DIVERSITY AND PHENOLOGY OF ORCHIDACEAE IN AN UPPER MONTANE FOREST, DEPARTMENT OF CUSCO, PERU

by

REBECCA ELAINE REPASKY

Bachelor of Science, 2004
Texas Christian University
Fort Worth, Texas

Submitted to the Graduate Faculty of the
College of Science and Engineering
Texas Christian University
in partial fulfillment of the requirements
for the degree of

Master of Science

May 2008

ACKNOWLEDGEMENTS

I would like to thank the Biology Department at TCU for allowing my research to take place in Peru and providing support and funding. I would like to thank Botanical Research Institute of Texas and AABP for making this project possible. I also want to thank the Moore Foundation and the Beneficia Foundation for providing the grants for this project.

I would like to also thank Eric Christenson for providing the expertise for orchid identification. I would like to acknowledge Keri McNew at BRIT and AABP for helping me survive my time in Peru and helping me with all facets of my research, from collecting data to writing. I would like to thank my Peruvian research team for doing the work of counting orchids and assisting in the orchid collection. Without them, I would not have completed my project.

I especially owe thanks to my major advisor John Janovec, whose vision and guidance created this project and led to its completion. I also owe a large thank you to John Pinder, professor of Biology at TCU, who helped get the most out of my data and led my thesis in directions I would have never thought possible without his advice.

Thanks are also owed to my parents, James and Karen Repasky, who allowed me to live in a foreign country for a year by myself in the cloud forest. I also appreciate the support Cole Luke has given me and I love him very much for it.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

INTRODUCTION

With an estimated 30,000 species in 800 genera, the Orchidaceae are often considered to be the largest family of flowering plants (Stebbins 1981, Gentry 1988, Gravendeel *et al.* 2004). Orchids are highly variable and can rapidly evolve into new species, with some developing into different species within the past several hundred years (Hirtz 2005). Hybridization is a common mechanism of new species formation within the Orchidaceae (Hollick *et al.* 2005), and individual species appear to be maintained by being pollinator specific (Christenson 2003). One of the more current checklists (Dressler 2005) contains 24,500 species of orchids, with still more to be found. For example, Luer and Dalström (2006) described six new *Masdevallia* species recently discovered in Peru.

The diversity of orchid species in cloud forests of the Andes Mountains of South America has been recognized and explored for years. In 1911, Hiram Bingham discovered Machu Picchu in the Andes Mountains of south-central Peru and commented on orchids he observed there (Christenson 2003), and these forests are known for their high diversity of epiphytes (Ingram *et al.* 1996). The high diversity of orchids in Andean cloud forests is due to a moist, cool climate that is favorable to epiphytes and to isolation and diversification on mountain ranges where genetic drift may lead to further genetic and morphological variation (Tremblay *et al.* 2005). Orchids require specific pollinators in order to reproduce and mycorrhizal fungi in order to germinate (Hollick *et al.* 2005, Otero *et al.* 2005, Shefferson *et al.* 2007). Because of these relationships, orchids are intimately intertwined with the ecology of their habitat. They have been shown to be excellent indicators of the overall biodiversity and health of habitats in an area (Christenson 2003).

There exists limited quantitative knowledge about the diversity and spatial distribution of orchid species between geographic sites and along elevation gradients in the Peruvian Andes. There are only a few detailed studies of orchid diversity and distribution at cloud forest elevations (e.g. Zambrano *et al.* 2003a and 2003b). More is known about all epiphytes in general. Cloud forests, or neotropical montane, forests feature abundant and diverse epiphytic plant species (Grubb *et al.* 1963, Cornelissen and Ter Steege 1989, Ingram *et al.* 1996,). The highest diversity of epiphytes occurs within an elevation range of several hundred meters (2200 to 3700 m above sea level). Epiphyte diversity is generally greatest in wet aseasonal forests on fertile soils at "middle elevations" (Gentry and Dodson 1987). Epiphyte diversity in montane forest appears to be affected by the tree diversity and the climate in the forests (Krömer and Gradstein 2003, Andersohn 2004), and also by the heights and diameters of the epiphyte hosts (Arévalo and Betancur 2006).

Besides the limited number of studies, the assessment of orchid diversity and distributions is further limited by potential problems in nomenclature that are difficult to resolve because of the lack of access to reference specimens, digital imagery, and detailed collection data. Even more complications arise from the timing of many studies. Generally, studies have been limited to only a few months of the year, and may overlook species flowering at other times. Due to the difficulties of travel and field work during the rainy season, it is often a challenge for researchers to visit or work in cloud forests throughout the year.

One of the difficulties facing conservationists is that without more extensive data, it is difficult to know the size and number of areas to protect. Would a few large areas protect the orchid diversity of a region? Or would it take multiple smaller areas to conserve their habitat? The issues for orchid conservation are the same that are being asked of lowland neotropical rainforests where there has been a recent emphasis on quantitative studies to evaluate the best strategies for conserving these forests (Phillips and Raven 1996).

The purpose of this study was to initiate quantitative analyses of the Orchidaceae in a Peruvian cloud forest habitat. This was accomplished by beginning a systematic collection of orchids across the field site and establishing permanent plots within the study site that were monitored monthly for one year. Data were collected from the plots to accomplish the objectives of (1) having a more expansive understanding of the local distribution and abundance of orchids and (2) addressing the question of how the relatively unknown flowering phenologies of the orchid community may affect assessments of orchid diversity over time and space. Some of the results of this study are described and discussed in the following three chapters.

The second chapter reports on the biodiversity of the orchid family at Wayqechas Biological Field Station (WBFS) and compares the orchid diversity of WBFS to that reported for other Andean cloud forests. The third chapter reports on the phenology of the orchid family at WBFS. The last chapter summarizes the results of Chapters 2 and 3 to develop a potential protocol for the conservation of orchid biodiversity in Andean cloud forests. It illustrates the potential importance of numerous small conservation areas and discusses methods that may be both sufficient and efficient to assess the orchid diversity of these reserves.

CHAPTER TWO

DIVERSITY AND ABUNDANCE OF ORCHIDS IN A PERUVIAN CLOUD FOREST

The Orchidaceae is often argued to be the largest family of flowering plants with an estimated 30,000 species in 800 genera and approximately 70 percent occurring as epiphytes (Stebbins 1981, Gentry 1988, Gravendeel *et al.* 2004). Orchid species diversity in cloud forests of the Andes Mountains of South America has been recognized and explored for years. A common orchid from the Peruvian Andes, *Epidendrum secundum*, was first characterized in 1760 (Rolfe 1916). In 1911, Hiram Bingham discovered Machu Picchu in the Andes Mountains of south-central Peru and commented on orchids he observed there (Christenson 2003). The high diversity of orchids in Andean cloud forests is due to a moist, cool climate that is favorable to epiphytes, isolation and diversification into different ecological niches on mountain ranges, and the ability of orchids to hybridize easily. While there are few genetic barriers to hybridization, orchid species remain diverse by having very specific pollinators (Christenson 2003).

There is limited quantitative knowledge of the species richness and spatial distribution of orchids between geographic sites and along elevation gradients in the Peruvian Andes. More is known about all epiphytes in general. Cloud forests or neotropical montane forests feature abundant and diverse epiphytic plant species (Grubb *et al.* 1963, Cornelissen and Ter Steege 1989). Most epiphytes occur within an elevation range of several hundred meters (2200 to 3700 m above sea level). Epiphyte diversity is generally greatest in wet aseasonal forests on fertile soils at "middle elevations" (Gentry and Dodson 1987). Detailed studies of orchid diversity and distribution at cloud forest elevations are limited to a 12-month study in a 143.5-hectare section of the Machu Picchu Historical Sanctuary (MPHS;

Zambrano *et al.* 2003a) and inventory of nine 100 m^2 plots in the Manu National Park (MNP; Zambrano *et al.* 2003b). Both studies report high diversity and abundance of orchid species, but there is limited overlap with the more complete species list compiled for MPHS (Christenson 2003). These studies demonstrate the diverse orchid flora of Andean cloud forests of southeastern Peru with numerous species of potentially limited distribution. However, because of the general paucity of such studies, the available information is clearly not sufficient to quantify the extent of orchid diversity across the range of the Peruvian Andes. Besides the limited number of studies, the assessment of orchid diversity and distributions is further limited by potential problems in nomenclature that are difficult to resolve because of the lack of access to reference specimens, digital imagery, and detailed collection data.

Orchids have also been shown to be excellent indicators of overall biodiversity in an area (Nadkarni 1992, Christenson 2003). Orchids are highly evolved with their pollinators and require a specific relationship with mycorrhizal fungi to germinate (Otero *et al.* 2005, Shefferson *et al.* 2007), therefore they are intimately intertwined with the ecology of their habitat. The conservation of orchids requires the conservation of their habitat. One of the difficulties facing conservationists is that without more extensive data, it is difficult to know the size and number of areas to protect. Would a few large areas protect the orchid diversity of a region? Or would it take multiple smaller areas to conserve their habitat? The issues for orchid conservation are the same that are being asked of lowland neotropical rainforests where there has been a recent emphasis on quantitative studies to evaluate the best strategies for conserving these forests (Phillips and Raven 1996).

The purpose of this study was to initiate a similar quantitative study of orchid diversity at a small cloud forest site in southeastern Peru, to compare the results to other Andean sites where orchids have been well-documented, and to use these data and comparisons to assess the potential conservation implications in order to maintain orchid diversity.

MATERIALS AND METHODS

The design of this study is in accordance with the protocol recommended by Phillips and Raven (1996) for assessing tree biodiversity in the Neotropics. This protocol was designed to establish a basic procedure for quantifying biodiversity on a local scale by obtaining: (1) a more complete inventory of plant species composition, (2) an assessment of the spatial distribution of species, and (3) basic phenological information. These objectives are accomplished by conducting surveys to document the species composition (= florula, *sensu* Phillips and Raven 1996) and monitoring of permanent plots for species distribution and phenology. These objectives were addressed through: (1) systematic collection of the orchid species present and (2) monitoring of 47 permanent plots over a 12-month interval to study orchid distributions and phenology.

STUDY SITE

All field research was carried out at the 560-ha Wayqechas Biological Field Station (WBFS, 13°10’40” S, 71°36’20” W), which borders the southeast margin of the Manu National Park in the Department of Cusco in southeastern Peru (Fig. 1). The field station is owned and operated by the Amazon Conservation Association (ACA) of Washington, DC, and its sister organization in Peru, the Asociación para la Conservación de la Cuenca Amazónica (ACCA). The elevation of the field station ranges from 2200-3200 m. The natural vegetation consists of upper montane forest (Young and León 2000), which is continually saturated with rain and fog. Temperatures average 11°C with little seasonal variation. Precipitation ranges from < 0.01 m in the months of June and July to > 0.10 m in the months of January, February, and March.

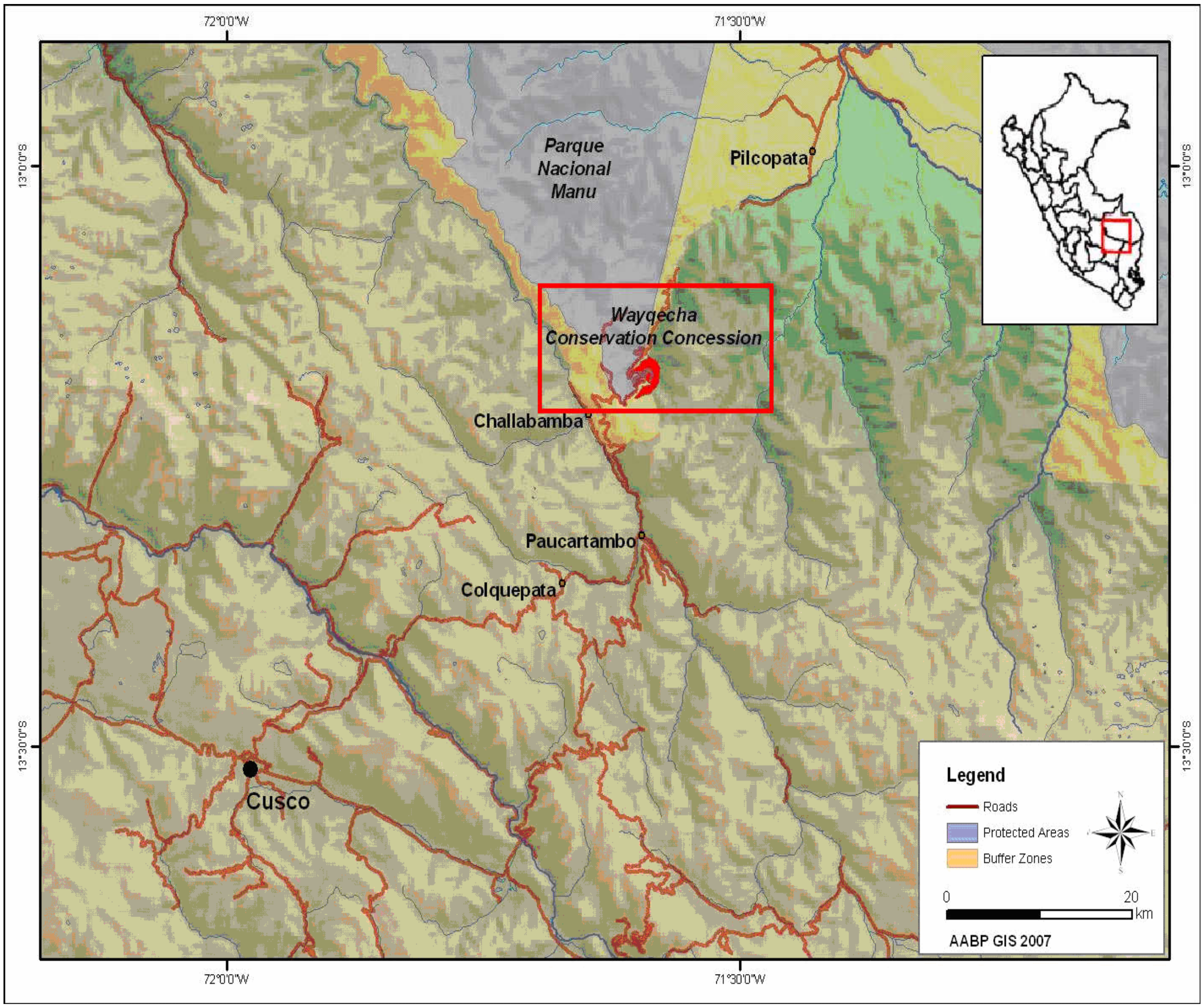

Figure 1. The location of Wayqechas Biological Field Station (labeled with box); inset is map of Peru.

SYSTEMATIC PLANT COLLECTION

To identify and document the orchid species occurring at WBFS, approximately 10 days of each month from September 2005 to August 2006 were devoted to collecting, photographing, and preserving specimens of both terrestrial and epiphytic orchid species. The majority of the WBFS was explored to include all local vegetation types. Epiphytes occurred up to heights of 20 m in the host trees. Collections were carried out on a regular basis along existing trails and in off-trail areas. At least one voucher specimen was collected for each potentially new orchid species encountered with up to six voucher specimens when individuals were abundant. The first specimen was deposited in the herbarium of the Museo Nacional Mayor de Historia Natural (USM) in Lima, Peru. The second specimen was deposited in the herbarium of the Botanical Research Institute of Texas (BRIT) in Fort Worth, Texas. The third specimens are being dispersed to orchid experts at different herbaria. All additional duplicate specimens of the orchid collections made on this project were deposited at USM in Peru. Auxiliary collections were made in the form of flowers preserved in a solution of 80 percent alcohol and 20 percent glycerine. Each potential new species, or morphospecies, for the project was assigned a unique identification number, and relevant data were recorded including: (1) date of collection, (2) location as determined by a Garmin Map76C global positioning unit, (3) description of relative location, (4) relative flower size (1 to 5, 5 being the largest), (5) habit (terrestrial or epiphytic), (6) color of the flower (particularly lip and column colors), and (7) height of the individual from its base to the top of the stem or the flower, whichever was taller. In addition, the habitat was recorded as one of the following types: (1) tall cloud forest with multiple tree species with heights > 15 m, (2) short cloud forest with multiple tree species with heights < 15 m, and (3) grass areas with few or no trees with heights < 3 m. Areas of grass most likely originated from human disturbance, such as logging, landslides, construction, grazing, or fire (Young and León 2000, Lozano *et al.* 2006). Digital photographs ($\geq$8 Mpixels) were taken of all collections to aid in species identification and documentation. Every orchid with unique vegetative and reproductive morphological characteristics was considered to be a potential new species to the project, unless it could be readily identified in the field as belonging to a previously collected morphological type. Those orchids in vegetative growth states that were considered potential new species to the collection were marked and revisited in following months until their point of flower production.

Upon completion of the plant collection, the morphospecies identified in the field were compared and either considered unique species or combined with other similar morphospecies to form one species. Identifications were made by Dr. Eric Christenson of Florida. Collected materials and data for all morphological types were placed in appropriate repositories. Specimens were documented with their identification number, transported, and mounted for processing and identification. Digital images, preserved flowers, and collection data are retained by BRIT. Collection data and associated digital images are accessible through the Atrium Biodiversity Information System monitored by BRIT (http://atrium.andesamazon.org).

QUANTITATIVE DATA COLLECTION

To measure the effects of elevation and habitat differences on species diversity, distribution, and phenology, 47 5 m x 5 m plots were established across elevation and habitat gradients within the Wayqechas. Because of steep gradients and dense vegetation, the plots were established at random locations along pre-existing trails that were strategically designed to allow access to the forest and grassland habitats characteristic of the area. Each plot was labeled and its latitude, longitude, and elevation were determined by GPS. The habitat was classified as tall cloud forest, short cloud forest, or grass areas, as described above.

During a period of 12 months from September 2005 through August 2006, each of the 47 plots was monitored between the 9th and the 13th of each month for orchids that were flowering and fruiting. Orchid species in flower were observed in every month. For the first occurrence of a species flowering in a plot, the species was catalogued using its identification number in the general collection. The number of individuals with flowers, total number of flowers, total number of fruits, the height of the tallest plant, and the height of occurrence relative to ground level were recorded. To prevent misidentification of species, fruits were only noted if the species had been previously observed to flower in that plot. A height of occurrence of 0 m indicated a terrestrial plant. Heights > 0 m indicated an epiphyte.

If a potential new species was first encountered in the plots instead of the general collection, it was added to the general collection using the previously described procedures using suitable specimens located outside of the plot. If the orchid was encountered only in the plot, it was not collected as a voucher specimen. Digital images were taken to assist in species identification. If the species could not be identified from the digital images, it remains an undetermined species.

For subsequent observations of a species in a plot, the number of individuals with flowers and the total number of flowers and fruits in the plot were recorded. If fruits were present without flowers, the total number of fruits was recorded and the number of individuals and flowers were defined as zero. General linear model tests of the effects of elevation and habitat type on the species richness and density of orchids were performed using the Statistical Analysis System (SAS; Der and Everitt 2001).

Certain limitations to the project were imposed by terrain and field conditions. Because these limitations prevented each individual plant being tagged, it is possible that the individuals counted as flowering in one month were also counted in subsequent months. For this reason, the term density used in subsequent discussions is a minimum estimate of density taken as the maximum number of individuals observed in that plot in any one month.

RAREFACTION ANALYSIS

To evaluate how well the plot data can estimate the species richness of the orchid flora, rarefaction curves (Gotelli and Colwell 2001) were computed using the non-parametric species richness estimators Chao 1 (Chao,1984), Chao 2 (Chao 1984, 1987), Jack 1 (Burnham and Overton 1978, 1979), Jack 2 (Burnham and Overton 1978, 1979), Abundance-based Coverage Estimator (ACE; Chao and Lee 1992; Chao *et al.* 1993), and Incidence-based Coverage Estimator (ICE; Lee and Chao, 1994). These non-parametric estimators use the occurrence of rarer species in the samples to adjust the number of observed species for the number of species that were likely missed in the sampling. The Chao 1, Jack 1, and ACE estimators define rareness as being represented by only a few individuals. The Chao 2, Jack 2, and ICE define rareness as being observed in only a few plots. Confidence intervals (CI) are available for Chao1 and Chao 2, but not the other estimators. Estimators and their 95% CI were computed from 10,000 randomizations of plot sequences using the software EstimateS 8.0 (Colwell 2006). Chao 1 and Chao 2 were computed using the classic rather than the bias-corrected methods as recommended by Colwell (2006).

RESULTS

SYSTEMATIC COLLECTIONS

A total of 239 orchid species were collected from 49 genera. The final list of species was compiled from a set of voucher specimens for 341 potential morphospecies. Over 134 species have been identified as previously described species, three species are considered new species to science (Christenson, 2008, *pers. comm.*), and 102 species remain as verifiable morphospecies to be identified with previously named species or described as new species. The number of species per genera ranged from one in genera such as *Altensteinia* to 42 for *Stelis*. Thirty-one genera were represented by only one or two species. It is important to note that species separations in the current project are based on structural characteristics which may not be supported by subsequent genetic analysis. However, it is also possible that subsequent genetic analyses may separate apparently similar flowers into distinct species. A complete species list organized by genera is presented in Appendix A.

PLOT CHARACTERISTICS

The elevation range of plots was from 2496 to 2993 m. When grouped into equal elevation intervals of 175 m, there were similar numbers of plots in each interval (Fig. 2; Appendix B) and all three habitats were present in each interval. The proportion of plots in grass habitats tended to increase with increasing elevation, possibly due to human interference such as logging and road construction (Young and León 2000). There was no apparent trend in the relative proportions of tall and short cloud forest with elevations.

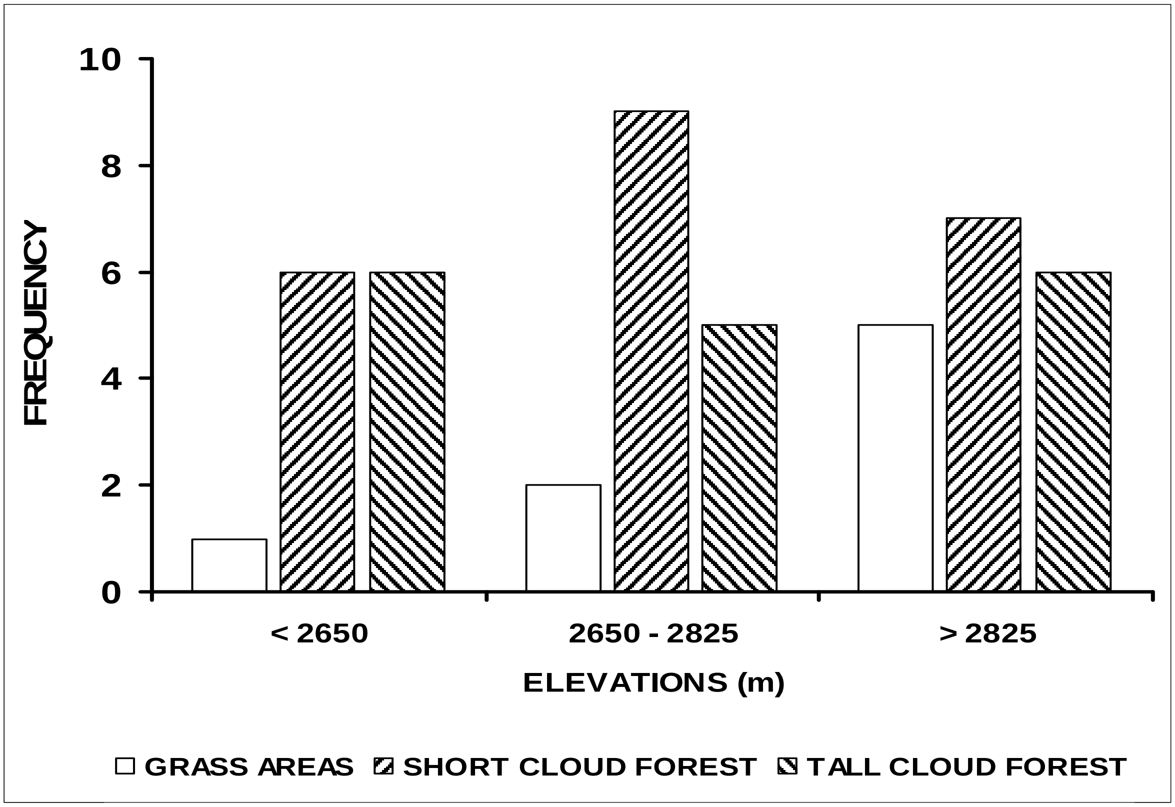

Figure 2. The total number of plots in each habitat and elevation interval in the study area.

ORCHID ABUNDANCE

The minimum number of individuals recorded in the all of the plots for the duration of the study was 2565, which is the sum of the maximum number recorded in any month for a species in each plot. The maximum number of individuals, 5591, was computed by summing the number of individuals across months for all species. The minimum number is a more realistic estimate of species abundance because the maximum number assumes that there was a new set of individuals in each plot for every species in every month with no overlap between months.

The minimum numbers of individuals per plot showed little variation with elevation or habitat type (F = 0.33; df = 5, 41; P > 0.10; Appendix B). Densities were approximately normally distributed (Kolmogorov-Smirnov test of normality; Conover 1971 and Field 2005). Mean (± Standard Deviation; SD) densities for grass areas, short cloud forest, and tall cloud forest were 2.2 (± 1.1), 2.4 (± 1.2), and 1.9 (± 2.1) individuals per m^2, respectively. The rate of change of density with elevation, which was not significantly different from zero, was a decline of 0.14 individuals per m^2 for every 100 m increase in elevation.

DISTRIBUTION OF ORCHID SPECIES AMONG PLOTS

A total of 128 species were observed across all plots throughout the course of the 12-month study period. The number of species per plot ranged from three to twenty-five (Fig. 3), with a median of nine species per plot. Twenty-five orchid species were found in only a single plot. Almost all orchid individuals observed in the plots flowered during the 12-month period of the study. It is possible that additional species occurred in the plots and never flowered, but the number of these species is assumed to be minimal. Most of the orchid species (72 %) occurred as epiphytes. Thirty-seven species occurred as both terrestrial and epiphytic forms.

There were significant differences in the number of terrestrial species per plot among habitats (F = 6.15; df = 2, 43; P < 0.01) and elevation (F = 14.09; df = 1, 43; P < 0.01). The mean (± SD) number of terrestrial species per plot for tall cloud forest was 1.8 (± 2.5). This was significantly (P < 0.05) less than the means of 4.0 (± 2.7) species per plot in short cloud forest and 3.4 (± 2.6) species per plot in grassland. There was a decrease of 0.81 (Standard Error = 0.22) terrestrial species per plot for each 100 m increase in elevation. The two terrestrial orchids with the highest frequency of occurrence, *Epidendrum secundum* and *Elleanthus sp.,* occurred in 17 of the 47 plots.

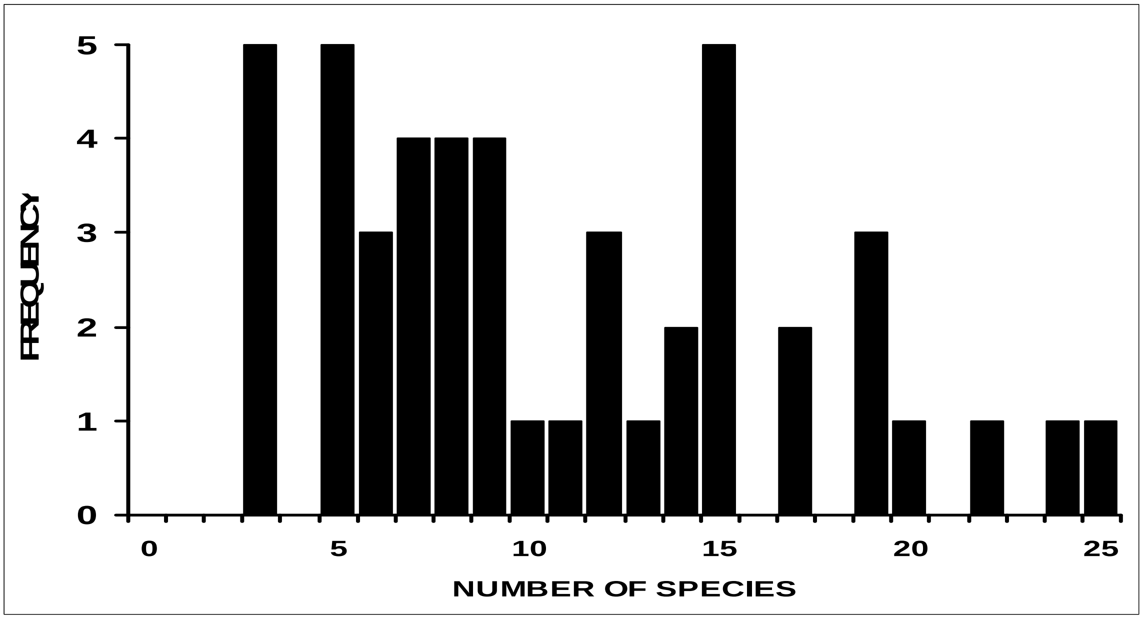

Figure 3. The frequency of species counts for individual plots.

For epiphytic species, there were also significant differences in the number of species per plot among habitats (*F* = 7.29; df = 2, 43; *P* < 0.01) and in elevation (*F* = 19.12; df = 1, 43; *P* < 0.01). The number of epiphytic species per plot for short cloud forest (10.1 ± 4.5) was significantly greater than that for the tall cloud forest (6.3 ± 3.7) and the grass areas (6.2 ± 4.0). For each 100 m increase in elevation, there was a decrease of 1.4 (SE = 0.3) epiphytic species per plot. The epiphytic orchid with the highest frequency of occurrence, 20 plots, was *Pleurothallis acuminata*.

The mean (± SD) heights (m) above ground level for epiphyte orchids were 1.0 (± 0.8), 1.4 (± 1.0), and 2.1 (± 1.6) for grass areas, short cloud forest, and tall cloud forest, respectively. The maximum observed height was 7.0 m for *Pleurothallis coriacardia* in a tall cloud forest plot at 2778 m elevation.

There was considerable variation among species in abundances and frequencies of occurrence. The range of abundance of orchid species was from one to 335 for

Pachyphyllum sp., with a median of six. There were 27 species with an abundance of one individual and 12 species with an abundance of two. The majority of orchid species had abundances of < 100 individuals. Only eight species had minimum numbers > 100. The next four most abundant species after *Pachyphyllum sp.*, in order of decreasing abundance, were *Pleurothallis vestigipetala aff.*, *Stelis breviracema, Elleanthus sp.*, and *Stelis grandibracteatum.*

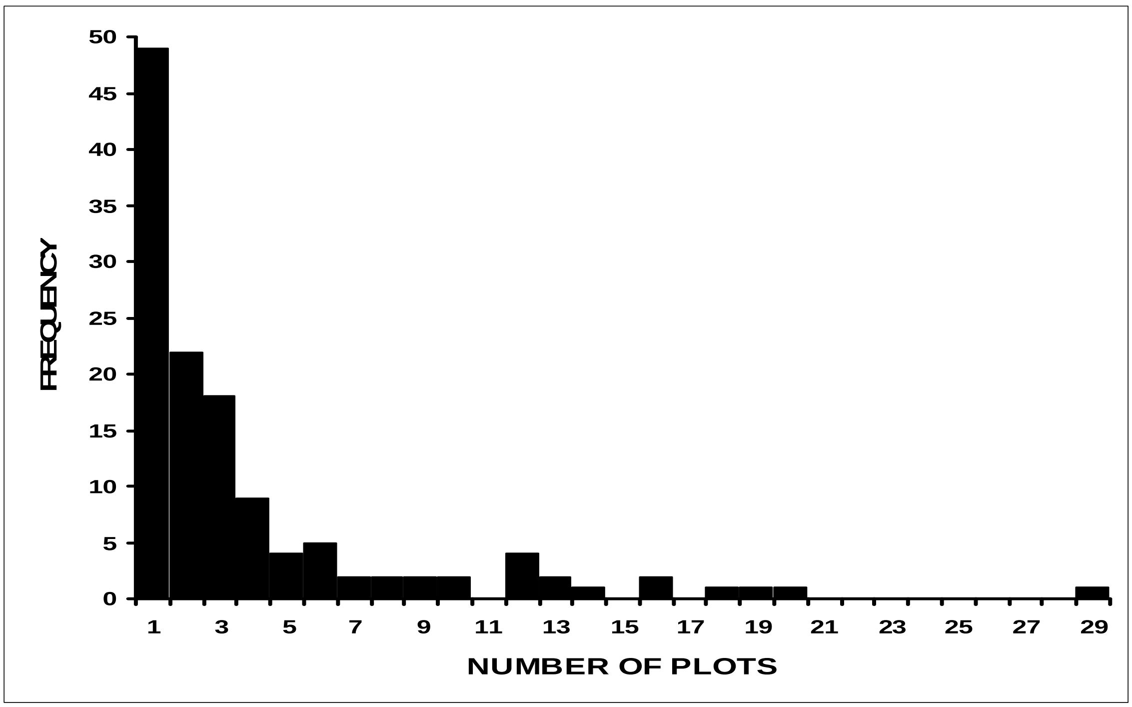

Figure 4. The number of species occurrences per plot.

The frequencies of occurrence in plots ranged from one to twenty-nine with a median of two (Fig. 4). Most species were observed in < 5 plots. Forty-nine species were found in only one plot, and 22 species occurred in only two plots. Thirteen species were observed in > 10 plots. Again, *Pachyphyllum sp.* was the species with the highest frequency of occurrence. The next four most frequent species were *Pleurothallis acuminata, Stelis breviracema*, *Epidendrum secundum*, and *Elleanthus sp.*

Rarefaction Analysis

All of the species richness estimators underestimated the number of species observed in the general collection (Table 1). Higher estimates of richness were produced by the ICE, Chao 2, and Jack 2 procedures that define rareness based on frequencies of occurrence than for the ACE, Chao 1, and Jack 1 procedures that define rareness based on the number of individuals sampled. Only the 95 % CI for the Chao 2 estimator included the number of species observed in the general collection.

Table 1. The mean and 95 % CIs for estimators of species richness from plot sampling. NA indicates not applicable.

Estimator	Mean Estimated Number of Species	95 % Confidence Interval Lower Bound	95 % Confidence Interval Upper Bound
ACE	148	NA	NA
Chao 1	158	140	206
Jack 1	176	NA	NA
Chao 2	183	155	240
ICE	183	NA	NA
Jack 2	202	NA	NA

A plot of the Chao 2 estimator (Fig. 5) as a function of the number of individuals sampled indicates a continuous and nearly linear increase in the estimated richness as the number of individuals increases beyond 1500. Recall that the number of individuals is a minimum estimate of the actual number of individuals contributing to the samples. The transient peak at approximately 100 individuals, which corresponds to the randomization of two plots, is a product of the rather patchy dispersion of orchid species.

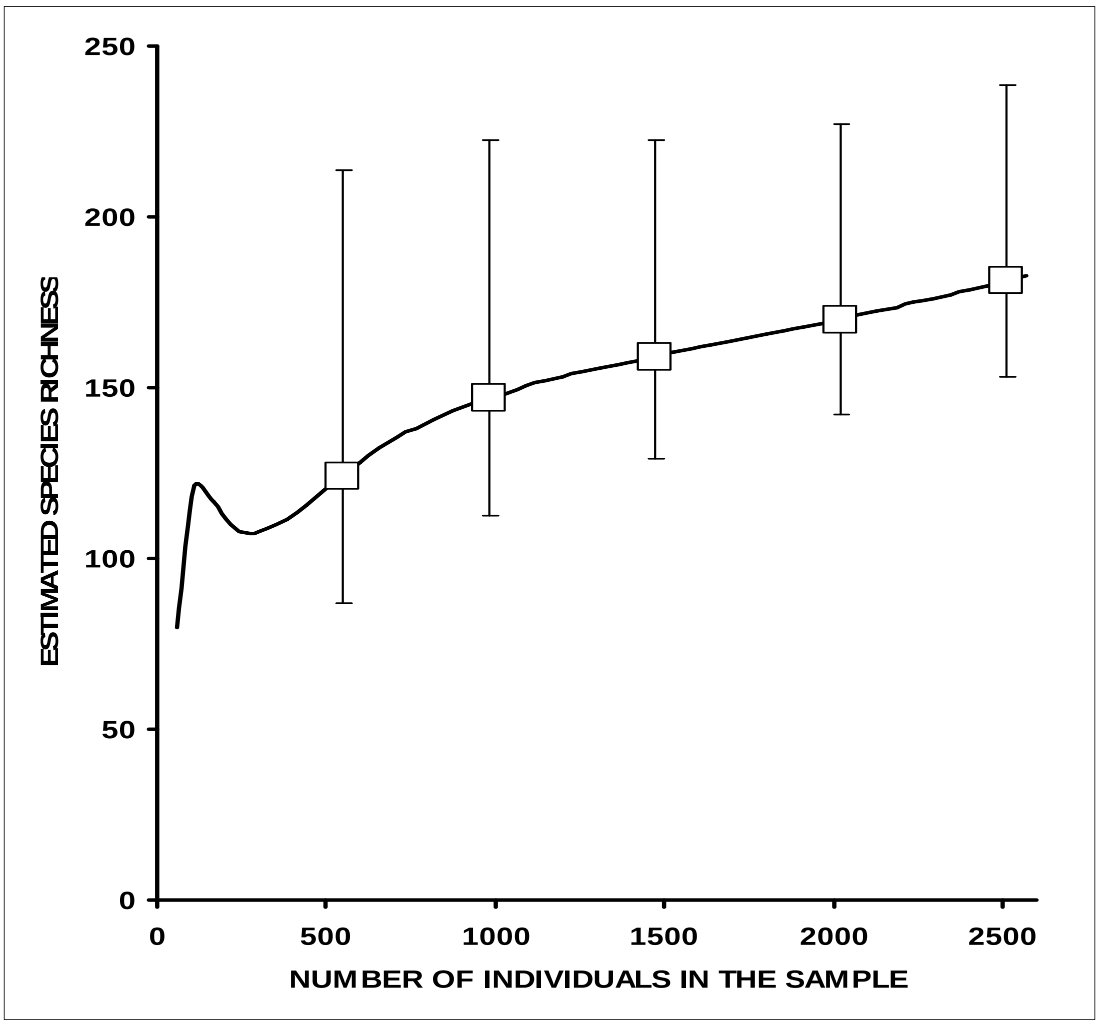

Figure 5. The rarefaction curve showing the estimated species richness for the Chao 2 estimator with 95 % CI at approximate intervals of 500 individuals sampled.

DISCUSSION

Other similar compilations of orchid species richness in Peru have been done for the MPHS (Christenson 2003), a 143.5-ha subsection of the MPHS (Zambrano *et al.* 2003a), and a portion of the Manu National Park (MNP; Zambrano *et al.* 2003b). Machu Picchu is located about 100 km to the west of Wayqechas. The locations sampled in MNP are approximately 30 km north of Wayqechas. A complete list of observed species has only been published for the MPHS. All of these studies included orchid species from cloud forest and lower elevation plant communities, complicating comparisons to the species observed at WBFS. Comparisons of species among sites are also complicated by variations in the use of nomenclature and taxonomic classification. Collections over a number of years have found 252 orchid species at MPHS, which is a similar number to the 239 species found at WBFS during the 12-month period at Wayqechas. Because MPHS is larger (32,592 ha) than Wayqechas (560 ha), it may be expected to contain more species due to more variation in habitats and variety of niches for orchids. However, the degree to which collections at MPHS have extended from readily accessible areas into the larger, more remote areas of MPHS is unclear. In the subsection of MPHS studied by Zambrano *et al*. (2003a), 179 species were found including 16 species not previously collected at MPHS.

The studies at MNP also report large numbers of species from relatively small areas with limited overlap with MPHS. In two separate study areas encompassing an elevation range from 1500 to 3000 m, Zambrano *et al.* (2003b) observed 212 species of orchids. Of these, 149 species had not been reported from MPHS. It is also remarkable that there was almost no overlap in orchid species between the study area that ranged from 1500 to 2100 m with the area that ranged from 2200 to 3000 m.

There are also differences in species compositions between Wayqechas and the MPHS. At Wayqechas, 49 genera were documented, which is less than the 75 genera collected at the MPHS. A total of 79 genera have been collected from both sites, but only 43 are common to both sites. Thirty genera collected at the MPHS were not found at Wayqechas, including *Aa*, *Bletia*, and *Brassia*. Twenty-five of the genera that occur at both Wayqechas and the MPHS were represented at Wayqechas by a single species. The other five genera were represented by less than four species at the MPHS. Six of the genera found at Wayqechas were not found at the MPHS and all of these were represented by only one species at Wayqechas. For those genera that were represented by numerous species at both Wayqechas and the MPHS, there were six *Epidendrum* species, 16 *Maxillaria* species, and six *Pleurothallis* species that were only collected at Wayqechas. Other genera found at both sites had species collected only at WBFS, and more species may be found to be unique to WBFS as more progress is made on accurate species identification of current morphospecies for difficult genera such as *Lepanthes* and *Stelis*. There are a number of species from Wayqechas that are new additions to the flora of Peru, and three species from Wayqechas with proposed names *Stellilabium cuscoense, Telipogon bettymooreana,* and *Telipogon gordonmooreana* have apparently never been previously described.

The species observed in plots represent less than 60 percent of the number observed in the general collection. This difference reflects the spatial distribution of species, with some apparently very rare or infrequently flowering species that were recorded only once during the course of study. Forty-nine of the 128 species present in the plots were only found in one plot. Examples included *Epidendrum laxicaule, Pachyphyllum gracillimum*, and *Maxillaria divaricata*. The monthly sampling intervals and nearly complete flowering of all orchid individuals in a plot ensures that the lower number of species in plots is not due to missing species that were present in the plots. To obtain a more complete sampling of the orchid flora using plots, there needs to be a larger area covered by the plots being monitored. Because of the continuous flowering throughout the year and the inability to identify orchids that are not in flower (Chapter 3), the plots still require visitation throughout the year to identify all of the species.

Attempts to determine the species richness of the orchid flora from only the plot data clearly underestimated the biodiversity observed in the general collection. Because both sets of data were the result of extensive field sampling throughout the year, this difference cannot be attributed to seasonal variation in effort. Instead, the results suggest that the differences are due to the infrequent spatial occurrence of many orchid species in the cloud forest. This is consistent with the estimators that define rareness using the frequencies of occurrence in plots producing greater estimates of richness. More than 50 percent of the species occurring in the plots only were found in only one or two plots, and almost 50 percent of the species in the general collection did not occur in the plots. Because of the slow increase in estimated richness with increasing numbers of individuals sampled for Chao 2 (Fig. 5) and the other estimators, it may require at least 70 plots or more to achieve an estimate of richness from that is comparable to that from the general collection sampling methods. But even this number of plots may fail to include all the species in the sampled area.

At present, the orchid species lists compiled from the various sites are analogous to the early tree species lists for the Amazonian rainforests, which suggested a small-scaled and relatively unpredictable structure to the forests. These quantitative studies indicated that despite the biodiversity of Amazonian rainforests, a few common species were abundant over broad regions (Pitman *et al.* 2001). As Pitman *et al.* (2001) demonstrated, this unpredictable structure became predictable once mere species lists were replaced by quantitative measures of abundances across locations for the various species. At present quantitative measures of orchid abundance are only available for WBFS, and it is unclear whether the abundant and commonly occurring orchids at WBFS such as *P. acuminata, P. vestigipetala, S. breviracema,* and *S. grandibracteatum* are regionally or merely locally abundant. Future studies of orchid communities must include at least semi-quantitative assessments of orchid abundances to achieve a level of understanding of orchid floras that is commensurate with that developing for neotropical rainforests.

Currently, only a fraction of the Peruvian cloud forests are protected in a few national parks and sanctuaries scattered along 1200 km of the Andes range (Young and León 1999). Only the southwestern corner of the MNP protects cloud forest. Large expanses of Peruvian cloud forests and their orchids remain unprotected. This study and those of Zambrano *et al.* (2003a, 2003b) have demonstrated that a larger number of species can be found in relatively small areas of Andean vegetation (< 1000 ha). The variation among these small sites may be due to the species ranges, forest structure, or climate, but the main factor that causes these differences is still unknown. The results of comparing these sites points to the need to establish numerous relatively small and manageable reserves to conserve orchid biodiversity, even though the reserves may be too small for conserving tree species or mobile fauna.

There are important factors to consider when determining not only the size but especially the structure of these reserves. First, because orchids have evolved highly specific relationships with their pollinators, the areas must be of a sufficient size to preserve the habitat resources required by pollinators (Dick 2001). The reserves must also have sufficient variation among them in forest structure in case epiphyte-host specific relations prove important (Watthana *et al.* 2006, Burns 2007). Similar recommendations have been made for the conservation of orchid biodiversity in Ecuador (Meisel and Woodward 2005) and on Reunion Island (Jacquemyn *et al.* 2007).

The extent to which these small reserves may expand or only duplicate the conservation potential of large reserves will require more complex analyses of orchid occurrences and abundances (Boecklen 1997). Using the design of this study to acquire the quantitative information needed to assess the importance of small reserves may require excessive time and effort. Instead, it may suffice to obtain semi-quantitative data using a simplified design. In this simplified design, each plot would be in a linear arrangement of contiguous 2 m x 2 m subplots. The linear arrangement and size of the subplots limits the potential damage that may be done to the habitat by reducing the need for intrusion into the plots. Monthly observations of relative abundance of each species in each subplot would evaluate the abundance and species composition of flowering individuals. The abundance would be recorded in an ordinal scale of zero (no individuals) to five ($\geq$ 50) individuals, and the median number of orchids in each of the subplots would be used to estimate the abundance in the full plot. This design would diminish the invasiveness and effort to inventory and monitor the plots over time and space. Potentially 150 of these 2 m x 2 m plots could be monitored per month with less effort than was required for the 47 plots of this project. The data from these linear plots can be used to estimate total species richness using the Chao 2 estimator, which requires only presence and absence data to assess species rareness and predict the number of unsampled species.

CHAPTER THREE

THE FLOWERING PHENOLOGY OF AN ORCHID COMMUNITY IN A PERUVIAN CLOUD FOREST, WITH IMPLICATIONS FOR THE ASSESSMENT OF LOCAL SPECIES DIVERSITY

The diversity of orchids in cloud forests of the Andes Mountains of South America has been recognized and explored for years. This high diversity of orchids is due to a moist, cool climate that is favorable to epiphytes, isolation and diversification into different ecological niches on mountain ranges where genetic drift may lead to further differentiation (Tremblay *et al.* 2005), and the ability of orchids to hybridize easily (Hollick *et al.* 2005). A complete assessment of the species diversity of cloud forest orchids in the Andes Mountains of Peru is currently limited by two main factors. First, there have been only a few intensively studied sites (e.g. Zambrano *et al.* 2003a, 2003b). Second, orchid species can be accurately identified only during periods of flowering, and few studies have been long-term and intensive enough to carry out systematic inventory across all orchid flowering phenologies in a given area. Moreover, it is not clear that the times when sampling has occurred are the times of maximum flowering.

Orchids are generally pollination limited (Tremblay *et al.* 2005) and pollinator specific (Tremblay 1992, Christenson 2003), therefore the flowering phenology of orchid species may be determined by these limitations. However, even closely related orchid species that do not have specific pollinators can exhibit different flowering patterns (Lehnebach and Robertson 2004).

Some Andean orchids, such as *Epidendrum secundum*, flower for most of the year (Zambrano *et al.* 2003a), but others flower for only brief periods. Zambrano *et al.* (2003a) report maximum flowering during times of maximum precipitation in February and March for an assemblage of 179 orchid species in southeastern Peru. These authors also report that few species flower in other months, but they do not provide details about potential overlap in species compositions between successive months. Without this information, it is not possible to decide if monthly sampling is required or if a less frequent sampling schedule would be sufficient to accurately assess diversity. If there is little overlap, then monthly sampling may be necessary. If considerable overlap occurs, then bimonthly sampling may suffice.

The purpose of this study, conducted in conjunction with an assessment of local orchid abundance and diversity, was to document the flowering phenology of an Andean cloud forest orchid community in southeastern Peru through an annual climatic cycle. The objectives of the study were to: (1) determine the proportion of the orchid species flowering in each month, (2) analyze the length and patterns of flowering for each species, and (3) assess whether collections to document orchid species diversity in an area over time must be carried out on a monthly basis or if a less-frequent sampling schedule is possible.

MATERIALS AND METHODS

All field research was carried out at the 560-ha Wayqechas Biological Field Station (WBFS, 13°10’40” S, 71°36’20” W), which borders the southeast margin of the Manu National Park in the Department of Cusco in southeastern Peru. The field station is owned and operated by the Amazon Conservation Association (ACA) of Washington, DC, and its sister organization in Peru, the Asociación para la Conservación de la Cuenca Amazónica (ACCA). The elevation of the field station ranges from 2200-3200 m. The natural vegetation consists of upper montane forest (Young and León 2000), which is continually saturated with rain and fog. Temperatures at WBFS average 11°C with little seasonal variation, and precipitation ranges from < 0.01 m in the months of June and July to > 0.10 m in the months of January, February, and March (http://atrium.andesamazon.org).

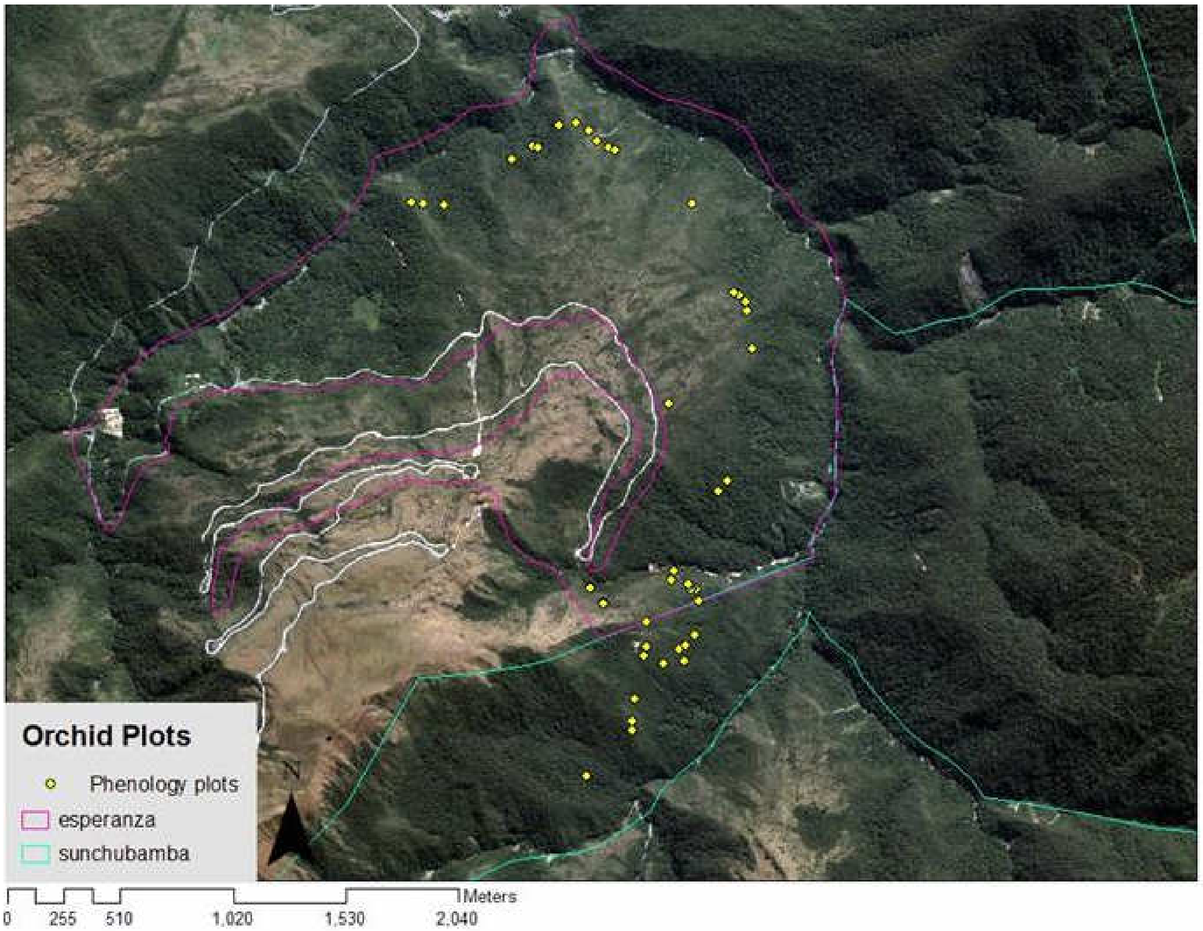

Figure 6. The location of plots at Wayqechas Biological Field Station.

Monthly assessments from September 2005 to August 2006 were conducted to document flowering phenology patterns of 128 orchid species occurring in forty-seven 25 m^2 plots (Fig. 6) established across an elevation gradient from 2200 to 3200 m within the WBFS (Chapter 2). Because of steep gradients and dense vegetation, the plots were established at random locations along pre-existing trails. Each plot was labeled, and its latitude, longitude, and elevation were determined by Garmin Map76C global positioning system. The plot's habitat was recorded as one of the following types: (1) tall cloud forest with multiple tree species with heights > 15 m; (2) short cloud forest with multiple tree species with heights < 15 m; and (3) grass areas with few or no trees with heights < 3 m. Areas of grass most likely originated from human disturbance, such as logging, landslides, cattle grazing, construction, or fire (Young and León 2000, Lozano *et al.* 2006).

A total 128 orchid species occurred in the plots and these were a subset of 239 species documented during simultaneous, general collecting activities in all habitats of the WBFS (Chapter 2) from September 2005 to August 2006. More than 130 of these were previously described species, and the rest are verifiable morphospecies whose voucher specimens have been deposited at the herbarium of the Museo Nacional de Historia Natural (USM) in Lima, Peru, with duplicates deposited at the Botanical Research Institute of Texas in Fort Worth, Texas (BRIT). Orchid identifications were made by Eric Christenson using herbarium specimens in the department of Cusco. Digital images of orchid species, flowers preserved in 80% alcohol and 20% glycerin, and collection data were deposited at BRIT. All digital images and appropriate collection data are available in the Atrium Biodiversity Information System at BRIT, at the following URL: http://atrium.andesamazon.org/.

RESULTS

During each of the 12 months of the study, the total number of flowers was counted for each species and expressed as a density of flowers per m^2. For each species, the flowers counted in subsequent months were presumed to be new flowers. However, a total number of flowers produced by a species in a plot cannot be computed because numerous flowers may have formed and senesced in the weeks between sampling.

Plots ranged in elevation between 2496 to 2993 m. When grouped into equal elevation intervals of 175 m, there were similar numbers of plots in each interval and all three habitats were present in each interval (Chapter 2).

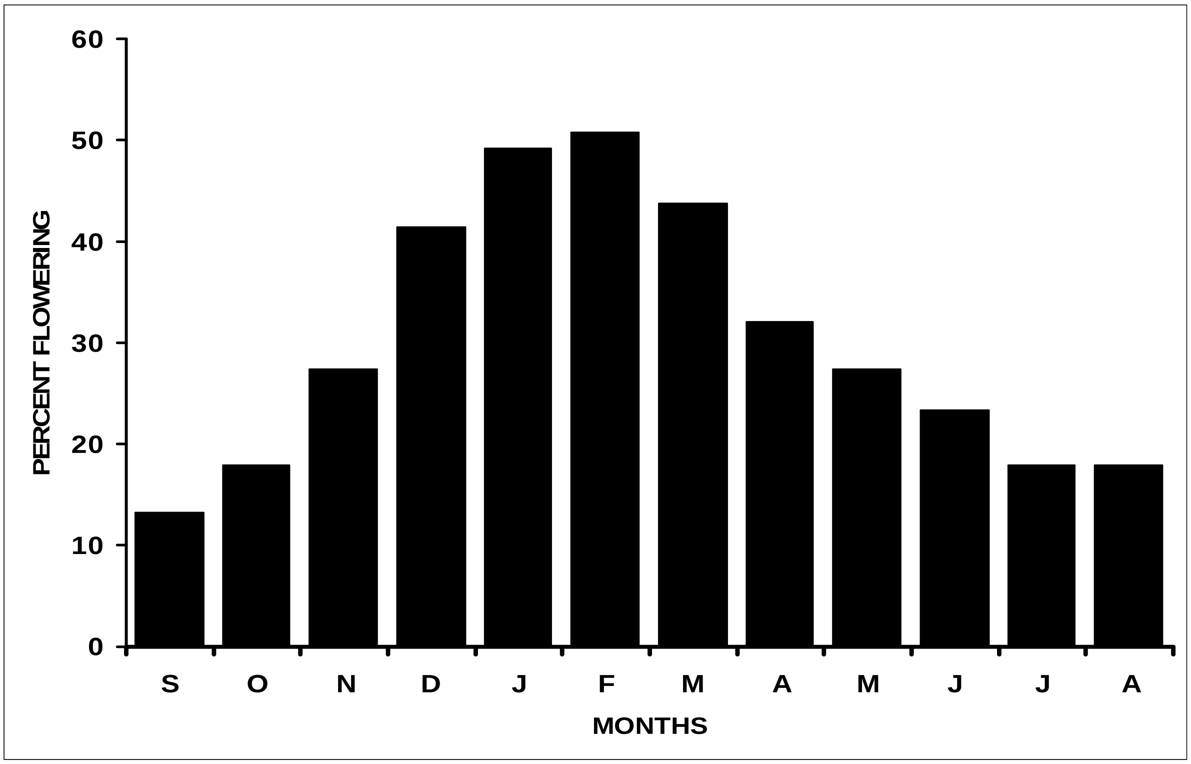

Figure 7. The percentage of species flowering in each month.

There were species flowering in every month (Fig. 7), and the percent of species in flower ranged from 13 percent in September to 51 percent in February. The density of orchid flowers was greatest during the months of January and February 2006 (Fig 8). The mean ($\pm$ SD) density of orchid flowers in the plots also ranged from a minimum of 0.647 ($\pm$ 1.06) per m^2 in September to a maximum of 25.7 ($\pm$ 37.6) per m^2 in February. Flowering density decreased rapidly after February. There were few differences in flowering phenologies among the three habitat types or across elevations.

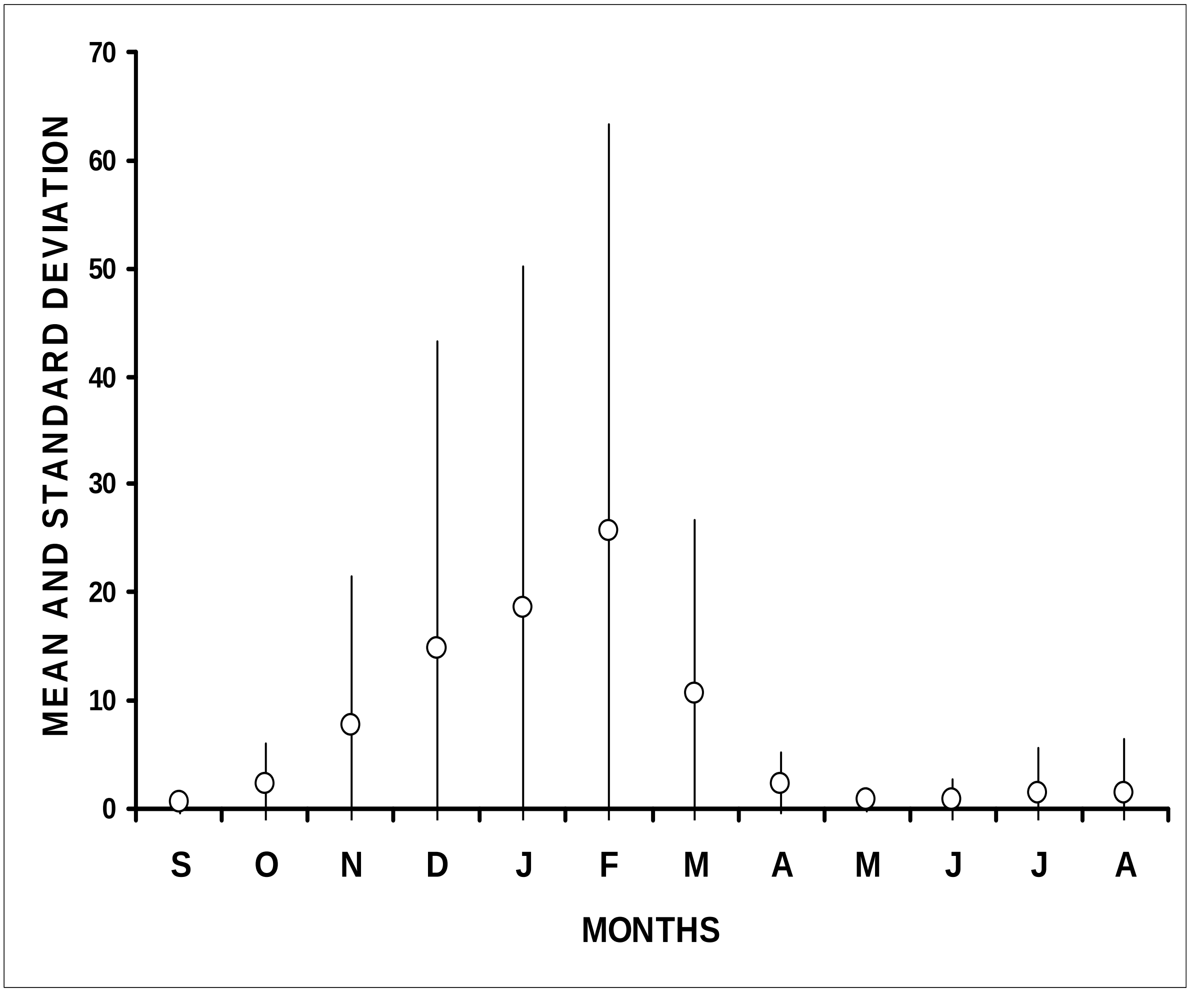

Figure 8. The mean and standard deviation of flower densities per m^2 across months.

The median duration of flowering exhibited by the 128 orchid species was three months. Over 80 percent of the species flowered for less than six months (Fig. 9). No species were observed to flower during all 12 months of the study, but six species flowered for at least ten months. These species included *Epidendrum secundum, Cyrtochilum cimiciferum,* and *Pleurothallis vestigipetala.*

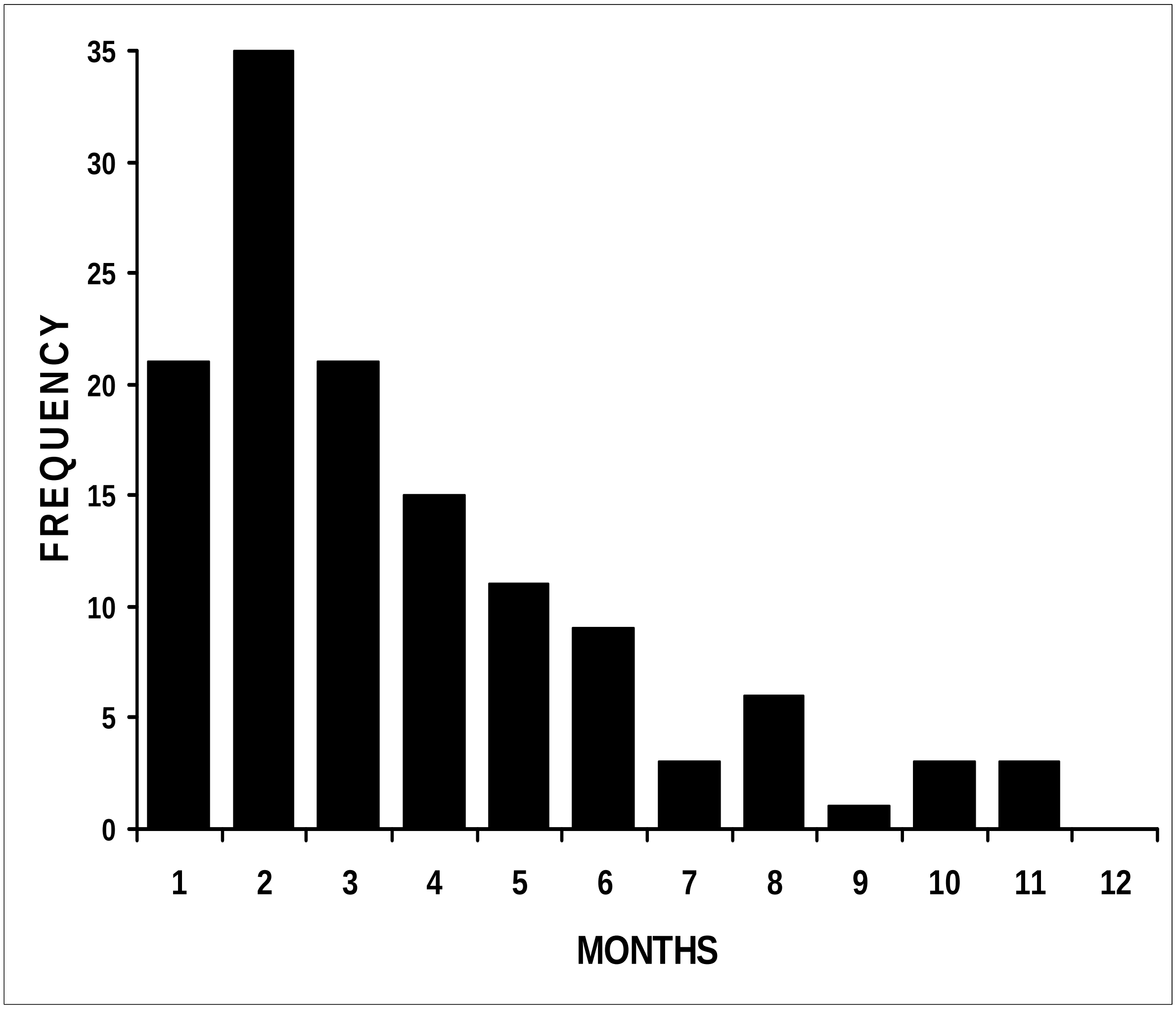

Figure 9. The frequency distribution of the number of months of flowering per species.

The proportion of flowering species shared between successive months ranged from 0.31 to 0.40 (Fig. 10). The pattern of variation showed little relation to the time of peak flowering. The proportion of orchid species flowering in successive months was similar during the months of limited flowering (i.e. July and August) and maximum flowering (i.e. January and February).

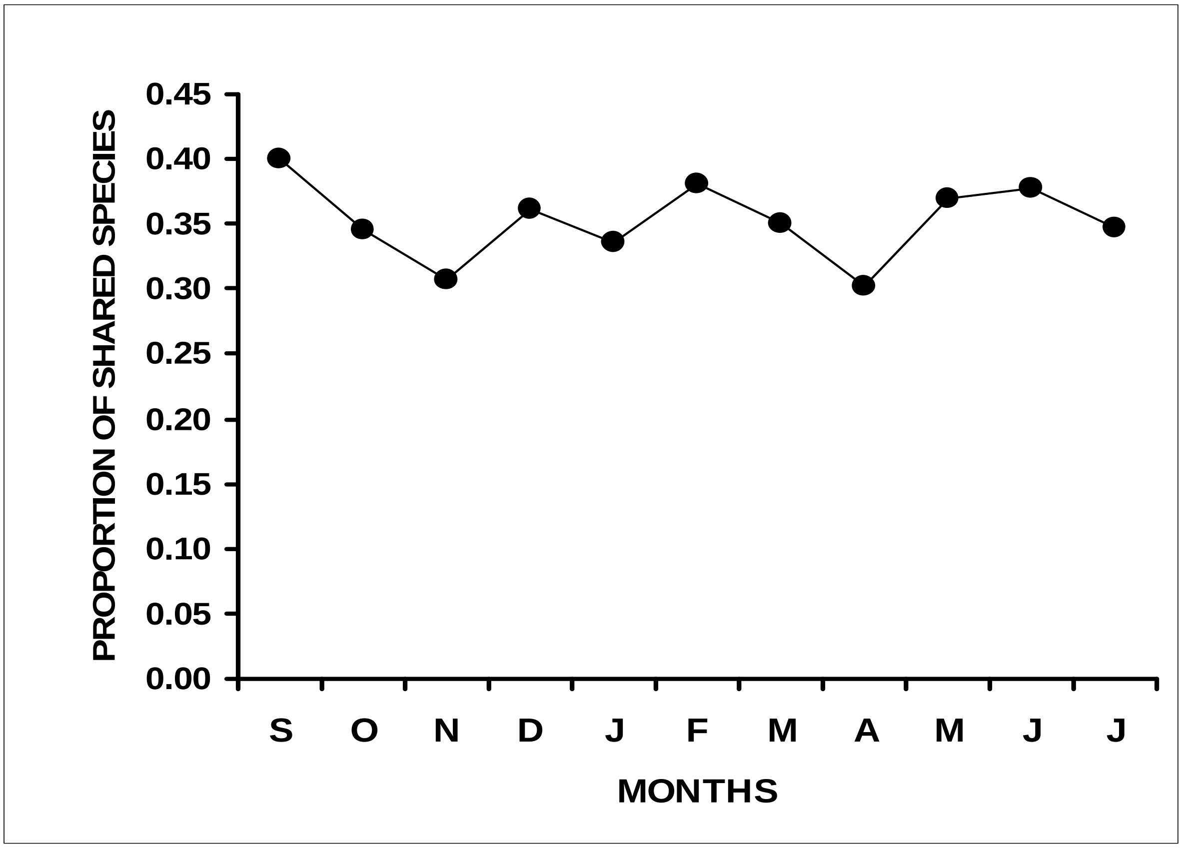

Figure 10. The proportions of flowering species that were the same in successive months.

DISCUSSION

The results indicated that to assess the orchid diversity of areas similar to WBFS, it is crucial to collect specimens and data in each month of the year, if not multiple times per month and over multiple years. Cloud forest orchids flower at different times of the year, but these data showed February to be the month of maximum flower densities and proportion of species flowering. While most flowering occurred in the months of January and February, there was continuous flowering of the orchid community throughout the entire year. Most species flowered for three months or less, and many of the species flowering in one month were not flowering in the following month. There were no peaks in Fig. 11, indicating that there were no major subsets of orchid species exhibiting synchronous flowering phenology. Had such peaks occurred, they may have been times of optimum sampling. The lack of these peaks indicates that research must be conducted throughout the annual climatic cycle to assess the true diversity of an area like the WBFS.

While February was the peak month of flowering in this study, another study conducted at a similar site in MPHS showed the peak flowering time to be in March. At both MPHS and WBFS, peak flowering occurred during months with > 0.25 m of rainfall, and minimum flowering occurred in months with < 0.05 m of rainfall. There was also a difference in the flowering patterns. Zambrano *et al.* (2003a) reported at MPHS in March there was a sharp peak in the number of flowering species, 45 percent with less than 16 percent of species were flowering in all other months. At WBFS there were five months of the year when > 30 percent of the species were flowering. The only species that flowered ≥ 10 months at both sites was *Epidendrum secundum*. The greater concentration of flowering in a single month at MPHS may be related to a broader range of elevations at MPHS or a difference in the amount and timing of precipitation between the sites. Whatever the cause of the differences, the Zambrano *et al.* (2003a) study indicates that the period of maximum flowering can be shorter and more intense than what was observed at WBFS.

Orchid species exhibit significant variations in flower longevity. For instance, in the genus *Lepanthes*, the duration of each flower can be on the order of 6-8 days (Tremblay *et al.* 2005). With the design of this study, it is not possible to determine the longevity of each orchid flower, but the flowering of more than 20 orchid species in this study was restricted to only one month. The potential for species to be in flower for only a week or so in a year suggests that even more frequent sampling than monthly may be required.

From the general collection of 239 species at WBFS (Chapter Two), more than 100 species were not present in the plots but did flower during at least one month during the year. Because once one of these a species flowered and was added to the general collection, it was not collected again. Thus, there are only records of when flowering was first noticed but no further records to indicate how long flowering may have continued. Data from these orchids would have enhanced the results of this study.

The data from WBFS and the results of Zambrano *et al.* (2003a) suggest that a complete assessment of orchid diversity in these cloud forests will require numerous periods of sampling within the year. At least monthly sampling may be required, but certain orchid floras may require more frequent sampling.

CHAPTER FOUR

CONSERVING CLOUD FOREST ORCHID BIODIVERSITY: THE POTENTIAL VALUE OF NUMEROUS SMALL RESERVES

In a 2004 address to the International Orchid Conservation Congress, Stuart Pimm (Pimm 2005) asked, “Do you know where your orchids are?” It was a question asked to raise the issues of how well orchid diversity is known and what is being done to conserve them. This question may be especially relevant to Andean cloud forest orchids. New species continue to be discovered in all of the Andean countries, including Peru (Luer and Dalström 2006). New surveys are also uncovering more data about species that remain poorly known or poorly studied (Zambrano 2003a, 2003b, Chapter 2). While all of these discoveries increase our knowledge of orchid diversity, that diversity is facing increased threats to its survival. Deforestation is one of the major threats to orchid diversity, with as much as 75 percent of montane forests in Ecuador (Meisel and Woodard 2005) and approximately 14 percent in Peru having been lost (Koopowitz *et al.* 1994, Young and León 2000). Global climate change poses another threat, with potential loss of habitat especially at lower elevations where species densities are greater (Chapter 2). Commercial harvesting to obtain more marketable hybrids is a considerable threat to the existence of wild orchids (Fernández 2005). Few preserves have been created to protect cloud forests, and those that do exist may not contain a wide diversity of orchid species (Young and León 2000, Meisel and Woodard 2005). Currently, only 15 percent of the orchid flora of Ecuador is protected in reserves (Meisel and Woodard 2005), and only a fraction of the cloud forests of Peru are protected in national parks and sanctuaries (Young and Leon 1999).

Unless rapid action is taken, Pimm's question may become, "Do you know where your orchids were?" As the habitats shrink or disappear at the current rates, many orchids may become extinct without ever having been described. Others may only exist as herbarium specimens, having disappeared from their natural environments. Some orchids may be found as commercialized hybrids having lost their original genetic structure in their native habitats. Artificially created orchid gardens, such as the one developed at Machu Picchu, may represent the only continuing outdoor existence for many species (Rolando 2005).

Recent intensive investigations of small areas of Andean cloud forests have all demonstrated high orchid species diversity. They include investigations in Manu National Park and the Machu Picchu Historical Sanctuary by Zambrano (2003a, 2003b), and investigations reported here for Wayqechas Biological Field Station (Chapter 2). The number of orchid species documented in these small study areas of less than 1000 hectares ranges from 179 to 239. Despite this local species diversity, there was often little overlap in species compositions between these areas. The studies at MNP reported few species in common with MPHS. Even within MNP, there was little overlap between two different study sites with different elevation ranges.

The results from these intensive orchid inventories focused on small areas suggest that the establishment of small reserves could play an important role in orchid conservation (Young and León 2000, Meisel and Woodard 2005). The ownership of these reserves could be private, government, or community-based. The WBFS is owned and operated by the Amazon Conservation Association (ACA) of Washington, DC, and its sister organization in Peru, the Asociación para la Conservación de la Cuenca Amazónica (ACCA). Similar reserves for the conservation of orchids have been developed in East Nepal (Shakya and Bajracharya 2005), in Ecuador (Meisel and Woodard 2005), and have been proposed for Réunion Island (Jacquemyn *et al.* 2007). A 1305-ha reserve in Brazil has protected at least 88 orchid species (Saddi *et al.* 2005).

Several considerations are important in the design of these reserves. The size of a reserve should be large enough to encompass several factors. First of all, it needs to contain the natural habitats of the orchid pollinators (Dick 2001). Because orchids may distributed in patches of only a few reproductive individuals, separated by 200 meters or more (Tremblay 1997), the reserves must be large enough to contain several such patches of a given species. A general size limit would need to be > 500 ha and preferably about 1000 ha. It is also crucial that the area consists of the range of habitats known for the region. Studies at WBFS have shown that different numbers of species occur in different habitats, such as short or tall cloud forest, grasslands, and at different elevation ranges (Chapter 2, Gentry and Dodson 1987). Some studies have shown specificity between epiphytes and their host trees, so reserves should contain variation in tree species to ensure the proper host trees are present (Watthana *et al.* 2006, Burns 2007). Tree sizes and growth forms may also be important, as these factors have been demonstrated to the distribution and abundance of epiphytes on their host trees (Krömer and Gradsetein 2003, Arévalo and Betancur 2006).

The relative importance of a single large or several small (SLOSS) reserves has been an area of considerable theoretical debate among ecologists (Diamond 1975, Simberloff and Abele 1982, Patterson and Atmar 1986, Fischer and Lindenmayer 2005, Moore and Swihart 2007). Smaller and more fragmented reserves such as those being described here may experience greater rates of species loss and may be redundant if their species composition is merely a subset of that in a larger reserve. Despite these theoretical debates, two practical factors remain. First, small reserves are better than no reserves at all. Second, in a number of studies, several small reserves have been shown to contain species that are absent from or underrepresented in larger reserves (McNeill and Fairweather 1993, Boecklen 1997, Virolainen et al. 1998, Benedick et al. 2006).

Once reserves are established, a uniform sampling protocol should be implemented to assess species composition, overlap with other reserves, and future needs. To assess the diversity of a reserve, a semi-quantitative sampling protocol should be implemented to allow the collection of sufficient data to document and compare orchid communities among reserves (Chapter Two). The protocol should include sampling of numerous permanent plots dispersed throughout the reserve and it should be designed to minimize within-plot disturbances such as trampling. A potential, simple design suggested by intensive quantitative sampling of orchids (Chapter 2) could be a linear arrangement of five contiguous 2 m x 2 m subplots. The size and arrangement of the subplots limits the potential damage by reducing the need for intrusion. With this size, species presence could be readily observed, and species abundances could be recorded in an ordinal scale of 0 (no individuals) to 5 ($\geq$ 50) individuals, with the median of the subplots estimating the abundance in the full plot. It would be possible to monitor 150 or more of these 2 x 2 m plots per month (Chapter 2). These plots should be inventoried monthly to ensure the discovery of orchids with different and sometimes brief flowering periods (Chapter 3). Continued long-term monitoring of the plots in subsequent years would also contribute to an understanding of population dynamics and climatic impacts (Light and MacConaill 2005) and assessment of the stability of orchid populations in the reserve.

The results and problems, especially taxonomic problems, of these separate reserves should be linked in a commonly available website. Digital images of flowers, fruits, and structures would enable researchers to resolve identifications, allow uniform morphospecies designations across sites, and permit remote access by taxonomic specialists to resolve similar nomenclatures. This imaged database could also assist in the recognition of orchid species by local authorities and the enforcement of local laws and regulations (Fernandez 2005). Recognition of rare or potential endemic species at reserves could focus attention on their scientific description and preservation.

These small reserves may be a sub-optimal solution to orchid conservation but may prove to be a more readily implemented, inventoried and managed solution, that may be integrated efficiently and effectively with local or regional, community-based conservation programs.

Appendix A. A list of orchid species collected at Wayqechas Biological Field Station between September 2005 and August 2006. Species listed as unidentified either remain to be classified as a previously described species or described as a potential new species. Additional information including date of collection, flower description, habit, habitat, digital images is located at http://atrium.andesamazon.org/.

Genera	Named Species	No. of as yet Unidentified Species
1 *Altensteinia*	*A. boliviensis*	
2 *Barbosella*	*B. cucullata*	
3 *Baskervilla*	*B. machupicchuensis*	
4 *Brachionidium*		1
5 *Cranichis*	*C. ciliata*	3
	C. engelii	
6 *Cyclopogon*		1
7 *Cyrtidiorchis*	*C. rhomboglossa*	
8 *Cyrtochilum*	*C. cimiciferum*	1
	C. minax	
9 *Dichea*		1
10 *Elleanthus*	*E. aurantiacus*	3
	E. capitatus	
	E. kermesinus	
	E. weberbauerianus	
11 *Epidendrum*	*E. Macrostachym aff.*	6
	E. anderssonii	
	E. farinosa	
	E. fimbriatum	
	E. funcii	
	E. goodspeedianum	
	E. gracillium	
	E. haenkeanum	
	E. jajense	
	E. laxicaule	
	E. marcapatense	
	E. mesomicron	
	E. renzii	
	E. roncanum	
	E. saxicola	
	E. schlimii	
	E. scutella	

	E. secundum	
	E. subliberum	
	E. syringothyrsus	
	E. trachysepalum	
12 *Erythrodes*		1
13 *Frondaria*	*F. caulesceus*	
14 *Gomphichis*		2
15 *Habenaria*	*H. corydophora*	
	H. dentifera	
	H. uncatiloba	
16 *Hapalorchis*	*H. pumilus*	
17 *Hofmeisterella*	*H. eumicroscopica*	
18 *Lepanthes*	*L. dictyota aff.*	12
	L. falcata	
	L. mesochlora	
	L. ptyxis	
	L. pumila	
	L. tracheia cf.	
19 *Lepanthopsis*		1
20 *Liparis*	*L. elegantula*	
	L. retusa	
21 *Lycaste*	*L. cobbriana*	
	L. gigantea	
22 *Malaxis*		4
23 *Masdevallia*	*M. picturata*	
	M. antonii	
24 *Maxillaria*	*M. alpestris aff.*	3
	M. brevifolia	
	M. brunnea	
	M. christobalensis aff.	
	M. cuzcoensis	
	M. deniseae	
	M. divaricata	
	M. floribunda	
	M. gigantea	
	M. graminifolia	
	M. haemathodes	
	M. meridensis	
	M. mungoschraderi aff.	
	M. notylioglossa	
	M. nubigena	
	M. nutans	
	M. procurrens	
	M. quitensis	
	M. rotunilabia	
	M. trigona	

	M. winaywaynaensis	
25 *Moretzia*	*M. peruviana*	
26 *Myoxanthes*	*M. frutex*	
	M. gyas	
	M. hirsuticaulis aff.	
27 *Neodraya*	*N. rhodoneura*	
28 *Odontoglossum*	*O. digitatum*	1
	O. machupicchuense	
	O. mystacinum	
	O. subuligerum	
	O. tetraplasium	
29 *Oncidum*	*O. retusum*	
30 *Pachyphyllum*	*P. breviconnatum*	3
	P. crystallinum	
	P. gracillimum	
	P. hispidulum	
	P. pectinatum	
31 *Pityphyllum*	*P. laricinum*	
32 *Pleurothallis*	*P. acuminata*	9
	P. angustilabia	
	P. cassidis	
	P. cordata	
	P. coriacardia	
	P. cyathioflora	
	P. imraei	
	P. lamellaris	
	P. melanostele	
	P. mesochlora	
	P. quadrata	
	P. rubens	
	P. ruberrina	
	P. vargasii	
	P. vestigipetala aff.	
33 *Ponthieva*	*P. cornuta*	
	P. diptera	
	P. garayana	
34 *Prescottia*	*P. petiolaris*	
	P. stachyodes	
35 *Prosthechea*	*P. farfanii*	
	P. fusca	
36 *Pterichis*		1
37 *Rusbyella*	*R. caespitosa*	2
38 *Sauroglossum*		1
39 *Scaphyglottis*	*S. punctulata*	
	S. summersii	
40 *Solenidiopsis*	*S. galianoi*	

41 *Stelis*	*S. antennata*	41
	S. breviracema	
	S. grandibracteatum	
	S. tricaridium	
	S. uninervia	
42 *Stellilabium*		(1 ined.)
43 *Stenoptera*	*S. acuta*	
	S. ciliaris	
44 *Telipogon*	*T. salinasii*	3 (2 ined.)
	T. vargasii	
45 *Trichoceros*	*T. armillatus*	
46 *Trichosalpinx*	*T. arbuscula*	1
	T. chamaelepanthes	
	T. intricata	
	T. teagueii	
47 *Vargasiella*	*V. peruviana*	
48 *Xylobium*	*X. elatum*	
	X. squalens	
49 *Unknown genus*		1

Appendix B. The elevation, habitat type, minimum number of individuals and number of species for 5 m x 5 m plots at the Wayqechas Biological Field Station.

Plot Number	Elevation above Mean Sea Level (m)	Habitat Type	Minimum Number of Individuals per Plot	Number of Species
1	2612	Short Cloud Forest	23	8
2	2609	Short Cloud Forest	48	12
3	2575	Tall Cloud Forest	9	3
4	2557	Tall Cloud Forest	52	15
5	2530	Tall Cloud Forest	107	17
6	2508	Tall Cloud Forest	67	13
7	2496	Tall Cloud Forest	28	11
8	2517	Short Cloud Forest	43	15
9	2501	Grass Areas	30	12
10	2524	Short Cloud Forest	66	22
11	2538	Tall Cloud Forest	19	9
12	2526	Short Cloud Forest	127	25
13	2808	Tall Cloud Forest	5	3
14	2787	Short Cloud Forest	35	5
15	2760	Tall Cloud Forest	62	5
16	2686	Short Cloud Forest	63	15
17	2662	Grass Areas	101	19
18	2666	Short Cloud Forest	68	19
19	2666	Short Cloud Forest	78	19
20	2665	Short Cloud Forest	83	24
21	2661	Short Cloud Forest	32	14
22	2606	Short Cloud Forest	63	20
23	2982	Short Cloud Forest	43	9
24	2993	Short Cloud Forest	102	14
25	2969	Grass Areas	59	6
26	2973	Grass Areas	50	7
27	2966	Grass Areas	21	3
28	2970	Grass Areas	32	3
29	2968	Short Cloud Forest	33	6
30	2946	Short Cloud Forest	16	5
31	2945	Short Cloud Forest	52	10
32	2954	Short Cloud Forest	31	9
33	2917	Tall Cloud Forest	202	3
34	2915	Tall Cloud Forest	11	7
35	2849	Short Cloud Forest	34	8
36	2820	Tall Cloud Forest	11	7
37	2844	Tall Cloud Forest	120	7

38	2839	Tall Cloud Forest	15	8
39	2831	Tall Cloud Forest	17	5
40	2819	Short Cloud Forest	90	17
41	2817	Short Cloud Forest	68	12
42	2802	Grass Areas	82	15
43	2817	Short Cloud Forest	103	15
44	2801	Tall Cloud Forest	12	5
45	2778	Tall Cloud Forest	45	9
46	2845	Tall Cloud Forest	37	6
47	2932	Grass Areas	70	8

REFERENCES

Andersohn, C. 2004. Does tree height determine epiphyte diversity? Selbyana 25: 101-117.

Arévalo, R., and J. Betancur. 2006. Vertical distribution of vascular epiphytes in four forest types of the SerranÍa de Chiribiquete, Colombian Guayana. Selbyana 27: 175-185.

Benedick, S., J. K. Hill, N. Mustaffa, V. K. Chey, M. Maryati, J. B. Searle, M. Schilthuizen, and K. C. Hamer. 2006. Impacts of rain forest fragmentation on butterflies in northern Borneo: species richness, turnover and the value of small fragments. Journal of Applied Ecology 43: 967-977.

Boecklen, W. J. 1997. Nestedness, biogeographic theory, and the design of nature reserves. Oecologia: 123-142.

Burnham, K. P., and W. S. Overton. 1978. Estimation of the size of a closed population when capture probabilities vary among animals. Biometrika 65: 623-633.

Burnham, K. P., and W. S. Overton. 1979. Robust estimation of population size when capture probabilities vary among animals. Ecology 60: 927-936.

Burns, K. C. 2007. Network properties of an epiphyte metacommunity. Journal of Ecology 95: 1142-1151.

.Chao, A. 1984. Non-parametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11: 265-270.

Chao, A. 1987. Estimating the population size for capture-recapture data with unequal catchability. Biometrics 43: 783-791.

Chao, A., and S. M. Lee. 1992. Estimating the number classes via sample coverage. Journal of the American Statistical Association 87: 210-217.

Chao, A., M. C. Ma, and M. C. K. Yang. 1993. Stopping rules and estimation recapture debugging with unequal failure rates. Biometrika 80: 193-201.

Christenson, E. 2003. Machu Picchu: Orchids. PROFONAPE, Lima, Peru

Colwell, R. K. 2006. EstimateS 8.0 User's Guide. http://viceroy.eeb.uconn.edu/estimates.

Cornelissen, J. H. C., and H. Ter Steege. 1989. Distribution and ecology of epiphytic bryophytes and lichens in dry evergreen forest of Guyana. Journal of Tropical Ecology 5:131-150.

Der, G., and B. Everitt. 2001. Handbook of statistical analyses using SAS. CRC Press, Boca Raton, USA.

Diamond, J. M. 1975. The island dilemma: Lessons of modern biogeographic studies for the design of natural reserves. Biological Conservation 7: 129-146.

Dick, C. W. 2001. Habitat change, African honeybees, and fecundity in the Amazonian tree *Dinizia excelsa* (Fabaceae). *In* Bierregaard, R. O., Jr., C. Gascon, T. E. Lovejoy, and R. C. G. Mesquita. Lessons from Amazonia: The ecology and conservation of a fragmented forest. Yale University Press, pp. 146-157. USA.

Dressler, R. L. 2005. How many orchid species? Selbyana 26: 155-158.

Fernández, R. 2005. Orchid conservation in Peru: the need for taxonomists to assist local authorities with plant identification. Selbyana 26: 335-335.

Field, A. 2005. Discovering statistics using SPSS. Sage Publications, London, England.

Fischer, J., and D. B. Lindenmayer. 2005. Perfectly nested or significantly nested – an important difference for conservation management. Oikos 109: 485-494.

Gentry, A. H. 1988. Changes in plant community diversity and floristic composition on environmental and geographical gradients. Annuals of the Missouri Botanical Garden 75: 1-34.

Gentry, A. H., and C. H. Dodson. 1987. Diversity and biogeography of neotropical vascular epiphytes. Annuals of Missouri Botanical Garden 74: 205-233.

Gotelli, N., and R. K. Colwell. 2001. Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4: 379-391.

Gravendeel, B., A. Smithson, F. J. Slik, and A. Schuiteman. 2004. Epiphytism and Pollinator Specialization: Drivers for Orchid Diversity. Philosophical Transactions: Biological Sciences 359: 1523-1535.

Grubb, P. J., J. R. Lloyd, T. D. Pennington, and T. C. Whitmore. 1963. A comparison of montane and lowland rain forest in Ecuador. I. The forest structure, physiognomy, and floristics. Journal of Ecology 51: 567-601.

Hirtz, A. 2005. The latest explosion in orchid evolution. Selbyana 26: 277-287.

Hollick, P. S., R. J. Taylor, J. A. McComb, and K. W. Dixon. 2005. If orchid mycorrhizal fungi are so specific, how do natural hybrids cope? Selbyana 26: 159-170.

Ingram, S. W., K. Ferrel-Ingram, and N. M. Nadkarni. 1996. Floristic composition of vascular epiphytes in a neotropical cloud forest, Monteverde, Costa Rica. Selbyana 17: 88-103.

Jacquemyn, H., O. Honnay, and T. Pailler. 2007. Range size variation, nestedness and species turnover of orchid species along an altitudinal gradient on Reunion Island. Biological Conservation 136: 388-397.

Koopowitz, H., A. D. Thornhill, and M. Anderssen. 1994. A general stochastic model for the prediction of biodiversity losses based on habitat conversion. Conservation Biology 8: 425-438.

Krömer, T., and S. R. Gradstein. 2003. Species richness of vascular epiphytes in two primary forests and fallows in the Bolivian Andes. Selbyana 24: 190-195.

Lee, S. M., and A. Chao. 1994. Estimating population size via sample coverage for closed capture-recapture models. Biometrics 50: 88-97.

Lehnebach, C. A., and A. W. Robertson. 2004. Pollination ecology of four epiphytic orchids of New Zealand. Annals of Botany 93: 773-781.

Light, M. H. S., and M. MacConaill. 2005. Long-term studies: a case for orchid species survival. Selbyana 26: 174-188.

Lozano, P., R. W. Bussman, and M. Kueppers. 2006. Landslides as ecosystem disturbance – their implications and importance in Southern Ecuador. Lyonia 9: 75-81.

Luer, C. A., and S. Dalström. 2006. Six new *Masdevallia* species (Orchidaceae) from Peru. Selbyana 27: 15-24.

McNeill, S. E., and P. G. Fairweather. 1993. Single large or several small marine reserves? An experimental approach with seagrass fauna. Journal of Biogeography 20: 429-440.

Meisel, J. E., and C. L. Woodward. 2005. Andean Orchid Conservation and the Role of Private Lands: A Case Study from Ecuador. Selbyana, The Journal of the Marie Selby Botanical Gardens 26: 49-57.

Moore, J. E., and R. K. Swihart. 2007. Toward ecologically explicit null models of nestedness. Oecologia 152: 763-777.

Nadkarni, N. M. 1992. The conservation of epiphytes and their habitats: summary of a discussion at the international symposium on the biology and conservation of epiphytes. Selbyana 12: 140-142.

Otero, J. T., P. Bayman, and J. D. Ackerman. 2005. Variation in mycorrhizal performance in the epiphytic orchid *Tolumnia variegata in vitro*: the potential for natural selection. Evolutionary Ecology 19: 29-43.

Patterson, B. D., and W. Atmar. 1986. Nested subsets and the structure of insular mammalian faunas and archipelagos. *In* Heaney, L. R., and B. D. Patterson (Eds.). Island Biogeography of Mammals. Academic Press, pp. 65-82, London.

Phillips, O. L., and P. H. Raven. 1996. A strategy for sampling neotropical forests. *In* Gibson, A. C. Neotropical Biodiversity and Conservation. University of California, pp. 141-165, USA.

Pimm, S. L. 2005. Keynote address: It's a new century: Do you know where your orchids are? Selbyana 26: 5-13.

Pitman, N. C. A., J. W. Terborgh, M. R. Silman, P. Núñez V., D. A. Neill, C. E. Cerón, W. A. Palacios, and M. Aulestia. 2001. Dominance and distribution of tree species in upper Amazonian terra firme forests. Ecology 82: 2101-2117.

Rolando, I. 2005. Fifteen years of in-situ conservation at Machu Picchu. Selbyana 26: 134-135.

Rolfe, R. A. 1916. IX - *Epidendrum Secundum* and *E. Elongatum*. Bulletin of miscellaneous information 1916: 49-49.

Saddi, E. M., R. C. Lopes, and R. H. P. Andreata. 2005. Floristics and conservation of Orchidaceae at Rio das Pedras Reserve. Selbyana 26: 318-325.

Shakya, L. R., and D. M. Bajracharya. 2005. Orchid sanctuary Raja Rani (Morang District), east Nepal: An effort toward habitat conservation. Selbyana 26: 236-239.

Shefferson, R. P., D. L. Taylor, M. Weib, S. Garnica, M. K. McCormick, S. Adams, H. M. Gray, J. W. McFarland, T. Kull, K. Tali, T. Yukawa, T. Kawahara, K. Miyoshi, and Y. Lee. 2007. The evolutionary history of mycorrhizal specificity among lady's slipper orchids. Evolution 61: 1380-1390.

Simberloff, D. S., and L. G. Abele. 1982. Refuge design and island biogeographic theory – effects of fragmentation. American Naturalist 120: 41-56.

Stebbins, G. L. 1981. Why are there so many species of flowering plants? BioScience 31: 573-577.

Tremblay, R. L. 1992. Trends in the pollination biology of the Orchidaceae. Evolution and systematics. Canadian Journal of Botany 70: 642-650.

Tremblay, R. L. 1997. Distribution and dispersion patterns in nine species of *Lepanthes* (Orchidaceae). Biotropica 29: 38-45.

Tremblay, R. L., J. D. Ackerman, J. K. Zimmerman, and R. N. Calvo. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. Biological Journal of the Linnean Society 84: 1-54.

Virolainen, K. M., T. Suomi, J. Suhonen, and M. Kuitunen. 1998. Conservation of plants in single large and several small mires: species richness, rarity and taxonomic diversity. Journal of Applied Ecology 35: 700-707.

Watthana, S., H. A. Pedersen, and S. Suddee. 2006. Substrate diversity, demography, and fruit set in two populations of the epiphyte *Pomatocalpa spicatum* (Orchidaceae) in Thailand. Selbyana 27:165-174.

Young, K. and B. Leon. 1999. Peru's humid eastern montane forests: An overview of their physical settings, biological diversity, human use and settlement, and conservation needs. Center for Research on the Cultural and Biological Diversity of Andean Rainforests, Technical Report No. 5. 97 pages.

Young, K., and B. León. 2000. Biodiversity conservation in Peru's eastern montane forests. Mountain Research and Development 20: 208-211.

Zambrano, D. A., N. S. Revilla, and W. N. Huari. 2003. La familia Orchidaceae L. en Wiñay-Wayna, Sanctuario Histórico de Machu Picchu. Lyonia 3: 273-282.

Zambrano, D. A., N. S. Revilla, and W. N. Huari. 2003b. Orquídeas del Valle de Cosñipata, Parte Alta de la Reserva de Biósfera del Manu, Cusco – Peru. Lyonia 3: 283-290.

VITA

Personal Background	Rebecca Elaine Repasky Dallas, Texas Daughter of James and Karen Repasky
Education	Diploma, Kilgore High School, Kilgore, Texas, 2000 Bachelor of Science, Biology, Texas Christian University, Fort Worth, 2004 Master of Science, Biology, Texas Christian University, Fort Worth, 2008
Experience	Research Assistant, Chemistry Department, Texas Christian University, 2001 Research Assistant, Biology Department, Texas Christian University, 2002 Teaching Assistantship, Texas Christian University, 2005-2007

ABSTRACT

DIVERSITY AND PHENOLOGY OF ORCHIDACEAE IN AN UPPER MONTANE FOREST, DEPARTMENT OF CUSCO, PERU

by Rebecca Elaine Repasky, M.S., 2008
Department of Biology
Texas Christian University

Thesis Advisor: John Janovec, Botanical Research Institute of Texas
Thesis Co-Advisor: John Horner, Professor of Biology

A study of orchids was carried out in a cloud forest in southeastern Peru, in the department of Cusco, between September 2005 and August 2006. A systematic collection found 239 different morphospecies of orchids which was compared to those found at two similar areas, Manu National Park and Machu Picchu Historical Society. A set of 47 plots were set up and monitored for orchid diversity, abundance, and phenology of the area. Based on habit (epiphytic or terrestrial), the number of species varied among habitats. Orchids were shown to be flowering at all times of the year with some species being rarer. Flowering times of the orchids were maximized in times of high precipitation levels (February). Recommendations are made for future research, including study length and design. A potential protocol for orchid conservation is also described, with attention to size of a reserve to include multiple variables.