) active inflorescence, flower continuously throughout the year, with two pollinia per flower and produce a capsule which holds approximately 2000–6000 seeds. Population sizes are small and distances between populations of *L. caritensis* are large (Tremblay 1997), characteristics that are potentially important in determining the likelihood of population survivorship. This species is closely related to *L. sanguinea* (Jamaica and Puerto Rico) and *L. eltoroensis*

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(Puerto Rico, endangered species) which share many derived characters (Tremblay, unpublished).

To determine the population growth rate, a demographic analysis using matrices was used. Information on growth rate (transition among stages), reproductive effort and success, recruitment and survivorship was gathered to determine the intrinsic growth rate of two populations of *Lepanthes caritensis*. Elasticity analysis was used to determine the critical life cycle transitions.

MATERIALS AND METHODS

Demographic Survey

On two trees, in the Charco Azul area of Carite Forest Reserve, all accessible individuals of *Lepanthes caritensis* were tagged on one tree (T_1) while all individuals were tagged on a second tree (T_2). Plants from both trees were followed for two years. Tagged individuals were placed in one of four categories, seedlings (baby plants, smaller than a millimeter, $T_1 = 6$, $T_2 = 13$ sample size, respectively), juveniles (larger than seedlings with no evidence of having had an inflorescence, $T_1 = 54$, $T_2 = 40$) and non reproductive adults on the first survey (evidence of an old inflorescence $T_1 = 24$, $T_2 = 6$) and reproductive adults (individuals with an active inflorescence, $T_1 = 12$, $T_2 = 12$). The orchids were visited once a month, from September 1994 to August 1996 (excluding July 1995, May and June 1996). The stage, survival, presence of active inflorescences, number of flowers, pollinaria removal and fruits were noted for each individual. A life history diagram is shown in FIGURE 1. S_i are the probabilities of moving from one stage to another stage, or while G_i are the probabilities of staying in the same stage. S_i was calculated as the number of individuals that moved to another stage over the total number of individual sampled stage (i.e. 8 juveniles were reproductive adults the next month over a total of 696 juveniles, thus the probability of stage transition to reproductive adult the next month is 0.011). G_i was calculated as the number of individuals which stayed in the same stage over the total number of individuals sampled from that stage (i.e. 579 juveniles were juveniles the next month over a total of 696 juveniles, thus the probability of remaining a juvenile the next month is 0.976). Reproductive success was calculated as the probability of seedling establishment (F = # of recruited seedlings/(# of reproductive plants (sum of S_{24}, S_{34}, was, G_{44})). All values collected from field data.

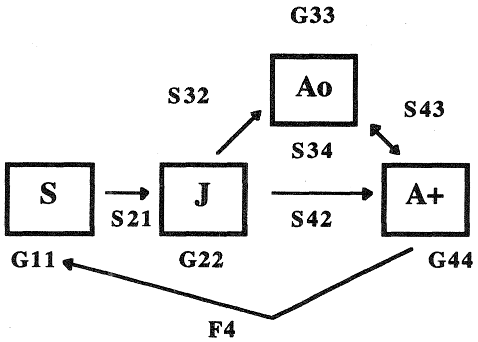

FIGURE 1: Flow chart of life cycle of *Lepanthes caritensis*. New individuals are introduced in the population only from reproducing individuals. *Figure Abbreviation:* S_{ij} = proportion of individuals moving from stage i to stage j, G_{ij} = proportion of individuals staying in the same stage, F_i = proportion of individuals reproducing from stage i. S = seedlings, J = juveniles, Ao = non-reproductive adults, A+ = reproductive adults.

Because of the low number of seedlings in both populations the G_{11} and G_{21} are a sum of the individuals in both populations. From the matrix analysis, I obtained the intrinsic growth rate, lambda (λ), which estimates the asymptotic population growth rate. When λ is below one, the number of individuals in a population decreases, if λ is above one population size would increase, when λ is one then the population remains stable from one generation to the other (Caswell, 1989). Analyses of elasticity and sensitivity were performed to determine the contribution of each stage and reproductive effort to λ.

Half-life of the Orchid

Estimates of half-life (Survival Analysis' from Statistica v.4.1; Statsoft, Inc.) are based on the survivorship of tagged individuals included in the census and the period of time needed for the population to lose 50% of the number of individuals in the populations. Three half-lives are estimated. The first includes all individuals (seedling, juveniles and adults), second and third estimates are based on juvenile and adult survivorship. Half-life, which is sometimes referred to as generation time, is an estimate of time required for 50% of individuals to die. Half-life on different life stages of an organism can differ drastically and may indicate a sensitive demographic stage. Because of the short period of time that these individuals were followed, few adults perished, and the estimate for the generation time is based on the assumption that survival is linear.

TABLE 1. The frequency of flowers produced by individuals with variable number of inflorescences. Monte Carlo simulation $p < 0.00001$, from 50,000 trials.

# of inflorescences	# of flowers		
	0	1	2
1	171	55	0
2	14	2	3

RESULTS

Flower Production and Pollinaria Removal

Thirty percent of adults had active inflorescences at any one time although only 7.2% of plants with active inflorescences had open flowers. A total of 70 open flowers were observed during the study period and only three of these had their pollinaria removed. Most of the flowers were produced by individuals with only one active inflorescence, although the probability of having more than one flower on a plant when two inflorescences are present is dramatically increased as shown by the regression between the number of secondary stems and number inflorescences ($y = 0.17 + 13x$, $R^2 = 0.076$, $F_{1,113} = 9.35$, $p = 0.003$). Individuals with more secondary stems have a higher probability of producing inflorescences, as a consequence it is likely that the larger the individual the higher the reproductive success.

Mean flower production per adult plant did not differ significantly between the two populations (unpaired t-test (2 tailed), $t_{66} = 1.269$, p. $= 0.21$, ; mean and s.e., $\bar{x} = 2.49 \pm 0.44$, $N = 45$, and 1.65 ± 0.35, $N = 23$, for T_1 and T_2 respectively). Flower production at the two sites was more or less continuous throughout the year, but did vary among months and sites. High and low flower production at T_1 did not correlate with T_2 (Paired t-test, 2 tailed: $t_{20} = 2.43$, $p = 0.0031$, FIGURE 2).

Reproductive Success and Recruitment

No fruits have been produced by surveyed plants, although five new seedling and two juveniles were recruited in the area surveyed on T_1, while on T_2 five seedlings and one juvenile were recruited after one year of survey.

Stage Distribution and Transition Matrix

The matrix analysis is shown (TABLE 2a and 2b). With no fruit production the matrix population model analysis is estimated at 0.994 for T_1, and 0.995 for T_2.

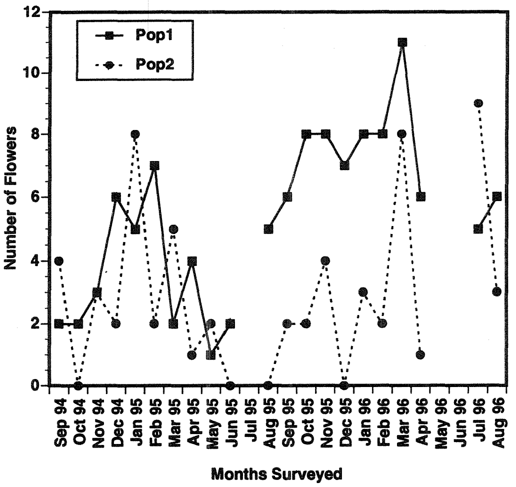

FIGURE 2: Number of flowers produced per month from two populations of *Lepanthes caritensis*. The months of July 95 and May and June 96 were not surveyed.

Since recruitment was observed, we can calculate F_4 as the proportion of recruitment per individual that belonged to the reproductive stage. F_4 is then estimated to be 0.017 and 0.047 for T_1 and T_2 respectively. Lambda is estimated as 0.995 and 0.999 for T_1 and T_2 respectively. The stable stage distribution of the population calculated from the matrix is skewed towards population composed of mostly adults. We would expect the population to be almost exclusively composed of adults, with very few seedlings or juveniles seedlings (seedlings 6.2%, juveniles 1.7%, non-reproductive adults 60.9% and 31.1% reproductive adults for T_1; and seedlings 10.2%, juveniles 13.2%, non-reproductive adults 57.4% and 19.3% reproductive adults for T_2).

The distribution of the reproductive value of the different life stages is skewed towards juveniles and adults (proportional reproductive value of seedlings 8.9%, juveniles 25.3%, non-reproductive adults 32.4% and 33.4% reproductive adults for T_1; seedlings 10.0%, juveniles 29.7%, non-reproductive adults 29.5% and 30.7% reproductive adults for T_2).

Elasticity Analysis

Determining which of the life stages is most crucial in the survivorship of the population can be done with an elasticity analysis (Caswell 1989). In this analysis, the question being asked is, how much effect would an error in estimating the transition in the matrix have on lambda? Calculating the proportional changes in lambda re-

TABLE 2. Projection matrix corresponding to the life cycle graph Figure 1. Note that S represent change in stage from time x to time x + 1, G represent staying in the same stage in the next time period and F represent reproductive success.

a.

G11	0	0	F4
S21	G22	0	0
0	S32	G33	S34
0	S42	S43	G44

b. Corresponding matrix with transition probabilities inserted in the matrix.

T1				T2			
0.911	0	0	0.017	0.863	0	0	0.047
0.030	0.888	0	0	0.045	0.976	0	0
0	0.072	0.821	0.335	0	0.012	0.856	0.417
0	0.011	0.169	0.665	0	0.012	0.137	0.583

c. Corresponding elasticity matrix.

T1				T2			
0.016	0	0	0.002	0.034	0	0	0.003
0.002	0.013	0	0	0.003	0.138	0	0
0	0.001	0.523	0.110	0	0.002	0.522	0.086
0	0	0.111	0.223	0	0.002	0.087	0.124

sulting from a proportional change in each of the reproductive and transitional pathways determines the most critical transition in the life history of the organism. Elasticity analysis is thus a way of determining the stage of the life cycle which has the most affect on the intrinsic growth rate. A proportional change in the frequency of non-reproductive adults staying non-reproductive the next month, G_{33}, has the highest effect on lambda in both population (TABLE 2c). This is followed by the reproductive adult stage, G_{44}, in population one and juvenile and reproductive stage in population two.

Generation Time

The estimated half life of *Lepanthes caritensis* is based on the proportion of individuals who died during the monitored period. A total of 78.2% of individuals were still alive at the end of the sampling period. The number of deaths among stage classes (juveniles and adults) differed (Cox's test, I=8.69, U=−10.74, test statistic −3.64, $p < 0.001$) (FIGURE 3). A total of 28.9% of juveniles perished while only 10.6% of adults suffered the same fate. Survivorship in both populations was equal (Cox's test, I=10.06, U=−0.599, test statistic −0.19, $p = 0.43$). If we assume that survival time is linear (linear probability of death), then we may predict the generation time of juvenile and adult plants. The half-life range approximately two and six years for juveniles and adults, respectively. Linear hazard rates appears to be an appropriate predictor of survival considering most deaths were distributed throughout the year apart from the high death rate which was monitored during February 1995. This high death rate incident was most likely the result of the dry spell which Puerto Rico experienced (FIGURE 4).

Estimates of population half-life using the matrix analysis with no fruit set of *Lepanthes caritensis*, is calculated at approximately seven and twelve years for population one and two respectively. While population half-life considering recruitment is estimated at approximately 9.5 and 47 years for population one and two, respectively.

DISCUSSION

Flower Production and Pollinaria Removal

Pollinators are unknown, but *L. caritensis* may be pollinated by small Diptera (i.e., *Drosophila*). Flower production and fruit set are not limited to a season in any *Lepanthes* of Puerto Rico (Ackerman 1995).

Reproductive Success and Recruitment

At least for T_2 , where all individuals on the tree were surveyed, the observation of recruitment can only mean one of two possibilities: a) that seeds are able to survive and be dormant for a long time. It is known that orchid seeds may survive long periods in unnatural conditions and germinate (refrigerated storage, Light, personnel communication), but in natural conditions no information is available, and long

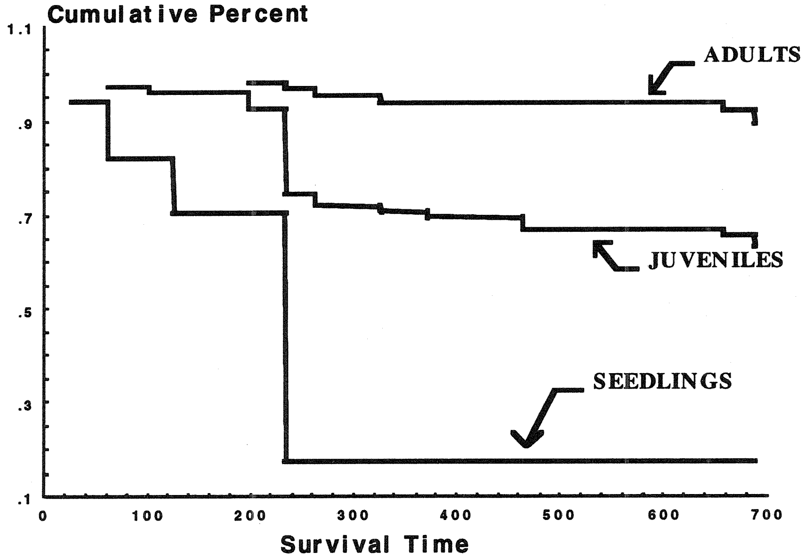

FIGURE 3: Kaplan and Meier Survivorship function for seedlings, juveniles and adults of two populations of *Lepanthes caritensis*. Cumulative percent survival time (in days) of adults (N = 67) and juveniles (N = 82) and seedlings (N = 17).

term viability of seeds is variable among orchids (Arditti *et al.* 1982). b) Seeds may have blown in from other individuals on other trees. The closest occupied phorophyte to tree #2 was at 75 m. The dispersal explanation is unlikely, although not impossible. Most observations of seed flow in nature have shown to be of short distance under most circumstances and highly skewed (Slatkin 1985, 1987, but see Stacy *et al.* 1996). Both possible explanations for these seedling recruitments in orchids are weakly supported by the literature.

MATRIX ANALYSIS. Considering the low recruitment rate, both populations will go extinct. The rate of extinction will depend on survivorship of

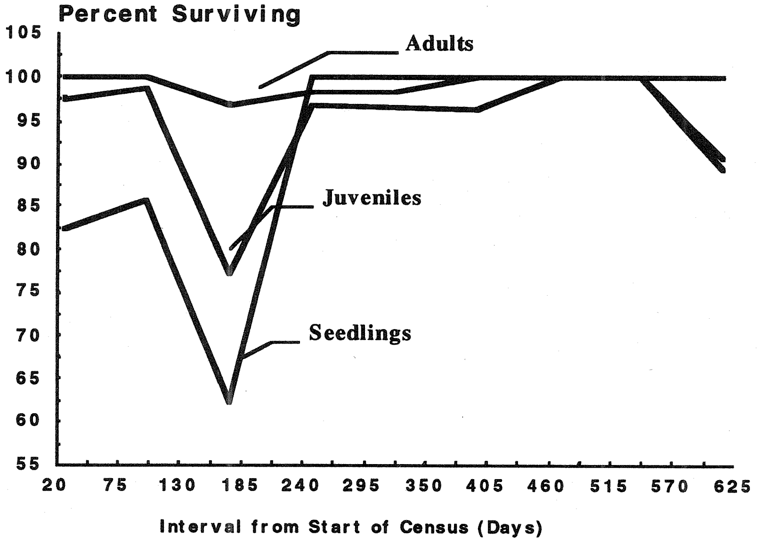

FIGURE 4. Percent survival from month to month of seedlings, juveniles and adults of *Lepanthes caritensis* from September 1994 to September 1996.

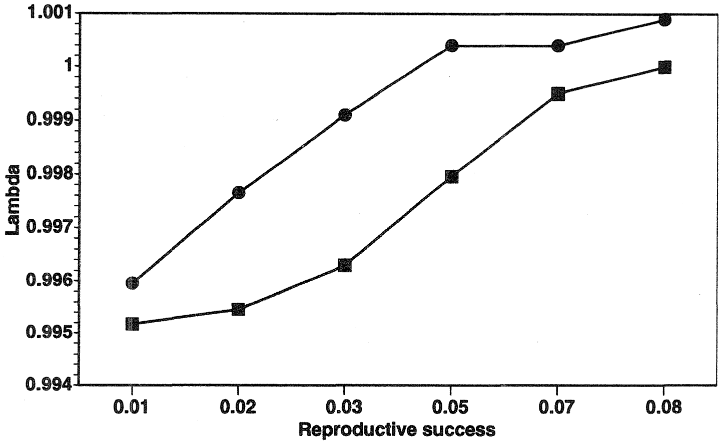

FIGURE 5: The relationship between the mean reproductive success of the population (F4) and lambda (graph as the intrinsic population growth rate). For the population to be stable, lambda should be equal to one. Lambda's that are lower than one suggest that the population will decline. Square = population two; Circle = population one.

individuals. We can estimate the amount of recruitment needed so that the population would be stable by proportionally increasing the probability of recruitment per reproductive individual. By increasing the mean reproductive success per adult we can observe at which point lambda is equal to one. Thus the mean reproductive success per reproductive individual needs to be close to 7–8% for lambda to equal one (FIGURE 5). Since the number of seeds in a *Lepanthes* fruit ranges from 600 to 10,000 in different Puerto Rican species (Tremblay unpublished data), it is possible to have a lower recruitment per plant than the model predicts and have sufficient recruitment for a stable population size.

The lack of fruit production in orchid populations is not rare and often only a small proportion of the flowering individuals account for most of the reproductive success (Fritz and Nilsson 1994). Fruit set in epiphytic orchids is frequently 5% or less. The lack of observed fruit set in of *Lepanthes caritensis* is not necessarily abnormal. Although I observed only 70 flowers in two populations over two years, a 5% fruit set is not statistically significantly different from zero (Rohlf and Sokal 1981). In three European orchids, *Orchis spitzelli, O. palustris* and *Anacamptis pyramidalis* fruit set in very small populations is frequently low and erratic (Fritz and Nilsson 1994).

Stochastic reproductive success may be the norm in small populations of orchids and should not necessarily be a cause for concern if it is associated with a long life cycle. If the half-life of population two, 47 years, is normal in *Lepanthes caritensis* then there is ample amount time for reproduction, and two years without fruit set may not be detrimental. Alternatively, if population half-life is closer to the population one (9yrs), then two years without reproduction may be worrisome.

Suggested Conservation Management for *Lepanthes caritensis*

In this survey none of the individuals reproduced. Without fruit production, this species is bound to go extinct in less than 100 yrs. The causes of low fruit set in this species have yet to be determined, but may include, insufficient pollinator activity, genetic incompatibility, stochastic variation in reproductive success, and inbreeding depression (Barrett 1988). To test for these conditions requires manipulation of plants, flowers and some population genetic analysis to determine the extent of inbreeding and gene flow among populations. In addition host specificity and microhabitat requirement (i.e. moss, fungal associate, Tremblay *et al.* in press) must be investigated thoroughly. Even if reproductive success can be increased or shown to be sufficient, without the appropriate host trees the number of populations may not increase.

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