DIVERSITY, NATURAL HISTORY, AND CONSERVATION OF *VANILLA* OF MADRE DE DIOS, PERU

by

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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 2007

ACKNOWLEDGEMENTS

This manuscript represents the hard work, sweat, thoughtfulness, guidance and generosity of many friends and colleagues. Without their assistance, there is good chance that I would have mysteriously vanished in the tangled maze of wetland vegetation or lost my way in the labyrinths of a massive dataset. Specifically, I must thank Dario Cruz and Javier Huinga for guiding me safely on expeditions. Their orienteering skills and sharp eyes never cease to amaze. I thank John Janovec, who has become over the years mentor, advisor, friend, colleague and inspiration. I thank Mathius Tobbler, Ranjan Muttiah, and John Horner for guidance on various aspects of data analysis. I thank Nigel Pitman for taking a sincere interest in all of the wonderful science, work and people filtering in and out of the Los Amigos Biological Station. I thank Alex Van Dam, Pesach Lubinsky, and Miguel Soto-Arenas for species identifications, stimulating conversation, and their shared passion for an incredible group of plants. I thank Jesus and all CICRA employees for obliging me and all other investigators with our strange requests and needs. I thank both Texas Christian University and the Botanical Research Institute of Texas for the generous financial support and grants that funded this research.

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SUMMARY AND GUIDE TO THESIS

The following work is the result of one solid year's worth of intense fieldwork concerning the diversity, natural history, ecology, and biogeography of the genus *Vanilla* in Madre de Dios (MdD), Peru. Due to the breadth of topics discussed in the following pages, the thesis has been broken into independent chapters. Each contains its proper Abstract, Introduction, Materials and Methods section, Results, Discussion, Conclusions, as well as its own pertinent tables, figures, and supporting literature.

Chapter 1, "Diversity and Natural History of *Vanilla* in Wetlands of Madre de Dios, Peru" serves as the basic foundation upon which the other chapters build. I describe in detail all five species of wetland-dependent *Vanilla*. Also, various aspects of the natural history observed over the course of an entire year are documented for each species. Important aspects of diversity, pollination biology and dispersal are discussed.

Chapter 2, "Diversity and Biogeography of *Vanilla* (Orchidaceae) in Wetlands of Madre de Dios, Peru" accounts for the bulk of fieldwork. Here I report species composition and species composition of *Vanilla* in sixteen wetlands covering over 200 km of the wetland system. I attempt to explain distribution and abundance by drawing on ecological aspects specific to each species as well as large-scale gradients and historical events that may affect many wetland species. I utilize *Vanilla* as a model to begin asking questions concerning the larger plant communities inhabiting MdD wetlands and how best to conserve them.

Chapter 3, **"**Fruiting and Flowering Dynamics of *Vanilla pompona subsp. grandiflora* (Orchidaceae) During a Single Flowering Season" takes a detailed look at a single *Vanilla* species. I document and analyze the habit, growth, pollination rates, flower production, and fruit production, discussing the results as they pertain to pollination ecology, demography, and life-history of this single species.

Chapter 4, "Mapping a Potential Non-Timber Forest Product in the Wetlands of Madre de Dios, Peru", offers an applied perspective on *Vanilla* in the region. Here, I classify the distribution of a single species of commercial *Vanilla* in Madre de Dios. Although vanilla culture and

economics are briefly reviewed, the heart of this chapter focuses on using remotely sensed data to predict where and how much *Vanilla* habitat occurs within the study region.

RESUMEN

El trabajo de tesis presentado a continuación refleja los resultados de un año de trabajo de campo sobre la diversidad, historia natural, ecología y biogeografía del género *Vanilla* en el departamento de Madre de Dios, Perú. Debido a la diversidad de temas que abarca, la tesis ha sido dividida en cuatro capítulos. Cada capítulo es independiente y enfoca aspectos específicos de la vainilla. De esta manera, cada capítulo cuenta con una Resumen, Introducción, Materiales y Métodos, Resultados, Discusión, Conclusiones, Figuras, Tablas y Referencias.

El primer capitulo, "Diversidad y Historia Natural de *Vanilla* en Humedales de Madre de Dios, Perú", sirve como base para los posteriores capítulos. En el capitulo mencionado, se describen con detalle las cinco especies de *Vanilla* que habitan los humedales estudiados. Además de las descripciones morfológicas, cada especie va acompañada con una breve reseña sobre algunos aspectos de su historia natural, observados durante el transcurso del año. Por ejemplo, aspectos sobre su diversidad, polinización y dispersión son incluidos y discutidos.

El segundo capitulo, "Diversidad y Biogeografía de *Vanilla* (Orchidaceae) en los humedales de Madre de Dios Perú", abarca gran parte del trabajo de campo. En este capitulo, se reporta la composición y frecuencias relativas de las especies de *Vanilla* distribuidas en dieciséis humedales, dentro de aproximadamente 200 Km de rio. El objetivo de este sección es el de explicar las distribución y abundancia de las especies de *Vanilla* en cuestión, utilizando información sobre su ecología, gradientes a escala regional y eventos históricos que hayan podido afectar la composición de la comunidad de *Vainilla* así como de plantas en general. En conclusión se propone utilizar al género *Vanilla* como modelo para la formulación de hipótesis que busquen responder preguntas sobre la composición de la vegetación que habita los humedales y sobre su conservación.

El tercer capitulo se titula: “Dinámica de floración y fructificación de *V. pompona* sp. *grandiflora* (Orchidaceae) durante una temporada”, y se enfoca describiendo en detalle a la especie *V. pompona* subespecie *grandiflora.* Se describe y analiza detalladamente su crecimiento, taza de polinización y la producción de flores y frutos. Los resultados se integran al entendimiento sobre la ecología de polinización, demografía, y historia natral de esta especie.

El cuarto y ultimo capitulo, “Mapeando un potencial producto no maderable en los humedales de Madre de Dios, Perú”, ofrece una perspectiva radicalmente distinta sobre la *Vanilla* en la región. En este capitulo se examina la distribución de *Vanilla* en Madre de Dios y sus potenciales usos. Aunque se presenta un resumen sobre la producción y economía de *Vanilla*, el corazón de este capítulo se centra en el uso de imágenes satelitales para predecir su localización y abundancia dentro de la región.

CHAPTER 1

DIVERSITY AND NATURAL HISTORY OF *VANILLA* IN WETLANDS OF MADRE DE DIOS, PERU

ABSTRACT

Five species of V*anilla* have been found in wetlands of Madre de Dios, Peru representing approximately 10% of neotropical *Vanilla*. Within wetland habitat Vanilla is the second most diverse orchid genus. Each species is described in detail and various aspects of its natural history discussed, including pollination system, bee interactions, dispersal, ecology, and habit. A key is offered for proper identification in vegetative states. Four species are widespread or fairly common in the Amazon. One species has as of yet uncertain taxonomy. All five species are exclusive to wetland habitat in Madre de Dios.

INTRODUCTION

Vanilla is a pantropical genus of orchid including over one hundred species of epiphytic, semi-epiphytic, and terrestrial herbs. It is noteworthy among the genera of tribe Vanilleae as the most species rich and widespread genus within the group (Stern and Judd 1999). Furthermore, recent molecular evidence suggests that the vanilloid orchids are an isolated, ancient lineage of orchid (Cameron et al. 1999). Such traits highlight the genus as key to understanding the most species rich plant family on the planet, calling for further studies of the group's taxonomy and natural history. However, taxonomic investigations are scarce or incomplete and the genus is plagued by misidentifications, synonymy and confusion (Chevailer 1946, Correll 1946). Although there may be abundant morphological studies pertaining to *Vanilla*, there has been little comparative vegetative anatomy and even less attempt to apply these findings to classification until recently (Stern and Judd 1999). Thus, from a botanical standpoint, there is a deficit of basic natural history, posing a major obstacle to our understanding of the group.

From an economic standpoint, cured vanilla fruit provide the world's major source of natural vanilla flavoring. In 1995, vanilla beans ranked as the leading U.S. spice import on par with black pepper (Buzzanell and Gray 1995). World production of approximately 2000 metric tons (1995 to 2000 estimates) is generally restricted to poor, tropical nations with a cheap labor force (Koekoek 2005). Thus, the crop is important to thousands of small farmers worldwide. However, the narrow genetic base of commercial vanilla (*Vanilla planifolia*) and its growing susceptibility to viral and fungal pathogens suggest that the industry has not fully explored the genetic resources harbored by potentially disease resistant wild species (Grisoni et al. 2004). Given the global economic importance of vanilla as a flavoring agent it is surprising that so few studies describing the natural history of this genus exist in the literature.

Several characteristics of the group make detailed investigation, documentation, and collection difficult. *Vanilla* species often occur as hyper dispersed populations with limited geographic ranges, a distribution pattern that has contributed to the lack of herbarium collections and accumulation basic natural history. Ephemeral, often tender flowers further exacerbate specimen collection and quality. However, despite these difficulties to the study and collection of V*anilla*, the number of species in herbaria has more than doubled since the latest revision of the genus by Rolfe (Rolfe 1896), suggesting that a more recent monograph would be both timely and valuable.

Nowhere is this need for a formal treatment and basic natural history more striking than in the Amazon, the heart of *Vanilla* diversity (Soto-Arenas et. al. 2003). Amazonian V*anilla* are poorly known compared to their Central American counterparts (Soto-Arenas pers comm.). In order to fill the voids existing in our understanding of the genus in the Amazon, the goals of this study were two-fold: 1) report the diversity of *Vanilla* in Madre de Dios, Peru and 2) document the ecology, habit, and reproductive biology of each species.

MATERIALS AND METHODS

Study Site

The department of Madre de Dios is situated in southeastern Peru, corresponding to the headwaters region of the southwestern Amazon. The region is well known as a mecca of biological and cultural diversity that enjoys at least some protective status in a series of large reserves.

Annual average rainfall is 2,600 mm, ranging between 2,152 and 3,498 mm during 2001 to 2005. Rainfall is unevenly distributed throughout the year, with greater than 80% falling between October and April (fig 1). Average daily temperature ranges from 21 to 26ºC. Southerly cold fronts from Patagonia, known as “friajes”, are common in June through August (Amazon Conservation Association, unpublished data). Friajes can decrease temperatures by 10ºC or less in minutes. Climate data since 2001 is publicly available and provided by the Amazon Conservation Association.

As part of a larger project to document the local flora, from July 2005 to August 2006, our

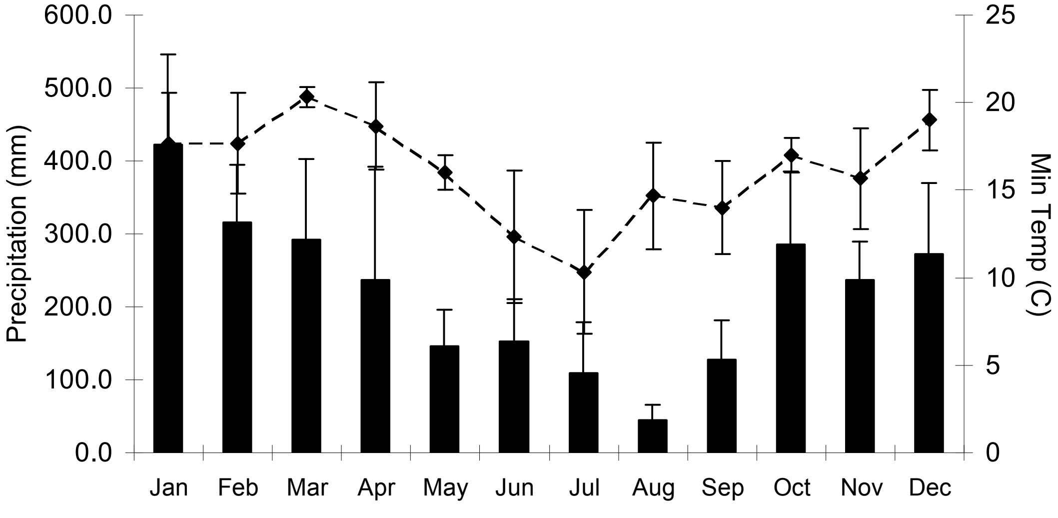

Figure 1. Climograph of CICRA biological station (ACA). Values are averages from 2001 -2005. Error bars are set at plus and minus one standard deviation. Darkened bars are average monthly precipitation. The dashed line is average monthly temperature.

team initiated intensive field campaigns into remote regions in search of *Vanilla*. These orchids occur almost exclusively in wetlands, known locally as “agujales”. This habitat is named after the common name of the dominant palm, “aguaje” or *Mauritia flexuosa* (Kahn 1988). These wetland systems are characterized by deep histosols, measuring up to 10 meters deep in some places, waterlogged soils, and monodominance by *M. flexuosa*. Soils are saturated year round with acidic, black waters due to leachate from palm material.

Transects of variable length were established within wetlands. Transect direction and length were determined by accessibility and size of the wetland. In total, 35 km of transect were surveyed in 16 separate wetlands (fig 2). Plant descriptions of vegetative parts were done from field specimens of climbing individuals. Flowers found in the field were photographed and preserved in 95% alcohol for later description. Pollination rates for all species were estimated at the end of the flowering season by counting the number of empty floral bracts, flowers and fruits of the maximum number of flowering individuals as possible - ranging from three to 148. Rates were calculated by dividing the total number of fruits by the sum of empty bracts and flowers. Bagging experiments were performed on two species thought to be auto-pollinating because of their high pollination rates. Entire racemes were covered with one millimeter mesh bags to exclude pollinators. Any observations of bees at flowers and/or fruits were opportunistic. If possible, photos and sample specimens were collected for later identification. Descriptions of floral scents were facilitated by enclosing the entire flower into a plastic film container for five minutes to intensify the fragrance.

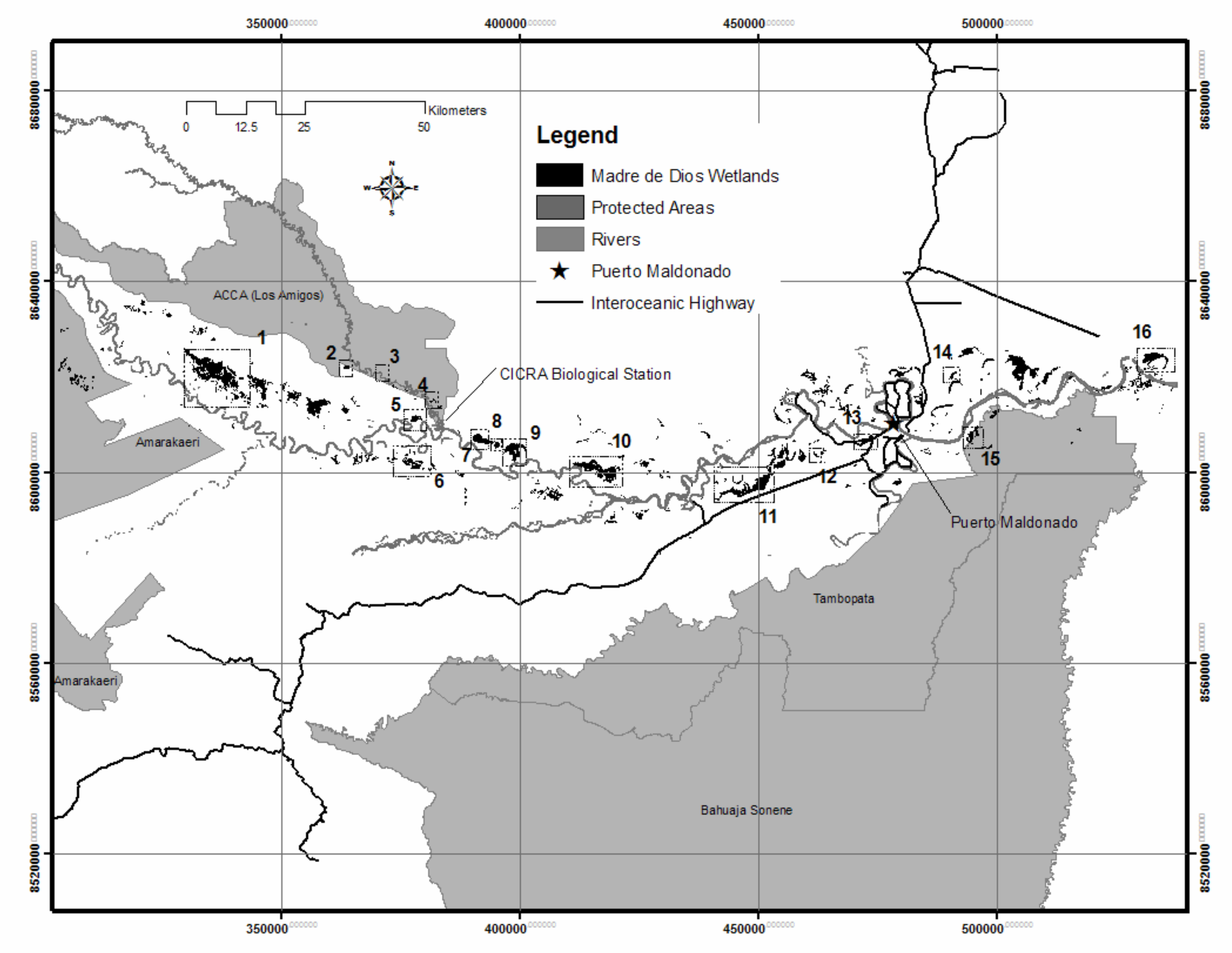

Figure 2. Map of study region illustrating the location and distribution of visited wetlands.

RESULTS

Five species of *Vanilla* occur in the wetlands of Madre de Dios. Following the key, each species is described and its natural history and nomenclature are detailed. Nomenclatural details and taxonomic placements follow Miguel Soto-Arenas.

KEY TO SPECIES

1a. Leaves solid green, succulent; stems ~1cm diameter or greater……………………………….…………2
    2a. Leaves lanceolate, dark green… ……………………………………………….…*V. riberoi*
    2b. Internode length less than 8cm for climbing individuals……………………………..*V. sp. 4*
    2c. Internode length greater than 8cm for climbing individuals………*V. pompona subsp. grandiflora*
1b. Leaves thin; stems less than 1cm in diameter……………………………………………….....3
    3a. Leaves with orange-purple margins, held parallel to host, petioles (~1cm), ……......…..*V. bicolor*
    3b. Leaves papery thin, no petioles………………………………………………*V. guianensis*

Vanilla pompona Schiede supsp. grandiflora (Lindl.) Soto-Arenas

Nomenclature

It is a member of the American clade of fragrant vanilla.

Description

Robust vine climbing up to 30 meters, one to many branched (fig 3). Climbs via aerial roots produced from each node; aerial roots short, stout, white, unbranched, ca. 0.5 cm in cross-section, ca. 3 – 5 cm long. *Terrestrial roots* emerging from lower stems and entering superficially into substrate; branched and extensive. *Stems* thick and succulent with irritating sap, 0.9 – 2.3 cm in diameter, internodes 9 – 13 cm for climbing individuals. *Leaves* alternate, produced from each node opposite to roots, highly plastic, ca 3 x 6 - 13 x 30 cm, succulent, held at 30 to 60 degrees below horizontal, sessile; blade elliptic to orbicular; apex acute to rounded; margin entire; texture smooth. *Inflorescences* racemose, axillary only, fleshy, green, sessile ca. 1 to 20-flowered; *bracts* 1 x 1.5 cm. *Flowers* resupinate, tender, fleshy, ca. 15 cm across, arranged spirally, flowers variably fragrant; *pedicel* green, with ovary; *buds* green; *dorsal sepal* yellow, elliptic-oblanceolate, reflexed backwards, ca 1.5 x 9.4cm; *lateral sepals* yellow, reflexed, c 1.5 x 8.5cm. *Petals* yellow, ca 1.1 x 9cm, with thickened midvein on dorsal surface. *Lip* uniformly yellow except for a characteristic white triangle on ventral surface, adnate to column for ca. 5 cm, margins involute, undulate; *callus* well developed and robust, c 0.8 x 0.6 cm, preceded by dark longitudinal line connecting margins and callus, ca. 2.2 cm long and 0.3 cm wide. *Column* 6.3 cm long, 0.4 cm wide, ventral surface hirsute near rostrellum, then glabrous, slight sinusoidal curve; *rostrellum* broad, thick, yellow. *Fruit* capsule, fleshy, triangular in cross-section, green when immature, ca. 2.6 x 15, extremely oily and slow drying when open.

Natural History and Reproduction

Flowers open singly, occasionally doubly during cool weather and generally last approximately 8 hours, opening as early as 3 am. The maximum number of flowers observed on a single individual was 200. Flowering phenology is extremely seasonal, and individuals tend to flower more profusely following small-scale temperature reductions. Flowering episodes occur in April and September, and generally last thirty days or less. Rapid temperature drops probably induce bud initiation several months before flowers begin to open.

Several observations of bees in and around *V. pompona subsp. grandiflora* flowers have been made. Two euglossini species, the pollinator, *Euleama meriana*, and non-pollinating *Euglossa imperialis* are attracted to particularly fragrant flowers. Neither nectar nor fragrance collection was observed during the only observation of a pollination event, suggesting that flowers are deceptively pollinated (Lubinsky et al. 2006). No nectar reward is provided. On September 15, 2005, two individuals of *Euleama meriana*, and three of *Euglossa imperialis* were observed near a single flower. All bees flew in circles around a single flower although two on the same raceme were open. No bee, however, landed on the flower. After three minutes of observation without a pollination event occurring, the fragrant flower was removed, hidden from view, and transferred 30 meters from the original site. The non-fragrant flower remained on the raceme. Within 20 seconds, all bees, plus an additional *E. meriana*, had returned to the transported flower. However, no pollination event occurred. Pollination rates are extremely low, measured at less than one percent in September 2005 (table 1). The number of fruits per individual ranges from one to four, the great majority of fruiting individuals producing only a single fruit during any given season.

This *Vanilla* species demonstrates a degree of myrmecology, especially during flowering. Small ant colonies anticipate the production of sugars exuded at the abscission layer of immature buds by building colonies in hollow spaces of axillary meristems.

Fruits mature and begin to dehisce approximately 9 months after pollination. Fruits slowly open for a period of approximately two weeks, revealing hundreds of thousands of tiny seeds. Mature (dehiscing) fruits have a sweet vanilla fragrance. The odor is attractive to a variety of bees, including *Euleama meriana*, *Euglossa imperialis*, *Melipone sp*., and *Xylocopa sp*. *Melipone sp*. has been observed to collect and fly away with small packets of twenty seeds on their hind tibia. Nothing is known about the fate of these seeds once collected, however, we have observed melipone bees to drop their seed packets often, suggesting they may be effective dispersers. Occasional herbivory of fruits suggests that larger vertebrates, such as bats or locally abundant marsupials, may also be important dispersers.

Seedling emergence is distinctly seasonal. Most seedlings surface in May, coinciding with the initiation of the dry season. However, seedlings are extremely rare and most reproduction is through vegetative means. The vine is extremely clonal and resilient to disturbance, capable of growing up to 1 meter in length monthly.

Figure 3. Flowers, fruits, and habit of *V. pompona subsp. grandiflora*.

Habitat

Occurs in open, boggy areas of *M. flexuosa* wetlands. *V. pompona subsp grandiflora* is the most abundant *Vanilla* species, present in all visited wetlands. While extremely opportunistic, it is generally restricted to a distinct wetland habitat characterized by open palm canopies, high light levels, and a well-developed herbaceous understory.

Table 1 Pollination rates for each *Vanilla* species. Rates were calculated as the total number of fruits, divided by the total number of bracts counted. Counts were timed at the end of the flowering season, or in the case of V. bicolor and V. guianensis, only individuals that had terminated flowering were included.

Species	Total Bracts	Total Fruits	Total Racemes	Total Individuals	Poll Rates
V. pompona subs grandflora	5405	50		148	0.9%
V. bicolor	692	301	141	43	43%
*V. bicolor***	31	22	13	13	71%
V. guianensis	163	127	38	8	78%
V. sp 4	110	4	8	5	3.6%
V. riberoi	88	1	12	3	1.1%

**** Racemes were bagged with cloth mesh. Self-pollination rates were estimated as the number of fruits divided by the total number of unpollinated flowers at the bottom of the bag plus the number of fruits (flowers remain attached to the ovary if pollinated).**

Vanilla bicolor Lindl.

Nomenclature

Possibly synonymous with *V. wrightii* Rchb. F (Soto-Arenas, pers com). It is a primitive member of the American clade of fragrant vanillas (Soto-Arenas et al. 2003).

Description

Plant is a semi epiphyte with the maximum length of a single branch reaching 3 to 4 meters (fig 4). Attaches to host via *roots* produced from each node, thin, unbranched, 3 – 100 cm in length, slightly tan. *Stems* thin, fleshy, ca. 0.4 cm in diameter, internodes 8.5 – 10.5 cm long, not succulent. *Leaves* alternate, produced from each node opposite to roots, ca. 2.5 X 5.1 cm – 4.5 X 11cm, held vertically hanging from petioles; *petiole* u-shaped in cross-section, ca. 1cm in length, red to purple; blade elliptic, apex acute, margin entire with red to purple outline, texture smooth. *Inflorescences* racemose, axillary and terminal, fleshy, green, sessile, 1 to 20 per individual, ca. 1 to 10-flowered; non-floriferous bracts generally 2; *floral bracts* red to purple ca. 0.3 x 0.6cm. *Flowers* trumpet-like, resupinate, fleshy, tender, 10cm across, arranged spirally on raceme, non-fragrant; *pedicel* with ovary, dark red when young, maturing to green, ca. 3 cm long and 0.2 cm in diameter; *buds* dark red; *dorsal sepal* elliptic-oblanceolate, orange 0.7 x 7.5 cm, *lateral sepal* orange, elliptic-oblanceolate, 0.9 x 7.5 cm; *petals* orange-red, oblanceolate-linear 0.6 x 6.5cm. *Lip* yellowish-orange adaxially for 4/5 of length, fading to pure white margins, adnate to column for three quarters of its length, thick hairs form distinct patch, c 0.7 x 0.3 cm, *callus* 0.7 x 0.3 cm; margins involute, undulate. *Column* straight, 4.2 cm long, 0.2 cm wide, ventral surface hirsute for about a third of its length, then glabrous; *rostrellum* thin, narrow, white; *anther sac* 0.3 x 0.4 cm. *Fruit* capsule, cylindrical, c 10 cm long, non-fragrant, quick drying.

Natural History and Reproduction

Vanilla bicolor may exhibit both epiphytic and terrestrial habits, however, the clear majority exist as epiphytes on *M. flexuosa* hosts. Epiphytic seedlings germinate where the frond sheaths the palm trunk. Long, slender roots reach deep into the hollow space between frond and trunk, tapping a rich source of nutrients and moisture. Fronds of adult palms generally last on the order of two years. As the palm fronds upon which *V. bicolor* grows are sloughed, older ramets of are discarded. Thus, epiphytic *V. bicolor* must continuously colonize young frond crotches as older ramets are torn away. Virtually all reproductive adults occur as epiphytes in *M. flexuosa* palms twenty to thirty meters high. These individuals are exceedingly difficult to study and all reproductive data was collected from individuals near or rooted in soil substrate. Results may differ for epiphytic individuals in the canopy.

Flowers of plants open singly at approximatley 5-day intervals, lasting themselves less than one day. The plant is auto-pollinating and somewhat cleistogamous. Occasionally flowers on several individuals are simultaneously fully open, suggesting that they are responding to some synchronizing environmental variable. Fully opened flowers auto-pollinate, however, cross pollination mediated by animal vectors may be possible. Auto-pollination is achieved by the excretion of excess stigmatic fluids that liquefy the pollen grains and facilitated by a thin, narrow rostrellum that forms a poor barrier between pollen and stigma (fig 5). Pollination rates are extremely high, falling between the ranges of 45% to 75% (table 1). No local pollinators have been observed at or near flowers, although this may be due to our bias of observations at ground level.

Figure 4. Flower, fruits, and habit of *V. bicolor*. Notice that the flower in this case if fully open.

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Figure 5. Photograph showing the ventral surface of the stigma (clear/white) with pollen deposited on either lateral extreme (yellow). The rostrellum has been removed for clarity.

Flowering phenology is fairly aseasonal, although there may be a slight peak during the height of the dry season in August. Fruiting phenology is also aseasonal. Mature fruits can be observed all year.

The mechanism of seed dispersal of *V. bicolor* is unknown. Ants often collect sugars from scale insects on developing fruits, but do not visit dry, dehisced fruits and are unlikely dispersers. Lack of fragrance, oils, or sugars, extremely small seeds, and canopy habitat (Kelly 1985) is suggestive of wind dispersal. Seedlings are abundant, germinating almost exclusively in the small openings between palm trunks and fronds.

Habitat

This species has a large distribution, previously being collected in Ecuador, Guyana, and Venezuela. This is the first documentation for *V. bicolor* in Peru, significantly extending it range southwards. In wetlands it is among the most abundant and frequent *Vanilla*. Terrestrial individuals are restricted to the most open areas where sunlight is often the most intense. Epiphytic individuals are more widespread, but favor areas where *M. flexuosa* crowns are well-spaced. The town of Puerto Maldonaldo roughly corresponds to the eastern limit of *V. bicolor* in our region (fig 2).

Vanilla guianensis Split.

Nomenclature and Taxonomy

This species has a complicated nomenclature, with no type specimen and several synonyms. The oldest name is *V. guianensis* Split and thus conserved. However, it may also be found as *V. acuta* Rolfe, *V. latisegmenta* Ames, and *V. surinamensis* Rchb.f. (Soto-Arenas, pers comm.) As a member of the membranaceous clade it is distinguished by its thin leaves, continuous endoderm comprised of a large number of row fibers in the stem, and little differentiation between vegetative and reproductive axes.

Description

Plant a vine, generally restricted to understories within shadier environments, however occasionally reaching 10 meters above ground in full sun (fig 6.). Climbs via *aerial roots*, short, thin, ca 0.2 X 3cm, unbranched. *Terrestrial roots* long, thin, superficial. *Stems* thin, brittle, 0.4 – 0.7 cm in diameter, internodes 8 – 10 cm, one to many branched. *Leaves* alternate, produced from each node opposite to roots, thin and membranaceous, ca 3.7 X 7.5cm – 11 X 22 cm; blade elliptical; apex acuminate; margins entire and undulate. *Inflorescences* racemose, axillary, sessile, 2 - 8 flowered, fleshy, green; *bracts* green, triangular, ca 1 x 1 cm. *Flowers* not resupinate, fleshy, very stiff, ca 7 cm wide, distichous on raceme, slightly fragrant (like cut grass or trunk slash); *pedicel* with ovary, pale yellow-green, straight, ca 0.3 X 3 cm (pedicel of auto-pollinating individuals are often longer because fruit development begins in bud stage). S*epals* green, stiff, ca 1.7 x 4 cm. *Petals* green, waxy, stiff, ca 1.2 x 4 cm, margins occasionally sinuate. *Lip* white, stiff, distally 3-lobed; lateral lobes arching over column, adnate to column for 0.4 cm of length, margins rounded; middle lobe broadly 3-angular, margins undulate, anterior surface with two thick, yellow longitudinal ridges, *callus* absent. *Column* 2.4 cm long, 0.4 mm wide, stiff, forks 0.4 cm from tip of stigma, rostrellum absent, if outcrossing then 1 – 2 mm space separates stigma

Figure 6. Flower, fruits, and habit of *V. guianensis*.

from another. Anther sac 0.2 x 0.2 cm, margins orange-red. Stigmatic surface with four lobes, yellow, ca. 0.05 x 0.2 cm. Fruit cylindrical, long ca 1 X 18 cm, disagreeable fragrance when moist.

Natural History and Reproduction

Flowering is somewhat sporadic. Various individuals flowered in January, April, May, and November. The number of flowers per individual ranged from 5 to about 50, depending on plant size. Flowers often open in triplets, and remain open for several days to a week as observed for other membranaceous species (Soto-Arenas et al. 2003). As more triplets open a relatively large floral display accumulates. Flowering occurs most gregariously on hanging ramets not supported by a host trunk similar to many cucurbits (Niel, pers. com.).

The population of *V. guianenis* in wetland five (fig 2) includes both auto-pollinating individuals and individuals needing insect vectors for pollination. Self-pollination occurs in the late bud stage as the stigma and anther sac grow to contact one another (fig 7). This seems to be genetically controlled, resulting when the column does not fork at its terminal end to separate anther sac and stigma. Pollination rates are approximately 78% for auto-pollinating individuals (table 1). Only a single outcrossing individual was confirmed, blooming in January. We suspect that in some wetland populations (wetlands one and six) non-crossing individuals dominate local populations.

Figure 7. Photograph illustrating an outcrossing individual of *V. guianensis*. The space separating stigma and rostrellum becomes severely reduced in auto-pollinating individuals.

No potential pollinators have been observed near the flowers. The rather stiff flowers and relatively closed throat of the flower must be opened by separating the lip from the column, a feat that could only be achieved by a large, strong pollinator. Soto-Arenas (2003) suggests that *Xylocopa* likely canidates. The rather large, flat landing surface may be especially suitable for large, ungainly pollinating vectors such as *Xylocopa*. However, no *Xylocopa* species have been observed near flowers of *V. guianensis* in the wetlands.

Unlike other *Vanilla* the flower and column rapidly abscise from the developing fruit. Nothing is known about seed dispersal. However, the fruits do have a rather unpleasant, putrid odor for one week to one month before the fruit completely desiccates, and this may be attractive to some animal dispersers. None, however, have been observed near the fruits.

The thin brittle stems do not readily propogate and sexual reproduction, rather than clonal reproduction, probably dominates measures of individual fitness.

Habitat

This species is fairly common throughout the Amazon basin (Soto-Arenas, pers. com.). In our region, it occurs in *M. flexuosa* wetlands. This species is generally associated with low-lying, shrubby trees common to these wetlands, such as *Ilex sp* (Aquifoliaceae), *Tapirira sp* (Anacardiaceae), and *Tabebuia sp*. (Bignoniaceae). The readily decomposed, leafy litter associated with these wetland shrubs may provide more suitable germination sites than palm fronds, the dominant litter source. The town of Puerto Maldonaldo roughly corresponds to the eastern limit of this species in our region (fig 2). It tends to occur much lower densities than the other species.

Vanilla sp 4.

Nomenclature

This species is possibly new to science. Most similar to *V. cristagallii* Hoehne, a possible synonym with *V. cristato-callosa* Hoehn. These represent a diverse group of poorly collected Amazonian species, and it is necessary to resolve the old names before this species can confidently be identified. Nonetheless, we can group this as a member of the American clade of fragrant vanilla.

Description

Plant a short vine climbing up to 3 to 4 meters (fig 8). Climbs via *aerial roots* produced from each node, white, unbranched. *Terrestrial roots* emerging from lower stems and entering superficially into substrate, branched and extensive. *Stems* succulent, 0.6 – 0.9 cm in diameter, internodes ca. 6cm for climbing individuals and ca. 9 cm for scandent individuals. *Leaves* alternate, borne opposite to aerial roots, held horizontal to ground, ca 3 X 9.5 cm – 7 X 17 cm, succulent; blade elliptic; apex acute to slightly acuminate; margin entire; texture smooth, venation somewhat apparent, sessile. *Inflorescences* racemose, terminal or axillary, fleshy, green, sessile, ca 1 to 40-flowered; *bracts* green, triangular. *Flowers* resupinate, tender, fleshy, arranged spirally, fragrance fabacious, like torn leaf of a bean; *pedicel* white for half its length, fading to green near perianth, with ovary, ca 0.4 cm wide, 4.9 cm long; *buds* green. *Sepals* yellow, oblanceolate, apex acute, ca 0.9 x 6.3 cm. *Petals* yellow, oblanceolate, apex acute, 0.8 x 5.6 cm. *Lip* yellow, adnate to column for 4 cm, trumpet-like, margins undulate, patch of thickened papillae ca 0.6 x 1 cm dominates lip surface, *callus* ca 0.4 x 0.3 cm. *Column* 4.6 cm long, 0.2 cm wide, *Anther* cap yellow, 0.2 x 0.4 cm. *Fruit* capsule, fleshy, cylindrical in cross-section, green when immature, approximately 1 x 15cm, mature dehiscent fruit produces abundant gelatinous substance, strong vanillin fragrance.

Figure 8. Flower, fruits, and habit of *V. guianensis*. Note the metallic green euglossini bee visiting the dried fruit.

Natural History and Reproduction

Flowers occur in mid January, during the height of the rainy season. Flowers open singly with an interval between subsequent flowers of a few days. Fruits are generally terminal on the inflorescence, suggesting that once pollinated, flower production ceases. Pollination rates are approximately 4% (table 1). The pollinator is unknown. Ants are often present during bud and raceme development, feeding on a sugary exudates secreted at the abscission line between bud and ovary.

Fruits persist for one year and remain fragrant. We observed a single species of metallic green *Euglossa sp* visiting old fruits in January and April, presumably collecting fragrance and possibly seeds.

Habitat

In our region *V. sp 4* occurs in *M. flexuosa* swamps. The distribution of *V. sp 4* is extremely localized, known to occur in only a single wetland in the center of our study area. All reproductive adults we encountered grow in much shadier conditions than other *Vanilla* species.

Vanilla ribeiroi Hoehne

Nomenclature

The only type specimen for this species is damaged. Central Amazonian member of the American clade of fragrant *Vanilla*.

Description

Plant a vine climbing up to 3 meters. Climbs via *aerial roots* produced from each node, short, stout, white, unbranched. *Terrestrial roots* emerging from lower stems and entering superficially into substrate, branched and extensive. *Stems* succulent ca 0.5 – 0.8 cm in diameter, zigzag pattern apparent, internodes 10 – 15 cm. *Leaves* produced from each node opposite to roots, succulent, dark green, held at 0 to 15 degrees below horizontal, ca. 1.5 x 8.5cm – 2 x 14 cm; blade lanceolate; apex acute; margin entire; texture smooth. *Inflorescences* racemose, axillary only, fleshy, green, sessile ca 1 to 20-flowered. *Flowers* resupinate, tender, fleshy, arranged spirally, non-fragrant; *pedicel* with ovary, white at base to three-quarters length fading to green towards perianth, ca 0.5 x 4.5 cm; *buds* green; *Dorsal* s*epal* green, oblanceolate, apex rounded, c 1 x 5 cm. *Lateral sepals* green, oblanceolate, apex rounded, ca 1.4 x 4.7 cm. *Petals* green, oblanceolate-linear, ca 1 x 4.6 cm, longitudinally ribbed on dorsal surface. *Lip* white, adnate to column for two-thirds length, margins involute, undulate, distinct patch of thickened hairs ca 1.5 x 1 cm, *callus* 0.5 x 0.4 cm. *Column* 2.7 cm long, 0.4 cm wide, ventral surface hirsute; rostrellum thick, wide, stigmatic surface with two lobes. *Fruit* capsule, fleshy, cylindrical in cross-section, green when immature.

Natural History and Reproduction

Flowers last a single day. The flowering season occurs in mid August, just prior to the main flowering season of *V. pompona subsp grandiflora*, and phenologies may partially overlap. Nothing is known about pollinators. Pollination rates are low, estimated to be about one percent based on three individuals (table 1), suggesting that pollinations are due to outcrossing by insect vectors.

Figure 9. Flower and growth form of *V. riberoi*.

Habitat

Previously collected from Mato Grosso, Brazil near the Bolivian border in inundated zones. In our region it occurs in open *M. flexuosa* wetlands. A single individual was observed in a seasonally inundated habitat dominated by shebón. In wetland habitat, it is generally associated with clusters of the leafy shrub, *Ilex sp*. (Aquifoliaceae). *V. ribeiroi* is very rare, appearing only in the eastern portion of our area.

DISCUSSION

Diversity and Distribution

At least five species of *Vanilla* occur in wetlands of MdD, accounting for approximately 10% of all neotropical *Vanilla* species. This makes *Vanilla* the second most speciose genus of orchid in the wetlands behind *Epidendrum* (Chocce, unpublished data). *Vanilla*, however, is perhaps the most conspicuous due to its large size and high abundance. Only one other study, located in the Caribbean islands, has documented several species of sympatrically occurring *Vanilla* (Nielsen and Siegismund 1999). The authors note, however, that the species tended to segregate according to favored substrate, and this seems to be the case in much of Central America (Soto-Arenas et al. 2003).

Patterns of *Vanilla* distribution in MdD swamps contrast those often found in Central America. In the former, several species occur sympatrically, often within meters of each other, and are quite abundant. In the latter, segregation to specific substrates and hyper dispersed populations are the general rules.

All five species, with the exception of *V. sp 4*, are known to be widespread or are fairly common within central to eastern Amazonia. None, however, are known to occur exclusively within wetlands in their ranges. The high diversity and exclusivity of *Vanilla* to wetlands in Madre de Dios suggests that these wetland systems are satisfying key biotic or abiotic requirements not met in the surrounding forests. While *Vanilla* can withstand the extended dry season from May to September, signs of water stress such as desiccated leaves, wilting, and reduced growth rates are expressed even in the humid wetlands. Although a number of factors likely contribute to the role of MdD wetlands as refugia for *Vanilla*, the permanently saturated soils point towards humidity an important environmental variable explaining its restricted distribution. Given the humid conditions within wetlands, it is surprising that the epiphytic and climbing communities in general are not more diverse. However, monospecific stands of smooth, straight-trunked *M. flexuosa* palms may provide few suitable niches or germination sites.

Pollination

Pollination rates of wetland *Vanilla* are among the lowest and highest for deceptively pollinated tropical orchids (Tremblay et al. 2005), typifying the great diversity in life-history and pollination strategies of Madre de Dios wetland *Vanilla* species. Fragrance has long been implicated in pollinator specificity and speciation in Orchidaceae (Dodson 1962, Dodson et al. 1969, Hills et al. 1972). In *V. pompona, subsp grandiflora*, floral fragrance seems to attract two species of euglossini bees, one of which is the pollinator. While this observation suggests that fragrance seems to be important in initial pollinator specificity and attraction, it is perhaps not the sole ingredient necessary for successful pollination by insect vectors, as attraction does not necessarily lead to a bee entering the gullet flowers to pollinate. In fact the pollinator of *V. pompona subsp grandiflora* does not display the brushing behavior typical of scent collection on the lip surface when pollinating (Lubinsky et al. 2006). This seems to be a general phenomenon among many outcrossing, fragrant *Vanilla*, such as *V. planifolia, V. insignis,* and *V. hartii*. These species are pollinated by both female euglossini bees not known to collect fragrances, as well as scent-collecting male euglossini bees (Soto-Arenas et al. 2003). A combination of visual and olfactory stimuli may be necessary, or alternatively fragrance may have a more complicated role in reproductive success than simple pollinator attraction. For example, fragrance may also be important in deterrence of non pollinating agents rather than attraction of pollinators, or perhaps anachronistic (Ackerman et al. 1997).

We did not detect any fragrance in flowers of *V. riberoi*, but this may be due to the small number of flowers available and the high variability in the strength of floral fragrance documented in other species, such as *V. pompona, subsp grandiflora*. Rolfe (1896) describes the flowers of *V. bicolor* as “deliciously fragrant”. We however, note a complete absence of floral fragrance in this wetland population even after examination of hundreds of flowers.

Two of the five wetland species auto-pollinate, *V.bicolor* and *V. guianensis*. The degree of auto-pollination in any group will depend on a range of factors, but floral morphology and the abundance of pollen vectors are thought to be the most important (Schemske and Llande 1985). If this is the case, orchids may be particularly prone to auto-pollination. Indeed, Catling (1990) estimates that 5 – 20% of orchids auto-pollinate. The fusion of male and female parts into a column and generally low pollination rates of deceptively pollinated orchids (Tremblay et al. 2005) may facilitate a transition towards auto-pollination. In our study, this shift always results in higher fruit production, suggesting that pollination limitation may be severe in crossing species unless background mutations remove costs of intensified sexual reproduction resulting from auto-pollination.

Two hypotheses attempt to explain the evolution of self-pollination. The ‘automatic selection’ (Jain 1976) hypothesis posits that auto-pollinating morphs will spread in populations as long as fitness of selfed progeny is one half or greater than that of progeny from outcrossing events (Fisher 1941), and inbreeding depression is not crippling (Holsinger 1988). If these conditions are met, selfing genes are transmitted to next generations at higher rates than outcrossing genes and can thus spread in future generations (Fisher 1941). The automatic selection hypothesis would be more likely to occur in an unsaturated, uncompetitive environment, where the consequences of inbreeding depression are minimal (Catling 1990). The ‘reproductive assurance’ hypothesis, on the other hand, posits that selective advantage of self-pollination lies in assured seed production when pollinators are rare or unreliable, usually the result of long distance dispersal (Baker 1955). The reproductive assurance hypothesis is manifested under conditions of pollination limitation due to intense pollinator competition, inefficiency of pollinators, or dearth of pollinators in a new environment (Schoen et al. 1996). Trends in Orchidaceae such as chronically low pollination rates and specificity of pollinators point toward reproductive assurance as a powerful selective force mediating the evolution of auto-pollination in *Vanilla* (Catling 1990, Johnson et al. 1994). We predict that auto-pollinating *Vanilla* in MdD wetlands are the result of colonization events and that a different pollinator community has provided a large selective force favoring this pollination mechanism.

It is interesting to note that the two auto-pollinating species, *V. guianensis* and *V. bicolor*, are both characterized by their non-fleshy, brittle stems. According to our own observations this greatly reduces the ability of the vine to propagate vegetatively after a disturbance. As a result, fitness for these two organisms is highly dependent on sexual reproduction and seedling establishment, much more so than their more clonal counterparts. The contrast is important. Fecundity, or the number of seeds produced, is only one component of total fitness, and in extremely clonal species, it may even be minor (Calvo and Horvitz 1990). In the case of *V. pompona subsp. grandiflora*, *V. sp. 4*, and *V. ribeiroi*, the adverse fitness consequences of low pollination rates during a single season decrease throughout the vine's life, and these robust vines may be incredibly long-lived. In the case of *V. bicolor* and *V. guianensis* reduced clonal reproduction may predispose these particular species to auto-pollinate, especially when confronted with novel pollinator communities and/or intense pollination limitation in wetland environments.

Dispersal

We suspect that fruit fragrance is coupled to dispersal strategies in *Vanilla*. In *V. pompona subsp. grandiflora* and *V. sp. 4,* surprisingly few organisms visit the deliciously fragrant fruits. Bees are by far the most common visitors to fragrant fruits and our observations of seed collection by at least one, a small melipone bee at the fruit of *V. pompona subsp. grandiflora*, suggest that bees may be responsible for seed dispersal. Especially intriguing is the fact that

Euglossa sp. and *Euleama meriana* are the only two bee species observed at both fruits and flowers of *V. pompona subsp grandiflora*. Likewise, only a single species of euglossini bee has been observed at fruits of the only other wetland vanilla species known to posses vanilla-scented fruits, *V. sp. 4*. Such specificity in fruit visitation by bees suggests intriguing parallels in both fruit and flower fragrance.

Vanillin is known to be a general attractant to a variety of euglossini species in southeastern Peru (Pearson and Dressler 1985). The few species of bees lured to fragrant *Vanilla* fruits suggests that it is the less abundant aromatics other than vanillin that may be responsible for the observed specificity (Hills et al. 1972). Future chemical studies of a variety of *Vanilla* species along with further observations of bee specificity at fruits as well as flowers may shed light on the role of euglossini bees in the evolution of the notoriously complex fragrance profiles of neotropical *Vanilla* fruit. Occasional evidence of slightly masticated fruits suggests that bats may also have a role in vanilla seed dispersal. The sclerotic seed coat of *Vanilla* species may protect ingested seeds passing through the mammalian digestive tracts (Cameron and Chase 1998).

Vanilla bicolor is expected to have fragrant fruits (Soto-Arenas et al. 2003). However we note the complete lack of fragrance, at least within Madre de Dios. The lack of fragrance is curious given its basal position in the American clade of fragrant *Vanilla* (Soto-Arenas et al. 2003). Lubinsky (2006) and Dressler (1989) suggest that succulent, possibly fragrant fruits may be the ancestral condition. However, Madison (1981) suggests that a transition from bee dispersal (associated with fruit fragrance) to wind dispersal (non-fragrant), would be an evolutionarily simple step. Tiny seeds of bee dispersed orchids would be pre adapted to wind dispersal. This is clearly the evolutionary trajectory followed by most advance orchids (Dressler 1993), with their dust-like, highly convoluted seeds capable of extremely long distance dispersal. The quick-drying pod of *V. bicolor* suggests wind may be the predominant disperser. Furthermore, the specific germination sites of seedlings of individuals in this population (within spaces produced by *M. flexuosa* petioles) are not likely to be visited by flying dispersal vectors. The evolution and loss of fruit fragrance in *Vanilla*, as well as the role of euglossini dispersers would be much enlightened by mapping its occurrence or disappearance on a phylogeny. Such phylogenies, however, do not yet exist.

CONCLUSIONS

We have documented five species of *Vanilla* occurring in Madre de Dios, all of which are virtually restricted to wetland habitat dominated by the palm *M. flexuosa*. Despite their high abundance within wetlands, these plants have never been documented by the scientific community in Madre de Dios and are virtually unknown to local peoples. We believe this status of affairs typifies the current understanding of the larger Amazonian flora, and especially of the wetlands where *Vanilla* is found. Our knowledge deficit is especially alarming given the increasing rate of human development and habitat destruction in and around these wetlands. The impending completion of an inter-oceanic highway in our study region will likely cause unprecedented changes in immigration, land use, and the economy, greatly threatening the conservation status of *Vanilla* and wetland habitat. Gold mining directly within wetlands particularly threatens the flora, fauna, and important ecosystem services provided by this understudied habitat (Hamilton et al. 2004).

This study complements the growing amount of natural history of Amazonian *Vanilla*. The high diversity, various life history traits, pollination systems, and dispersal strategies of *Vanilla* in MdD wetlands make this an intriguing assemblage of organisms for further study. The incredible abundance would facilitate detailed observational and experimental studies. *Vanilla* is the most speciose genus within its subtribe, but our limited knowledge of its natural history limits our ability to ask begin asking important questions and make meaningful comparisons. More studies on the pollination, dispersal and biogeography *Vanilla* would be especially important to elucidate important questions concerning the incredible diversity of the genus, as well as orchids in general. Lastly, the exclusivity of *Vanilla* to wetland habitat suggests this group as a model to investigate broader vegetation patterns in MdD wetlands.

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CHAPTER 2

DIVERSITY AND BIOGEOGRAPHY OF *VANILLA* ORCHIDS (ORCHIDACEAE) IN WETLANDS OF MADRE DE DIOS, PERU

ABSTRACT

Amazonian wetlands and the organisms that inhabit them are little understood. Most botanical collections have been limited in geographical extent despite important biogeographical questions resulting from these studies. To begin collection of biogeographical data at larger spatial scales, thirty-six kilometers of transect through 16 wetlands dispersed along the Madre de Dios (MdD) river in Madre de Dios, Peru were laid in order to document to the distribution of the genus *Vanilla* within MdD wetlands. The study region covers over 200 km of wetland habitat along an east-west course.

Five species of *Vanilla* are known to occur in MdD. One species is widespread and very abundant, occurring in all wetlands throughout the study region. Two lesser-abundant species occur only in the eastern half. Two rare species are restricted to single wetlands. All of the species are known to occur in different phytogeographical regions and this may be a general pattern of wetlands vegetation communities. Thus, MdD wetlands may be serving as a common refuge for *Vanilla* and other members of the plant community. High humidity in wetlands is likely the common factor that restricts many species to wetlands.

Relative species frequencies are in part explained by latitude, suggesting that a significant east-west environmental gradient exists over the study region. Once this pattern is taken into account, however, relative frequencies seem to be erratic and we cannot determine whether historical or ecological factors are more important explanatory factors. However, at local scales there does not seem to be much ecological segregation of species, and historical events

such as wetland development and seed dispersal probably account for variation in relative frequencies of *Vanilla* species between wetlands.

INTRODUCTION

In Amazonia, seasonal and permanently flooded forests comprise approximately 20% of forested land (Klinge et al. 1990, Junk 1993). Amazonian wetlands encompass a wide variety of reoccurring habitats and plant communities (Prance 1979, Kalliola et al. 1991, Ancaya 2002) embedded within a matrix of the most diverse forests world-wide (Gentry 1988, Spichiger et al. 1996, de Oliveira 1999). Within wetlands, long periods of inundation and anoxia are often the limiting factors, and many species demonstrate various mechanisms to deal with adverse wetland conditions (Kozlowski 1984, Scarano et al. 1997, Casanova 2000, Ferreira 2000, Lenssen et al. 2000). As a result, wetland plant communities are generally less diverse than surrounding *terra firme* forests (Richards 1952, Terborgh and Andresen 1998), and often tend towards canopy monodominance (Richards 1952, Kahn 1987, Kahn and Mejia 1990, Terborgh and Andresen 1998). Despite lower diversity, wetland plant communities form distinct patches within *terra firme* forests, contributing greatly to habitat heterogeneity and beta diversity at landscape scales (Campbell et al. 1986). It is to no small measure the extreme diversity of the surrounding *terra firme* forests that Amazonian wetlands have largely been ignored by the scientific community. Little information exists in the literature and basic questions must still be asked (Frey 1995, Junk and Piedade 2004).

Our knowledge deficit of Amazonian wetlands is alarming for three reasons. First, their role as refugia for habitat-dependent, rare, endemic, or endangered species remains largely undetermined (Crawshaw and Quigley 1989, Campos and Magnusson 1995, Goulding et al. 2003, Brightsmith and Bravo 2005). Secondly, the relevance of Amazonian wetlands in water management, nutrient cycling (Kern and Darwich 1997, Whigham et al. 1999), carbon cycling (de la Cruz 1986, Castillo and Lòpez 2003), and greenhouse gas emmissions (Wassmann and Martius 1997, Smith et al. 2000) are not yet firmly established (Junk and Piedade 2004). Finally, Amazonian wetlands are becoming severely and rapidly degraded due to deforestation, logging (Ayres 1993), agriculture (Junk and Piedade 2004), gold mining (Nogueira and Junk 2000), dike construction, river channalization, hydroelectircal operations (Fearnside 1989, 1995), and unsustainable harvesting practices (Padoch 1988, Vasquez and Gentry 1989). Local and regional conservation efforts in Amazonia have largely bypassed wetlands, and more detailed accounts of their distribution, ecology, and botany are needed to guide successful wetland conservation.

Much of the existing floristic information on Amazonian wetlands focuses around the cities of Belém, Santarém, Manuas, and Tefé along the great floodplains of Central and Eastern Amazonia (Junk and Piedade 2004). Furthermore, most studies have been limited in geographical extent (Keel and Prance 1979, Campbell et al. 1986, Guillaumet 1987, Kubitzki 1989, Campbell et al. 1992, Worbes et al. 1992, Amaral et al. 1997, Junk and Piedade 2004) with the result that no wetland classification system is yet available that could be used consistently on a regional scale (Klinge et al. 1990). Indeed, every investigator seems to develop his/her own classification scemes (Parodi and Freitas 1990)

Wetlands of upper Amazonia receive far less attention despite their ubiquity and large coverage. In the Peruvian Amazon, for example - representing the southwestern headwaters region of the Amazon drainage basin – wetlands cover between 24,000 – 47,000 km^2 (ONERN 1986, Kalliola et al. 1991). Our study focuses specifically on the wetlands fringing the Madre de Dios (MdD) river in southeastern Peru. The Peruvian MdD region is well-known as a mecca of biodiversity which receives some protective status in a series of large reserves – Parque Nacional Manu, Los Amigos Conservation Rerserve, and Parque Nacional Bahua-Sonene (Goulding et al. 2003). No large wetland, however, is under protective status along the MdD river- an alarming circumstance given the rise in gold mining directly within MdD wetland habitat due to increasing international gold prices.

Wetlands of the department of MdD, Peru are generally confined to the floodplains of larger rivers (Kalliola et al. 1991). The arborescent palm, *Mauritia flexuosa* tends towards complete monodominance, sharply delineating wetland habitat from surrounding *terra firme* and giving the impression of simplicity. However, recent floristic studies of MdD wetlands have revealed intriguing patterns in the distribution and composition of plant communities in wetlands. Wetland habitat seems to be much more structurally and compositionally complex than previously thought (Janovec et. al, in press). As found in other Amazonian wetlands, (Ferreira 2000) distinct vegetation associations form complex mosaics within wetlands, contributing to local diversity and creating new niches for species with a variety of life history traits and abiotic requirements. Floristic comparisons between wetland and non-wetland vegetation communities show that there is virtually no overlap at the generic level, suggesting a degree of habitat specificity for many wetland inhabitants (Ancaya 2002). Several studies note floristic similarities between Andean cloud forests and lowland wetlands with the prevalence of such genera as *Hedyosmum*, *Clusia*, *Erythroxylum*. This is in stark contrast with flooded forest of central and lower amazonia, where such habitats are floristically more similar to immediately surrounding non-flooded terra firme (Kubitzki 1989). Such patterns point towards interesting questions concerning the biogeography and origins of the plant assemblages found in MdD wetlands.

However, to date no study has sufficiently sampled a large enough geographic extent to begin asking important biogeographical questions concerning MdD wetlands. Do species that occur in one wetland occur in all wetlands throughout MdD? Are some species restricted to single wetlands? Does the composition of species remain stable across all wetlands or is there high species turnover? Knowing the distribution of organisms throughout the MdD wetland system is essential for understanding the processes and factors that influence species composition within wetlands. Also, such geographic information has real implications in the conservation of MdD wetlands and inhabitants.

With these questions and motivations in mind, we designed a study focusing on a single genus, *Vanilla* (Orchidaceae), with the main objective of describing its regional distribution. Previous studies documented that *Vanilla* in MdD wetlands may serve as a good model group because of its abundance, conspicuousness, and its diversity of pollination systems, dispersal mechanisms, and habits. Such points of comparison in life-history traits allow considerable insight in explaining geographic patterns. Here we report information on the distribution, diversity, and ecology of *Vanilla* as it occurs throughout MdD wetlands. We then discuss our findings in light of the species' world-wide distributions, life-histories, pollination systems, dispersal strategies, and habitats.

MATERIAL AND METHODS

Study Site

The department of Madre de Dios, named after the Madre de Dios river, is situated in southeastern Peru. The MdD river, a tributary of the Madeira river, is in the headwaters region of the southwest Amazon drainage basin. The Madre de Dios river drains a portion of the Sub-Andean Fold and Thrust Belt along the Eastern Cordillera and displays meander scroll morphology with rapid rates of lateral channel migration in the study region (Puhakka et al. 1992).

In our study area, over two hundred wetlands occupy floodplain habitat along the MdD river, ranging is size from one to 2000 ha. Wetlands are known locally as "aguajales", after the common name of the palm, *M. flexuosa*, "aguaje", that dominates wetland canopies. Covering over 30,000 ha in less than 200km - from the mouth of Rio Colorado to Rio Heath - MdD wetlands cover a significant portion of floodplain habitat. They are best characterized by the monodominance of *M. flexuosa*, histosol soils up to ten meters deep (Householder, pers obs), and permanently saturated substrate (Ancaya 2002).

Satellite Imagery of Wetlands

LandSat TM imagery was used to determine which swamps to visit and points of access. Two images with path/row of 3/69 and 2/69, taken on May 2000 and August 2001 respectively, cover the entire study area. Wetlands appear as blue-purple areas on thematic mapper band combinations 3 (red), 4 (near infrared), and 5 (mid infra-red) for the red, green and blue display bands in ENVI (ITT Visual Systems Inc, Boulder Colorado). A vector file of MdD wetlands based on a supervised classification proved to be extremely accurate in guiding expeditions and was used to create maps. Both satellite images and the vector file are publicly available from www.andesamazon.atrium.

Field Data Collection

Data were collected along transects at 100-meter intervals. Transect length and direction was determined by the size of the wetland and point of access. The following data were collected at each point along a transect 1) presence or absence of all species 2) the number of visible stems of each species; 3) images of the habitat in the four cardinal directions; 4) GPS coordinates; 5) collections of *Vanilla*. Data were transferred from field notes to excel spreadsheets and a geodatabase based in ArcMap (ESRI, Redlands, California).

Data Analysis

Species frequencies were determined as the total number of points where each species was present. Relative frequency values were used to describe the species composition within a single wetland by dividing the number of occurrences of a single species by the total number of occurrences of all *Vanilla* species in that wetland (table 2). Relative frequency values were used in principle coordinate analysis to account for the unequal number of points in wetlands due to wetland size and sampling effort. This methodology also removes points where no *Vanilla* occurred, since we are strictly interested in compositional patterns. Principle component analysis was performed on a correlation matrix of the Manhattan distances between relative frequencies of species within wetlands. This procedure was used to analyze patterns of *Vanilla* compositions between wetlands. A linear regression model was used to investigate relationships between the resultant principle coordinate axes and the distribution of wetlands. All statistical procedures and graphics were accomplished using R statistical package. Maps were produced using Arc GIS software.

RESULTS

Wetlands

In total we put approximately 35 km of transect in 16 different wetlands spanning a wide range of areas and geographic locations. The largest swamp covered 3,592 ha, the smallest 13 ha. Over 200 km separates wetland 1 from 16. We made point counts of all *Vanilla* species at 292 separate points. Table 1 gives a numeric summary of the distribution of points, area, and location of each wetland.

Point Data: Frequency and Abundance

In 16 wetlands we found a total of 5 *Vanilla* species (table 2). *Vanilla pompona subsp. grandiflora* is the most frequent species, present at 55% of points, followed by *V. bicolor, V. guianensis, V. sp. 4*, and *V. ribeiroi*, which were present at 40, 18, 1, and 1% of points respectively (n = 292; Fig 1). Up to three species occurred at any single point, but generally no more than two species were present (Fig 2).

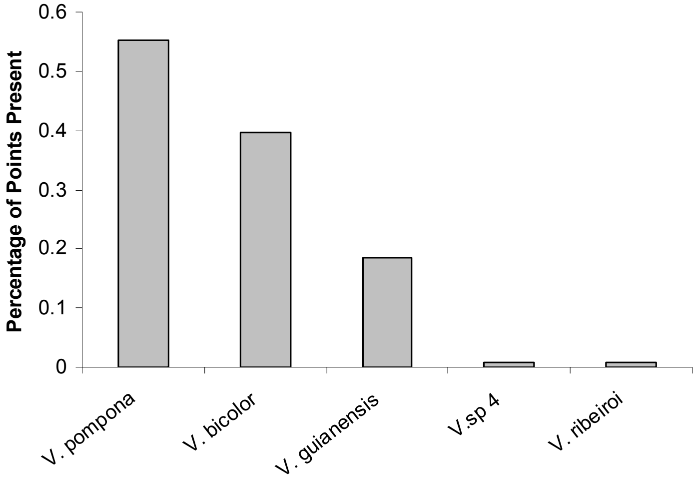

Figure 1. Frequency of all five Vanilla species across all wetlands (n = 292).

Table 1. Summary of transect data

ID	Centroid X	Centroid Y	Area (ha)	Points in Transect	Total Observations	Species Richness
1	335660	8621153	3592	38	80	3
2	363601	8621954	62	12	12	3
3	370580	8621685	32	12	4	2
4	377943	8615413	13	6	8	2
5	378137	8611267	235	27	31	3
6	377434	8601873	529	17	18	3
7	391872	8606825	531	21*	14	3
8	395087	8605703	128	13*	19	3
9	398820	8604628	850	18*	15	3
10	416556	8600408	1657	39	51	3
11	448378	8597000	1681	33	57	4
12	463931	8602081	33	9	3	1
13	472231	8606905	208	11	7	1
14	490746	8620257	112	15	10	1
15	494980	8607784	362	11	2	1
16	533021	8623982	755	10	4	1

Note: Points designated with a "*" were measured at 200, rather than 100 m intervals

Table 2. Summary of species data across all wetlands.

	Species				
	V. pompona	*V. bicolor*	*V. guianensis*	*V. sp. 4*	*V. ribeiroi*
Number of Wetlands	16	11	9	1	1
Range (km East-West)	215	93	93	NA	NA
Frequency	161	116	54	2	2
Abundance	1548	354	193	7	4
Range of Stems/Point	0 - 48	0- 13	0 - 16	0 - 3	0 - 5

Exactly 2,106 stems were counted, 74% of which belong to *V. pompona subsp. grandiflora.* This species often occurred in very dense clusters, sometimes reaching 40 or more vines at a single point. Other species were not nearly as abundant and did not form dense clusters to the same extent as *V. pompona subsp. grandiflora* (Fig 3).

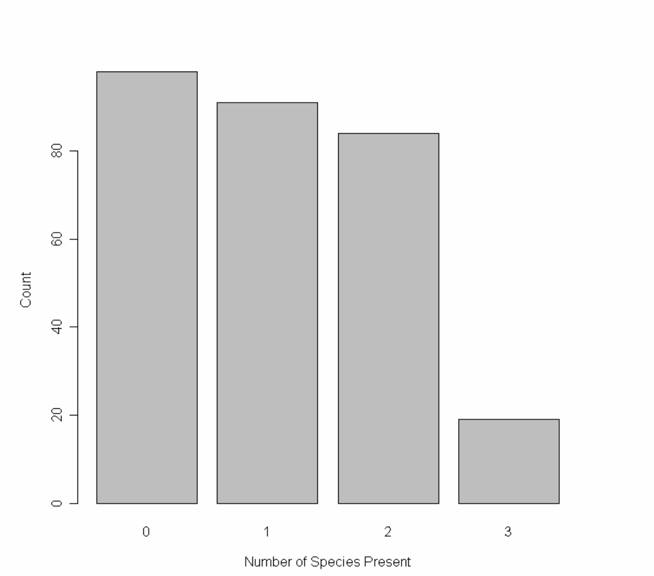

Figure 2. Histogram of the total number of Vanilla species present across all wetlands (n = 292)

Species Composition

The mode number of species in a single wetland was two, ranging from one to four (Fig 4). Eleven wetlands had more than one species, while five had only a single species. A single wetland, number 11, had four species. The most common species association within wetlands was *V. pompona subsp. grandiflora*, *V. bicolor*, and *V. guianensis*, all of which occurred sympatically in nine wetlands. The only two species that did not occur together in the same wetland were *V. sp. 4* and *V. ribeiroi* however, these two species did co-occur with all three other *Vanilla* species.

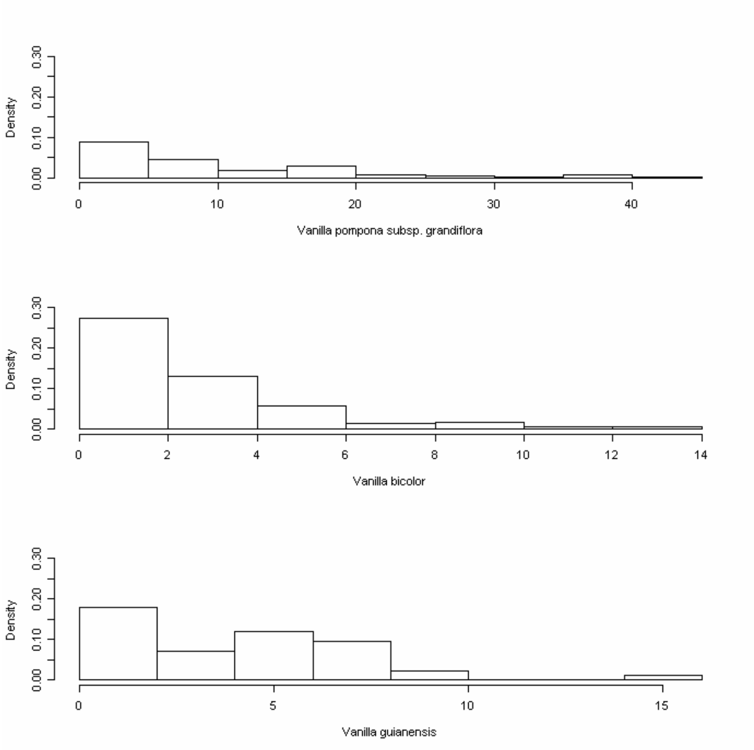

Figure 3. **Histogram of abundances (number of stems) at each point across all wetlands for *V. pompona subsp grandiflora* (n = 161), *V. bicolor* (n = 116), and *V. guianensis* (n = 54). Y-axes are relative frequencies. X-axes are the number of stems at each point. *V. sp. 4* and *V. ribeiroi* are not present due to their extremely limited frequency.**

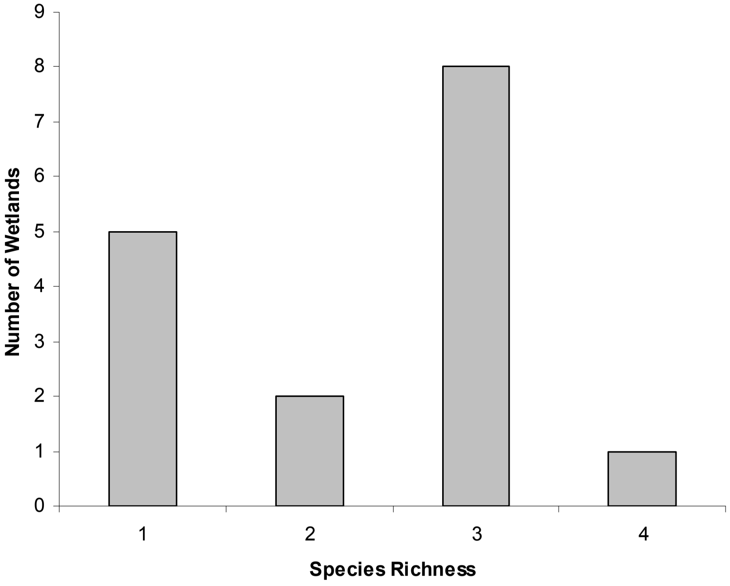

Figure 4. **Histogram of the total number of species occuring in wetlands**

The first two eigenvalues of the principle components on Manhattan distances accounted for 80% of the variation (table 3). The first axis (Fig 5) is interpreted as a contrast between the most frequently occurring vanilla, *V. pompona subsp grandiflora* (positive correlation with PCO1), and the two less frequent, *V. bicolor*.and *V. guianensis* (negative correlations with the first component). *Vanilla sp. 4* and *V. ribieroi* had almost no influence on the analysis due to their limited frequency and restricted ranges. Wetland 2, 3, 4, 5, 7, 9, 10, and 11, more or less sit on a spectrum along axis one. Wetlands 13, 14, 15, 16 represent a clearly differentiated cluster along this same axis because of the complete dominance of *V. pompona subsp. grandiflora*.

The second axis is interpreted as a contrast between *V. guianensis* (high positive correlation with PCO2) and *V. bicolor* (high negative correlation) relative abundance. Wetlands 1, 6, and 8 form a distinct cluster due along this axis because of the high relative frequency of *V. guianensis*.

According to the linear regressions, longitude of the wetland centroid explains 68% ($r = 0.82$) of the variation in the first principle coordinate axis ($F = 30.06$, $p > 0.001$) meaning that a shift in the relative frequencies between *V. pompona subsp. grandiflora* and both *V. bicolor* and *V. guianensis* occurs moving along an east-west trajectory.

Species Distribution

Only a single species, *V. pompona subsp. grandiflora*, was present in all 16 wetlands visited, followed in order by *V. bicolor*, *V. guianensis*, *V. sp 4*, and *V. ribeiroi*, occurring in 11, 9, 1, and 1 wetlands respectively (Fig 6). *Vanilla bicolor* and *V. guianensis* are present only in the western section of the study area, disappearing east of an imaginary vertical line running through the department capital, Puerto Maldonado. The loss of the former is quite abrupt, while the relative frequency of *V. guianensis* gradually tapers - it is also noticeably absent from two extremely small wetlands in the western section (3 and 4). Two species, *V. sp. 4* and *V. ribeiroi*, are restricted to single wetlands, 10 and 11 respectively.

Table 3. Matrix of Manhattan distances calculated from relative frequencies within wetlands

Wetland ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	0.73														
3	0.70	0.67													
4	0.80	1.17	0.50												
5	0.51	0.44	0.23	0.73											
6	0.27	1.00	0.78	0.78	0.70										
7	0.59	0.95	0.43	0.21	0.51	0.63									
8	0.39	1.12	0.95	0.95	0.82	0.17	0.80								
9	0.63	1.00	0.47	0.17	0.56	0.64	0.05	0.81							
10	0.62	0.73	0.16	0.52	0.28	0.70	0.37	0.87	0.41						
11	0.66	0.61	0.12	0.62	0.17	0.75	0.52	0.91	0.55	0.22					
12	1.30	1.67	1.00	0.50	1.23	1.11	0.71	1.16	0.67	1.02	1.12				
13	1.30	1.67	1.00	0.50	1.23	1.11	0.71	1.16	0.67	1.02	1.12	0.00			
14	1.30	1.67	1.00	0.50	1.23	1.11	0.71	1.16	0.67	1.02	1.12	0.00	0.00		
15	1.30	1.67	1.00	0.50	1.23	1.11	0.71	1.16	0.67	1.02	1.12	0.00	0.00	0.00	
16	1.30	1.67	1.00	0.50	1.23	1.11	0.71	1.16	0.67	1.02	1.12	0.00	0.00	0.00	0.00

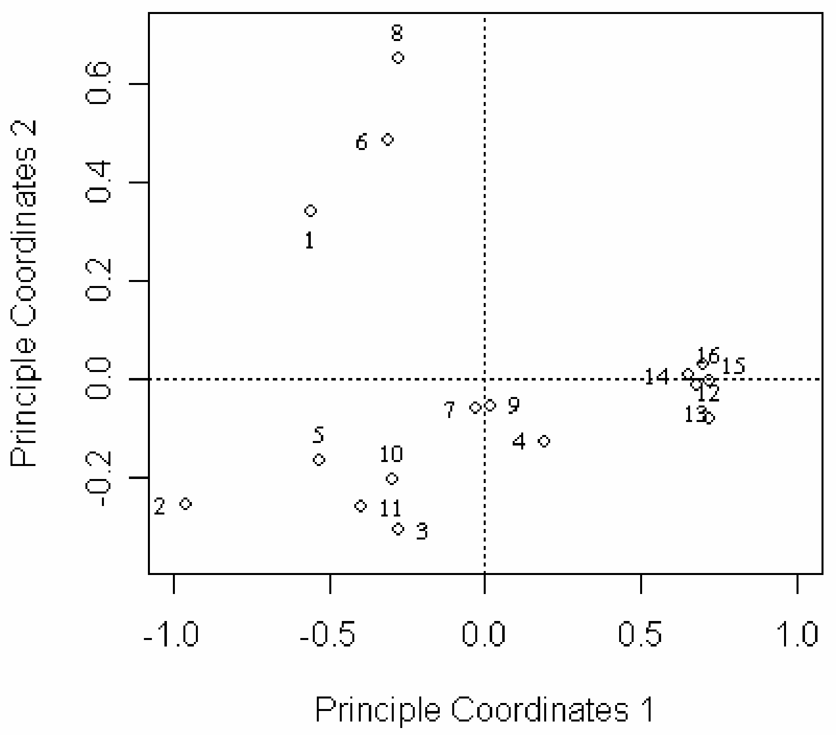

Figure 5. Scatter plot of the first two principle component scores of the correlation matrix of Manhatan distances calculated from the relative frequencies of *Vanilla* in individual wetlands. Each number corresponds to its particular wetland mapped in figure 6. Wetlands grouped in the center right were slightly jittered for clarity. The first axis is interpreted as a contrast between the most frequently occurring *Vanilla*, *V. pompona subsp grandiflora,* and the two less frequent, *V. bicolor*.and *V. guianensis*. The second axis is interpreted as a contrast between *V. guianensis* and *V. bicolor*.

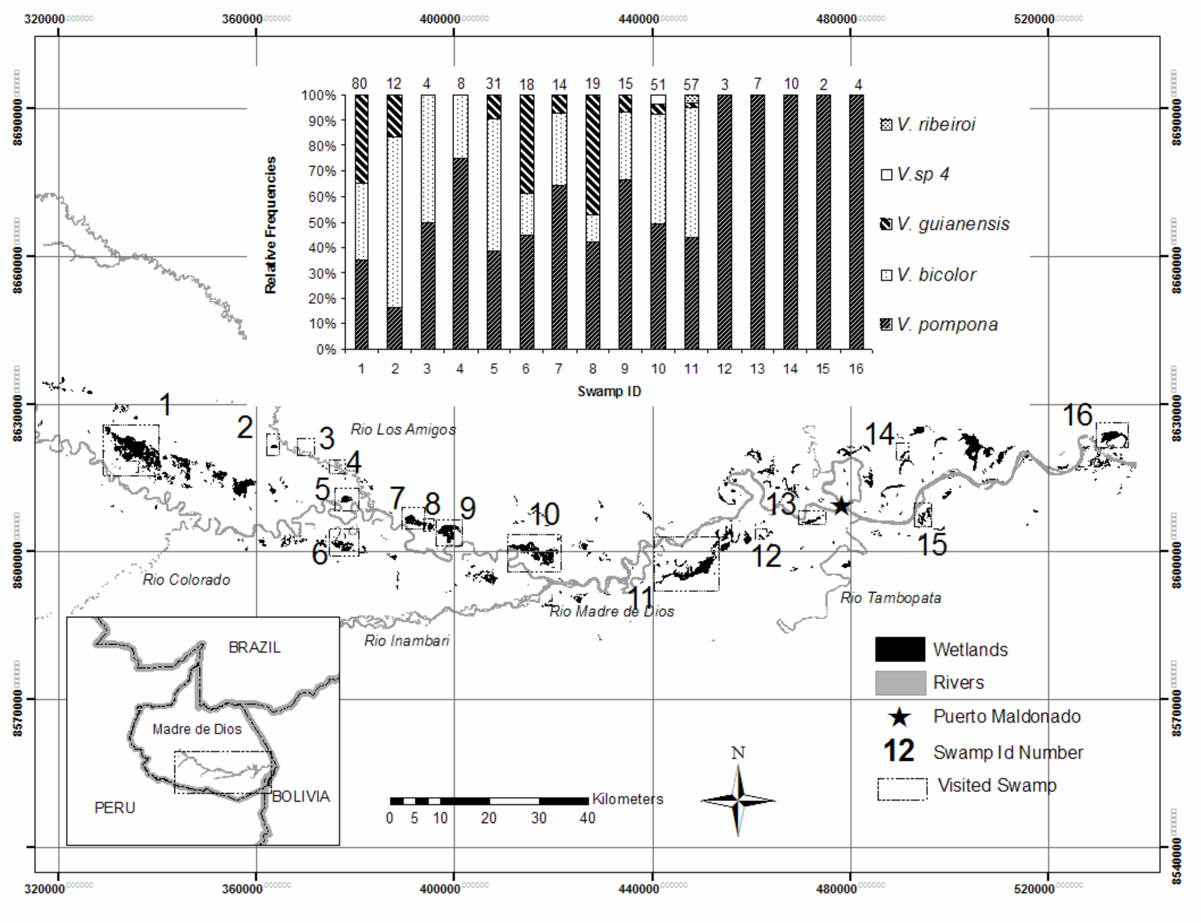

Figure 6. Map of wetland disturbution along the Madre de Dios River (n = 16). The inset bar graph illustrates relative frequencies of *Vanilla* species in all visited wetlands. Numbers at the top of each bar indicate the total *Vanilla* frequency within that wetland.

DISCUSSION

Wetlands of Madre de Dios represent a unique and little understood system. The few existing florstic studies have revealed sharp floristic differences between MdD wetlands and the surrounding matrix of *terra firme* forests, floristic connections with distant phytogeographical regions, and an abundance of rare species represented by single individuals (Ancaya 2002). Such patterns raise intriguing questions about the composition and origins of wetland flora and call for more detailed biogeographic studies on individual, wetland-dependent plants (Prance and Schaller 1982).

However, floristic studies have been limited by their geographic extent, an ever-present problem in the collection of botanical data in the Amazon (Janovec, pers comm). No study for example, has included any wetlands down river of wetland 10 (Fig 6). The limited geographic extent of these studies and the paucity of biogeographical information on wetland organisms severely limits our ability to effectively conserve wetlands and their inhabitants.

To our knowledge this is the most extensive study, including sixteen wetlands and spanning over 200 km, of any MdD wetland organism. *Vanilla* may be an excellent model group to begin understanding biogeographical patterns in wetlands. It occurs exlusively in wetlands and, as we have shown, occurs throughout the entire region. There are at least five species, making it the second most speciose orchid in the wetlands behind *Epidendrum* (Chocce, unpublished data) and is by far the most abundant orchid within wetland habitat. Furthermore, MdD wetland *Vanilla* demonstrate a wide variety of life-history traits, habits, pollination systems, and dispersal mechanisms (Householder, chapter 1).

Point data

Vanilla species are not equally abundant or frequent (Fig 1 and Fig 3). A variety of factors probably produce this pattern, including ecological variables, life-history traits, and historical events. Of the five *Vanilla* species, *V. pompona subsp. grandiflora* is clearly the most abundant and frequently seen. This species is a particularly robust vine. Fragmentation of the vine by disturbance stimulates clonal reproduction, and clusters of up to 48 individual ramets may occur at a single point. Frequent disturbances by falling palm fronds, rapid growth rates, and quick maturation rates most likely contribute to the species' ubiquity. That said, *V. pompona subsp grandiflora* tends to occupy specific habitats structurally characterized by low canopy coverage, high light conditions and dispersed *M. flexuosa* palms. Common plants associated with this habitat include *Ilex sp*. (Aquifoliaceae), *Tabebuia sp*. (Bignoniaceae), *Luhea sp*. (Tilliaceae), *Tapirira sp.* (Anacardiaceae), *Hedyosmum sp*. (Chloranthaceae), and *Clusia, sp*. (Clusiaceae). The ubiquitiy, habitat preference, and conspicuousness of *V. pompona subsp. grandiflora* would provide support for its use as an indicator species (Householder chapter 4).

Vanilla bicolor is the second most frequent species of wetland *Vanilla* (Fig 1), but much less abundant at any given point than *V. pompona subsp. grandiflora* (table 2). It generally occurs as an epiphyte within pockets created by *M. flexuosa* petioles. Rarely does more than one individual occupy a single palm host, limiting local abundance to the number of palms present. Its high abundance is most likely due to its high fruit set and abundant number of germinating seedlings within *M. flexuosa* petioles.

At times *V. guianensis* can dominate a local area but it is generally neither frequent overall, nor abundant where it is found. Most often it occurs as fairly dispersed individuals or in small groups. Unlike *V. pompona subsp. grandiflora*, its brittle stems do not easily propagate after a disturbance, and reproduction is probably dominated by seedling germination (Householder, chapter 1). However, not much is known about dispersal mechanisms and seedling establishment of this species.

Vanilla riberoi and *V. sp. 4* were only found at a few points. However, like *V. pompona subsp. grandiflora*, they often occur in dense clusters. All three species have succulent stems and leaves, and attempts to propagate have revealed that these species readily produce new ramets after fragmentation. We have observed that clonal reproduction of *Vanilla* is greatly facilitated in MdD wetlands because water levels do not fluctuate greatly (also Zimmerman, unpublished data). Fragments of succulent stems can withstand months of drought, but rapidly decompose when affected by flooding. Ability of *Vanilla* in MdD wetlands to reproduce clonally and withstand droughts probably contributes to the incredible abundance of *Vanilla* in MdD wetlands, contrasting with the hyperdispersed populations more commonly encountered elsewhere (Soto-Arenas et al. 2003).

Species composition and distributional patterns within wetlands

Clearly, relative frequencies of species vary drastically between wetlands (Fig 6). The most clear-cut pattern emerging from the distribution of relative frequencies within wetlands is that *V. bicolor* and *V. guianensis* are absent in the eastern half of the study area. The linear model indicates that differences in the relative frequencies of the most abundant species, *V. pompona subsp grandiflora*, and two lesser-abundant species, *V. bicolor* and *V. guianensis*, change with longitude. The lack of the latter two species east of Puerto Maldonado clearly drives this relationship.

Although we can only speculate on the ultimate cause(s) of the disappearance of *V. bicolor* and *V. guianensis*, similarities in the two species may prove illustrative and suggest an ecological explanation. Both species have slender, brittle stems and thin leaves. The thin leaves and high stomatal density of *V. guianensis* are known to limit this species to humid locations (Soto-Arenas et al. 2003), and this may be true for *V. bicolor* as well. In any case, the epiphytic habit of *V. bicolor* surely makes this species prone to drought stress (Benzing 1973). Water stress may be crippling between June and August, when less than 12% of precipitation falls (Amazon Conservation Association, unpublished data). The distribution patterns of *Vanilla* across MdD, however, may be driven by regional-scale patterns in rainfall seasonality. Sombroek (2001) places MdD at one of the most precipitous rainfall gradients in the Amazon basin. According to Sombroek's analysis of long-term climate data, the number of consecutive months receiving less than 100mm of rainfall increases from zero, in the southwestern foothills, to four at the Bolivian border. Eastern portions of our study area may receive substantially less rainfall at key times during the dry summer months of April to September than the western section, possibly driving the drop out of these two species. The humid conditions within wetlands surely ameliorate drought stress, but their leaf morphology and habit would make them susceptible to dry spells during critically dry summer months.

Once this longitudinal relationship on species composition and relative frequency is taken into account, no clear patterns emerge. Relative frequencies seem to fluctuate randomly. Why, for example, is *V. guianensis* so frequent in wetland 1, 6, and 8, and virtually absent in 2,3,5,7 and 9? Casual observers of wetland vegetation often conclude that every wetland is different. Indeed, complex hydrological patterns can create complex vegetation patterns and each wetland consists of a mosaic of several distinct vegetation communities (Casanova 2000). One hypothesis is that *Vanilla* species may segregate themselves by the distinct ecological conditions each wetland provides. In other regions, some *Vanilla* are apparently specific to certain substrates (Nielsen and Siegismund 1999, Soto-Arenas et al. 2003).

However, assigning an ecological explanation of seemingly random changes in *Vanilla* composition would be difficult to support. Often, three species of *Vanilla* can be in sight from a single point (fig 2). Rather, historical factors of wetland development and random dispersal to new sites may play a role. Active fluvial dynamics and channel migration ultimately create wetlands where drainage is impeded. As successional processes, sedimentation, and weathering proceed, drainage conditions change, creating a dynamic wetland landscape over long periods of time (Kalliola et al. 1991). Dispersal to new sites thus becomes critical, and those species arriving first may dominate by virtue of their early arrival.

The mechanism of *Vanilla* dispersal remains elusive. Ninety-nine percent of orchids seeds are wind dispersed (Dressler 1993). However, the oily mesocarp and extremely smooth seeds of vanilla have led many authors to speculate that euglossini bees attracted to fruit fragrance are the major dispersers (Madison 1981, Lubinsky et al. 2006). Although flight patterns of euglossini bees are largely unknown (Kroodsma 1975), several investigators hypothesize that they are capable of flying distances of ~20km per day (Janzen 1971, Williams and Dodson 1972). Thus, wetland-dependent *Vanilla* populations may be viewed as metapopulations sustained by occasional migration and maintenance of connectivity between wetlands via propagule dispersal by large bees (Hanski and Gilpin 1991, Semlitch 1998). The exclusivity of *Vanilla* to small patches of wetland habitat within a larger matrix of uninhabitable forest may mean that effective populations within wetlands are small, and thus vulnerable to local extinction (Møller and Rørdam 1985, Dodd 1990, Sjögren 1991, Young et al. 1996, Templeton et al. 2001) especially if human alterations of wetland habitat begin to decrease connectivity (Gibbs 2000). Restricted species such as *V. sp. 4* and *V. ribeiroi* would be extremely susceptible to local extinction with increased human habitat alteration, especially if the location of MdD wetlands is such that immigration events from source populations are rare.

Vanilla sp. 4 and *V. ribeiroi* are both localized within single wetlands despite extensive searching. *Vanilla sp. 4* seems to be closely allied to *V. cristagalli*, a member of a little known Central Amazonian complex. Nothing is known about its wider distribution. *Vanilla ribieroi*, on the other hand, has been collected in riparian areas of Mato Grasso, Brazil. Precipitation patterns in Mato Grasso, as MdD, are seasonal with only 20% of precipitation occurring between April and September (Prance and Schaller 1982). Much of Mato Grasso is pantanal, a diverse and species-rich complex of permanent swamp, seasonal swamp, and *terra firme* covering 160,000 km^2 (Frey 1995). The wetlands of MdD, with permanently saturated soils, may simply be an extension of its former habitat. The absence of *V. ribeiroi*, as well as *V. sp 4* in easternmost swamps however, is puzzling, and the extremely localized distribution of these two *Vanilla* populations remains to be explained. These species may be recent immigrants, representing rare colonization events. Alternatively, they may persist in the eastern portion of our study area as dispersed, rather than clumped individuals. The reasons driving such a shift in population dynamics, however, are unclear.

Examination of the larger distribution of wetland-dependent *Vanilla* of MdD reveals that these five species come from varied phytogeographical regions. There is clearly a strong Central Amazonian element in wetland *Vanilla* in *V. riberoi* and possibly *V. sp. 4*. This contrasts with the Andean elements other investigators have noted (Ancaya 2002). Interestingly collections of *V. bicolor* are concentrated along the equator in Ecuador, Venezuela, and Guyana. Its collection in MdD is the first in Peru, greatly expanding its range southward. *Vanilla pompona* is a widespread species throughout the neotropics occurring from southern Mexico to southern Peru. Humidity is likely the common limiting factor that restricts such a diverse assemblage of *Vanilla* species to wetlands.

However, the occurrence of five sympatric *Vanilla* from very different phytogeographical regions raises interesting questions of the origins of wetland vegetation in general. Are wetland communities predictable assemblages of highly adapted species or a hodge-podge of distantly derived colonizing species that have found refugia within hospitable wetlands? If *Vanilla* may serve as a model for other groups, wetlands may be serving as a common refuge for plants from surrounding or distant phytogeographical zones. The affiliations between wetland and Andean genera reported in previous studies, and the prescence of numerous amazonian *Vanilla* in this study, create an intriguing vegetation community that begs further investigation.

One possible explanation for the biogeographical patterns of wetland vegetation is suggested by recent paleogeographical models of the amazon basin. Although still controversial, the interpretation of sedimentary strata and molluscan fauna suggest that an extensive wetland complex with frequent marine incursions may have dominated the heart of the Amazon basin during the Miocene (~5 - 10 ma). Marine and freshwater environments fluctuated with global changes in sea levels and Andean orogenesis, drastically altering regional drainage patterns (Nuttal 1990, Hoorn et al. 1995, Rasanen et al. 1995, Hoorn 2006). Pollen cores in upper Amazonia suggest that these wetland/tidal complexes were dominated by *Rhizophora* and *Mauritia*, the latter being consistent with modern wetlands. Furthermore, pollen of gymnosperm species in the same strata suggest that these wetland complexes persisted even as highland species began colonizing the emerging Andes (Hoorn 2006). The thick Ipiruro clay layer in southeastern Peru suggests that this massive Amazonian wetland complex may have extended as far south and west to MdD (Antoine et al. 2003, Hovikoski et al. 2005). The evolution of these Miocene tidal/wetland complexes have been implicated in the great faunal exchange between North and South America by creating a corridor through which plants and animals could disperse (Rasanen et al. 1995, Campbell et al. 2000). With the continued uplift of the Andes, wetland habitat may have become restricted to narrow bands along floodplains of major rivers and blocked valleys as the modern amazon drainage basin was formed, isolating once larger, more connected populations of wetland organisms during the Miocene. Thus, the wetland *Vanilla* may represent relict, isolated fragments of once much larger populations. Alternatively, long-distance dispersal may be the primary mechanism by which wetland organisms arrive.

A rather large proportion (40%) of *Vanilla* species are auto-pollinating; both *V. bicolor* and *V. guianensis*. The degree of auto-pollination in any group will depend on a range of factors, but floral morphology and the abundance of pollen vectors are usually the most important (Schemske and Llande 1985). This pollination system is argued to be advantageous when pollinators are rare, unreliable, or where the population exists in a stable, unsaturated environment where any reductions in fitness due to inbreeding effects are rendered neutral (Catling 1990, Johnson et al. 1994). Trends in the occurrence of auto-pollination suggest that colonizing organisms - those frequently inhabiting geographically isolated habitats - are more prone to auto-pollinate (Baker 1955). For example, in the Caribbean region, populations of *Turnera ulmifolia* on small islands are strictly self-pollinating, while larger islands supported individuals with both selfing, and crossing pollination mechanisms. Repeated colonizing episodes and the need for establishment after long-distance dispersal seem to favor self-pollination during island colonization events (Barrett 1988). Recently colonized, geographically isolated areas may not have similar pollinator communities, environmental conditions, or sufficient individuals to sustain cross-pollination. The presence of multiple auto-pollinating *Vanilla* species suggests that these populations may be fringe or isolated populations originating from different biotic and abiotic environments.

Fringe populations are often fertile grounds for evolutionary processes to unfold rapidly due to genetic isolation and novel selective pressures. Geographic isolation of small, isolated populations create conditions where genetic drift is a strong evolutionary force (Ellstrand and Elam 1993). This, coupled with pollinator limitation and specificity lead to rapid speciation (Gentry and Dodson 1987, Tremblay et al. 2005). Here we note the subspecies status of *V. pompona subsp. grandiflora*. The parent species, *V. pompona*, is extremely widespread and highly plastic throughout its range (Soto-Arenas, pers. com.). The subspecies distinction, then, is significant, suggesting a high degree of genetic isolation for this wetland population.

CONCLUSIONS

We have used *Vanilla* as a model group to begin asking important biogeographical questions concerning wetland organisms. Several trends have been suggested by this study. Species composition of wetland *Vanilla* is probably affected by region-wide climatic patterns and site-specific, ecological and successional processes. These processes, acting on different spatial scales, likely contribute to the floristic complexity of wetland vegetation in general. Future floristic studies should take this into account, visiting wetlands over a broader region. Furthermore, wetlands may serve as refuge for *Vanilla* from different phytogeographical regions, making MdD wetlands cauldrons of unique plant communities and interactions. Humidity is likely the common denominator that restricts *Vanilla* to wetlands. The spatial distribution of MdD wetlands is such that many wetland-dependent *Vanilla* live in multiple, small, local populations sustained by occasional migration. Maintaining high connectivity between wetlands would be essential in any conservation initiative and wetlands should be understood as an integrated system, rather than treated as isolated entities.

Explaining the presence of *Vanilla* in MdD wetlands is difficult from an analysis of their distributions only. We feel that molecular studies in particular may shed light on several important questions on *Vanilla* distribution patterns as well as give a broader perspective of wetland communities. What is the degree of connectivity and gene flow between wetlands for *Vanilla* and organisms with different life histories? To what extent is the MdD wetland system acting as refugia and isolating mechanism at the same time? Molecular studies of a variety of wetlands organisms, including plants, fish, insects, mammals, and amphibians may begin to elucidate on the origins, evolution, and distribution of wetland organisms throughout the MdD wetland system. Such information is critical to further understand of the complexities of MdD wetlands, as well as how best to protect them.

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CHAPTER 3:

FRUITING AND FLOWERING DYNAMICS OF *V. POMPONA* SCHIEDE *SUBSP. GRANDIFLORA* (LINDL.) SOTO-ARENAS (ORCHIDACEAE) DURING A SINGLE FLOWERING SEASON

ABSTRACT

Over one hundred marked individuals of *Vanilla pompona subsp. grandiflora* were studied following a single flowering season in a forested wetland in southeastern Peru. Individuals were composed of overlapping generations of one to six ramets, with up to ~300 leaves and reaching ~25m in height. Fifty percent of the individuals flowered during the season. Fruiting failure was high, as only 26% of individuals produced fruit. Most individuals produced only a single fruit, creating a highly right skewed fruiting distribution. The absolute number of flowers on a given individual slightly increases the probability of producing at least one fruit. Pollination rates are among the lowest for neotropical, deceptively pollinated orchids, calculated at 0.9%. It is hypothesized that low pollination rates and high fruiting failure can be sustained if clonal reproduction represents a large component of individual fitness.

INTRODUCTION

Vanilla (Orchidaceae: Subtribe Vanillinae) is a genus 100+ species of herbaceous vines, epiphytes, and semi-epiphyte (Soto-Arenas et al. 2003). Its pantropical distribution (Cameron 2000), basal position within Orchidaceae (Cameron et al. 1999), and relative high diversity compared to other members of the subtribe (Dressler 1993, Stern and Judd 1999) highlight this key group of orchids. Further underscoring this important group is the fact that *Vanilla* is the only orchid producing a commercially valuable product (besides ornamental orchids) in the form of vanilla flavoring (Correll 1953). From 1995 to 2000, demand exceeded two thousand tons of processed vanilla fruit, most of which is used by the ice-cream industry (Koekoek 2005).

Despite its botanical and economic importance, few detailed accounts of basic natural history of vanilla exist outside of managed plantations. Natural populations are often hyper dispersed and flowers ephemeral (Soto-Arenas et al. 2003), characteristics that have surely contributed to the paucity of collections and accumulation of basic natural history. The motivation of this study is to document various aspects of reproduction of a single vanilla species occurring in southeastern Peru, *Vanilla pompona subsp. grandiflora*.

Vanilla pompona subsp. grandiflora is a fast growing (up to one meter per month) vine climbing its host via short aerial roots opposite to the large, succulent leaves at each node. The plant may be one- to several-branched, composed of several connected ramets. A single, physiological individual can become quite large, reaching heights of 30 meters with several ramets at varying degrees of senescence and hundreds of leaves. Populations are maintained largely through clonal reproduction, which frequently occurs when any part of the stem is physically disconnected by external disturbance (Householder, pers. obs.). It occurs sympatrically with at least four other vanilla species, but is clearly the most widespread and abundant *Vanilla* species, occurring at densities up to 150 ind/ha (Householder, chapter 1).

The pollinator, recently documented by Lubinsky et al. (2006) is a large euglossini bee, *Eulaema meriana.* The bee is clearly attracted to floral fragrance, but scent collection was not observed during the only pollination event witnessed (Lubinsky et al. 2006). The large, yellow flower forms a large gullet into which the pollinator must completely enter to effect pollination or remove pollen masses. Mealy pollen is shed as monads on the bee's scutellum whereby it is transported to another flower. Flowers open as early as 3 am and the stigmas remain receptive during a four- to eight-hour window of cooler morning temperatures. Flowers open sequentially at two to three day intervals on racemes of one to twenty-five buds (Householder, chapter 1).

V. pompona subsp. grandiflora occurs exclusively in wetland habitat in Madre de Dios. Wetlands of Madre de Dios are known as “aguajales”. The term is derived from the local name for the arborescent palm *Mauritia flexuosa*, or “aguaje” that dominates wetland habitat.

Here I propose to compliment the growing natural historical data concerning this species by examining size relationships, and flowering and fruiting dynamics of a natural population during a single season.

METHODOLOGY

Study Site

All data were collected from a single swamp located two kilometers from the Concession de Investigación y Capaticación Rio los Amigos (CICRA) biological station managed by the Amazon Conservation Association (fig 1). Wetlands are characterized by monodominance of the palm *M. flexuosa*, deep histosols (up to 10 meters), and perennially saturated substrate. *Mauritia flexuosa* is the most common host for *Vanilla*. Common tree and shrub species associated with *Vanilla* include *Ilex* sp. (Aquifoliaceae), *Hedyosmum* (Chloranthaceae), *Tabebuia sp.* (Bignoniacae), and *Luehia sp.* (Tilliaceae), all of which may serve as alternate hosts of *Vanilla*. These species coexist with *Vanilla* in areas of low palm coverage and higher light conditions. At least four other *Vanilla* species occur sympatrically with *V. pompona subsp grandiflora* but this species is the most abundant and wide spread *Vanilla* species in wetlands (Householder, chapter 2).

The surrounding forest is classified as humid tropical, receiving between 2,152 and 3,498 mm of annual precipitation between 2001 and 2005. Average rainfall over this 5-year period is 2,653.2 mm. Rainfall is seasonal. On average less than 23% of annual precipitation occurs during the long five-month dry season persisting from May to September. Monthly temperatures fluctuate minimally around 23 ºC, but ephemeral cold fronts, known locally as “friajes”, are common in the dry season and can reduce temperatures to a low of 10 ºC for a period of several days (Amazon Conservation Association, unpublished data).

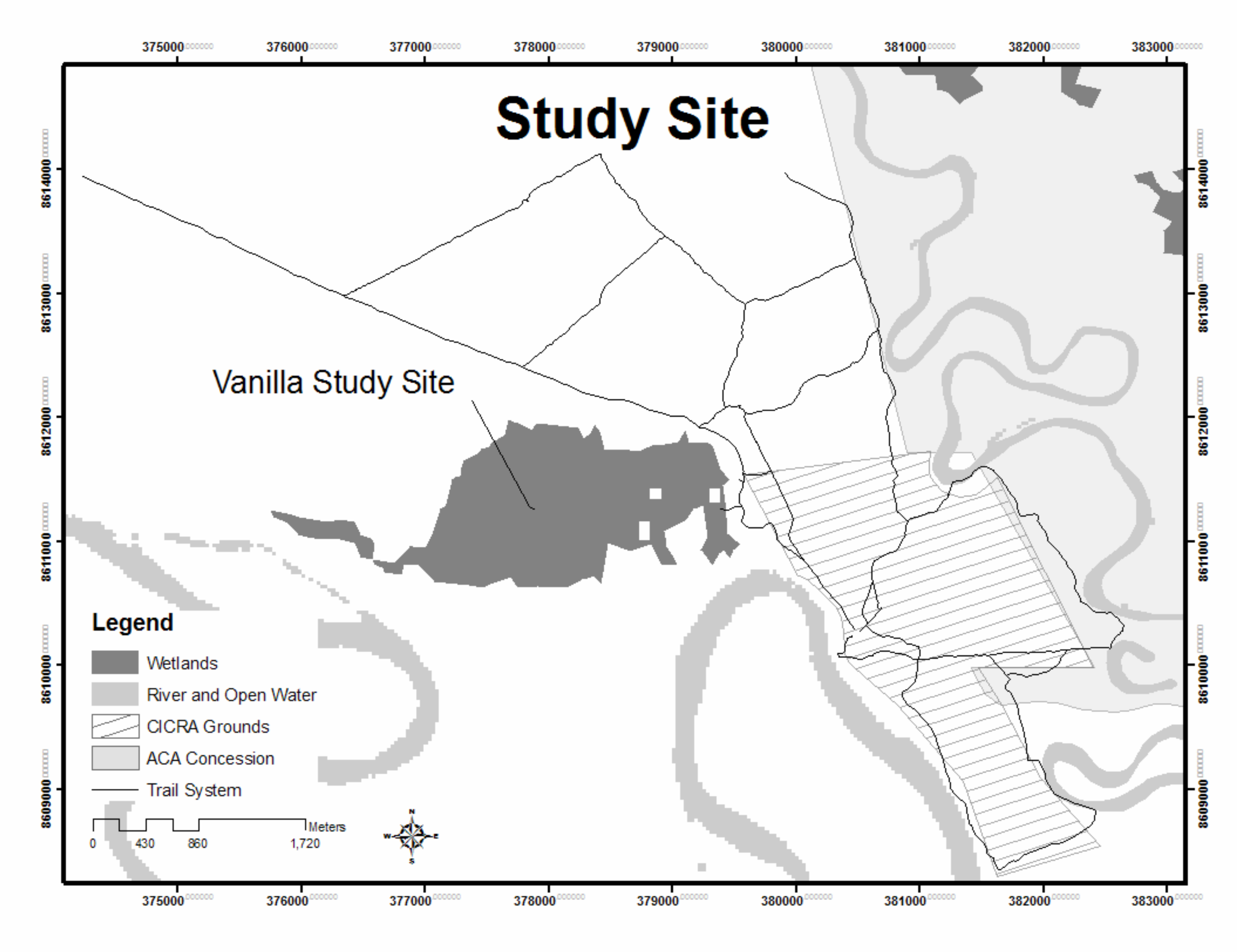

Figure1. Map of the study site and local surrounding

Individuals

One hundred and twenty five individual vines along a 1.5 km transect within a single swamp were marked in the month of July, three months prior to the flowering season. Only potentially reproductive (flowering) vines were chosen. Based on prior knowledge, climbing vines do not flower unless at least one ramet has reached a minimum height of two meters, and this criterion was used to select vines. A number of vegetative attributes were chosen to describe this population. First, the total number of leaves on a given individual was counted using binoculars for particularly large vines. Leaves of reproductive individuals are quite large, c. 13 x 30 cm and extremely succulent, representing the greater part of plant biomass and possibly a significant storage reservoir for sexual reproduction and plant metabolism in times of stress (Dressler 1993). Second, the maximum height obtained by any ramet was measured with a 10 m measuring tape and a 5 meter ladder for individuals less than 9 meters high. For taller individuals, height was estimated visually using the three-meter ladder sections as a reference to reduce error. The maximum height attained by an individual may affect the amount of incoming solar energy within different canopy strata (Koop and Sterk 1994). Finally, the total number of ramets was also quantified. Connections between ramets are long-lived and a single genet consists of one to several overlapping generations.

Immediately following the flowering season of September 2005, the number fruits and flowers was quantified for each individual. Empty bracts and persistent ovaries developing into fruits were used as evidence of recently past flowers. In this species, the corolla separates from the ovary within two to three days of blooming, leaving behind the ovary for another two to three days. If pollinated, the corolla persists on the ovary, becoming hardened and dry so that developing fruits were easily recognized by the persistent corollas and swelling ovaries. Fruits and flowers of eighty additional flowering, unmarked vines were quantified in order to better examine fruiting patterns.

Statistics

Relationships between all untransformed variables (number of flowers, leaves, ramets, and maximum height) were examined using Spearman's correlation statistic. Pollination rates for the entire population were determined by dividing the total number fruits of all tagged individuals into the sum of the number of fruits and flowers of all individuals. A binary logistic regression model was created to examine whether the number of flowers influence fruit production. All statistical procedures were performed using SPSS software. Graphics were produced in R–Stastical Package.

RESULTS

Growth and Habit

The number of leaves range from zero to 286, with a mean of 62.86 ± 4.36 (all mean values reported as mean ± standard error). Mean height is 6.57 ± 0.331, ranging from two to sixteen meters. Mode number of ramets is two, ranging from one to six (fig 2). The number of leaves is positively correlated to maximum height ($r = 0.600$), and the number of ramets ($r = 0.563$) of individual vines; maximum height is also correlated with the number of ramets ($r = 0.375$) (all $p < 0.01$; fig 3).

Vines often double over, growing up and over smaller hosts once the maximum height of that host is reached. Multi-branched individuals are composed of overlapping generations at various degrees of growth and senescence. Ageing ramets, which often have many leafless nodes, will produce new ramets at their bases, generally in October immediately following the September flowering season. New ramets generally grow vertically up the same host as the parent ramet but occasionally find unoccupied hosts by scrambling along the ground.

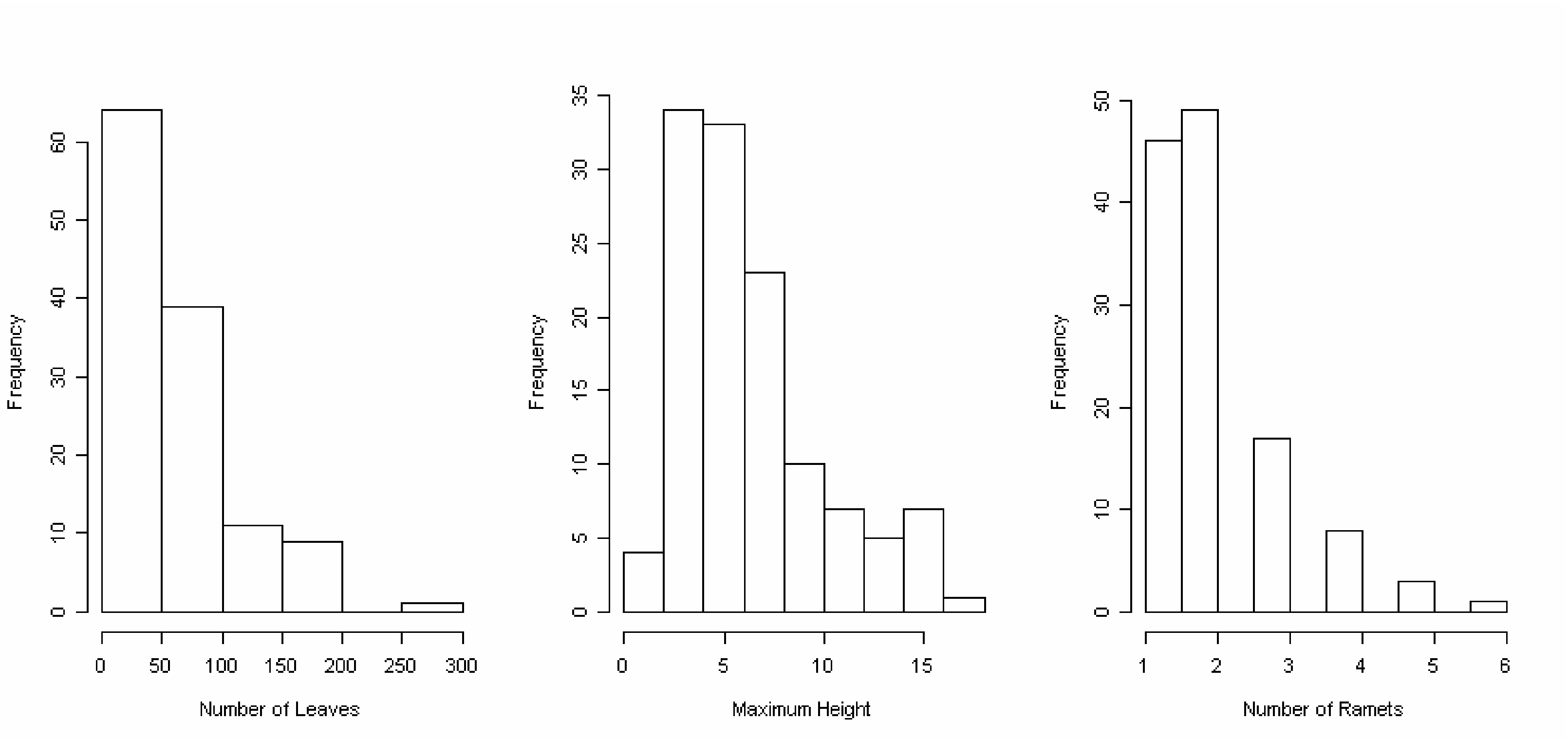

Figure 2. Histograms of the number of leaves, maximum heights, and number of ramets per individual for all marked indivuals (n = 124).

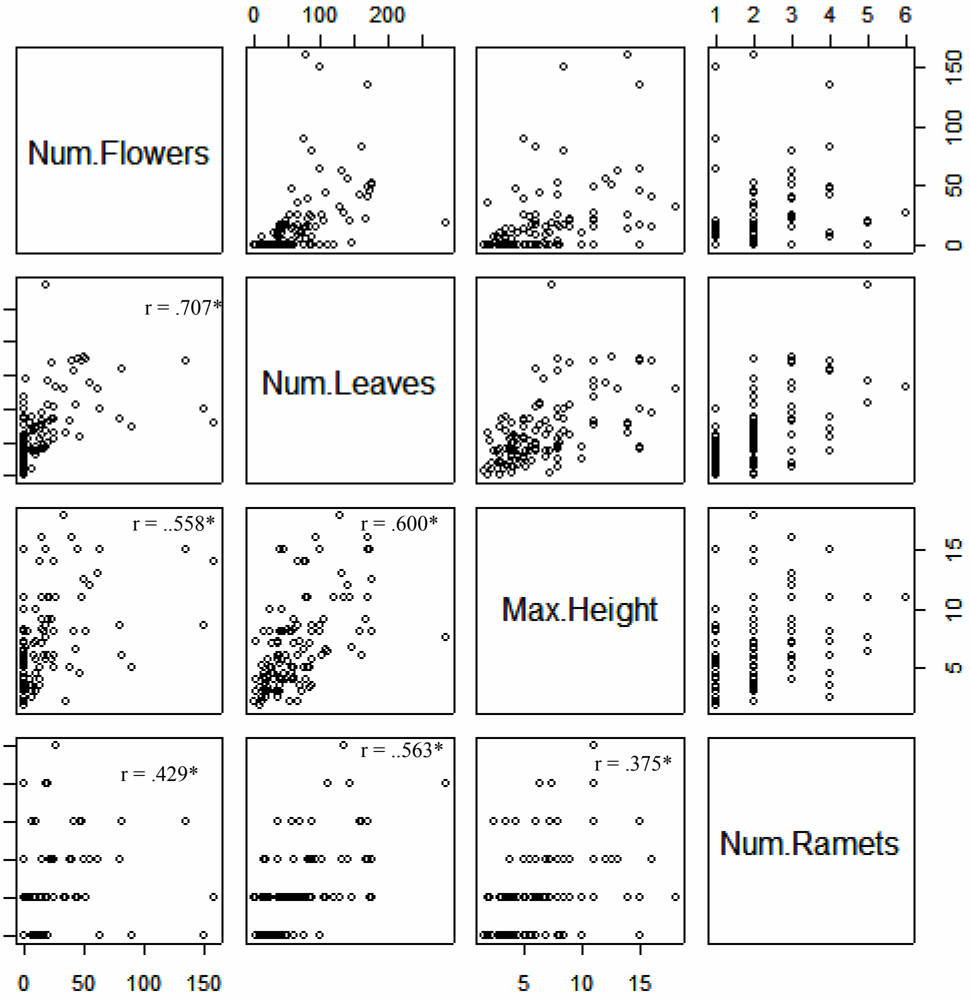

Figure 3. Scatter plot matrix of all measured attributes of vines (n = 124). Spearman's correlation statistic is also given. An "*" denotes significance at $p < 0.01$ level.

Flowering Dynamics

Flowers mainly bloom singly at several-day intervals, however on particularly cool mornings they often bloom in pairs on racemes. Flowers are only open and receptive during the cooler morning hours. At this study site, flowers opened around 3 am and often wilt by midday heat. However, during cold fronts, when temperatures do not rise above 10-16 °C flowers may continue to be functional for two days. Current temperature seems to be a deciding factor in flower longevity and the number of flowers opening per raceme on any given day.

Approximately fifty percent of individuals marked three months prior to the flowering season bloomed in September 2005. Heavy flowering occurred between the eighth and thirtieth of the month. The shortest flowering individual was two meters in vertical height, the tallest 18 meters. The flowering individual with the least number of leaves was eleven; the greatest number of leaves on a flowering individual was 286. Flowering individuals had one to six ramets. The distribution of flowers per individual was positively skewed, with skewness value ± standard deviation of 2.24 ± 2.04 (fig 4).

Fruiting Dynamics and Pollination

Thirty-seven of 141 flowering individuals (26.2%) produced fruit, the majority of fruiting individuals producing only a singe fruit. Fruiting individuals ranged in height from three to sixteen meters in height; had eleven to 141 leaves; and one to six ramets. The distribution of fruits per individual was positively skewed, with a skewness value ± standard deviation of 2.916 ± 0.204 (fig 4).

In total, 5,148 flowers and 47 fruits were counted on 141 individuals. Pollination rates are calculated as 0.9%. According to the binary logistic model, there is a significant increase in the probability of more heavily flowering individuals to produce at least a single fruit (log liklihood = 5.214, $p < 0.05$). Exp (B) is calculated as 1.011. A Man-Whitney U test comparing mean number of flowers of fruiting and non-fruiting individuals confirmed that fruiting individuals produced significantly more flowers ($U = 2.818_{140,36}$, $p < 0.01$; fig 5).

DISCUSSION

Size and habit

The viny, climbing habit is generally interpreted as a means of reaching light without investing heavily in supportive infrastructure. However, mean height of potentially reproductive individuals (6.57 m) is well below the canopy height at twenty to thirty meters. Few individuals maximize vertical growth on their hosts, an observation also noted by Soto-Arenas (2003). Often, the lowest, oldest sections of the individuals begin to senesce before the vine can reach canopy level, preventing nutrient transport to actively growing tips and arresting upward growth before the vine reaches the canopy. At our study site, frequent disturbances of falling palm fronds that sever ascending ramets may also reduce maximum potential heights. Both decay of lower sections and frequent disturbances that limit maximum attainable height may be factors that limit wetland *Vanilla* to predictable habitats with lower canopy coverage and higher light penetration.

Connections between individual ramets are fairly long-lived in *Vanilla* as a single individual may be composed of up to six ramets. However, the degree of integration of differently aged ramets is unknown. Ramet integration may increase the ability of clonal species to respond to local disturbance, especially in plants with limited storage capacities (Marshal and Sagar 1965; Pitelka and Ashmun 1985). This may be especially important for *Vanilla* species, which both lack the specialized storage organs common to other orchid species and inhabit a dynamic canopy habitat. Resources such as light and hosts may be patchy in both time a space, favoring a strategy focused on exploration of suitable habitat and integrated ramet connections able to funnel resources to ramets occupying favorable microsites (Grime 1979).

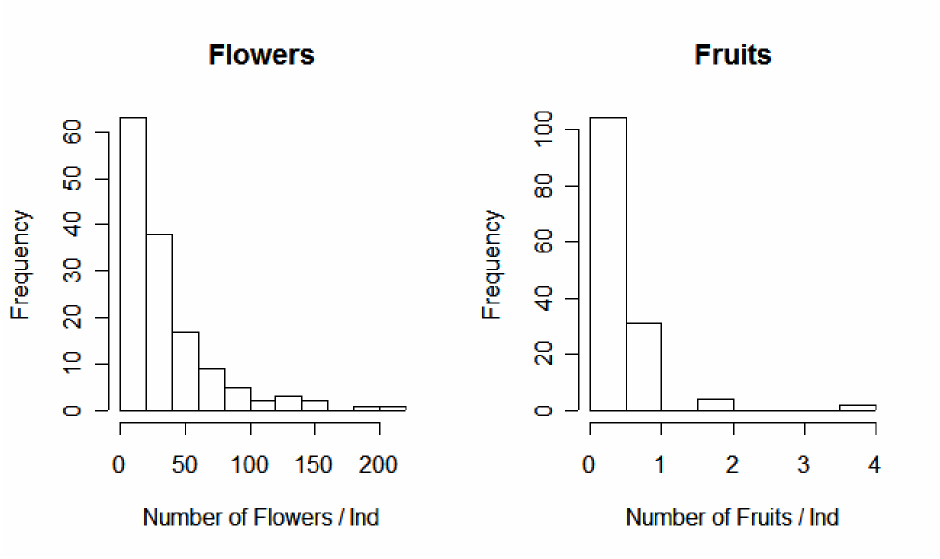

Figure 4. Histograms showing the number of flowers and fruits per individual (n = 124).

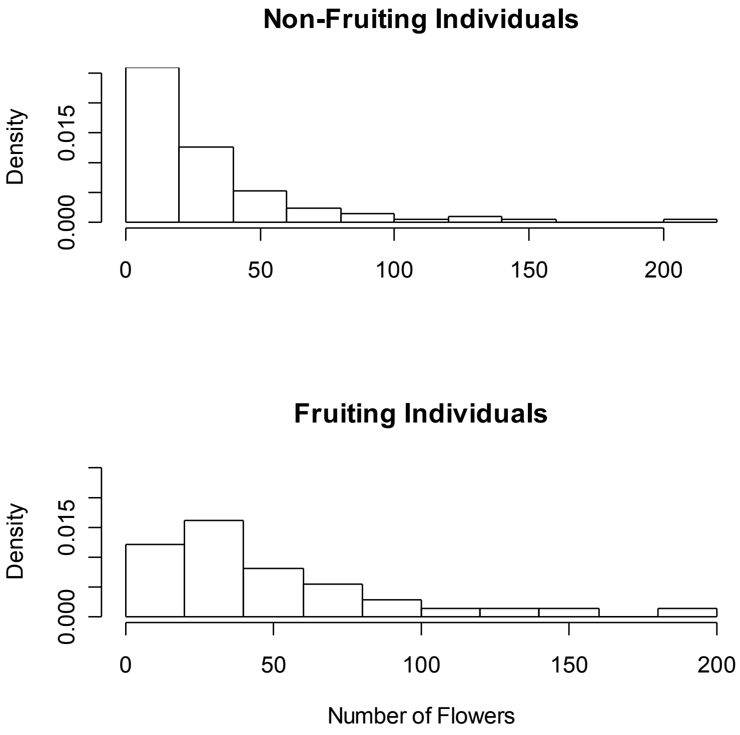

Figure 5. Histograms of the number of flowers on non-fruiting (top) and fruiting (bottom) individuals. Differences are slight, but significant (n = 141).

Fragmentation after disturbance stimulates clonal reproduction. Scandent ramets or fragments at ground level reach reproductive status rapidly once a host is found; vines only two meters in height can produce flowers. Frequent disturbance coupled with the vine's ability to reproduce vegetatively and rapidly reach sexual maturity may be explanatory factors in this particular species' widespread occurrence and abundance in regional wetlands (Householder, chapter 2). Furthermore, the propensity of *Vanilla* to reproduce clonally means that this species may be extremely long-lived and that clonal reproduction amounts to a large component of individual fitness (Caswell 1985).

Flowering dynamics

Only 50% of potentially reproductive vines flowered. Bud induction in orchids can be extremely complex and is little understood. Traditionally, drought has been thought to be the primary flowering stimulus of commercial *Vanilla*. Indeed, in *V. pompona subsp. grandiflora*, the September flowering season is preceded by a long dry season. However, the high degree of synchrony and relatively abrupt flowering season suggests that a single, well-defined environmental stimulus is responsible rather than a gradual drying. Thus, we suspect that cold fronts during June-July may also be implicated in bud initiation. A long- term study of flowering phenology is being conducted to answer this question.

During this study several flowering vines were severed by natural disturbances into two- to three-node sections. Inflorescences on these fragments continued to develop and produce fruit despite the lack of terrestrial roots. Selective abortion of flowers or buds has not been observed, even after severing. This suggests that racemes are tapping an internal energy source that has been previously allocated towards sexual reproduction. Furthermore, development of sexual organs is a long process. Inflorescences become visible two to three months prior to flowering, and initiation of bud development may occur well before, suggesting that resources may be allocated and reserved to sexual reproduction well before flowering begins. Thus, unmeasured variables such as past growth, flowering, or environmental stresses may affect the pool of available resources that determine current sexual reproduction. Furthermore, the lack of psuedobulbs in *Vanilla* may exacerbate tradeoffs between vegetative plant activities and sexual reproduction.

Fruiting dynamics and Pollination

Complete fruiting failure was high since approximately 74% of flowering individuals produced no fruit and fruiting distribution was highly right skewed (fig 4). Of the 37 individuals (26%) that produced fruit, 31 produced only a single fruit. High fruiting failure and right skewed fruiting distributions seem to be a common phenomenon in the family (Janzen et al. 1980, Schemske 1980, Merhoff 1983, Ackerman 1989, Gill 1989, Zimmerman and Aide 1989).

In *V. pompona subsp. grandiflora*, individuals producing more flowers have greater chances of producing at least one fruit, albeit a very small one. Figure five illustrates the slight but significantly different distribution of flower number between fruiting and non-fruiting groups. Similarly, other investigators have shown that a larger number of flowers arranged in floral displays at the inflorescence (Montalvo and Ackerman 1987) or local level (Sabat and Ackerman 1996) have no or little effect on pollination in orchids. That flower number and large visual displays play minimal or no roles at all in pollination might be expected in plants pollinated by deception. Deceived pollinators are not likely to pollinate multiple fruits on the same plant, a strategy that may result in a greater number of offspring being fathered by outcrossed pollen.

Six individuals produced multiple fruits (fig 4). Multiple developing fruits on a single individual almost always occur in pairs, have the same size, and hang from the same raceme. These fruits were probably pollinated during the same event when paired buds opened simultaneously in response to a sudden temperature reduction.

Pollination rates of this species are incredibly low, even compared against other neotropical, deceptively pollinated orchids (Tremblay et al. 2005). Low pollination rates are common in Orchidaceae, and many have been shown to be capable of sustaining higher pollination rates during a given season (Schemske 1980, Horvitz and Schemske 1988, Campbell and Halama 1993), leading many authors to conclude that orchids, in general, are pollinator limited. However, artificially increasing pollination rates has been shown to affect future vegetative growth for many orchid species (Montalvo and Ackerman 1987, Ackerman 1989, Snow and Whigham 1989, Zimmerman and Aide 1989, Ackerman and Montalvo 1990, Primack and Hall 1990), suggesting that throughout its lifetime, orchids are resource limited. Zimmerman suggests that orchids may be pollination limited during any given season, but resource limited during their lifetime.

This may be the case for *Vanilla*. Individuals of *Vanilla* are extremely dynamic as ramets grow and senesce. Constant regeneration of all organs (stem, leaves, roots) and the vine's modular construction means that individuals, under hypothetical conditions, are essentially eternal. Genetic individuals are likely extremely long-lived and clonal reproduction probably dominates measures of individual fitness. Because fitness accumulates over the lifetime of the plant, resources devoted to sexual reproduction may be conservative at any time t. Rather than devote limited resources to flowers and fruits, they may be better allocated to growth or maintenance. Essentially, survival to time $t + 1$ may be more beneficial to lifetime fitness than immediate seed production. In other words, low pollination rates may be sustained as long as clonal reproduction represents the larger component of individual fitness.

CONCLUSIONS

Vanilla pompona subsp. grandiflora occurs abundantly in the wetlands of Madre de Dios, Peru. The species is widely distributed yet few details of its natural history outside of managed plantations exist in the literature. In Madre de Dios wetlands, *V. pompona* is an abundant, fast growing, and extremely clonal vine. Fruiting and flowering dynamics are similar to other deceptively pollinated orchids, characterized by high fruiting failure, skewed fruiting and flowering distributions, and low pollination rates. Although such studies as this are important, they offer only a brief window into the reproductive biology of this important group of plants. Orchidaceae is the most speciose family on the planet, but the question of why still looms large (Dressler 1993, Tremblay et al. 2005). The majority of 30,000 + species remain to be described, and very few of these have received even fleeting attention by natural historians (Dressler 1993). More short-, and especially long-term studies of a variety of orchids are needed to better elucidate important patterns in the life history of this diverse group of plants.

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CHAPTER 4:

MAPPING A POTENTIAL NON-TIMBER FOREST PRODUCT IN THE WETLANDS OF MADRE DE DIOS, PERU

ABSTRACT

We are currently investigating a species of *Vanilla* (Orchidaceae) as a possible non-timber forest product (NTFP) in Madre de Dios, Peru. Its commercial potential is clear, as vanilla is among the most popular flavors worldwide and a vibrant global trade already exists. However, as an NTFP, collection, harvest, and maintenance of *Vanilla* in its natural habitat will largely depend on a favorable spatial distribution. We performed extensive groundtruthing and classified *Vanilla* habitat using a single LandSat TM image. In the region studied, including over 130 km of floodplain habitat along the Madre de Dios river, over 7,000 ha of high-density *Vanilla* habitat exists. Furthermore, there are over 100 locations of 5 ha or larger where vanilla occurs in densities ranging from 25 to 150 individuals / ha. These "natural plantations" of vanilla are extremely common in Madre de Dios and might be used as a tool in the management and conservation of important wetland habitat in the future.

INTRODUCTION

The conservation and sustainable use of the large wetlands of Madre de Dios, Peru (MdD), is receiving increasingly more attention from local governmental and non-governmental organizations (Rojas et al. 1995, Instituto Nacional de Recursos Naturales 2000, Conservation International 2001). Although little information exists in the literature about upper Amazonian wetlands, wetlands in general are noted for the myriad ecosystem services they provide, especially concerning their roles in hydrological and carbon cycles (de la Cruz 1986, Junk and Piedade 2004). Wetlands in MdD are important for several wide-ranging animal species. Large mammals such as tapir, peccaries, and a variety of monkeys feed on abundant *M. flexuosa* fruits during lean dry-season months when fruit abundance in forest habitat reaches a seasonal low (Tobler, pers com.). Additionally, wetland habitat is the exclusive nesting grounds for the green and blue macaw (*Ara ararouna*) in the region, around which much eco-tourism has been developed (Brightsmith and Bravo 2005). The need for some kind of protective standing is clear. However, current conservation status of MdD wetlands is poor, an unfortunate circumstance given their potential ecological, biological and environmental significance.

Madre de Dios wetlands are particularly vulnerable to over exploitation by humans. Traditional harvesting techniques destructively extract palm products by felling entire female trees. Fruits of *M. flexuosa* are in high demand in local markets and are exclusively harvested from wetlands. For example, 20 to 40,000 kilograms of fruit are extracted daily in the department of Loreto, Peru where wetlands have been severely degraded as a result (Kahn 1988, Vasquez and Gentry 1989, Berjarano and Piana 2002). In MdD the degradation is not so severe. However, there is abundant evidence of unsustainable palm fruit harvesting surrounding the department capital, Puerto Maldonado. Some wetlands immediately surrounding the city have few reproductive female palms.

Perhaps the most immediate threat to wetlands is gold mining. Since the 1980s gold mining along flood plain habitat has become a predominant economic activity in the region (Altamirano 2003) and mining directly within wetlands is not uncommon. A reliable source of energy from Cusco via the newly refurbished interoceanic highway and rising gold prices have contributed to the switch towards more mechanized and destructive mining techniques in the past decade. High-powered water pumps operating day and night can process tons of wetland sediment per day and may be altering hydrological patterns within fragile wetland habitats (Hamilton et al. 2004).

Extraction of non-timber forest products (NTFP) is important for millions of inhabitants in tropical developing countries (Anderson 1988, Pinedo-Vasquez et al. 1990, Phillips et al. 1994, Gram et al. 2001). In increasingly human-dominated landscapes, non-timber forest product (NTFP) extraction may be the best alternative to traditional conservation strategies, and has the potential to be ecologically sound under intelligent management schemes (Fearnside 1989, Peters et al. 1989, Peters 1990, 1994, 1996).

However, in the case of MdD wetlands, extractive activities are generally unmanaged and destructive. The main purpose of this study is to call attention to *Vanilla pompona subsp grandiflora* (Orchidaceae), an alternative extractive product that occurs abundantly in local wetlands. We believe this previously undocumented species (Householder, chapter 1) to be of potential economic value as a marketable flavoring product.

Peters (1994) suggests that first and foremost, the distributional pattern of the extracted product is perhaps the decisive factor that will ultimately determine future success and viability of conservation efforts driven by NTFP extraction. Grass-root efforts to sustain natural products extraction in the tropics have high failure rates in part because of dispersed distributions of raw materials leading to inefficient harvesting, uncompetitive pricing, and over harvesting (Peters 1994, 1996). Efficient harvesting in natural settings demands that distributions of harvestable plants be densely clustered -- a condition rarely encountered in tropical floras. We specifically examine the spatial distribution of *V. pompona subsp. grandiflora* in wetland habitat using a variety of remote sensing techniques and groundtruthing. We discuss vanilla's potential role in the conservation and management of wetlands within the MdD basin. Furthermore, delineation of high-density vanilla habitat and the classification of distinct wetland vegetation types serves as a guide for prioritizing future conservation efforts of important wetland habitats.

Vanilla Culture

Three species of *Vanilla* are produced commercially: *V. planifolia, V. tahitiensis, and V. pompona* (Rolfe 1896). All are succulent, herbaceous vines that climb a variety of hosts via short aerial roots. Individual vines can be highly branched and reach heights of twenty to thirty meters in natural settings, but are kept below 2 meters in managed plantations by guiding the vine up and down its host. Vines are easily propagated vegetatively (Augstburger et al. 2000). *Vanilla* fruit is mainly consumed as a flavoring agent in ice creams (Correll 1944). Cured capsules from the fruit produce the prized vanilla fragrance and flavor - the second most expensive agricultural product as of 1995. *Vanilla planifolia* dominates the vanilla market; world production in 1995 was approximately 2000 metric tons, with the U.S. being the chief consumer (Koekoek 2005).

Vanilla fruit production is a laborious, year-long endeavor. Pollination must be performed manually within a four- to eight-hour window during cooler morning temperatures. As afternoon temperatures rise, flowers begin to senesce and rapidly become unreceptive. Fruits are hand harvested at an exact ripeness when the terminal section of the capsule begins to turn yellow. Manual pollination and well-timed harvests demand a close spatial distribution of the vines. Subsequent curing to produce vanillin and a marketable product lasts several months (Sabater 1903, Correll 1944, Augstburger et al. 2000).

Vanilla culture is native to Southern Mexico and Central America, but is now practiced throughout the tropics worldwide (Correll 1944). Madagscar is best known as the largest producer of high quality fruit and has historically controlled the vanilla market (de Melo et al. 2000). Despite the traditional hold of the vanilla market by Madagascar, vanilla fruit from other producing countries are quickly becoming accepted alternatives (Rain 2004, Koekoek 2005). Although *Vanilla* is found naturally in MdD and elsewhere, I am not aware of any current production in Peru.

Vanilla pompona subsp. grandiflora differs from *V. pompona* in minor floral traits. The grandiflora subspecies' fruit is highly fragrant with a distinctly sweet aroma. Fruits are larger than *V. planifolia*, weighing up to 80 grams before curing. We hand pollinated over 800 flowers, and produced about 400 cured fruits in the vine's natural habitat, demonstrating that harvests of fruits from the wetland subspecies is possible at least in the short term inside its natural habitat. Flowering phenology is bimodal with peaks in April and September. Flowering seasons are short, lasting approximately 30 days. Fruits reach full maturity at eight to nine months, dehiscing in April-June. The species occurs exclusively in the wetlands of MdD, but nothing is known about its abundance and distribution within wetland habitat; two primary factors that will ultimately determine the success or failure of possible conservation schemes driven by this potential NTFP.

MdD Study Site

The department of Madre de Dios, named after the MdD river, is situated in southeastern Peru. The MdD river, a tributary of the Madeira river, is in headwaters region of the southwest Amazon drainage basin. The Madre de Dios river drains a portion of the Sub-Andean Fold and Thrust Belt along the Eastern Cordillera and displays meander scroll morphology with rapid rates of lateral channel migration in the study region (Puhakka et al. 1992). A single Landsat ETM scence (path 3, row 69) including parts of the Inambari, Colorado, and Los Amigos rivers as well as the Madre de Dios river covered the entire study region (fig 1). Much of our investigations were based out of the Centro de Investigación y Capacitación Rio Los Amigos (CICRA) biological station operated by the Amazon Conservation Association as part of the Los Amigos Conservation Concession (http://www.amazonconservation.org/). It is located near the confluence of the Los Amigos river with the MdD river.

Lowland MdD has a humid tropical climate with annual average annual rainfall during 2000-2006 ranging between 2,700 and 3,000 mm. Rainfall is seasonal: a long dry season with less than 200mm of monthly rainfall dominates during April to September. Average temperature fluctuates minimally between 21-26°C. However, southerly cold fronts from Patagonia, known as friajes, are common in June-August (Amazon Conservation Association, unpublished data). These friajes are known to decrease temperatures to 10°C or less in minutes. Vegetation is mainly evergreen or semi-evergreen forest.

The MdD region is a mecca of biological and cultural diversity that enjoys at least some protective status from the Government of Peru as part of a series of large reserves (including the Manu and Bahaja-Sonene National Park systems) (Goulding et al. 2003). Unfortunately, wetland habitat is considerably underrepresented in reserves, a condition which provides the motivation for this study.

Wetland habitat is referred to locally as “aguajales”, after the common name of the dominant palm *Mauritia flexuosa* (Arecaceae), “aguaje”. *M. flexuosa* is the most common host of *V. pompona subsp. grandiflora*. Wetland vegetation is less diverse than surrounding forests but is home to several unique species adapted to the saturated, anoxic soils (Ancaya 2002). The wetlands of Rio Madre de Dios are generally located near the terrace escarpment, ranging in size from one to 2,000 hectare(s). Over two-hundred wetlands covering over 30,000 ha dot the floodplain of the Rio Madre de Dios from the mouth of the Rio Colorado to the border with Bolivia at Rio Heath, covering a significant proportion of floodplain habitat. These wetlands are underlain by an impermeable clay layer (Ipiruru), and fed by perennial springs that maintain soils permanently saturated year-long. Soils are histosols, with organic material measuring up to 10 meters deep (Householder pers. obs.). Few investigators have dealt specifically with this unique system and few descriptions exist in the literature, a condition that may explain the lack conservation initiatives of wetland habitat.

Table 1. Locational information of visited wetlands. ID's correspond to the labels in the map below. Numbered wetlands correspond to wetlands used in the groundtruthing survey, while lettered wetlands were used in post-classification analyses.

ID	Centroid X	Centroid Y	Area (ha)	Points in Transect
1 - A	335660	8621153	3592	38
2	363601	8621954	62	12
3	377943	8615413	13	6
4	378137	8611267	235	27
5	377434	8601873	529	17
6	416556	8600408	1657	39
B	391872	8606825	531	21*
C	395087	8605703	128	13*
D	398820	8604628	850	18*

"*" indicates that abunadance values were taken every 200, rather than 100 m along the transect.

MATERIALS AND METHODOS

Ground Truthing

Data were collected along transects in 6 different wetlands from August 2005 to August 2006. Wetlands of various size, shape, and geographic location were visited (table 1). Transects varied in length according to the size of the wetland. Wetland 4 was more extensively surveyed with multiple north-south transects spaced approximately 200 meters apart. *Vanilla* abundance was estimated every 100 meters along a transect by counting the total number of potentially reproductive vines in a circular area approximately 30m in radius. Reproductive vines were defined as climbing stems having a two-meter minimum length. A total of 226 ground truth points in 6 wetlands were visited (fig 1). Canopy coverage was estimated at 120 of the 226 points using an optical densitometer to assess the role of canopy coverage in *Vanilla* distribution. The average of two measures taken 5 meters apart was recorded for each point.

A separate, independent data set collected in August 2003 was used for an assessment of post-classification accuracy. The same methodology to estimate *Vanilla* abundance was used for the 2003 data, except data were collected every 200, rather than 100 meters along transects and canopy coverage was not assessed. The 2003 data consisted of 75 points in four wetlands.

One wetland (number4 in fig 1), near the CICRA biological station was visited almost daily from July 2005 to August 2006 during which many observations of the hydrological regime of this particular wetland were noted, although not quantified.

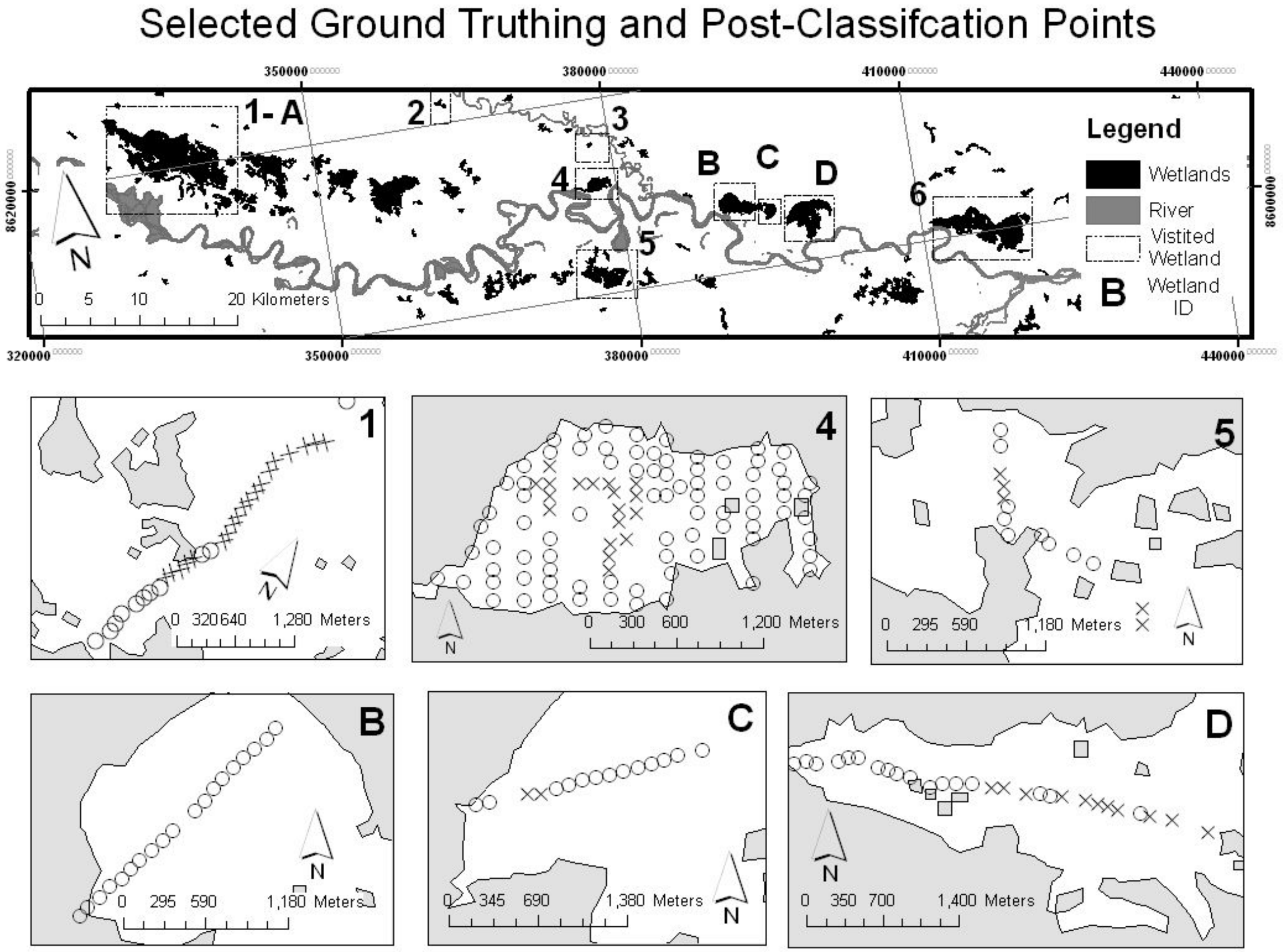

Figure 1. Map of study region and selected wetlands depicting points used for groundtruthing and post-classiciation. Numbers correspond to wetlands used for groudtruthing, while letters correspond to wetlands used in post-accuracy assessments. Not all wetlands are shown. An “x” represents high density vanilla points, while “o” represents low density vanilla points. Apparent gaps in transects reflect intermediate vanilla densities that were not used (see below).

Supervised Classification

ENVI 4.3 (ITT Solutions Inc., Boulder, Colorado) was used for image processing, classification, and post classification accuracy assessment. Groundtruthing points were classified into two groups: high density vanilla habitat (HDVH), and low density vanilla habitat (LDVH). HDVH included observation points with more than eight potentially reproductive stems. LDVH was defined where the number of stems counted was two or less. We chose to omit medium density values ranging between 3 and 7 *Vanilla* stems so that there was a clear distinction (no confusion from the ground) between the HDVH and LDVH classes. Our class definitions resulted in 57 HDVH points, 139 LDVH points, and 30 points of intermediate density not used in the analysis. The independent data set used for post-classification accuracy was classified similarly, resulting in 24 HDVH, 41 LDVH points, and 10 intermediate density points (not used).

LANDSAT Themmatic Mapper (TM) images were accessed from University of Maryland's Global Land Cover Facility (access date June 2003). The image for Path 03, Row 069, dated May 2000 was selected, orthorectified and projected to UTM Zone 19 S (WGS 1984). Image analysis was performed on six bands, excluding the thermal band.

Selection of groundtruthing pixels was accomplished by centering each point on the TM pixel overlaying it. Points were moved 1-15 meters. Centering was performed so that neighboring pixels had an equal chance of being selected with the groundtruth point. A circular buffer with a radius 50 meters was generated around each ground truth point. A fifty-meter buffer limited overlapping between successive points since points were taken at 100 meter intervals. The TM pixels contained by the buffers were used in the classification procedure, resulting in 537 HDVH pixels, and 1,875 LDVH pixels.

Non- wetland habitat was masked out for the classification procedure. A vector file delineating wetland habitat is publicly available from www.andesamazon.org. No clouds covered any portions of the wetlands, so no cloud or shadow classes were created; nor was the image atmospherically corrected. A Maximum Liklihood algorithm set at a probability threshold of 0.95 was applied to the image. Training sites corresponded to the 2005/2006 data set comprising 226 ground truthed points in six wetlands. Post-classiciation accuracy was assessesed with the 2003 dataset of 75 groundtruthed points in four wetlands, reporting the overall accuracy, the kappa statistic, errors of commission and omission, and the producer’s and user’s accuracy.

RESULTS & DISCUSSION

Ground Truthing and Habitat Observations

Within wetland habitat, light seems to be one critical factor determining the presence and abundance of *Vanilla*. *Vanilla* will neither grow in dense shade, nor full sunlight. It is most abundant and healthy in slightly filtered light with about 50% coverage (fig 2). Dense shade reduces growth rates and leaf size, while full sun causes leaves to sunburn, turning yellow, black, and eventually senescing. In proper conditions, *Vanilla* can occur in extremely high densities of up to 150 potentially reproductive individuals/ha.

On the ground, HDVH and LDVH are clearly two distinct vegetation types that also seem to segregate according to light regimes. LDVH is best characterized by a homogenous and dense canopy of *M. flexuosa* and dark understory conditions. Palm density may reach over 200 trunks per hectare (Householder, pers obs.). Shrub and herb layers are often relatively less developed and dark-leaved aroids dominate the vine and epiphytic communities. The vegetation is most likely adapted to low light conditions present in LDVH.

HDVH, on the other hand, is best characterized by lower palm coverage and higher light conditions at ground level. Shrub and herb layers are well developed. Common shrub and tree species occurring with *Vanilla* include *Ilex sp*.,(Aquifoliaceae), *Hediosmum sp* (Chloranthaceae), *Tabebuia sp* (Bignoniaceae), and *Luehia sp*. (Tilleacae).

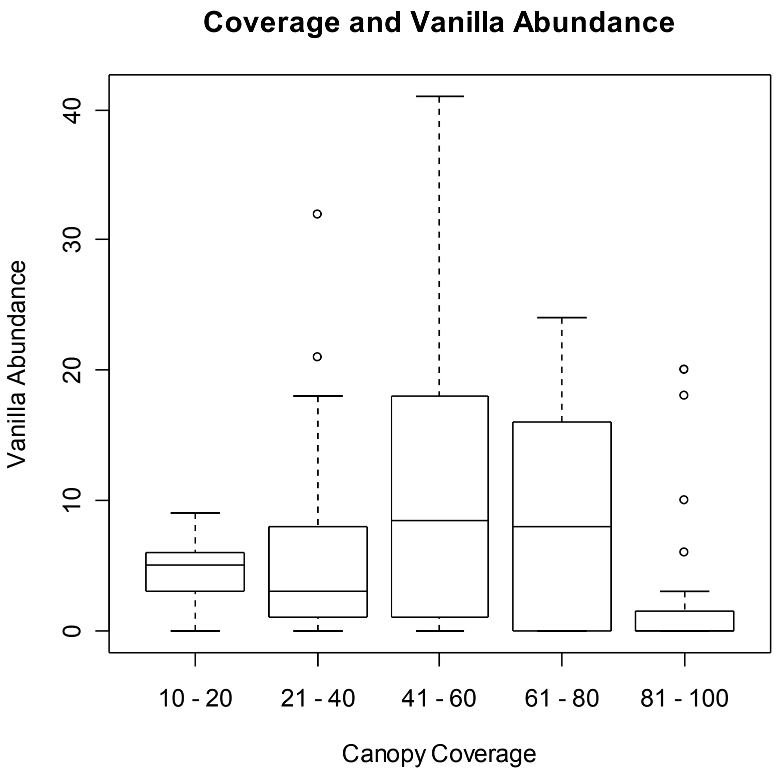

Figure 2. Boxplot illustrating relationship between *Vanilla* and light (n = 120). Data obtained from transects in six separate wetlands.

Our groundtruthing observations suggest that although defined by *Vanilla* abundance, HDVH and LDVH provide a meaningful classification of wetland habitat based on vegetation structure and composition. Thus, here, the concept of *Vanilla* as an indicator species allows a preliminary exploration into the complex habitat variation within MdD wetlands.

Figure 3 illustrates the transition from LDVH on the wetlands fringes, to HDVH located centrally. We suspect that the large and often abrupt changes in canopy coverage are due to differing hydrological regimes between HDVH and LDVH. Such drastic and often abrupt changes in large vegetation communities are rare in the region, perhaps equaled only by the large stands of bamboo that are common in the MdD region. If this is the case, then we may expect the distinct vegetation units within wetland habitat to show unique spectral signatures allowing reliable classifications over broad areas

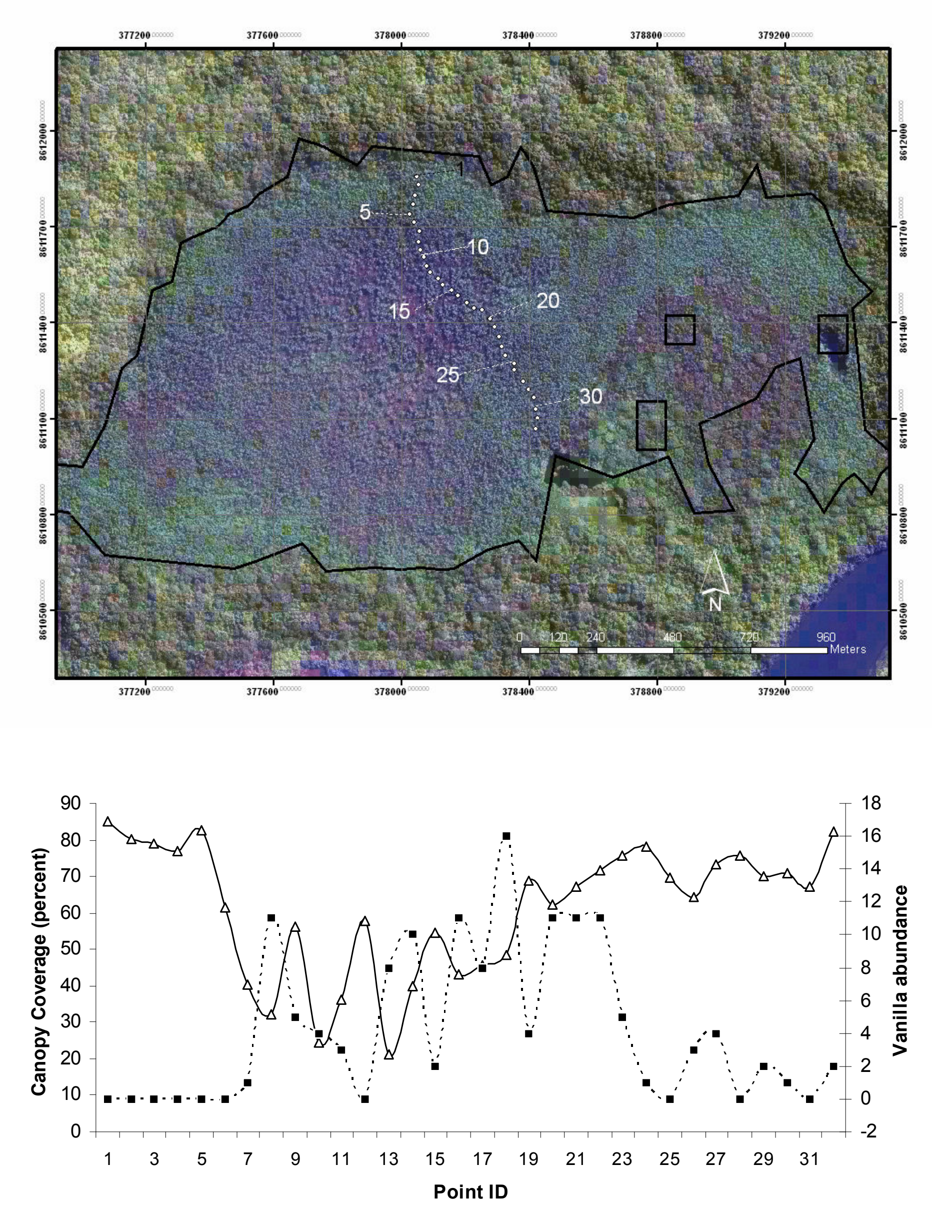

Figure 3. Transect demonstrating the rather abrupt ecotonal boundary between HDVH and LDVH. Open triangles represent coverage, while closed squares represent vanilla abundance. As coverage declines, vanilla abundance increases. Differences in coverage are easily visualized in the map above. A high spatial resolution Iknonos image is overlaid by a more spectrally resolute LandSatTM image. High density vanilla habitat is restricted to areas of dispersed, patchy palm coverage occurring in the purplish areas.

Assesment of Classification

The post-classification accuacy assesment is summarized in table 2. Error of commision is the percentage of extra pixels is class x, while error of ommision is the percentage of pixels left out of class x. The higher commission and ommission errors for LDVH and HDVH repspectively, suggest that the classification may be an underestimate of HDVH. Producer accuracy is the probability that a pixel in the classification image is put into class x given the ground truth is class x.

User accuracy is the probability that the ground truth class is x given a pixel is put into class x in the classifcation image. Overall accuracy is calculated by dividing the sum of correctly classified pixels (the diagonals of the classification matrix) by the total number of pixels. The kappa coefficient is an alternative post-classification measure of accuracy that also takes into account the non-diagonals. This measure of accuracy ranges between –1 to +1.

The user's, producers, and overall accuracy were surprisingly high. That there were no unclassified pixels indicates that our limited number of ground truthing pixels was sufficient to capture most of the spectral variability within wetlands. The largest problem seemed to be a high commission error for LDVH and a high omission error for HDVH (table 2), suggesting that our predictions may be slight underestimates of potential HDVH. An overall accuracy of 82% is particularly high, especially for tropical floras.

A likely cause for the good classification between HDVH and LDVH habitats is the large structural differences between HDVH and LDVH. The open canopy of LDVH exposes open water and saturated substrate more so than the closed canopy of HDVH, which is essentially a homogenous blanket of *M. flexuosa* tops. That said, however, the spectral resolution of LandSat TM does not provide convincing evidence supporting these differences. Class separability was low and comparisons of the average spectral curves of each habitat shows little difference (fig 4). This is a surprising conclusion given the high post-classification accuracy and large structural differences on the ground. Investigation into the spectral differences between LDVH and HDVH will be much enlightened when more spectrally resolute images become available.

Table2. Summary of post-classification assessments

	Ground Truthing Points			Errors		Accuracy	
Classification	**High Density**	**Low Density**	**Total**	**Commission**	**Omission**	**Producers**	**Users**
High Density	80% (32)	12.5% (3)	54.5% (35)	8.57%	20%	80% (32/40)	91% (32/35)
Low Density	20% (8)	87.5% (21)	45.31% (29)	27.59%	12.50%	87% (21/24)	72% (21/29)
Total	100% (40)	100% (24)	100% (64)				

Overall Accuracy = (53/64) 82.8215%   Kappa Coefficient = 0.6480

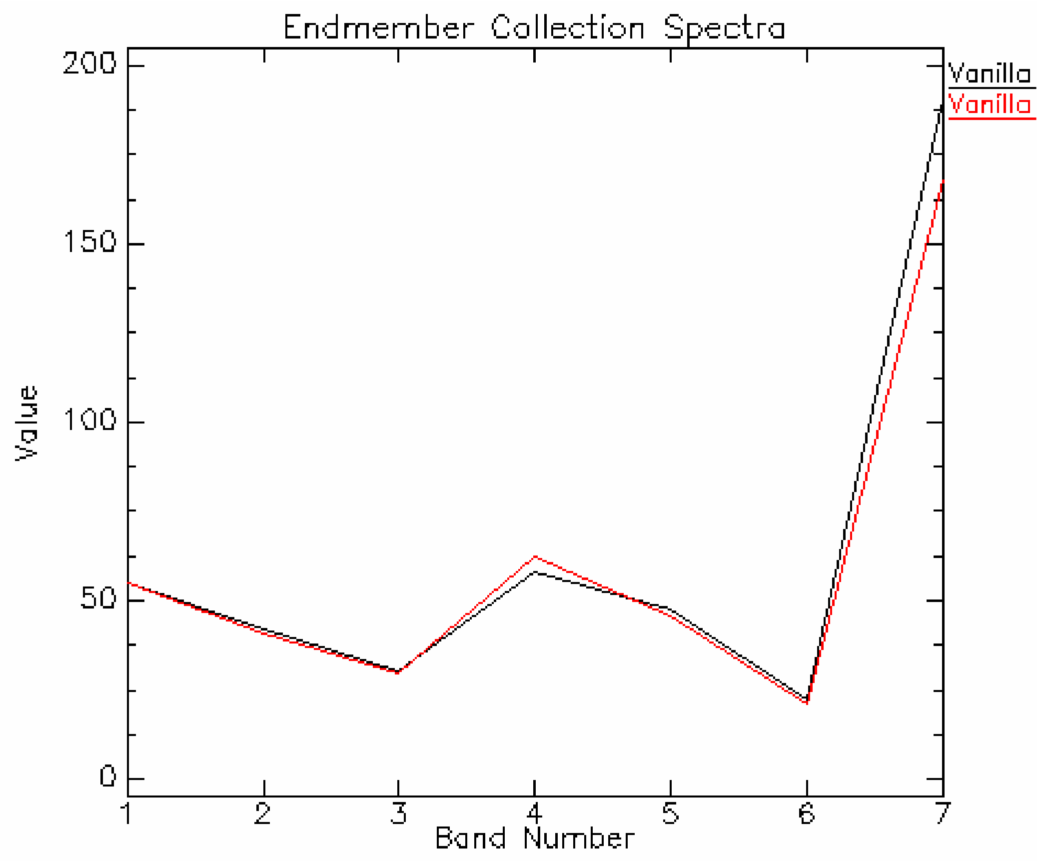

Figure 4. End member plots for HDVH and LDVH across all bands.

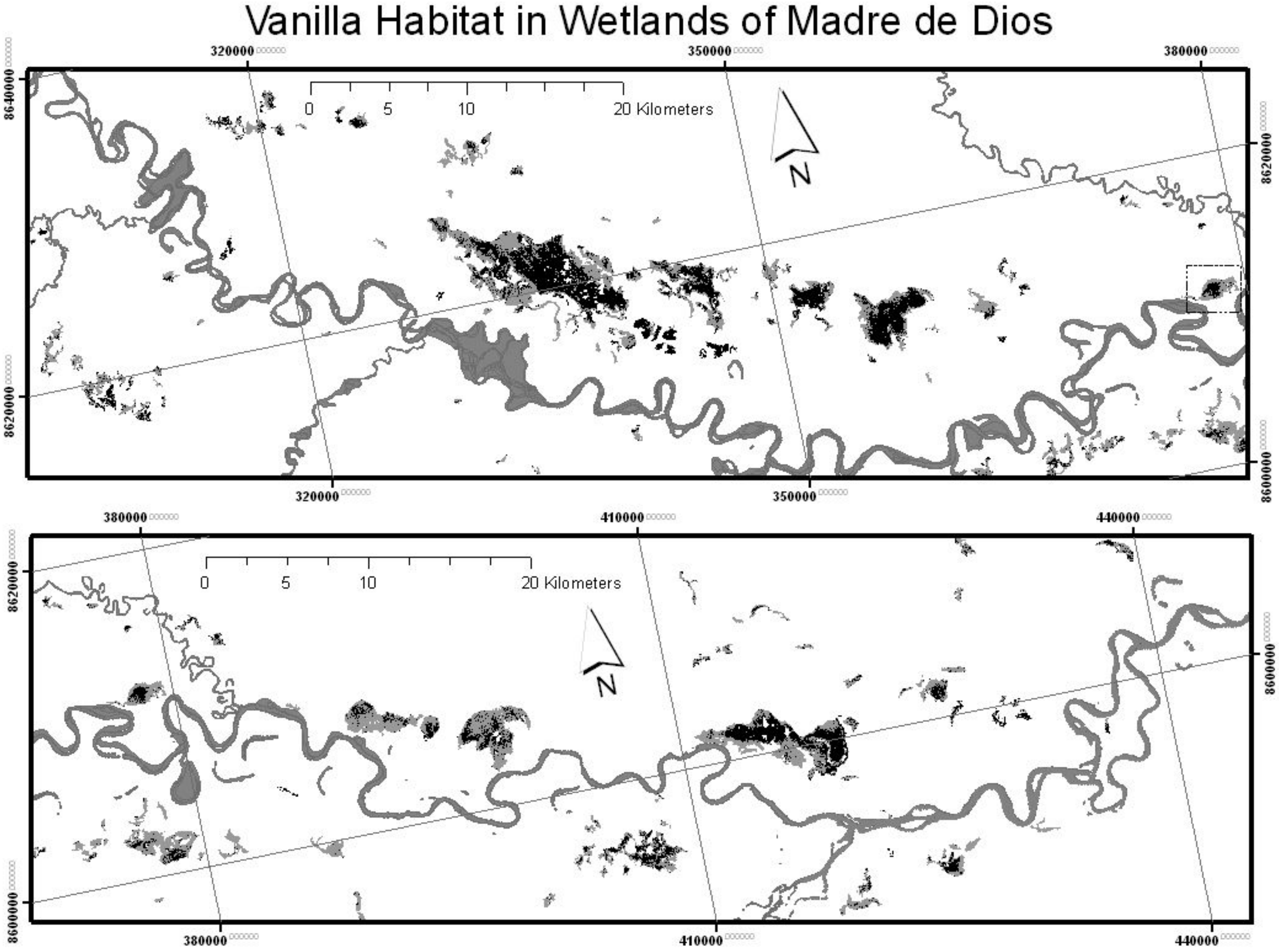

Figure 5. Results of the classification of high density vanilla habitat (dark areas).

Supervised Classification

Our classification predicts that 7,152 ha of high density vanilla habitat occurs in the , study region, representing 48% of wetland habitat (fig 5). According to our classification, the average wetland is 31% HDVH. A visual inspection reveals that pixels classified as HDVH are highly clumped. Our field observations also support this finding. Extremely open areas with few, dispersed palms, high light levels, and abundant *Vanilla* create a structurally and compositionally unique habitat that can cover large expanses. Next to nothing is known of ecological, hydrological, or biological importance of this wetland habitat despite its ubiquity.

The implications of this finding to harvesting schemes seems clear. *Vanilla* culture is laborious, requiring hand pollination, manual harvesting, and meticulous care of individual vines. Efficiency of harvesting and maintenance activities demand clustered spatial distributions, rather than populations of dispersed individuals. We often found large areas where *Vanilla* occurred in natural densities of over 150 individuals/ha that could be easily augmented with human management.

Most wetlands have little HDVH, but at least 19 wetlands have more than fifty hectares and 36 swamps have more than 20 hectares of *Vanilla* habitat (fig 6). However, because vanilla culture is such a laborious endevour, production methods are intentensive, rather than extensive. Small wetlands, with few hectares of vanilla habitat may be more economically productive and sustainable than attempting to convert large areas of *Vanilla* habitat into massive operations. Such habitat is abundant throughout the study area and often occurs near human population centers. Intensive management would minimize detrimental human impact on wetland habitat as well as provide more employment per land area than the dominant form of land management in the region (cattle production). Underemployment is high in Madre de Dios, partly explaining why gold mining continues to rise in popularity, further jeopardizing wetland habitats. Intelligent management of the MdD wetlands is vital to their conservation as humans increasingly dominate the MdD region.

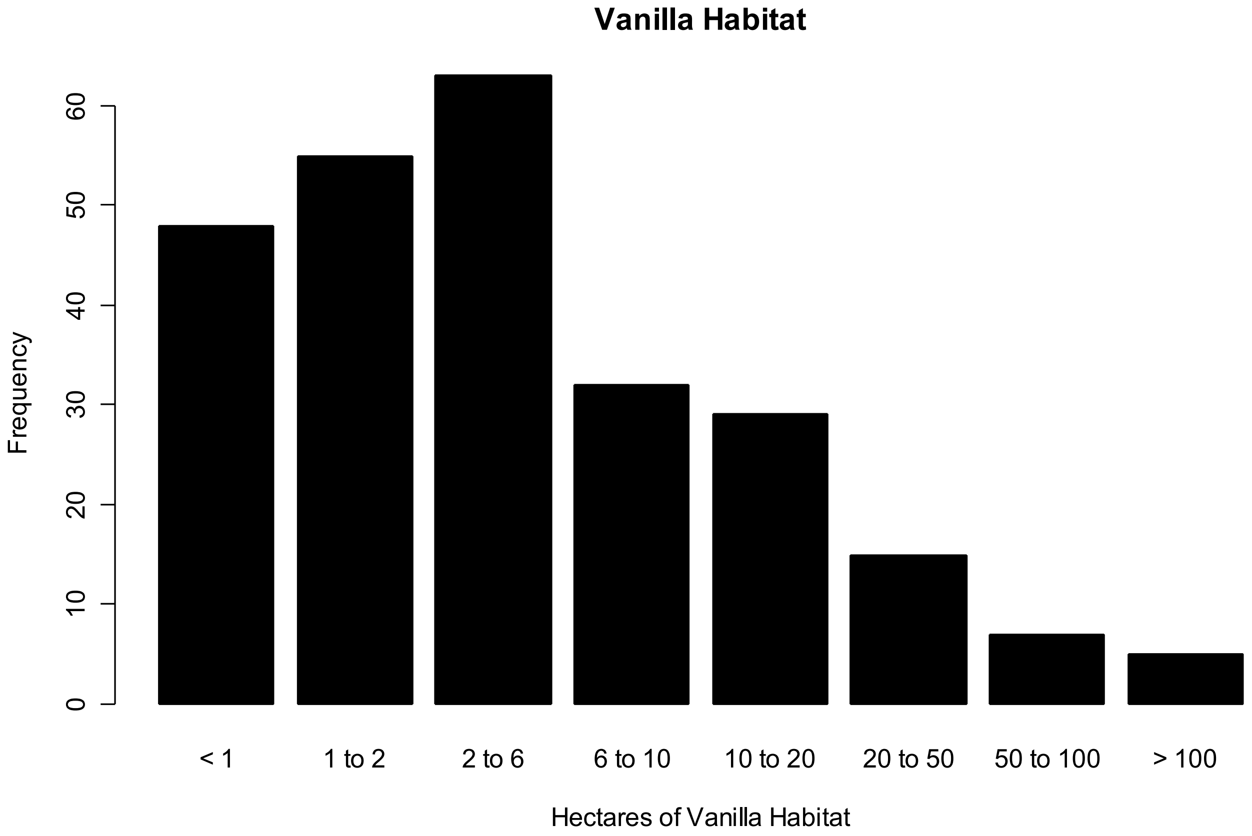

Figure 6. Histogram of HDVH hectarage.

Colonists originally began moving from distant urban areas to the MdD in the 1960s because of political and economic problems in the cities (Aguilar 1896, Alexiades and Peluso 2003), and are coming at ever higher rates with the lure of rising gold prices. The impending completion of an inter-oceanic highway spanning the length of Madre de Dios will increase connectivity with new markets and change the economic landscape in which the region has historically operated. Unprecedented changes in immigration, land use, social relations, and the economy are expected to occur (Hiraoka 1982).

NTFP extraction for the conservation of biodiversity faces social, and biological obstacles (Fearnside 1989, Browder 1990, 1992). The lack of markets, overexploitation from mismanagement (Peluso 1992), and harvesting strategies that ignore impacts on population dynamics, reproduction, and regeneration of targeted species (Peters 1996) are all potential pitfalls. Phillips (1993), studying in the nearby Tambopata Reserve, notes that extractive activities will not work as a safety valve for problems in distant urban areas because of the inherently low production of non-timber forest products. However, populations are exploding in Madre de Dios, and wetlands are a prime target for unsustainable activities. Their patchy distribution and proximity to human dominated landscape along the river margins makes them extremely difficult to protect via traditional fence and forget strategies.

Vanilla production within natural habitat may be able to bridge the gap between economic and conservation needs. Pollination, curing, and vine care would provide year-round employment. The well-established international market of vanilla and the complete absence of vanilla production within Peru suggest that such an endeavor may be economically viable. Furthermore, the curing process is a low-tech and relatively simple method of adding value to the raw product, increasing economic benefits to small producers.

Finally, I would like to note that vanilla culture may be of historical interest also. Both *Vanilla* and macaws (*Ara sp*.) were popular trade items between lowland natives and the highland Incan civilization. *Vanilla pompona subsp grandiflora* as well as *Ara arauna* are wetland specialists. While *Vanilla* climbs up *M. flexuosa* palms from their base, *Ara arauna* nests in hollow cavities at their tops. All three species, orchid, palm, and bird, are and were economically important. These three species, plus scattered individuals of culturally important plants such as coca (*Erythroxylum)* and mate (*Ilex sp*.), beg the intriguing question of whether wetland management in MdD is an ancient practice.

CONCLUSIONS

LDVH and HDVH are different in both the structure and composition of vegetation. LDVH has densely-spaced palms, high canopy coverage, reduced herb and shrub layers, and an abundance of aroid climbers. HDVH, on the other hand, occurs where *M. flexuosa* palms decrease in density, providing limited canopy coverage that encourages growth of light-loving wetland herb and shrub species. HDVH is often located centrally within wetlands, covers large areas, and has a rather abrupt ecotonal boundary with LDVH. Further attempts at classification should use images with higher spectral resolution. Despite the good post classification assessment, the spectral resolution of LandSat TM did not allow a more convincing exploration into why this might be the case.

Vanilla's location in ecologically important habitat and its favorable, highly clumped distribution suggest this plant's role in an innovative management plan for the conservation of wetland biodiversity and ecosystem services in MdD. Long-term concessions granted to local communities plus the economic incentives provided by vanilla production may encourage more sustainable and benign use of wetland resources than current, uncontrolled economic activities occurring within wetlands presently. Clearly, more investigations are needed to ascertain the economic, biological, and social viability of vanilla production in a natural setting.

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VITA

Ethan Householder was born in North Carolina on May 11, 1981. He has had a love affair with the tropical regions of the world since spending much of his adolescent years in Southeast Asia. Upon returning to the western hemisphere his sense of adventure has drawn him to the tropical regions of the Americas time and time again.

Ethan earned his Bachelor degree from Texas Christian University in 2004. During this period he spent considerable amount of time in Costa Rica, performing research in plant ecology, guiding eco-tours, and developing tours for an eco-lodge on the Golfo Dulce.

Ethan received his M. Sc. from Texas Christian University in 2007. These studies brought him to the headwaters of the Amazon river, where he spent one year performing intense field work in the wetlands of Madre de Dios, Peru.

ABSTRACT

DIVERSITY, NATURAL HISTORY, AND CONSERVATION OF *VANILLA* OF MADRE DE DIOS, PERU

by John Ethan Householder, M. Sc., 2007
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Five species of sympatric *Vanilla* (Orchidaceae) occur in the wetlands of Madre de Dios, Peru. The majority of species emanate from very different phytogeographic regions but one seems to be a previously undocumented species. All species are fully described and their habits, life histories, pollination strategies, dispersal strategies, and ecologies are detailed and discussed.

Little is known concerning the vegetation communities of these upper Amazonian wetlands and this system continues to be severely understudied. Sixteen wetlands spanning over 200km of floodplain habitat were visited in order to document the local distribution of each species. This marks the most geographically extensive study of this wetland system to date and *Vanilla* is used as a model in order to create hypotheses concerning larger vegetation patterns in the region.

Habitat of a single, potentially commercial species is classified using satellite imagery and the conservation implications discussed.