DRAFT TRANSPLANTATION DESIGN, ENHANCEMENT, AND ADAPTIVE MANAGEMENT PLAN (TEAM) FOR YADON'S PIPERIA FOR THE PEBBLE BEACH COMPANY'S DEL MONTE FOREST PRESERVATION AND DEVELOPMENT PLAN

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EXECUTIVE SUMMARY

This report is designed to aid evaluation of the potential for transplantation and enhancement projects to mitigate the loss of *Piperia yadonii* plants and habitat through the Pebble Beach Company's Del Monte Forest Preservation and Development Plan. It is the result of an intensive two-month effort to examine the rare plant's biology, site characteristics of current and future conservation areas in the Del Monte Forest, and the factors that influence success of the transplantation and enhancement projects. Using a scientific, integrated approach to design and implement the projects, transplantation and enhancement might be valuable components of larger programs to mitigate the loss of *Piperia yadonii* habitat and individuals associated with development

Examination of previous research on *P. yadonii* biology reveals that several important aspects of the plants ecology that would likely influence transplantation and enhancement project success are unknown, including the plant's habitat specificity and regeneration niche. Inferring *P. yadonii* habitat specificity from analyses in a quantitative study in this project has not to date been possible because the study occurred during the species' dormant period. However, significant gradients in plant community composition suggest that aspects of *P. yadonii* habitat requirements may be revealed through a quantitative study to examine habitat characteristics that has been designed as part of this plan.

Though quantitative information about what constitutes suitable *P. yadonii* habitat is currently unavailable, efforts to quantify acreage of habitat potentially suitable for these projects revealed 293 acres for transplantation. An additional 113 acres for habitat was identified as having a likely impediment to *P. yadonii* distribution therefore providing potential location for enhancement. Additional impediments will be discovered through future studies on the rare plant's biology as part of the proposed program. This information will .be used in further identification and prioritization of enhancement areas.

Evaluation of important considerations for transplantation and enhancement projects and identification of critical gaps in knowledge of *P. yadonii* biology lead to the proposal of a comprehensive program for successful transplantation and enhancement projects. Described in this plan, the proposed program combines observational studies and experiments to examine aspects of *P. yadonii* habitat specificity, regeneration niche, and horticultural requirements. Project implementation as proposed, would also utilize adaptive management. This scientific approach can further understanding of the biology of the rare plant while at the same time facilitating project success.

SECTION 1. INTRODUCTION

Background

As part of their effort to evaluate the potential for transplantation and enhancement projects to mitigate the loss of *Piperia yadonii* plants and habitat through the Pebble Beach Company's Del Monte Forest Preservation and Development Plan, the County of Monterey requested that the first phase of a "Transplantation, Enhancement, and Adaptive Management Plan (TEAM Plan)" be developed. As originally scoped (Appendix A), the product was to include two components: a site assessment which would determine appropriate sites for transplantation and enhancement (task 1.1) and a draft TEAM Plan providing a detailed protocol for transplantation and enhancement projects (task 1.2).

To complete these tasks, separate yet concurrent efforts were initiated for each task. The County of Monterey's consultant (Ecosystems West) collaborated with Pebble Beach Company's consultant (Zander Associates) to design a protocol to identify potential sites and evaluate their suitability for transplantation and enhancement. At the same time, Ecosystems West consultants worked independently to evaluate the aspects of the biology of the rare plant and factors of transplantation and enhancement projects that must be considered in the design of successful projects.

Unfortunately, two significant constraints were revealed early in the project: 1) there is insufficient biological information available about the plants to design protocols for project implementation, and 2) characterization of *P. yadonii* habitat for the site assessment was not possible at this time of year due to the phenology of the plant.

Overview

As a result, the County of Monterey and its consultants agreed that the scope of the Draft TEAM Plan as outlined in Phase I of the suggested outline (Appendix A) should shift. The goal of the site assessment changed from evaluating site suitability for transplantation and enhancement to identifying and quantifying the acreage of potentially suitable habitat that could be further evaluated using a quantitative study examining habitat specificity. The scope of the draft plan shifted from creating detailed protocols for transplantation and enhancement projects to designing the elements of a program that could address transplantation and enhancement efforts for *P. yadonii*.

Reflecting these changes, this report contains the following elements:

- A summary of the biology of the rare plant, which focuses specifically on the characteristics of its life history and ecology that would influence the feasibility of transplantation and enhancement (Section 2),

- An identification and initial evaluation of potential sites for transplantation (i.e. receiver sites) and enhancement (Section 3)

- A critical assessment of the factors that would influence the success of transplantation and enhancement projects for *P. yadonii* (Section 4)
- An outline of the components of a comprehensive program that uses scientific approaches to design and implement transplantation and enhancement projects (Section 5)
- A detailed protocol for a study to determine specific characteristics of suitable habitat for *P. yadonii, which* can be used in the design of the transplantation and enhancement program (Section 6).

SECTION 2. BACKGROUND INFORMATION ON YADON'S PIPERIA

Taxonomy

In the classification scheme of Dressler (1993), the genus *Piperia* is included in the subtribe Orchidinae of the tribe Orchideae in the subfamily Orchidoideae. Ackerman (1977), in the most recent treatment of the genus (published, however, before Yadon's piperia was described), summarizes earlier treatments of the genus. Although *Piperia* was first described by Rydberg (1901), most previous treatments had included the genus in either *Habenaria* or *Platanthera*, large genera distributed more or less worldwide.

Ackerman (1977) argued that *Piperia* should be maintained as a separate genus on the basis of several morphological features as well as on the basis of an ecological preference for relatively dry sites (as opposed to the preference of most *Habenaria* and *Platanthera* species for relatively wet sites). Morphological features distinguishing *Piperia*, according to Ackerman, include ovoid (vs. fusiform) tubers; leaves basal and withering before or during anthesis; caudicles very short and inconspicuous; and auricles at the base of the anther absent. In addition, in a survey of the seeds of four taxa of *Piperia* and three taxa of *Platanthera*, Healey et. al. (1980) found that the periclinal testa walls of *Piperia* are transversely reticulate, while those of *Platanthera* are smooth.

However, a recent molecular phylogenetic study of the tribe Orchideae and the related tribe Habenariinae (Bateman et. al. 2003) indicated that *Piperia* is nested well within, and therefore appears to be derived from, *Platanthera*. These authors reduce *Piperia* to section *Piperia* of *Platanthera*, and propose the new combination *Platanthera yadonii* (R. Morgan and Ackerman) R. M. Bateman for Yadon's piperia. Their results also strongly indicate that the inclusion of *Piperia* within *Habenaria* in older treatments (e.g. Munz and Keck 1973) is incorrect. *Habenaria* is now placed in the related but distinct tribe Habenariinae (Dressler 1993).

Yadon's piperia was first described by Morgan and Ackerman (1990). The previous treatment of Ackerman (1977) recognized four long-spurred *Piperia* taxa but did not attempt to subdivide the short-spurred forms, all of which were treated as belonging to the widespread, polymorphic species *Piperia unalascensis*. In two subsequent papers, Morgan and Ackerman (1990) and Morgan and Glicenstein (1993) segregated five additional short-spurred taxa from *P. unalascensis*: Yadon's piperia, white-flowered piperia (*Piperia candida* R. Morgan and Ackerman), chaparral rein orchid (*Piperia cooperi* (S.Watson) Rydb.), Coleman's piperia (*Piperia colemanii* R. Morgan and Glicenstein), and Point Reyes rein orchid (*Piperia elegans* (Lindley) Rydb. ssp. *decurtata* R. Morgan and Glicenstein). These authors note the strong morphological distinctness of these taxa from each other and from *P. unalascensis*. They also note the rarity and localized distribution of Yadon's piperia and Point Reyes rein orchid.

Morphology

Yadon's piperia is a small perennial herb. It perennates by means of a small ovoid tuber. Generally, two tubers are present per plant. This tuber is an unusual structure, found only in members of subfamily Orchidoideae, which is referred to as a "root-stem tuberoid" and consists of a sheath of root tissue surrounding a core of stem tissue with an apical bud (Dressler 1981, 1993; Rasmussen 1995). The tuber produces a short rhizome and a leafy shoot. The leaves are basal, lanceolate to oblanceolate and 10 to 15 cm (4 to 6 inches) long and 2 to 3 cm (0.8 to 1.2 inches) wide. One or two leaves, less commonly three, are produced each season; most individuals that flower have two leaves (Doak and Graff 2001). The leaves whither at or shortly after the flowering stalk bolts in May or June. A single scapose flowering stalk is produced; this may be up to 80 cm (31 inches) in height in exceptionally large individuals, although it is usually smaller. The inflorescence is a densely flowered, narrowly cylindrical, spikelike raceme. There are between 10 and 120 flowers per flowering plant (Morgan and Ackerman 1990), with an average of about 56 (Doak and Graff 2001).

The flowers of orchids have a distinctive, somewhat stereotyped (although often much modified), specialized, evolutionarily advanced structure (Dressler 1981). Although relatively small and inconspicuous, the flowers of Yadon's piperia and other *Piperia* species display more or less typical orchid floral morphology. There are three petal-like sepals and three petals (sepals and petals collectively are often referred to as tepals), all approximately 2-4 mm (0.08-0.16 inches) long in Yadon's piperia. The flowers are bilaterally symmetrical (one and only one imaginary line can be drawn such that it divides the flower into two mirror-image halves), and one petal, referred to as the lip or labellum, is differentiated from the other two. In Yadon's piperia, the lip is narrowly triangular. As in most orchids, the flowers are resupinate; that is, the flower twists 180 degrees in the course of development, such that the lip, which is on the adaxial (upper) side of the bud, comes to be on the lower side of the mature flower. The flowers of Yadon's piperia are conspicuously bicolored, with the upper three tepals green with a white margin and the lower three tepals predominantly white.

In *Piperia* and related genera, the base of the lip is elongated to form a slender, backward-projecting spur. The spur accumulates nectar, which serves as an attractant to pollinators. Yadon's piperia is one of the short-spurred species of *Piperia*, with spurs less than 5 mm (0.2 inches) long; spurs in the long-spurred species are generally 6-18 mm (0.24-0.7 inches) long (Ackerman 1977). Spur length in those orchids with spurs may be related to the identities of the principal pollinators. It has been shown that longer-spurred species are pollinated primarily by larger lepidoptera (moths or butterflies), while shorter-spurred species may be pollinated by a greater range of lepidoptera as well as other insect groups. This tendency is especially well documented in the genus *Platanthera* (Catling and Catling 1991, and references cited therein), within which *Piperia* apparently belongs (Bateman et. al. 2003). Based on relatively limited observations, Doak and Graff (2001) found moths to be the principal pollinators of Yadon's piperia, although they also observed mosquitoes and a bumblebee (*Bombus* sp.) as potential pollinators (Pollination Biology section, below).

As in most orchids, the stamen and pistil of Yadon's piperia are completely united into a single structure, the column or gynostemium, with a highly specialized architecture. The ovary is inferior (located below the other flower parts). In Yadon's piperia, as in all but the more primitive orchids, there is only a single fertile stamen, bearing an anther. Because stamen and style are completely united, the anther is in close proximity to the stigma. The pollen grains are aggregated together by a sticky substance into clusters called pollinia, which, in many orchids (including Yadon's piperia) are subdivided into smaller clusters called massulae. This allows a single pollinium to potentially pollinate a number of flowers, since only one or a few massulae may be left behind on a given stigma in a single pollination event. The stigma includes a specialized portion, the rostellum, which is usually interpreted as part of the median stigma lobe (Dressler 1981). The pollinia are attached to a sticky disk, the viscidium, which is part of the rostellum. In *Piperia* species and many other orchids an extension or tail of the pollinium, called a caudicle and produced by the anther, attaches the pollinia to the viscidium. Short and inconspicuous caudicles are characteristic of *Piperia* species. The pollinium, caudicle, and viscidium together are referred to as a pollinarium; this is the actual package detached from the flower and carried by the pollinator. In flowers of Yadon's piperia, a pollinarium consists of two pollinia attached to a single viscidium, and there are two pollinaria per flower.

The fruit of Yadon's piperia and other *Piperia* species is an elongate capsule, approximately 5-8 mm (0.2-0.31 inches) long. As is typical of orchids, the seeds are tiny and dust-like, and several hundred are produced per capsule. Doak and Graff (2001) estimated that healthy plants of Yadon's piperia that successfully set fruit produce an average of approximately 7,000 seeds per plant in a season.

Yadon's piperia is distinguished from other species of *Piperia* by a distinctive suite of morphological characters. These include: the short spur; the dense, condensed raceme; the falcate (sickle-shaped) upper petals; the narrowly triangular lip; the white and green tepals; and the distinctly bicolored appearance of the flower (Morgan and Ackerman 1990; Wilken and Jennings 1993).

Distribution

Yadon's piperia is endemic to northern Monterey County, within four to six miles of the coast (Allen 1996; U.S. Fish and Wildlife Service [USFWS] 1998, 2002). In surveys conducted in 1995 and 1996, a total of 82,712 individuals were counted on a total of approximately 355 acres of occupied habitat at all known locations (Allen 1996). The center of its distribution is the Monterey Peninsula, where the largest populations and the greatest number of populations occur. The largest populations, collectively accounting for at least 67 percent of all known plants, are in the larger undeveloped tracts of Monterey pine forest in the Del Monte Forest area, mostly on land owned by the Pebble Beach Company and the Del Monte Forest Foundation. Smaller populations occur in patches of remnant habitat elsewhere on the Monterey Peninsula, including parks and open space areas of Pacific Grove and Monterey, at and near the Monterey Peninsula Airport, and at the Naval Postgraduate School in Monterey.

Outside the Monterey Peninsula, the largest known populations are to the north and slightly inland, on chaparral-covered ridges north of Prunedale, where about 22 percent of known plants occur (Allen 1996). Some of these plants are at Manzanita County Park and The Nature Conservancy's Blohm Ranch preserve. North of the Monterey Peninsula, the northernmost known populations are in the Las Lomas area, northwest of Prunedale. South of the Monterey Peninsula, Yadon's piperia occurs east of Point Lobos State Reserve, primarily on lands owned by the California Department of Parks and Recreation (CDPR) or on lands that will be turned over to CDPR in the future. The southernmost known occurrence, a small population containing less than 50 individuals, is about 15 miles south of the Monterey Peninsula near Palo Colorado Canyon.

Habitat

Yadon's piperia is usually found in two distinct types of habitats: Monterey pine forest and maritime chaparral (Allen 1996; USFWS 1998, 2002; Doak and Graff 2001). Although the microhabitat characteristics of the two habitat types are quite different, it is not known whether distinct ecotypic races of the species inhabit these two different habitats.

In Monterey pine forest Yadon's piperia generally grows under varying canopy densities in areas with a sparse herbaceous understory, although it also sometimes occurs with fairly dense cover of annual grasses, especially the non-native species rattlesnake grass (*Briza maxima*). The soils are generally more or less sandy but shallow and underlain by a clay hardpan that holds moisture during the rainy season but becomes very dry during the flowering season. In Monterey pine forest, the species is also capable of colonizing certain types of disturbed areas, but only after a decade or more has passed following disturbance. (Allen 1996). In maritime chaparral, Yadon's piperia grows on shallow soils overlaying sandstone, often but not always on exposed ridge tops. In this habitat the plants grow up through the foliage of shrubs and flower in full sun. It is principally associated with prostrate mats of Hooker's manzanita (*Arctostaphylos hookeri* ssp. *hookeri*) (Allen 1996; USFWS 1998, 2002; Doak and Graff 2001).

Even within those areas where Yadon's piperia is relatively abundant, its distribution is very patchy. This patchiness may reflect small-scale variability in suitability of habitat within those areas, and the important microhabitat characteristics may not be readily observable. Considerable small-scale variation is observed within Monterey pine forest habitat in general in such readily observable characteristics as shrub layer density, herb layer density, and herbaceous species composition, and a number of subtypes can be defined (see Zander Associates 2002). While Yadon's piperia clearly occupies only a subset of all Monterey pine forest habitat, the microhabitat factors most closely correlated with the occurrence of Yadon's piperia have not been well studied.

Threats to Persistence

The primary threats to the persistence of Yadon's piperia have been identified as fragmentation and destruction of habitat due to urban and recreational (primarily golf course) development; competition from invasive non-native plants; and herbivory from large deer populations (USFWS 1998, 2002). It is believed that Yadon's piperia was once much more widespread on the Monterey Peninsula, before large tracts of Monterey pine forest were lost to development. At present, some populations of Yadon's piperia are on lands that are afforded varying degrees of protection, as, for example, at Manzanita County Park, Blohm Ranch, the Samuel F. B. Morse Botanical Reserve and Huckleberry Hill Natural Area on the Monterey Peninsula, the Naval Postgraduate School, and on CDPR lands and future CDPR lands near Point Lobos. However, the largest populations, and largest contiguous blocks of suitable undisturbed habitat, are on unprotected private land in the Del Monte Forest, primarily owned by the Pebble Beach Company. These lands include over half (184 acres) of the known occupied habitat of the species. Most of the remaining land supporting Yadon's piperia in the Del Monte Forest has been or will be set aside as part of the open space preserve progrgam. However, a portion of such land zoned for development has been proposed for development of a golf course and new residential subdivisions. Many smaller populations of Yadon's piperia, both on the Monterey

Peninsula and elsewhere, are on private land proposed for various types of development. Other specific proposed projects that threaten populations of Yadon's piperia include a proposed roadway circulation improvement project at the Monterey Peninsula Airport, a proposed realignment of U.S. Route 101 near Prunedale, and proposed residential subdivisions in the Prunedale area (USFWS 1998, 2002).

In addition to direct loss of plants and their habitat, development often results in fragmentation of populations and habitat, a subtler, but potentially serious, threat (USFWS 1998; Doak and Graff 2001). Large-scale ecosystem processes, such as the natural fire regime, may be altered by fragmentation. Detrimental microhabitat changes may occur around the edges of fragmented blocks of habitat, due to altered light, moisture, and wind regimes. Fragmentation may make the habitat more susceptible to invasion by non-native species dispersing in from the margins. Fragmentation may interfere with both gene flow between populations and pollinator abundance and activity. Doak and Graff (2001) found lower pollen deposition and fecundity in two populations of Yadon's piperia occupying small, disturbed, and isolated habitat areas compared to two populations occupying relatively large areas of undisturbed habitat. They also found evidence of inbreeding depression at the level of seed set in Yadon's piperia (less viable seeds per fruit following self pollinations vs. outcross pollinations), suggesting that both large population sizes and gene flow between populations may be important in maintaining high fecundity in this species.

Competition from invasive non-native species and herbivory by deer are also potentially serious threats to Yadon's piperia. Aggressive non-native species frequently observed to invade habitat supporting Yadon's piperia include French broom (*Genista monspessulana*) and purple pampas grass (*Cortaderia jubata*) (USFWS 1998; pers. obs.). Allen (1996) and Doak and Graff (2001) found that significantly lower proportions of uncaged plants than caged plants successfully flowered, indicating that herbivory reduces reproductive success. Quantitative data on deer population levels on the Monterey Peninsula is lacking, but anecdotal evidence indicates that population levels are high (USFWS 1998).

Disease may represent an additional threat to Yadon's piperia. Doak and Graff (2001) found that many plants of Yadon's piperia were infected with a fungal pathogen tentatively identified as *Rhizoctonia* sp. (*Rhizoctonia* is a form genus consisting of asexual forms only; thus, the true identity of this fungus is unknown). Infected plants exhibit tip wilt and sometimes die. Fewer diseased plants set fruit compared to healthy plants, and fruit and seed set were lower in diseased plants that did set fruit, compared to healthy plants.

Regulatory Status

On August 12, 1998, Yadon's piperia was listed as Endangered by USFWS under the federal Endangered Species Act (USFWS 1998). It is not formally listed by the State of California, but it is listed on List 1B (Plants Rare, Threatened, or Endangered in California and Elsewhere) of the California Native Plant Society's (CNPS) *Inventory of Rare and Endangered Vascular Plants of California* (Tibor 2001; CNPS 2003). All species on List 1B of the CNPS *Inventory*, including Yadon's piperia, are recognized by state agencies as falling under the regulatory authority of the California Environmental Quality Act (CEQA) under the provisions of Section 15380 of the CEQA Guidelines.

Life History

Little research has been conducted on the life history and reproductive biology of Yadon's piperia, or other *Piperia* species. The following information is based on general knowledge of orchid biology (Rasmussen 1995) and may or may not apply to Yadon's piperia.

Seed

Seeds of orchids are sometimes referred to as "dust" seeds, and are among the smallest of all vascular plant seeds. Terrestrial or temperate-zone orchids like Yadon's piperia, however, tend to have somewhat larger seeds than epiphytic or tropical species. Data on seed size and weight in Yadon's piperia are lacking, but seeds of several other *Piperia* species examined by Healey et. al. (1980) averaged between 0.43 and 0.77 mm (0.02 to 0.03 inches) long and 0.15 to 0.23 mm (0.006 to 0.009 inches) wide. Terrestrial orchid seeds typically weigh approximately 2 to 8 micrograms (Rasmussen 1995). The bulk of the volume of the seed consists of an air-filled, often elongated testa covered with a water-repellent lipoid layer; the embryo occupies a much smaller volume than the testa. Consequently, orchid seeds have a relatively high volume to weight ratio. Orchid seeds lack endosperm, and the embryos contain only small amounts of reserve nutrients, primarily lipid and protein bodies, and generally do not contain starch.

The small size and high volume to weight ratio of orchid seeds make them well suited for wind dispersal. In addition, because relatively little resources are invested in each seed, seeds can be produced by the plant and dispersed in large numbers. Dispersal by wind of large numbers of seed allows for potential colonization over relatively long distances, and also allows the seeds to "sample" a wide variety of microsites.

Orchid reproduction by seed is successful despite the small size of the seeds and their low reserve nutrient content because all orchids, especially their seedlings, have a unique mycorrhizal relationship with certain fungi. In contrast to most mycorrhizal relationships between higher plants and fungi, which are mutualistic (beneficial to both partners; generally, the fungus facilitates the uptake of water and mineral nutrients from the soil and the plant provides the fungus with photosynthetic products for the nutrition of the fungus), the relationship between orchids and their mycorrhizal fungi, in all orchid species that have been studied, is entirely, or almost entirely, parasitic, with the orchid as the parasite and the fungus as the host. This has been shown by radioactive tracer studies demonstrating translocation of carbohydrates and other organic compounds from fungus to orchid, but not vice versa (Rasmussen 1995: Chapter 8, and references cited therein). Although seedlings of terrestrial orchids are difficult to study in the field because they are small and germinate underground, it is believed that, in all cases in nature, the seedling is dependent its fungal associate to provide it with necessary nutrients (although, in culture, orchid seedlings can often grow to maturity if they are supplied with carbohydrates and/or other organic substances in the culture medium).

Seedling

Seedlings of terrestrial orchid species develop underground. In fact, in many orchid species germination is inhibited, sometimes completely, by light. Because underground seedlings are difficult to study, orchid seedlings have been much more intensively studied *in vitro* than in the field. Orchid species differ considerably (at least *in vitro*) in the extent to which, strictly speaking, they require a fungus to be present in order to germinate. Germination requirements for *Piperia* species have not been investigated. However, even those orchids that can germinate successfully without a fungus are believed to require fungal infection at some time after germination if the seedlings are to grow and develop normally.

Seed dormancy may be a factor in regulating germination. If one or more dormancy mechanisms are present in Yadon's piperia seeds, then specific conditions would be required to break dormancy. Several types of dormancy mechanisms have been shown to operate in various orchid species (Rasmussen 1995), including physical mechanisms (e.g. the impermeability of the testa to water), physiological mechanisms (requiring a specific light regime, temperature regime, atmospheric conditions, or certain external chemical signals to break dormancy), and morphological mechanisms (e.g. a requirement for after-ripening of the seeds). Possible seed dormancy mechanisms have not been investigated in Yadon's piperia.

The regeneration niche (the sum total of all microhabitat factors important in ensuring regeneration of the species, from germination of the seeds to growth of the mature plant) of Yadon's piperia has also not been investigated. Factors potentially important in determining the regeneration niche could include, but are not necessarily limited to, soil conditions (e.g. depth, content of organic matter), amount of leaf and twig litter, moisture regime, temperature regime, and disturbance regime.

When conditions are appropriate, the seed imbibes water and germinates. In nature this apparently first requires weathering of the testa, a process that could also involve microorganisms. After germination, the apical meristem located at one end of the seedling (the chalazial end, ultimately the shoot end) extends the seedling to form a leafless, nongreen, relatively undifferentiated structure called a protocorm. This structure is, however, physiologically polarized into two ends corresponding to the hypocotyl (shoot pole) and radicle (root pole) of other higher plants. The seedlings of orchids are apparently unique among higher plants in that the radicle end has no meristem and is entirely modified into mycotrophic (interacting with, and obtaining nutrition from, a fungus) tissue. Orchid seedlings thus do not produce any primary roots; all orchid roots arise from shoots and are, by definition, adventitious.

If the appropriate fungus is not present at germination, at some point the embryo or protocorm must become infected by an appropriate fungus if the seedling is to survive. The fungus may enter through the suspensor (a stalk of cells attached to the radicle end of the embryo) or its remnants (since the suspensor has often degenerated by the time the embryo is mature). The fungus may also enter through rhizoids, rootlike cellular outgrowths (not true roots) that are present on the protocorms of many but not all orchid species. Infection may also occur through the epidermis of the protocorm, although this has not been confirmed for any orchid species. Nothing is known about the morphology or development of *Piperia* protocorms, about whether or not they produce rhizoids, or about their mode of fungal infection.

Eventually the apical meristem begins to produce a true shoot with scale leaves, termed a mycorhizome to emphasize its dependence on fungal nutrition. The mycorhizome is not distinct from the rhizome of the older plant; rather, this term denotes the earliest and most heavily infected portion of the rhizome. The mycorhizome also generally produces roots, which are usually infected with the mycorrhizal fungus either from the mycorhizome or from the soil. The protocorm may persist after the mycorhizome forms and begins to grow, so that both stages may be present in the seedling at the same time. Nothing is known, however, about the developmental sequence of *Piperia* seedlings.

The fungal hyphae (filaments) penetrate the cell walls of orchid mycotrophic tissue cells and grow into the cell. The plant plasmalemma (cell membrane) is not penetrated, however; rather, the plasmalemma recedes and elaborates to accommodate the growing hypha. Eventually the hypha branches and curls up to form a tightly coiled structure called a peloton. Observations of pelotons in orchid cells are considered definitive evidence of a mycorrhizal relationship between the plant and that particular fungal species. Eventually the pelotons are digested by the orchid cell; this is the main means by which the orchid receives nutrition from the fungus. Orchid protocorms and mycorhizomes generally show a zonation of cortical tissue behind the meristem, with younger cortical tissue and the outer cortex in older tissue containing mainly living pelotons, while the older inner cortical tissue contains pelotons that are being digested.

Vegetative and flowering plant

The orchid seedling may remain underground for an extended period of time, growing as a purely heterotrophic organism entirely dependent on its fungal symbiont. This period of time is not known for most orchid species, but Wells (1981) summarizes data indicating that it is generally longer than one year and may be up to 15 years. Eventually, however, a leafy shoot is produced above ground. Even after the shoot appears, the young plants of some orchids apparently are not able to sustain themselves by photosynthesis alone, and continue to depend on the fungal symbiont for some time.

Plants may produce only vegetative growth for several years before flowering. In addition, plants that have previously flowered may revert to vegetative growth in subsequent years. In a study of three British orchid species, Wells (1981) found that there was little relationship between flowering and the age of the plant. It is likely that Yadon's piperia behaves similarly. Only a small percentage of aboveground Yadon's piperia plants flower in a given season (Allen 1996; Doak and Graff 2001).

In addition, plants of some orchid species that have previously produced aboveground parts sometimes remain underground for one or more seasons, and then reappear above ground in subsequent seasons (Rasmussen 1995 and references in his Table 8.2). This phenomenon has not been well studied, but it is presumed that the orchid is depending on a fungal symbiont for nutrition and/or is surviving on nutrients stored in a tuber or other underground storage organ in those seasons when it does not produce an aboveground shoot. It is not known to what extent this may occur in Yadon's piperia or other *Piperia* species.

Members of the subfamily Orchidoideae, such as Yadon's piperia, perennate solely by means of the tuber, which also functions in nutrient storage. As noted previously, this "root-stem tuberoid" is an unusual structure consisting of both root and stem tissue. It originates as an axillary shoot from the rhizome that produces a basal extension resembling an adventitious root, which then grows to more or less surround the shoot tissue. The rhizome in orchidoid species is short and essentially constitutes connecting tissue between old and new tubers and the leafy shoot.

Like other *Piperia* and *Platanthera* species, Yadon's piperia is summergreen, producing leaves in the spring (in the case of Yadon's piperia, leaves are generally produced sometime after fall and winter rains) (USFWS 1998, 2002). Rasmussen (1995) outlines several life histories for tuberous orchids, differing in the order in which roots, aerial shoots, and tubers are produced. *Piperia* has not been studied in this regard, but, in many *Platanthera* species, roots develop first (and are implied to have a purely mycotrophic function), followed by an aerial shoot and then a new tuber.

Although complete dependence on a fungal symbiont appears to be universal among orchid seedlings, apparently there is considerable variation among species in the degree to which the adult plant depends on the mycorrhizal association, and this is an area of orchid biology that has been relatively little studied. Fungal symbionts can be isolated from roots and sometimes from rhizomes or tubers, although root-stem tuberoids are generally infected only in superficial tissue layers. On the basis of limited information, Rasmussen (1995) believes that all shades of intermediacy exist between orchids that remain heavily dependent on their fungal symbiont throughout their lives and those whose mature plants rely almost entirely on photosynthesis. It is not known to what degree mature *Piperia* species depend on fungal symbionts.

<u>Fungal Symbionts</u>

Most fungi known to form mycorrhizal associations with orchids have been referred to the form genus *Rhizoctonia*. Because these fungi are almost entirely asexual when they function as orchid symbionts, and because sexual structures are generally required for fungal identification, the true relationships of many of these fungi are not known. Formation of sexual structures by orchid symbionts can occasionally be induced *in vitro*, in which case they can be assigned to sexual genera and species; in addition, there are a few studies (e.g. Taylor and Bruns 1994; Kristiansen et. al. 2001; Shan et. al. 2002) in which molecular methods were used to assign orchid symbionts to sexual taxa. Fungal symbionts of Yadon's piperia and other *Piperia* species have not been studied.

Some of the fungi that form mycorrhizal associations with orchids are pathogenic in other groups of plants; for example, *Rhizoctonia solani* (the asexual form of *Thanatephorus cucumeris*), which is a common pathogen of crop plants like potatoes and soybeans. It is not known, however, if these same fungal species can be pathogens of orchids under certain circumstances. It was noted previously (Threats to Persistence section) that Yadon's piperia is often infected with a fungal pathogen tentatively identified as *Rhizoctonia* sp. (Doak and Graff 2001). Since the true identity of this fungus is not known, and since nothing is known about the identity of the fungal symbionts of Yadon's piperia, it is unknown whether this fungus might also be capable of forming a mycorrhizal association with the species.

Specificity between orchid species and fungal symbiont species is another area of orchid biology about which relatively little is known. In part this is because of the difficulty of studying the symbionts in the field, and the fact that orchid-fungus associations formed *in vitro* may not reflect what happens in the field. Most field studies that have been done have investigated mature plants because of the difficulty of finding and studying underground seedlings; however, it is known that the fungal symbionts of orchid seedlings (protocorms) can be different, and sometimes represent fewer species, than those of the mature plant. In a study of four North American orchid species Taylor and Bruns (1994) found strong, although indirect, evidence of specificity: neighboring orchids of different species never shared symbionts; and, although a particular species would utilize different symbionts in different habitats or geographic areas there were no symbionts in common, even between closely related species, for these four species over a wide geographic area.

Phenology

Doak and Graff (2001) outline the aboveground phenology of Yadon's piperia, and USFWS (1998, 2002) provide a less detailed account. The plant appears to be dormant in late summer and fall. Leaves emerge sometime after fall and winter rains, generally beginning around December. Bolting of flowering stalks generally occurs in late April, and leaves generally wither in May or June. Flowering occurs from mid-June to August, and fruits mature from August to early October.

Little is known about underground events in Yadon's piperia phenology, including the timing of seed after-ripening (if it occurs), seed germination, seedling growth, and growth and development of roots. Seed of most temperate Northern Hemisphere orchids germinates in spring, although some species germinate in summer or fall (Rasmussen 1995).

Reproductive Biology

Mating System

Flowers of *Piperia* species are protandrous (functionally male before they are functionally female) by age-dependent lip movement (Ackerman 1977). Initially the lip is horizontal and restricts access to the stigma, but allows the proboscis of the pollinator to contact (and remove) a viscidium with attached pollinia. As the flower ages the lip curves downward, widening the opening to the spur. At this stage, an insect carrying pollinia will probably contact the stigma and effect pollination, but will probably not remove pollinaria because the viscidia are then positioned well above the lip. Protandry serves to increase outcrossing and prevent or restrict self-pollination. In addition, because pollinators tend to move from lower (older) to upper (younger) flowers, they thus generally encounter female phase flowers before male phase, decreasing the likelihood of self-pollination by transfer of pollen from one flower to another in the same inflorescence (geitonogamy).

Doak and Graff (2001) used experimental pollinations to investigate the mating system of Yadon's piperia. The breeding systems they tested included outcrossing (pollination of one plant by another plant); geitonogamy (in insect-pollinated plants, this type of self-pollination in nature requires the intervention of a pollinator); and autogamy (self-pollination within one flower). Fruit set and proportion of viable seeds per flower (a measure of seed set) was compared between treatments; seed set within treatments was also compared with seed set of naturally pollinated flowers. Their results showed relatively high fruit and seed set for both outcrossing and geitonogamy treatments, but very low fruit and seed set with autogamy. The outcrossing treatment also showed significantly higher seed set than either the geitonogamy treatment or natural pollination. These results are generally congruent with those reported earlier by Ackerman (1977: Figure 2) for other *Piperia* species.

These results indicate that Yadon's piperia is self-compatible and has a mixed mating system, being capable of both outcrossing and (geitonogamous) selfing, but that autogamy rarely or never occurs in nature. Yadon's piperia is, thus, entirely dependent on insect pollinators to set seed. The fact that self-pollination resulted in lower seed set than outcrossing indicates that inbreeding depression occurs at the level of seed set in this species (Doak and Graff 2001).

Pollination Biology

Doak and Graff (2001) identified sixteen species of insect visitors to Yadon's piperia, all but one of them nocturnal. Most visitors were moths in four different families, and most visitations occurred between 8:30 and 10:00 PM. Mosquitoes were the only other nocturnal visitors. The only diurnal visitor was a bumblebee (*Bombus* sp.). About half the visitors, including a mosquito and the bumblebee, were observed with pollinaria attached to their proboscis. It thus appears that Yadon's piperia is largely dependent on nocturnal moths for pollination, although Doak and Graff indicated that bumblebees could potentially effect a large number of pollinations. Furthermore, as noted previously, short-spurred orchid species, such as Yadon's piperia, are often less dependent on lepidopteran pollinators than long-spurred species (Catling and Catling 1991). Ackerman (1977) noted that moths have been the principal pollinators observed visiting other *Piperia* species.

Most of the moth species observed pollinating Yadon's piperia are known to be common, and none are known to be rare (Doak and Graff 2001). The life history and ecology of most of them are, however, unknown. Doak and Graff (2001) suggest that it may be more important to determine the effects of population size and habitat quality on pollinator visitation rates and plant fecundity than to be concerned with conservation of rare and specialized pollinators.

Doak and Graff (2001) found that pollen deposition rates in Yadon's piperia differed significantly between populations, although no explanation for this was readily apparent. In 2000, although not in 1999, population differences in deposition rate generally paralleled those in fecundity (measured by proportional fruit set), despite the lack of a significant correlation between the two variables.

SECTION 3. SITE ASSESSMENT: IDENTIFICATION OF POTENTIAL RECEIVER AND ENHANCEMENT SITES

Purpose

This section describes efforts to evaluate suitability of habitat for *P. yadonii* transplantation and enhancement, and the results of study to quantify the availability of sites within the Del Monte Forest that merit further evaluation when more information about the rare plant's biology including habitat preferences is available.

Introduction

The goal of Task 1.1 of the TEAM Plan was to determine whether adequate sites are available to receive Yadon's piperia *(Piperia yadonii)* transplants as well as appropriate sites for resource enhancement. To achieve this goal, two objectives were identified: 1) to determine the physical and biotic characteristics of habitat suitable for *P. yadonii,* and 2) to identify the location of suitable sites for transplantation and enhancement projects.

CHARACTERIZING SUITABLE HABITAT FOR *P. YADONII*

There has been no previous research designed to characterize *P. yadonii* habitat. Previous studies to census *P. yadonii* (Allen 1996) and to examine reproductive success (Doak and Graf 2001) as well as the recovery plan for the species (USFWS 2002) have attempted to characterize preferred habitat of *P. yadonii* based on observations of where the species occurs (Section 2 "Habitat Preferences"). However, these general, qualitative descriptions, while helpful, need to be refined to allow determination of the habitat conditions required for transplanted populations or enhancement efforts.

As part of this project, Pebble Beach Company's (PBC's) consultants and Monterey County's consultants collaborated in the development of a protocol to determine the physical and biological characteristics of *P. yadonii* habitat that could allow assessment of the suitability of habitat currently unoccupied by *P. yadonii* for transplantation and enhancement. These studies included: assessment of edaphic conditions, including aspects of soils and hydrology, and examination of plant community structure.

<u>Edaphic Conditions</u>

Physical and Chemical Characteristics of Soils

To determine soil conditions that might influence suitability of sites for transplantation and enhancement, soil scientist David Kelley was contracted to examine the characteristics of soils in habitat with and without *P. yadonii*. Summarized here, the methods used are more fully described in Appendix B. Twenty-six soil pits were non-randomly located in areas with and without *P. yadonii* to examine characteristics revealed in the soil profiles including the dimension and characteristics of the various soil horizons, the ped structure, and distribution of roots within the soil. In addition, soil samples were collected using soil augers near high-density *P. yadonii* patches used as permanent plots by Pebble Beach Company consultants for population sampling to examine the physical and chemical characteristics of soils via laboratory analysis.

Analyses of the soil samples are ongoing, and only a brief summary of preliminary analyses of soil development was available at the time of this report (Appendix B). However, their author suggests that “soils supporting strong populations of *Piperia*, with a couple of exceptions, have permeability and penetration constraints in the upper 12 to 20 inches; they are generally sandy (in the upper part of the profile in which the *Piperia* corms [tubers] are found), and they are widespread throughout the forest” (D. Kelley, Appendix B).

Soil Hydrology

Observations of *P. yadonii* distribution and soil conditions within the Monterey pine forest suggest that *P. yadonii* may be restricted to areas with saturated soils during the winter months yet dry in the summer months (USFWS 2002). Shallow wells (16) established adjacent to the *P. yadonii* permanent plots can be used to sample soil water levels within the forest.

Plant Community Structure

Biological characteristics of *P. yadonii* habitat were examined through a study to correlate the presence of *P. yadonii* with gradients in plant community composition in Monterey pine forest habitat. Summarized here, the methods, analyses, and results of this study are completely described in Appendix C. Multivariate analyses of plant community composition sampled in 20 plots randomly located in the Del Monte Forest revealed heterogeneity in plant composition within the forest. This variability could be summarized through gradients extracted from the data that suggest that environmental conditions within the forest may explain variability in the plant species composition. Unfortunately, specific data on soil characteristics, hydrology, and other potential physical factors were not available for inclusion in the analyses.

In addition, because the study was conducted outside of the growing season for the plant, the distribution and abundance of *P. yadonii* was also not quantified in the study. Analyses correlated the variability in plant community structure with the presence or absence of *P. yadonii* based on its previously mapped distribution (Allen 1996). However, given the small-scale variability in the distribution and abundance of the Yadon’s piperia, these data were too imprecise to allow examination of potential habitat characteristics. As a result of these limitations of the study, the conclusion was reached that the present analyses could not provide a statistically justifiable, quantitative description of *P. yadonii* habitat characteristics (Appendix B). Given the importance of understanding the habitat characteristics necessary for *P. yadonii* population persistence for success of both transplantation and enhancement projects, the County of Monterey and its consultants deemed a more comprehensive study necessary to analyze piperia habitat. A detailed protocol for this study is described in Section 4 of this report.

Identifying Suitable Sites for Transplantation

Given the lack of quantitative data on *P. yadonii* habitat characteristics, the goal of Task 1.1 shifted to determining the acreage of habitat potentially suitable for transplantation and enhancement. This newly defined objective is essential to ongoing efforts to design mitigation projects as it identifies potentially suitable acreage for projects and the resulting sites for quantitative evaluation in the spring 2004 habitat assessment study. Following are descriptions of the methods and results to determine potential locations for each project.

<u>Methods</u>

An initial list of suitable receiver sites for *P. yadonii* transplantation was developed applying the following criteria: 1) must be on land owned by the Pebble Beach Company, 2) must support Monterey pine forest, 3) must currently be on or have the potential to be on protected land, and 4) must have *P. yadonii* nearby. The extent of potentially suitable habitat on each site was then determined by starting with the total acreage of existing Monterey pine forest, subtracting the acreage of occupied *P. yadonii* (based on the 1996 survey data and information provided in the ADEIR), and then subtracting unsuitable habitat areas. Although what constitutes suitable habitat for piperia has not yet been systematically determined, informed assumptions were made based on previously recorded observations about what could be considered unsuitable habitat for the initial screening effort. In order to provide an estimate of the area available for transplantation, previously mapped wetland, riparian, and remnant dune habitat in each site was excluded as was Spruance meadow in PQR because it is a large open area with a discontinuous canopy of pine and little or no duff layer. The extent of riparian area to be excluded was calculated using the linear footage designated for each site in the DEIR and assuming a 100-foot-wide corridor in all areas except PQR where a 200-foot-wide corridor was assumed.

<u>Results</u>

A total of 293 acres of potentially suitable habitat available to receive *P. yadonii* transplants was identified by Zander and Associates (Table 1). Additional information regarding the protection status of each site, criteria for determining unsuitable habitat and other relevant information for the sites included in Table 1 is provided below.

Protection Status

All of the transplantation sites selected are on property owned by the Pebble Beach Company and are designated "Preservation Areas" in the Del Monte Forest Preservation and Development Plan. None of these sites is currently protected but with implementation of the DMF/PDP, all areas will be dedicated open space through the recordation of conservation easements to be held by the Del Monte Forest Foundation or an equivalent organization. A Resource Management Plan will be prepared to guide the long-term ecological management, maintenance and enhancement of the open space components of the DMF/PDP, including the Preservation Areas. Monitoring protocol and success criteria will be established and regular, systematic field review will be conducted to evaluate the effectiveness of management measures over time.

Criteria for determining Unsuitable Habitat

P. yadonii has been found to occur in Monterey pine forest, as well as maritime chaparral habitats with dwarfed shrubs (Allen 1996). The preserved areas addressed herein are all dominated by Monterey pine forest with chaparral occurring in the understory in some areas. P. yadonii is found growing through pine needle duff in filtered sun and is typically found in open areas free of competing vegetation (Doak and Graff 2001). It is rarely found where dense ruderals occur or where the understory is dominated by poison oak as is common on better soils (Allen 1996). It favors a well-drained soil that retains moisture during the rainy season but is not subject to inundation (Allen 1996).

Based on these observations of preferred habitat, large open meadows, dunes, areas of high-density pine seedlings, wetlands, and riparian corridors were initially identified as unsuitable habitat and were excluded as potential transplant areas. Large open meadows lack a Monterey pine overstory, resulting in a lack of pine duff and no filtering of light. Dune areas have not been found to support *P. yadonii* in previous surveys (Allen 1996 and Zander 2001), and are likely unable to retain enough moisture during the rainy season to provide suitable habitat. Areas of dense pine seedlings were also excluded because the habitat precludes the establishment of a diverse understory until the trees mature and begin to thin. Wetlands are seasonally inundated and in the Del Monte forest are commonly occupied by high densities of *Carex* spp., *Juncus* spp., and *Calamagrostis nutkaensis*. Riparian corridors tend to be dominated by a dense understory with very few open areas. Based on field observations, riparian corridors within the Del Monte forest are commonly densely vegetated with *Rhamnus californica, Rubus ursinus, R. discolor,* and *Toxicodendron diversilobum.* Riparian areas also tend to retain moisture for longer periods of time, likely making them unsuitable as *P. yadonii* habitat.

Excluding large open meadows, dunes, high density pine seedling areas, and wetland and riparian areas provides a gross screen of available habitat for *P. yadonii* transplantation in the preserve areas. However, there are other elements constituting unsuitable habitat that this gross screen does not capture. For instance, localized patches of dense shrubs where there is no break in the shrub cover. These patches occur throughout the forest but have not been mapped and quantified. Other elements not captured include numerous stands of dense pine seedlings, dense thickets of blackberry, poison oak and *C. nutkaensis*, and secondary drainages. For these reasons the actual acreage of suitable potential habitat may be less than what is represented in Table 1.

Table 1: Estimated Acreage Available for Transplantation of *P. yadonii*

	ESTIMATED ACREAGES					
Site	**Site**	**Monterey Pine Forest**	**Piperia**	**Non-Piperia**	**Unsuitable Habitat**	**Transplantation**
Area G	47.92	47.92	11.76	36.16	16	20.16
Area H	53.15	53.15	9.12	44.03	1.3	42.73
Area I-1	40.48	40.48	14.96	25.52	6	19.52
Area J	0.8	0.8	0.21	0.59	0.4	0.19
Area K	3.94	3.94	0.23	3.71	1.35	2.36
Area L	18.15	18.15	0.52	17.63	1.1	16.53
Area PQR	233.05	233.05	48.19	184.86	11.5	173.36
Area B	22.14	22.14	0.56	21.58	2.6	18.98
Total Estimated Acreage for Transplantation						**293.83**

Notes Regarding Acreage of Unsuitable Habitat
Area G: 16 acres burned area with dense shrub and MP seedlings - may be suitable when trees mature
Area H: 1.3 acres wetland
Area I-1: 5.3 acres riparian; 0.7 Hickman's Onion
Area J: 0.2 acre wetland and 0.2 acre riparian
Area K: 0.35 acre wetland; 1 acre riparian
Area L: 0.05 wetland; 0.5 riparian; 0.5 remnant dune
Area PQR: 1.7 acres wetland, 1.8 acres riparian, 8 acres Spruance Meadow
Area B: 2.6 acres riparian

An effort was made to more thoroughly delineate potential habitat suitable for *P. yadonii* transplantation by conducting a visual assessment in the field and using GPS to record the boundaries of suitable habitat. The suitable habitat criteria used for this assessment included

1. Presence of Monterey pine forest canopy

2. Absence of wetland or riparian indicators (such as more than 50% cover of *Carex* and/or *Juncus* species, presence of *C. nutkaensis*, dense thickets of *T. diversilobum* or *R. ursinus*)

3. Absence of dense shrubs with few openings

4. Exclusion of dense pine seedling patches

5. Exclusion of slopes over 40% (based on field observations of high density piperia locations).

A thorough delineation applying these criteria was completed for Areas PQR, G and H. The boundaries of suitable habitat areas delineated in PQR were recorded using GPS and areas in G and H were drawn onto an aerial photograph of the sites (scale 1" = 200')

Other Relevant Information by Site:

AREA G : This area is largely occupied by dense shrubs with few openings, mostly shaggy barked manzanita and huckleberry. On the sloping east side of this site, a large stand of *C. nutkaensis* has established in an area that could otherwise be considered potential habitat for *P. yadonii*. Approximately 16 acres in the northern third of Area G burned in 1987 and currently supports dense stands of Monterey pine seedlings as well as Monterey clover. This area was considered unsuitable habitat for transplantation at this time but could become suitable over time once the trees mature and the understory develops.

AREA H: This area contains large polygons of potential *P. yadonii* habitat. There are some areas of dense shrubs that tend to occur around the large drainage running through the middle of the site. These areas may not be suitable for transplantation but they were not included as unsuitable habitat at this time.

AREA I-1: There are two drainages that run east to west at the southern portion of this site. A one-hundred-foot-wide corridor associated with each of these drainages was assumed to be unsuitable transplantation habitat. The 0.7-acre area of open meadow in the southeast portion of the site where Hickman's onion occurs was also assumed to be unsuitable habitat.

AREAS J and K: Wetland and riparian areas were excluded in both these sites. There may be less area suitable for transplantation due to the presence of dense blackberry and *C. nutkaensis* areas.

AREA L: There is about 0.5 acre of remnant dune in the western portion of Area L that was excluded from potentially suitable habitat. The site also contains wetland and riparian areas that were excluded.

AREA PQR: Areas identified as unsuitable habitat for transplantation for the initial screening in PQR include Spruance Meadow, and delineated wetland and riparian habitats. There are other areas that may be unsuitable but were not excluded at this time. For instance, PQR contains some very steep slopes leading down into 4 drainages that run north to south through the area. Most of these slopes appear to be unsuitable for *P. yadonii* due to their riparian nature, consisting of a dense understory. There are many patches of dense pine seedlings throughout PQR, some quite large, whose acreage have not been determined and were not included in the unsuitable habitat estimation. On the east side of PQR, along 17 mile drive, there is a large area, dominated by coast live oak, where there is a dense understory of *Mimulus aurantiacus* and poison oak*,* appearing unsuitable for *P. yadonii*.

AREA B: This is the only site identified as potentially suitable for transplantation that contains Baywood Sands. Although P. yadonii occurs here, it is not abundant and that may be a factor of the Baywood Sands as well as the dense stands of blackberry and other shrubs common throughout this site.

Identifying Suitable Sites for Enhancement

Methods

Defining Enhancement

In order to identify potential sites for enhancement, it is necessary to determine what constitutes enhancement. For the purposes of this project, enhancement is defined as activities within or immediately adjacent to previously mapped *P. yadonii* habitat that improve habitat quality, increase the size and extent of the existing population, reduce the threat to the existing population or otherwise contribute to the long-term sustainability of the population within the Del Monte Forest and throughout its range. Types of enhancement activities include: control of non-native invasive plant species, restoration of roads and trails, creating access controls, directed management actions (e.g. selective vegetation/duff clearance, spot fire treatment, deer exclusion, seasonally-adjusted weed-whacking).

Criteria for Site Selection

The criteria used in determining potential areas for enhancement include: 1) land owned by the Pebble Beach Company, 2) currently managed for conservation purposes (or to be so by the time of the project inception), and 3) supporting natural populations of *P yadonii*. Applying those criteria, attention was focused on the following areas: 1) designated Preservation Areas within the Del Monte Forest, 2) the Old Capitol site, and 3) areas within the proposed new golf course development that would remain unaltered forested areas between fairways or that would be graded, recontoured and landscaped with native plant materials.

There are other populations of *P. yadonii* outside of the Del Monte Forest that could benefit from enhancement. Except for the Old Capitol site, these areas were not considered in this initial plan, owing to time constraints in this initial phase of work, the lack of precise data on *P. yadonii* distribution in these other areas, and the limitations of this current season, which prevented determinations of populations locations with respect to potential sites for enhancement. Areas outside the Del Monte Forest could be considered in subsequent enhancement site determinations outlined in this plan.

Site Evaluation and Acreage Quantification

A field reconnaissance was conducted within the designated Preservation Areas of the Del Monte Forest and at the Old Capitol site to evaluate and locate potential impediments to the piperia populations therein. The primary impediments identified were "hot spots" of non-native species (primarily French broom and acacia) within the Preservation Areas, roads and trails bisecting areas supporting piperia, and absence of management and access controls (Old Capitol site). The non-native hot spots, roads and trails were field located, approximate limits were identified on scaled aerial photographs, and the extent of the impediments/enhancement opportunities was estimated as a percentage of the total area of the site. In two locations (Area O/Bristol Curve Preserve & Old Capitol), impediments were so widespread that the entire area mapped as occupied by *P. yadonii* was considered degraded and in need of enhancement.

The areas proposed for grading, recontouring and introduction of native plant materials to create new forested landscapes around the new golf course were identified, established as a separate map layer and quantified. The remaining unaltered forested areas around the new golf course fairways, greens, tees and clubhouse complex were also mapped and quantified.

<u>Results</u>

The areas and associated acreages potentially suitable for enhancement based on this initial screening by Zander and Associates are provided in Table 2. As the table indicates, the largest potential enhancement area occurs at the Old Capitol site. Hot spots and road/trail restoration opportunities occur in scattered locations throughout Area PQR and in other designated Preservation Areas. Remaining or reforested areas within the proposed new golf course provide further opportunity for introduction and management of piperia.

It is important to note that these acreages do not include habitat that might be enhanced by removing more widespread impediments to *P. yadonii* populations including deer herbivory, European annual grasses (e.g. *Briza maxima*), excess duff accumulation, and other currently unknown impediments that may exist throughout the plant's distribution and thus add greatly to acreage estimates. Determinations of impediments will be a main focus of the habitat characterization and other studies outlined in Section 5. Upon identification of other enhancement measures, the sites will be reassessed for enhancement opportunities and priorities evaluated. The following are descriptions of the current enhancement opportunities identified in each site.

Old Capitol

In the absence of directed management and access controls, *P. yadonii* habitat has been degraded. Broom and other invasive species have colonized extensive areas. Large patches of poison oak have become dominant in the understory in areas previously mapped as occupied piperia habitat. Vegetation clearing, unauthorized use (e.g. dumping, homeless encampments, motorized trail bike use) and general lack of focused habitat management have all contributed to the degradation of the Monterey pine forest habitat in formerly mapped piperia areas. All of the 57.3 acres of piperia habitat mapped by Allen (1996) would benefit from enhancement activities.

Area PQR

Only scattered patches of weedy hot spots occur over this large, relatively intact block of Monterey pine forest habitat. French broom has become established in actual and potential piperia habitat in the easterly portion of the area to the south of the adjacent residential neighborhoods along Sunset Lane and Sunridge Road. Another hot spot occurs as an approximately 30-foot wide linear strip along the unpaved fire road from the most northerly point of the area just east of the Rhonda Road/Mora Lane/Sunridge Road neighborhoods. These and other small hot spots and disturbed areas cover an estimated 6% of Area PQR.

Area I-1

The primary hot spots in this area occur along the drainages toward the southerly portion of the site and in a relatively large, isolated patch along Lopez Road near its intersection with Forest Way. Broom, acacia and pampas grass have extensively colonized drainages. Though these areas have limited potential to support piperia and were not included as potential sites for transplantation, eradication of broom and acacia in the patch along Lopez Road and elsewhere could provide an expansion area for piperia over an estimated 9% of Area I-1.

Area G

Much of Area G is dominated by a dense understory of shrubby species (or regenerating forest from the 1987 burn) that would probably require thinning to create openings suitable for expansion of piperia. However, large patches of acacia also occur, especially along the fire road leading into the site from Sunridge Road. An estimated 2% of the area could be recovered for piperia expansion by the elimination of patches of acacia and broom.

Area H

Patches of acacia and broom are scattered through Area H and could be eliminated to encourage piperia expansion into those areas. An unpaved portion of Spruance Road also bisects the site. If the road were reduced to a twenty-foot width, an average of fifty feet for the entire length could be available for habitat restoration and enhancement. These activities combined could result in enhancement over an estimated 8% of the acreage of Area H.

Area O Preserve

Broom has become established in the entire area of the Bristol Curve Preserve (Area O) and habitat quality has also been compromised as a result of several roads and trails through the area. Enhancement and ongoing management (weed eradication and control, trail/road restoration, educational/directional signage) of the entire area could preserve the area occupied by piperia and possibly increase the density of the population at this location.

Reforested Golf Open Space

Approximately 15 acres of land area that is currently occupied by the Pebble Beach Equestrian Center, Collins Field or areas that will be graded to create the new golf course, will be available for reintroduction of native plant materials to create a forested setting for the golf course. These areas could provide opportunity for the introduction of piperia. While these areas may not meet the primary criteria for the long-term maintenance of a viable Monterey forest habitat (large, intact, contiguous blocks of forest habitat), they could provide suitable habitat for piperia colonies (similar to other existing isolated locations in the DMF) and facilitate the persistence of populations in the area of MNOUV.

Unaltered Golf Open Space

Approximately 13 acres of existing forested land will remain unaltered (i.e. no grading or understory clearing) when the proposed new golf course is constructed. Much of this area supports piperia and is the basis for Jones & Stokes indirect impact calculation. If appropriate management and maintenance activities were implemented in these areas including imposing access restrictions, limiting understory clearing, conducting seasonally-timed mowing or weed-whacking, piperia populations could be sustained in these areas.

Table 2: Estimated Areas Available for Enhancement

	Estimated Area			
Site ID	**Total Site (acres)**	**Existing or Potentially Suitable Piperia Habitat (acres)**	**Area Suitable for Enhancement (%)**	**Total (acres)**
Old Capitol	57.3	57.3	100	57.3
Area PQR	233.05	221.6	6	13.8
Area I-1	40.48	34.48	9	3.1
Area G	47.92	31.92	2	0.75
Area H	53.15	51.85	8	4.1
Area O Preserve	6.72	6.72	100	6.72
			Sub-total	*85.8*
Reforested Golf	15	15	15	15
Remaining Golf	13	13	13	13
			Sub-total	*28*
			Total	**113.8***

* Acreage estimate does not reflect habitat that might be enhanced by removing/reducing widespread impediments to *P. yadonii* distribution and abundance including deer browsing and other factors that may be determined through proposed studies (Section 5).

SECTION 4. IMPORTANT CONSIDERATIONS FOR THE DEVELOPMENT OF TRANSPLANTATION AND ENHANCEMENT PROJECTS

Purpose

This section identifies many of the important considerations for successful transplantation and enhancement projects and current gaps in knowledge for *Piperia yadonii*. These considerations and data gaps provide the basis for studies and approaches in the transplantation and enhancement program outlined in section 5.

Introduction

In addition to the avoidance, minimization, and reduction components of the mitigation plan, transplantation and enhancement are proposed to mitigate for direct and indirect impacts to current populations and habitat of *Piperia yadonii*. Unlike other aspects of the mitigation, transplantation and enhancement require a high degree of knowledge about the biology of the species and the ecology of its habitat, extensive planning involving careful consideration of numerous factors that will influence success, substantial efforts to implement projects, and extensive and protracted monitoring and adaptive management to facilitate ultimate success.

Given these requirements and the current lack of understanding of critical aspects of the biology of *P. yadonii* that are necessary to design a specific methodology for these projects, development of plans for project implementation, we were not able at this time to develop a plan as originally scoped in the TEAM Project (Appendix A). Instead, the present effort has focused on three goals: 1) identifying the essential components of such projects and acknowledging their complexity; 2) identifying crucial gaps in knowledge that must be filled to facilitate project design, implementation, and success; and 3) outlining the steps of transplantation and enhancement programs.

This section identifies the essential components of the projects and critical gaps in knowledge. Section 5 outlines steps in comprehensive plans for transplantation and enhancement programs.

TRANSPLANTATION

Definitions

Transplantation is the process by which plants are moved from one location to another within the wild. It is often considered synonymous with *translocation*, which is a more general term for moving individuals from one place to the other, regardless of the source or destination. If transplantation occurs where the species was previously known to occur, it is referred to as *reintroduction*, while placing individuals where they are not previously known to occur constitutes an *introduction* (Berg 1996).

General Background

In the state of California, over 175 known transplantation projects designed to establish new populations of endangered plants have been implemented since 1980, many of which were designed as mitigation (Hickson 2003). Since their inception, a series of reviews has been conducted to evaluate the success of these projects (Hall 1987, Fiedler 1991, Howald 1996, Hickson 2003). Unfortunately, evaluating success is difficult owing to the lack of consistent performance standards (Howald 1996) and the short time following project establishment. However, based on available information, these authors have evaluated the factors which may contribute to the likelihood of success (Hall 1987, Fiedler 1991, Howald 1996). This information can be combined with our knowledge of *P. yadonii* biology (Section 2) as well as previous transplantation trials conducted on *P. yadonii* to inform design of the transplantation project.

Piperia yadonii Transplantation Project Overview

As one component of a larger mitigation plan, transplantation has been proposed for *P. yadonii* individuals currently found at site MNOUV, which supports 27 acres of *P. yadonii* habitat estimated to include approximately 10,000 individuals (Jones and Stokes 2003). Based only on counts of aboveground individuals, these estimates do not include belowground plants including seedlings that have yet to emerge as well as vegetative or adult individuals that did not produce leaves the year of the census (see "Biology" section). Proportional estimates based on acreage and relative population density estimate the project to impact 12% of the remaining population.

Transplanting a large number of individuals representing a substantial proportion of individuals of an rare species requires not only extensive resources for implementation, but extensive planning prior to transplantation and a large commitment to monitoring and adaptive management to promote project success. The following section outlines crucial components of a transplantation project.

Project Design

The design of a transplantation project includes several important components that can be broadly addressed under two main considerations: 1) receiver site location, and 2) horticultural processes. The following sections outline specific considerations and the gaps in knowledge of P. yadonii that must be filled to design these components of a transplantation project.

Receiver Site Location

A receiver site is the area into which the transplantation occurs. Choosing appropriate sites for transplantation of salvaged individuals requires consideration of numerous criteria grouped into the following categories: logistical, historical, physical, and biological (Fiedler and Laven 1996).

Logistical Considerations

Logistical considerations include factors that influence whether the newly established population(s) will be protected from future human impacts and can be accessed and monitored. These concerns are adequately addressed during the site assessment (Section 3), which identified protected habitat in which transplantation projects could be implemented as the land is owned and is (or would be prior to project inception) protected by conservation easements.

Historical Considerations

Within the unoccupied area, consideration should be given to whether the plant was historically present at the potential receiver site(s). This has implications for the potential success of the transplantation, and will determine the potential for negative impacts on extant, natural populations. Concerns in transplantation to areas where plants have not been known to occur (introductions) include: 1) cascading impacts of species additions to natural communities already present at the site (i.e. competition, introduction of pests, pathogens, etc.), and 2) lower probability of transplantation success due to unsuitable habitat at the site.

Lack of historical knowledge of *P. yadonii* distribution may make it impossible to determine whether unoccupied sites have ever been occupied, rendering it difficult to evaluate the significance of these considerations for potential receiver sites. However, these may be important considerations for *P. yadonii* transplantation. Like other orchids, *P. yadonii* produces abundant, wind dispersed 'dust' seeds capable of sampling the suitability of habitat over large areas (Rasmussen 1995), suggesting that areas where *P. yadonii* has not historically occurred cannot support self perpetuating populations. Efforts to increase likelihood of transplantation success by incorporating soils from the donor site which contain both the cryptic propagules as well as such requisite symbionts, may introduce pathogens, pests, and/or competitive plants (including the transplanted species) which may alter natural community structure and function at the introduction site.

Transplantation into currently unoccupied sites known to have supported *P. yadonii* populations historically would constitute *reintroduction*. Important considerations regarding site selection in reintroductions include: 1) suitability of site for population persistence, and 2) potential to alter the evolutionary trajectory for the population. Based on their patchy distribution, *P. yadonii* populations within the Monterey pine forest have been hypothesized to function as a metapopulation, in which individual habitat patches undergo periodic, localized extinctions and are subsequently recolonized by immigration (dispersal) from nearby populations. There is no current evidence for metapopulation dynamics in *P. yadonii*. However, if currently unoccupied patches historically have supported subpopulations within a metapopulation, currently unoccupied sites may be suitable, increasingly the likelihood of success in transplantation and/or enhancement. However, the high dispersability of *P. yadonii* seeds suggests, once again, that if unoccupied sites were indeed suitable, they would have been colonized by natural processes.

There is no current research on the genetic structure of *P. yadonii* populations. The patchiness of remaining *P. yadonii* populations and the habitat heterogeneity between patches creates the potential for evolution of localized co-adapted genetic complexes. Such localized adaptation is important for population persistence within a population; meanwhile, maintenance of this genetic diversity between populations can be key to species persistence and constitutes the basis of continued evolution. Therefore, transplantation of propagules from one patch to another within the Monterey pine forest may be problematical on two fronts. First, it may fail to establish persistent populations if the propagules are not well-adapted to conditions of the receiver site. Second, such movement of genetic material from one site (i.e. MNOUV) could possibly threaten extant *P. yadonii* populations near the receiver sites population by promoting genetic exchange which could possibly dilute the co-adapted gene complexes on which persistence of these intact populations may rely (Falk et al. 1996).

Physical and Biological Considerations

The physical and biological factors relevant to site selection include the environmental characteristics that will influence whether self-sustaining populations of *P. yadonii* can be established in receiver sites. As described in section 2, little is know about the specific physical and/or biological characteristics of habitat required by *P. yadonii.* However, previous research with other species of orchids has established that the biology of orchids is complex, owing to the obligate, symbiotic relationships with fungi, and cryptic, due to the belowground and otherwise less visible biological processes and stages involved in orchid life histories (Section 2). As described in the preliminary results of the site assessment (Section 3, Appendix C) and the proposed study for habitat assessment for *P. yadonii* (Section 4), quantitative analysis of physical and biological characteristics associated with the distribution and abundance of *P. yadonii* can provide an essential method for identifying habitat factors that may influence transplantation success. However, other characteristics of sites that cannot be readily sampled yet may play a crucial role in transplantation success include: the natural enemies (i.e. herbivores, pathogens), mutualists (mycorrhizal fungi, pollinators), and the regeneration niche.

<u>Natural Enemies:</u> Natural enemies are biological agents that negatively impact plants. Broadly referred to as herbivores and pathogens, diverse organisms from viruses to large mammals eat a variety of plant parts including roots, photosynthetic structures (e.g. leaves), and reproductive tissue (incl. seeds). These agents can act independently or interactively (with other organisms or environmental factors) to reduce the demographic performance of individual plants and thus the population performance. Under natural conditions, such enemies keep populations of plants at lower levels than when they are removed, however in rare instances natural enemies (i.e. pathogens) have been shown to completely restrict the distribution of plants.

Research on *P. yadonii* has found that both the pathogenic fungus *Rhizoctonia* and deer reduce plant fecundity (seed production; (Doak and Graf 2001). While research linking reduced individual reproductive output to reduced population performance renders it difficult to assess the impacts of these natural enemies, these agents may reduce population growth and thus influence success of transplantation.

<u>Mutualists:</u> Just as natural enemies of *P. yadonii* can negatively impact transplanted populations, the presence of mutualists in receiver sites may enhance population persistence. Research has shown that pollinators, including a variety of moths and bumble bees, can enhance seed production (Doak and Graf 2001). As a result, the presence of these mutualists within the potential transplantation sites may be key to population persistence. Little is known about the factors that influence the distribution and abundance of these pollinators, making it difficult to assess suitability of receiver sites based on these potentially important mutualists; however, population censuses can be used to determine their presence and abundance during site selection.

Mycorrhizal fungi likely play an essential role in *P. yadonii* seed germination, seedling establishment, growth, and survival (Rasmussen 1995). However, there is presently no information available about the identity, distribution, or habitat requirements of fungal associates of *P. yadonii.* This lack of information represents a substantial gap in knowledge of *P. yadonii* biology in terms of designing a successful transplantation project. The distribution of fungal mutualists may limit the distribution of *P. yadonii*, thus explaining the absence of this rare plant from unoccupied sites deemed suitable based on readily observable physical and biological conditions. While translocation of the fungal mutualists along with soils from the donor sites may facilitate plant success initially, populations may ultimately fail to persist if sites conditions are unsuitable for fungal population persistence.

<u>Regeneration Niche:</u> Efforts to evaluate potential transplantation of *P. yadonii* have thus far focused on the survivorship of *P. yadonii* tubers excavated, transported, and replanted. While sufficiently high survivorship of transplanted propagules is requisite to the success of transplantation projects, it is not sufficient. Individuals transplanted as tubers will eventually die, and in order for a self-sustaining population to result from transplantation, new seedlings from these individuals must be able to establish in the receiver site.

Many plants have complex requirements for seedling establishment which, when considered together, are referred to as their 'regeneration niche'. Unfortunately, there is no empirical information on the requirements for *P. yadonii* seedling establishment. Extensive research for orchid species suggests a variety of physical and biological factors are required for germination and seedling establishment, including various seed germination cues (darkness, temperature), the presence of fungal symbionts, absence of pathogens, among others (Rasmussen 1995). As with many species, characteristics of the regeneration niche for *P. yadonii* may differ from conditions conducive to individual plant persistence. For example, while adults may prefer high canopy conditions to avoid mortality due to desiccation, seedlings may require greater light availability to produce sufficient energy when aboveground structures are produced. Given the high dispersability of *P. yadonii* seeds, the lack of suitable conditions for seedling establishment may explain to the absence of *P. yadonii* in habitat adjacent to current populations which otherwise appears suitable. Understanding the characteristics of the regeneration niche of *P. yadonii* may be essential to ensuring the establishment of self-perpetuating populations in receiver sites and thus transplantation success.

Horticultural Processes

Horticultural processes activities in transplantation include salvage, propagation, planting, and handling and storage. General considerations important for the success of transplanted populations are outlined below; however, there is little specific information available for *P. yadonii.* Elements of a study to determine horticultural requirements are outlined in section 5.

<u>Salvage</u>

Efforts to maximize the size of transplanted populations and therefore the likelihood of population persistence should begin by maximizing the number of propagules (seeds and tubers) that are salvaged from the donor site. Successful salvage requires locating and removing individuals prior to development.

<u>Locating Propagules:</u> Determining where to target removal efforts requires locating individuals prior to development. Because only aboveground individuals can reliably be detected and because many individuals do not produce aboveground structures in any given year, intensive surveys should be conducted a minimum of two seasons prior to development. Depending on the

method(s) of excavation (below), though, systematic surveys should be used to mark plants and/or circumscribe patch perimeters.

<u>Removing Propagules:</u> Not mutually exclusive, three approaches to removing plant material from the site of development include seed harvesting, individual removal, and soil excavation. Each involves several important logistical considerations.

Seed harvesting: Collecting the *P. yadonii* seed within the area to be developed may enhance transplantation population persistence, by adding a source of seedling establishment in the receiver site before transplanted tubers are established and reproduce, thus diversifying the age structure of the transplanted population. Unfortunately, little is known about seed survivorship or germination requirements, necessitating study prior to salvage.

Individual removal: Individuals can be located and excavated individually (Doak and Graf 2001). This requires detection of aboveground biomass and could only be used to obtain vegetative and reproductive individuals (i.e. not seedlings). Tubers should be excavated when they are least susceptible to damage, which is when they are dormant between August and October (Allen 1998), though this has not been empirically tested.

Soil excavation: Volumes of soil within the perimeter of *P. yadonii* colonies could be excavated intact. Depth of individuals within the soil profile should be examined, and excavation occurs to a conservative depth in order to maximize salvage. Previous trials have excavated 5-6" of soil (Zander 2003). As with individual removal, the precise method of removal and need to be examined to determine effects on transplant. Sieving has been used to remove individual tubers from the soil; however, study methods rendered it difficult to evaluate success (Zander 2003).

<u>Propagation</u>

Prior to planting, all or some fraction of the salvaged plant material (tubers and/or seeds) might be used to propagate more individuals in order to augment the size of subsequent transplantation populations. The constraints, costs, and benefits of using propagation as an intermediate step should be carefully weighed.

<u>Constraints:</u> While there is no known information about propagating *P. yadonii* or other members of *Piperia,* research has examined *in vitro* propagation of a closely related species of *Platanthera* (Stoutamire 1996). Propagation of terrestrial orchids in general is known to be extremely difficult; however, extensive research and efforts have generated successful techniques with other species that could be tested for *P. yadonii.* Research prior to salvage would be needed to determine basic elements of successful propagation techniques.

<u>Benefits:</u> Propagating additional individuals of *P. yadonii* as part of a transplantation process could increase the number of individuals available. This would be a necessary step if mitigation requirements specify that transplantation must achieve "no net loss" (given that some plants will inevitably die).

<u>Costs</u>: Knowledge of propagation methods will need to be developed, likely requiring fairly elaborate facilities and extensive effort. If excavation must occur during the course of a single

year, at least some competency in propagation would need to be developed prior to excavation to avoid loss of all transplantation material due to poor handling.

Planting

Designing steps for planting salvaged or propagated material, whether via seed, tubers, or entire soils, involves several important horticultural considerations in addition to the critical attention to characteristics of receiver sites described above. These include seasonality of planting, site treatments

Seasonality: The season during which propagules are transplanted to the receiver site can determine their success. Though the precise impacts are unknown, the seasonality of transplantation should consider natural ecosystem processes and the phenology of the plant to maximize success. Seed sowing should mimic the natural dispersal season (i.e., mid to late summer). Given that growth is initiated from dormant tubers following the first significant rains in fall, tubers should be transplanted prior to that time.

Treatments A variety of treatments implemented to enhance plant performance at the various life stages (e.g. tuber survivorship, plant growth, seedling germination and establishment, etc.) may be essential to the success of transplanted populations. Unfortunately, little is known about the requirements of *P. yadonii,* necessitating a targeted effort to learn about horticultural treatment as well as the habitat requirements (section 5). Types of treatments that may be necessary can be categorized as handling treatments and site treatments.

Handling Treatments: Treatments may be necessary to alleviate the negative impacts of handling and planting on propagules, and thus enhance survivorship following planting. Types of treatments that should be investigated include watering, fertilization, and/or the application of plant hormones, among others.

Site Treatments: Several treatments might be used to enhance site suitability by addressing potential constraints to *P. yadonii* population establishment outlined in the discussion of receiver site location. Examples of potential site treatments include: soil inoculation to ensure mutualists are present, removal of competitors (e.g. weeds), and reducing litter thickness to enhance seedlings establishment. Such treatments should be informed by knowledge gained through the habitat suitability study and examined through experimental research to examine the habitat requirements for this plant (section 5).

Handling and Storage

As an intermediate step in the horticultural process of transplantation, handling and storage might greatly impact success of transplanted propagules and thus the growth and persistence of transplanted populations. Handling includes aspects of the physical treatment that propagules receive, both from human hands and the variety of equipment and conveyances in which they come in contact. Propagule storage, which may be necessitated by the sheer magnitude of this salvage project (i.e., over 10,000 aboveground individuals), can also greatly influence transplantation success. Given that orchid propagules are living organisms, storage conditions must be suitable for plant survival. Utmost care during excavation and intensive site preparation and maintenance can be foiled by improper storage between these steps. Section 5 outlines the steps that must be taken to determine proper storage and handling conditions.

Enhancement

Piperia yadonii Enhancement Project Overview

As part of the larger mitigation package, enhancement is proposed to mitigate the indirect effects of development on *P. yadonii* individuals that remain within the development site at MNOUV. Current estimates suggest that 13 acres of habitat containing approximately 3,000 *P. yadonii* individuals will be indirectly, negatively impacted by the development (Jones and Stokes 2003).

Definitions

The general goal of enhancement in mitigation is to compensate for the loss of habitat, population(s), or individuals of an endangered species by enhancing the same in another area. There is no single definition for enhancement, likely because the specific threats to species vary. In the case of *P. yadonii,* the primary threat to this species is loss of habitat (USFWS 2002). Therefore, enhancement in this project should increase the availability of suitable habitat and thus expand the distribution of the rare plant.

Increasing population density within currently occupied habitat by reducing or eliminating known or hypothesized impairments to population density is one possible definition of enhancement. In the case of *P. yadonii*, such efforts may do less to truly mitigate for the proposed loss of habitat. This is because the threat of environmental stochasticity on species persistence exacerbated by habitat loss cannot be alleviated by increasing population density within remaining habitat (Falk et al. 1996). However, removing habitat impairments in sparse populations may enhance population density and therefore probability of persistence, which should be considered enhancement.

Potential Enhancement Strategies

Efforts to devise effective enhancement projects confront similar issues faced in attempts to design the transplantation project: there is minimal information available on the biology of *P. yadonii*, specifically with regards to aspects of the plant's ecology, to inform enhancement treatments. Woody exotic plants including French broom (*Genista monspessulana)* and *Acacia* spp. are hypothesized to restrict the distribution of *P. yadonii* from otherwise suitable patches of Monterey pine forest habitat (USFWS 2002). Their removal may enhance the rare plant's distribution as well as overall abundance. Deer browsing of *P. yadonii* inflorescences reduces reproductive output (Doak and Graf 2001), suggesting that efforts to reduce herbivore pressure (i.e. erecting fences) may increase population density within occupied patches. However, such measures are less likely to increase the distribution of this rare plant as propagule availability does not likely limit the distribution of this wind dispersed species, at least not at the periphery of its distribution.

Lacking knowledge of factors that influence the distribution and abundance of the rare plant, it is difficult to devise treatments at this time that would expand the distribution and/or increase abundance. Instead, a plan is proposed for filling the important gaps in knowledge of the species ecology. Given the similar barriers to their thoughtful design, transplantation and enhancement projects can be informed through by the same studies, as described in section 5.

SECTION 5. TRANSPLANTATION AND ENHANCEMENT PROGRAMS: DEVELOPING INFORMED MITIGATION

Purpose

This section outlines the components of an informed enhancement and transplantation program for *Piperia yadonii*, describes how the components can be integrated within a framework of an informed mitigation, and discusses potential timelines for a program.

Introduction

Previous sections of this plan have identified the essential components of enhancement and transplantation project and highlighted the crucial gaps in knowledge that must be filled to facilitate project design, implementation, and success. Here we outline components of informed transplantation and enhancement programs that, owing to their scientific approaches, will have increased likelihood of success and contribute greatly to knowledge of *P. yadonii* biology.

The following section provides a detailed protocol for a crucial component in the development of both projects within the overall program[1]: a habitat specificity study to examine the habitat specificity of *P. yadonii*. Detailed protocols for the other steps outlined should be developed concurrently with the next phase of the project (i.e. the habitat specificity study) as identified in the timeline for the overall program.

General Approaches in an Informed Program

Given the lack of knowledge in crucial components of this enhancement and transplantation project, a scientific approach in which a series of methods will be used for building knowledge throughout project implementation to inform mitigation. Knowledge of *P. yadonii* biology and ecology crucial for project success should be developed prior to and during project implementation via an array of scientific studies including observational studies, experimental manipulations, and adaptive management. While all three approaches are based in the scientific method and incorporate quantitative, statistically analyzable assessments of patterns and/or processes that allow inferential knowledge to be gained, they differ in important ways that will determine their ultimate utility in these projects.

Observational Studies

In observational studies, aspects of a system are measured through carefully designed sampling protocols to gain knowledge of the system. They form the backbone of research in ecosystem and landscape ecology as well as many aspects of plant ecology where observing patterns can be crucial to understanding processes. Given the dearth of biological knowledge in advance of these projects, observational studies can be used to take "snap shots" of the system from which hypotheses can be generated. Often referred to as correlational studies, many observational

[1] The term "project" is used to refer to the specific mitigation projects (e.g. transplantation "project") The word "program" is used to refer to the proposed program that integrates both projects.

studies are designed to determine whether a given variable (e.g. *Piperia* abundance) is correlated with one or more factors (e.g. litter depth). Because correlation cannot be used to infer causation (e.g. litter cannot be said to *cause* the pattern of *Piperia* abundance), observational studies cannot be solely relied upon to determine the transplantation and enhancement treatments; thus the additional need for manipulative experiments.

Experiments

Manipulative experiments, or studies in which the factor thought to cause an effect in the system is actively changed through a carefully designed treatment which allows causation to be determined and thus hypotheses about transplantation and enhancement treatments to be tested. Below, an observational study is proposed to generate hypotheses for what constitutes suitable habitat for *P. yadonii*, and experiment tests used to determine whether the conditions identified as conducive to transplantation and enhancement actually facilitate the establishment, growth and increase of *P. yadonii* individuals and populations. Though immensely powerful means of building knowledge, experiments may require monitoring for long time periods, especially for *P. yadonii*; thus requiring the application of adaptive management

Adaptive Management

Adaptive management allows learning through doing the management (Lee 1999, Elzinga et al. 2001). By incorporating the basic components of scientific experiments in the implementation of management, adaptive management can be used to test hypotheses about treatments and gain information. Because management often proceeds in the absence of knowledge of treatment impacts, adaptive management employs quantitative monitoring of treatments applied at small spatial scales to avoid significant impacts of implementing inappropriate large-scale management treatments before we know their suitability or effectiveness.

Components of a Transplantation and Enhancement Program

These tools can be integrated into a comprehensive transplantation and enhancement program for *P. yadonii* based on the following approach: knowledge gaps are filled through studies conducted prior to project design and through project implementation using adaptive management which provides a mechanisms for attaining project success as defined by rigorous success criteria. Using this approach, this plan proposes that components varying in duration be used to inform ongoing and future studies as part of the cohesive program. As a result, discrete, incremental "steps" are not readily identified. Instead, a schematic is provided to illustrate how knowledge from the various components will be integrated within the framework (Figure 1).

The proposed program is based on current knowledge. A functional program of this length must itself be adaptive and incorporate new information, as it is developed, to reevaluate goals and objectives. The components of the transplantation and enhancement program are organized according to the gaps in knowledge or impediments to project design and success that they will address.

General Habitat Requirements

One of the major data gaps for implementing transplantation and enhancement projects is the lack of knowledge of habitat requirements for *P. yadonii*. The following components will address this issue prior to and through implementation.

1A: Habitat Sampling Study: An observational study can be used to examine *P. yadonii* distribution and abundance patterns in potential enhancement and transplantation areas, thereby generating hypotheses about environmental factors that may influence suitability of *P. yadonii* habitat (Section 6). These hypotheses would form the basis for determining potential sites and designing treatments that will be examined through adaptive management in transplantation and enhancement projects.

1B: Adaptive management in transplantation project: Using adaptive management, transplantation treatments can test factors influencing individual and population performance that are hypothesized to be important as determined through the habitat suitability study, the mycorrhizae study, and the horticultural experiments.

1C: Adaptive management in enhancement project: Initiated following preliminary results from the transplantation study, the enhancement project can be informed both by the initial studies as well as the transplantation study. Using adaptive management, effects of enhancement treatments not examined in transplantation projects (e.g. reducing deer herbivory, removing exotic plants) can be examined to determine whether they increase population abundance and distribution.

Regeneration Niche

Information on the regeneration niche of *P. yadonii,* crucial to success of both transplantation and enhancement projects, concerns the role of mycorrhizae in germination and seedling establishment.

2A: Laboratory mycorrhizae research: The role of mycorrhizae in seed germination and seedling establishment will be examined *in vitro*. If mycorrhizal specificity is thought to limit plant establishment within the Del Monte Forest and therefore suitability of receiver sites, mycorrhizae host specificity studies will isolate the fungal species from protocorms and tubers.

2B: Adaptive management within transplantation project: As described in 1B (above), transplantation treatments manipulating factors thought to influence individual and population performance based on mycorrhizae research can be used to tested through adaptive management in the transplantation project.

Horticultural Requirements

Horticultural requirements for *P. yadonii* must also be determined to design aspects of the transplantation project.

3A: Laboratory and/or field tests of horticultural techniques: The relative effectiveness of horticultural processes for *P. yadonii* should be examined using experimental trials that test available techniques for salvage, propagation, storage, handling, and planting of seeds and tubers. The details of such an experiment will need to be developed as part of a study plan for the transplantation program.

3B: Adaptive management in transplantation: Treatments to test techniques for salvage, propagation, storage, handling, and planting (as in 1B and 2B above).

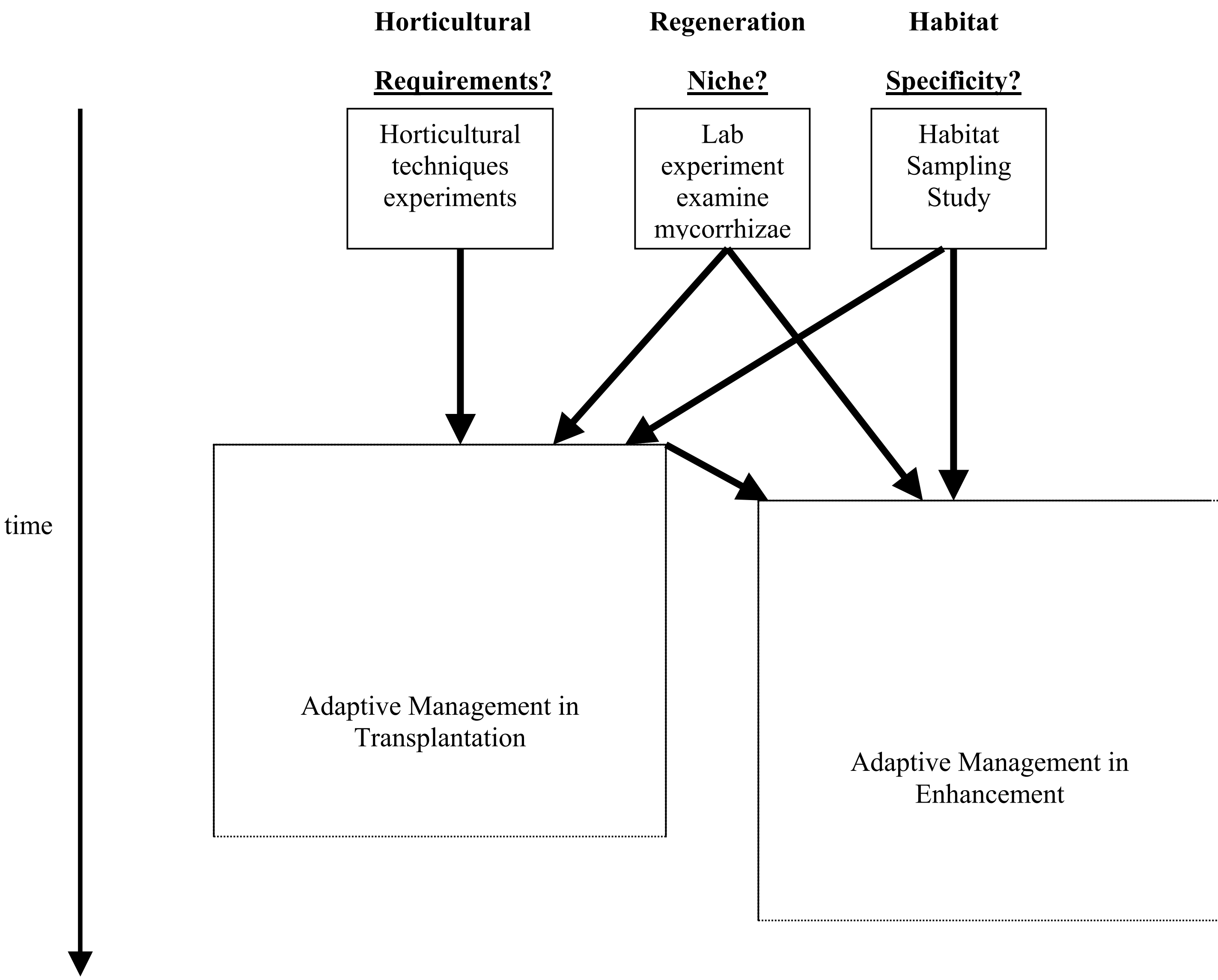


Figure 1: **Schematic showing a coordinated approach to obtaining biological information about *P. yadonii* prior to and through mitigation projects. Boxes represent actions (studies, management) to address the current gaps in knowledge, with box length proportional to time and projects ending when success criteria are reached. Arrows represent transmission of knowledge among actions. Specifics provided in text.**

Timelines

Components of transplantation and enhancement projects (section 4) combine with characteristics of the biology of *P. yadonii* biology (Section 2) to suggest that a substantial time commitment, both in terms of years and effort, will be required to complete a successful transplantation and enhancement program. The longevity, cryptic life stages, and complex ecology of the life history of *P. yadonii* will necessitate intensive monitoring efforts (e.g. demographic models) as well as extended monitoring periods to determine treatment impacts and evaluate project success. Depending on specific success criteria developed as part of the steps in the program, and the effectiveness of the treatments implemented, the number of years required to attain success in these projects may also be extensive.

Specific timelines for the components of this program should be developed as part of the study plan for the project. The study plan should balance the need to obtain as much information as possible from prior studies with desires to expedite projects and allow development to begin. The following is a working timeline for what has been proposed in this plan.

January-February 2004: Develop study plan for program that provides individual proposals for the studies that outline their goals, methods, personnel, costs, and timelines.

March-August 2004: Begin studies, including habitat requirements study (section 6), horticultural experiments, and regeneration niche study.

September-December 2004: Draft detailed protocol for transplantation that integrate findings to date from studies (habitat requirements study will be complete, others ongoing). Draft enhancement plan.

NOTE: Enhancement treatments can begin upon completion of plan; timing will depend on treatments

March 2005: Locate material for salvage (i.e. mark individuals or circumscribe colony perimeters).

September-October 2005: Salvage and plant and/or begin propagation of tubers.

(Site available for development)

SECTION 6. PROTOCOL FOR THE HABITAT SPECIFICITY STUDY

Purpose

This section provides a detailed protocol for a study to determine habitat characteristics associated with the distribution and abundance of *Piperia yadonii*.

Background

The initial site assessment indicated that more than 293 acres of Monterey Pine Forest may provide suitable habitat for population establishment and persistence following transplantation (Table 1). Using the current definition of what constitutes habitat enhancement, 113 acres were determined to have barriers to *P. yadonii* that could likely be enhanced (Table 2). In the absence of previous research or sufficient data to identify the factors that influence *P. yadonii* distribution and abundance, these site assessments used initial gross screens to determine potential areas for transplantation and enhancement, based in part on observations of preferred habitat that have been reported in previous studies of the plant (Allen 1996, Doak and Graf 2001). In order to determine actual locations for transplantation and enhancement and facilitate project success, greater knowledge of the specific habitat requirements of *P. yadonii* is needed.

Introduction

Many factors can influence where plants occur, including land use history, disturbance history, and dispersal. However, the ultimate determinant of where a plant *can* occur is its inherited tolerance to environmental factors. Both physical conditions of the site (e.g. climate, geology, soils, etc.) and biological characteristics (competitors, herbivores, pathogens, pollinators, etc.) can directly and indirectly influence habitat suitability. As a result, current plant distribution and abundance patterns can provide important insight into the biology of the species including potential habitat characteristics required for population persistence and thus the success of transplantation and enhancement efforts.

The patchy distribution of *P. yadonii* in remaining intact habitat within the Del Monte Forests indicates that the species exhibits some degree of habitat specificity while the highly variable density in areas where the plant occurs suggests certain conditions may be more favorable than others (Allen 1996). Variability in plant community structure in remaining patches of Monterey Pine forest was documented in a recent characterization of the remaining forests patches (Zander 2002). However, prior to the inception of the TEAM Plan, there were no known attempts to correlate the distribution and abundance of *P. yadonii* with the existing habitat heterogeneity within the Monterey Pine forests.

Realizing the potential for such analyses to identify suitability of sites for transplantation and enhancement, a study was conducted to correlate habitat factors in the Monterey Pine forest with *P. yadonii* during the course of the TEAM project. As described in section 3, analyses revealed significant gradients in plant assemblage composition; however, lack of reliable data on *P. yadonii* distribution and abundance due to the seasonality of data collection (during the plants belowground phase) thwarted attempts to correlate this variability with *P. yadonii* and thus infer potential characteristics of suitable habitat.

Despite the limitations of the data set, preliminary analyses suggested that the potential for a comprehensive study conducted during the growing season of *P. yadonii* to suggest potential indicators of habitat suitability was deemed high. As a result, the County of Monterey requested the preparation of a detailed protocol for additional study to determine more precisely the habitat characteristics associated with the distribution and abundance of *Piperia yadonii* to facilitate identification of suitable habitat for *P. yadonii* transplantation and enhancement.

Methods

This study proposes that habitat sampling of potential sites for transplantation and enhancement be used to examine the following questions:

1. What factors are correlated with the distribution and abundance of *P. yadonii*?

2. Do sites with populations of *P. yadonii* significantly differ in habitat characteristics from those without *P. yadonii* (i.e. potential transplantation and enhancement sites) and, if so, in what characteristics distinguish sites?

Sampling Design

The design of the sampling regime, which specifies how data are collected, greatly influences the validity and reliability of the study as well as fundamental aspects of how results can be interpreted and used to guide transplantation and enhancement project design. Five crucial elements of the sampling design are stratification, randomization, replication, sample area, and variables measured.

Stratification

In order to compare proposed sites as well as habitat with and without *P. yadonii*, a stratified random sampling design should be used in which sampling is stratified by site and by the distribution of *P. yadonii*. That is, replicate samples should be randomly located within areas with and without *P. yadonii* at each of the proposed sites for transplantation and/or enhancement. These strata have been delimited as part of the site assessment in this project (SECTION 3), which identified 10 sites, each of which has a region with *P. yadonii* present, and a region without *P. yadonii* but where transplantation or enhancement appears feasible (where "region" refers to the sum total of all patches meeting that criteria).

Randomization

The strata are chosen deliberately by the investigator who defines the universe of interest of the study. These include the 10 sites selected and the areas with and without *P. yadonii* present. However, the individual replicate samples must be randomly located in order to meet the assumptions of inferential statistical analyses and avoid investigator bias. A variety of methods can be used to insure that any area within each stratum has an equal probability of being sampled.

Replication

Allocation: Because the strata vary greatly in their overall acreage, the allocation of replicate samples in each stratum should be roughly proportional to acreage it comprises (relative to the total study area). For example, if a stratum comprising 50 acres is to be sampled in the overall 1000 acres universe of interest (i.e. study area), then about 5 percent of the replicates should be located in the area. However, to insure replication within each stratum is adequate for assessment, a minimum number of replicate samples should be located in each. This is especially important for adequate sampling of the areas with *P. yadonii* as these represent a small proportion of the overall study area at several sites.

Quantity: It is difficult to determine in advance how many replicate samples will be needed to address the questions; however, preliminary analyses of the fall 2003 habitat assessment study (Section 3) suggest that a minimum of 100 replicates could capture the variability in habitat within the areas identified as potentially suitable for transplantation. Within each stratum (e.g. area with *P. yadonii* present at PQR), a minimum of 5 replicate samples should be allocated.

Sample Area

The size of the area sampled can play an important role in determining results. Sample plots or "quadrats" should be large enough to incorporate variability in habitat characteristics that may vary at larger spatial scales, yet small enough to capture microsite variability. This balance can be hard to achieve. Nested sampling, whereby features varying at larger scales (e.g. trees) are measured in larger quadrats within which are nested small quadrats used to sample smaller features (e.g. herbs), can help capture variability in these different levels; however, relativization of the data is required *prior* to analyses and interpretation of results requires careful consideration of the different spatial scales at which the different variables were sampled.

Results from the preliminary study (Section 3) suggest that the 10 m x 10m quadrats allowed identification of gradients in species composition that exist in the Monterey Pine forest and captured variability in the abundance (cover) of shrubs, trees, and many perennial herbs. Several small-statured and/or uncommon perennials attained only low cover in these plots, indicating that the quadrats were too large to sample the variability in their abundance and perhaps similarly, that of small and/or uncommon annual herbs. Resulting analyses of such species proceeds based on their presence/absence, which may still provide valuable information about habitat suitability. Sampling in smaller quadrats (e.g. 5m x 5m) may make it difficult to capture meaningful variation in the abundance of trees, shrubs, and more abundant herbs (e.g. grasses etc.) and is not recommended.

Variables Measured

The data collected at each sample should include measures of *P. yadonii* performance and the hypothesized (or potential) habitat characteristics that either influence or indicate *P. yadonii* performance. The following lists variables currently known to be important.

Piperia yadonii *data*

1. Density: number of individuals within the sample broken down into the following categories:
   - **a.** Small vegetative: non-reproductive individuals with only one leaf
   - **b.** Larger vegetative: non-reproductive individuals with more than one leaf
   - **c.** Reproductive: individuals that produce reproductive structures that year
2. Reproduction: the length of each inflorescence on reproductive individuals
3. Distance to nearest *P. yadonii*: linear distance to the nearest individual if one is not in the plot (for replicates located in non-piperia habitat only)

Habitat Characteristics

Plant Community Composition

1. Plant cover (absolute) by species estimated occularly, using cover classes[2], [3]
2. Tree canopy cover (via spherical densiometer)
3. Litter cover (% cover using cover classes)
4. Litter depth (in cm based on 5 randomly located point measurements)

Soil Characteristics

1. Nutrient availability (NO_3^-, NH_4^+ P, K, Ca, Mg)
2. pH
3. Texture (by field method or particle distribution)
4. Soil saturation in winter

Other

1. Slope (%)-measured with a clinometer
2. Aspect
3. Location- UTMs can aid examination of spatial patterns

Data Analysis

A variety of univariate and multivariate statistical analyses can be used to explore and analyze the data.

Univariate Analyses

Univariate analyses, which examine the effects of one or more independent variables on a single dependent variable, that would be appropriate for the data include: analysis of variance (ANOVA), linear regression, and logistic regression. Factors hypothesized to explain the

[2] 1= <1%; 2= 1-5%, 3= 6-10%, 4= 11-25%, 5= 26-50%, 6= 51-75%, 7= 76-90%, 8= 91-95%, 9=96-99%, 10= >99%

[3] for trees and shrubs, separate basal area and canopy cover estimates would be useful

presence and density of *P. yadonii* can be explored through logistic or linear regression, respectively, while differences in specific habitat characteristics amongst the strata (i.e. locations with and without *P. yadonii* at each site) hypothesized to influence suitability of the sites can be examined using ANOVA.

Multivariate Analyses

Many of the factors hypothesized to independently or interactively influence or be predictive of *P. yadonii* distribution and abundance patterns can covary spatially, rendering univariate analyses to correlate independent factors with *P. yadonii* abundance not only time consuming, but potentially misleading. Fortunately, multivariate analyses, which examine the influence of multiple independent factors on multiple dependent variables simultaneously, provide a tool by which several factors hypothesized to influence plant distribution can be analyzed, allowing a more comprehensive assessment of habitat specificity that can be used to generate hypotheses for habitat requirements in transplantation and design enhancement strategies.

Several multivariate statistical tools can be used to examine habitat characteristics that may influence suitability for *P. yadonii*. These are outlined and described below. Several were used in the preliminary habitat analysis study (Section 3 and Appendix C).

Ordination

Preliminary analyses during the habitat assessment completed Fall 2003 indicated that plant species distributions within the Monterey Pine forest are not homogenous; rather, that plant species' abundance and distribution spatially covary along predictable gradients (Section 3). However, at present, we do not know how the distribution and abundance of *P. yadonii* fits into these associations. Ordination of the data derived from a spring study including *P. yadonii* abundance information can be used to extract the gradients in plant community composition that exist in the Monterey Pine forest and evaluate *P. yadonii* distribution with respect to these gradients. Ordination can also allow the relative role of environmental characteristics in influencing the gradients to be examined. Finally, analyses can be used to determine whether the gradients reflecting variability in species composition are associated with *P. yadonii* performance (density, reproduction, etc.).

Multi-response Permutation Procedure and Discriminant Analysis

Multi-response permutation procedures (MRPP) can be used to address the question: Do plots in the different strata (sites, presence/absence of piperia) differ consistently in habitat characteristics (species composition, soils, etc.)? If significant differences are observed via MRPP, Discriminant Analysis (DA) can be used to determine the factors that are most indicative of the different groups. This analysis can be used to understand potential limitations for sites without *P. yadonii* and thus design transplantation and enhancement treatments and choose the most appropriate sites.

Application of Results

Results of the separate analyses should be summarized in the context of the original goals of the study: to determine whether significant differences exist between sites with and without current populations of *P. yadonii*, and if so, to identify the habitat characteristics that differ. The precise methods of applying the results of this study to design and implement transplantation and enhancement projects will depend, to a certain extent, on the results themselves (i.e. whether there are indeed differences between piperia and non-piperia habitat, and what those differences might be). If habitat differences are identified and quantified, the logical next step for transplantation would be to use field reconnaissance to identify the location of suitable receiver sites for transplantation within the sites identified in the initial site assessment (Section 3). For enhancement, habitat characteristics for *P. yadonii* would be used to identify areas that can be enhanced via management to facilitate increases in population distribution and abundance.

Study Limitations

As with any associational research, correlates with *P. yadonii* distribution and abundance should not be inferred as having causative effects on *P. yadonii* performance. For example, if *P. yadonii* abundance is positively associated with high cover of *Briza maxima*, one might hypothesize that sites with *Briza maxima* represent good habitat for *P. yadonii* or even that *B. maxima* facilitates *P. yadonii.* However, in the absence of experimental evidence to confirm these hypotheses, one must also consider the following alternative explanations for the positive association between the species. First, it could be that *P. yadonii* is not at all affected by *B. maxima*, but that both species are tracking a third component of the habitat where they co-occur. For example, *B. maxima* and *P. yadonii* could be positively associated because both prefer light gaps within the canopy. While knowledge of their positive correlation could be cautiously used to identify potential sites for transplantation, one would certainly not want to infer anything about the effects of *B. maxima* on *P. yadonii* performance for use in enhancement. *B. maxima* and *P. yadonii* could be competitors within these light gaps, such that sites with *B. maxima* might indicate suitable habitat, however the abundance of the exotic *B. maxima* should be removed, not increased.

It is also important to note that results of the analyses from a habitat study are limited to the data included. This may seem obvious, but the point merits important consideration during interpretation of research results. For example, while *P. yadonii* may be positively correlated with soils higher in clay content, clay alone may not influence transplantation success. Instead, there may be a third (or fourth or fifth) factor associated with clay soil that was not measured in the habitat study that influences *P. yadonii* performance, such as the presence of important mutualistic fungi that are preferentially found in clay soils where soil moisture availability is higher.

Thorough, quantitative sampling studies are an important first step in the effort to sort through the complexity of habitat variability and to generate hypotheses about factors that may influence the distribution and abundance *P. yadonii.* Carefully designed scientific experiments to test the hypotheses generated from quantitative sampling are a logical and often times crucial next step. Study sites identified as having habitat characteristics hypothesized to represent favorable conditions for *P. yadonii* may indeed ultimately support transplanted populations of Yadon's piperia. In addition, enhancement treatments designed to create these conditions may indeed promote the growth and expansion of naturally occurring populations.

LITERATURE CITED

Ackerman, J. D. 1977. Biosystematics of the genus *Piperia* Rydb. (Orchidaceae). Botanical Journal of the Linnean Society 75: 245-270.

Allen, D. W. 1996. Results of two consecutive years of surveys for Yadon's Piperia (*Piperia yadonii*): 1995 and 1996. *in* David W. Allen Consulting, Port Angeles, WA.

Allen, D. W. 1998. Second year evaluation of Yadon's rein orchid transplanting trials. *in* E. Y. Brown, editor., Port Angeles, WA.

Bateman, R. M., P. M. Hollingsworth, J. Preston, L. Yi-Bo, A. M. Pridgeon, and M. W. Chase. 2003. Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). Botanical Journal of the Linnean Society 142(1): 1-40.

Berg, K. S. 1996. Rare plant mitigation: A policy perspective. Pages 279-292 *in* D. A. Falk, Millar, C.I. and M. Olwell, editor. Restoring Diversity: Strategies for reintroduction of endangered plants. Island Press, Washington, D. C>.

Doak, D. and A. Graf. 2001. Reproductive Biology and Pollination Ecology of the Federally Endangered Yadon's Piperia (*Piperia yadonii,* Orchidaceae) in Monterey County, California. Pages 45 pages *in*, Biology Board, University of California, Santa Cruz, CA.

Elzinga, C. L., D. W. Salzer, J. W. Willoughby, and J. P. Gibbs. 2001. Monitoring plant and animal populations. Blackwell Science, Malden, MA.

Falk, D. A., C. I. Millar, and M. Olwell. 1996. Restoring Diversity: Strategies for reintroduction of endangered plants. Island Press, Washington, D.C.

Fiedler, P. L. 1991. Mitigation-related transplantation, relocation, and reintroduction projects involving endangered and threatened, and rare plant species in California. Pages 82 *in*, Sacramento, CA.

Fiedler, P. L., and R. D. Laven. 1996. Selecting Reintroduction Sites. Pages 157-169 *in* Restoring Diversity: Strategies for reintroduction of endangered species. Island Press, Washington, D.C.

Hall, L. 1987. Transplantation of sensitive plants as mitigation for environmental impacts. Pages 413-420 *in* T. S. Elias, editor. Conservation and management of rare and endangered plants. California Native Plant Society, Sacramento, CA.

Hickson, D. 2003. Translocation data base. *in*. California Department of Fish and Game, Natural Heritage Program.

Howald, A. 1996. Translocation as a mitigation strategy: lessons from California. Pages 296-329 *in* D. A. Falk, Millar, C.I. and M. Olwell, editor. Restoring Diversity: Strategies for reintroduction of endangered plants, Washington, D.C.

JSA. 2003. Pebble Beach Company's Del Monte Forest Preservation and Development Plan. Jones and Stokes Associates Administrative Draft Environmental Impact Report.

Lee, K. N. 1999. Appraising adaptive management. Conservation Ecology **3**.

Rasmussen, H. N. 1995. Terrestrial orchids: from seed to mycotrophic plant. Cambridge University Press, Cambridge.

Rydberg, P. A. 1901. Studies on the Rocky Mountain flora. Part 5. Bulletin of the Torrey Botanical Club 28: 266–284.

Shan, X. C., E. C. Y Liew, M. A. Weatherhead, and I. J. Hodgkiss. 2002. Characterization and taxonomic placement of Rhizoctonia-like endophytes from orchid roots. Mycologia. 94(2): 230-239.

Stoutamire, W. 1996. Seed and Seedlings of Patanthera leucopheae (Orchidaceae). Pages 53-61 *in* North American Native Terrestrial Orchids-Propagation and Production, Washington, DC.

Taylor, L., and T. D. Bruns. 1994. A view of specificity in orchid mycorrhizae using molecular symbiont identification. Poster presented at The Fifth International Mycological Congress, Vancouver, British Columbia, Canada. http://plantbio.berkeley.edu/~bruns/text/poster.html.

Tibor, D. P. (ed.). 2001. Inventory of rare and endangered vascular plants of California. California Native Plant Society Special Publication No. 1 [6th edition]. California Native Plant Society, Sacramento, CA.

U.S. Fish and Wildlife Service. 1998. Endangered and Threatened wildlife and plants; final rule listing five plants from Monterey County, CA, as Endangered or Threatened. Federal Register 63(155): 43100-43116.

____________. 2002. Draft recovery plan for five plants from Monterey County, California. U.S. Fish and Wildlife Service, Portland, Oregon. x + 108 pp.

Wells, T. C. 1981. Population ecology of terrestrial orchids. Pp. 281-295 in: Synge, H. (ed.), The biological aspects of rare plant conservation. John Wiley & Sons, Ltd. 558 pp.

Wilken, D. H., and W. F. Jennings. 1993. Orchidaceae. Pp. 1211-1218 in: J .C. Hickman (ed.), The Jepson manual: higher plants of California. University of California Press, Berkeley, CA. 1,400 pp.

Zander Associates. 2002. Biological resources of the Del Monte Forest. Monterey pine and Monterey pine forest habitat. Unpublished report prepared for the Pebble Beach Company. 95 pp. + appendices.

Zander, M. 2003. Summary of Plant Counts in subplots where tubers excavated and replanted in 1999. *in* E. West, editor., Novato, CA.

APPENDIX A SUGGESTED OUTLINE OF TEAM PLAN

DRAFT

Pebble Beach Company's Del Monte Forest Preservation and Development Plan

Suggested Outline/Contents of Transplantation Design, Enhancement, and Adaptive Management Plan (TEAM Plan) Outline for Yadon's Piperia (*Piperia yadonii)*

Monterey County P&BI

October 28, 2003

If transplantation and/or resource enhancement are adopted by Monterey County as some of the means of mitigating adverse effects of the development associated with the DMF/PDP on Yadon's piperia, the following outline presents suggestions for a four-phased process to assess, design, and implement transplantation, enhancement, and adaptive management. "Transplantation" means the relocation of plants from development sites to new locations. "Enhancement" refers to identification and implementation of measures to address health and productivity impairments (such as excessive deer herbivory or competition from non-native vegetation) to allow for the expansion of existing piperia population boundaries in preservation areas.

This is a draft document that is advisory in nature and does not represent evaluation criteria to determine the adequacy of transplantation or enhancement as mitigation.

The four suggested phases include:

- Phase 1 (Prior to release of DEIR) – assessment of potential transplantation receiver sites and enhancement sites and development of a draft transplantation design, enhancement, and adaptive management plan (TEAM PLAN);
- Phase 2 (Prior to project implementation) - receiver site/enhancement site selection, transplantation and enhancement program specific design, and final TEAM PLAN;
- Phase 3 (Per TEAM PLAN schedule) - implementation of transplantation, enhancement, monitoring, and adaptive management; and
- Phase 4 (After success criteria met, likely minimum of 10-15 years) – development and implementation of long-term management plan.

Phase 1 - Site Assessment and Draft TEAM PLAN

<u>Task 1.1 Site Assessment</u>

Pebble Beach Company (PBC) shall retain a qualified team of resource specialists (botanist, soils scientist, horticulturalist) to determine if there are adequate sites available to receive Yadon's piperia transplants as well as appropriate sites for resource enhancement. As part of this task, The County's consultant and PBC's consultants shall develop a detailed protocol for determining appropriate sites for transplantation and enhancement. The protocol shall be based on edaphic and biotic characteristics of occupied habitat supporting healthy populations of Yadon's piperia. The minimum size of the receiver sites identified for transplantation must be greater than the number of plants and occupied habitat that would be directly affected by the proposed project. The minimum size of the enhancement sites must be greater than the number of plants and occupied habitat that would be indirectly affected by the proposed project, which is defined as 13 acres (approx. 2,900 plants) in the analysis prepared by Jones & Stokes or as determined by post-construction monitoring of the indirect effect areas. After the required size of suitable habitat and enhancement areas is determined based on this impact calculation, PBC's consultants shall conduct surveys to identify, characterize, and quantify potential transplantation receiver sites and enhancement sites. Modification of habitat to accommodate piperia should not be at the expense of other native species. Receiver sites shall be limited to the Monterey peninsula, while enhancement sites can be selected throughout the entire range of the species. Transplantation and receiver sites must be located in areas where long term protection can be assured through deed restrictions, public ownership, or other mechanisms.

The following data shall be gathered on each potential transplantation receiver site and each enhancement site and evaluated as part of this task:

- edaphic and biotic conditions;
- size of the receiver/enhancement site;
- land ownership;
- surrounding land uses;
- potential threats (human and natural) to the long-viability of the species at this location; and
- the size of buffers that could be maintained between outplantings and nearby existing structures, utilities, roads, and pathways or proposed construction.

The locations of the potential receiver sites and enhancement sites shall be identified using GPS and plotted on a map or recent aerial photograph. This data shall be presented and analyzed in a site assessment report. The report shall be provided to the County and resource agencies (DFG and USFWS) for their review. The County's consultant, in cooperation with PBC's consultant, shall rate the sites as to potential for translocation receiver sites.

<u>Task 1.2 Draft Transplantation Design, Enhancement, and Adaptive Management Plan</u>

The purpose of this task is to identify a variety of transplantation and enhancement methods and to develop an adaptive management program for the transplantation receiver sites and existing populations based on the results of these tests. The long-term and short-term management of receiver sites and existing populations shall depend on the results of the tests that are implemented as part of design.

The draft TEAM PLAN shall be prepared by a qualified third party, in cooperation with PBC's consultants, and provided to the County and resource agencies (DFG and USFWS) for their review prior to the release of the DEIR. The County shall review the TEAM PLAN as part of the proposed project in the draft EIR. The TEAM PLAN shall be incorporated by reference as part of the proposed project and shall be available for public review at the County Planning and Building Department office in Marina, along with other project references.

The report shall, at a minimum, contain the following chapters:

Chapter 1. Introduction/Goals. This chapter shall provide an introduction to the plan and clearly describe the goals and objectives of the plan. The objectives shall be indicator specific, quantitative (i.e. measurable), and biologically appropriate. For example, transplantation of x% more tubers than are estimated to be lost due to current development proposal, and the planting of seedlings compensating for an expected x% mortality, with transplants and seedlings establishing an increasing population that is self-sustaining. Progress toward the goals and objectives shall be evaluated through the proposed success criteria and plan monitoring, both of which are discussed below.

Chapter 2. Background Information on Yadon's Piperia. This chapter shall provide a summary of the biological, horticultural, and distribution information that is relevant to the transplantation and management of the Yadon's piperia. The information presented in this chapter shall form the basis for the design and monitoring program.

Chapter 3. Design. This chapter shall present a design for identifying and testing 1) various transplantation, storage, and outplanting methods, and 2) methods for managing existing populations to increase the numbers and health of individual plants (this may include herbivory, reproductive, and pollination studies). The receiver sites and existing populations shall be adaptively managed over time, depending on the results of the design.

Elements that may be tested as part of the design for transplantation include:

- seasons of year for marking, salvaging, and outplanting plants,
- measures for salvaging and minimizing damage to tubers,
- measures for storing plants, native soil, and mycorrizae,
- types of excavation tools,
- planting density and depth,
- different microhabitats for outplanting,
- measures for preparing the receiver sites (e.g., excavating the topsoil, roughening the subsoil, pre-soaking the subsoil, and removing weeds from the surrounding area),
- measures for protecting transplants from human and animal disturbances (i.e. herbivory)
- the propagation and use of seedlings in the transplantation, and
- the timing of transplantations, including the potential of starting with pilot plots and test plantings before the implementation of large scale transplantation.

Specific donor sites shall be identified, including estimates of salvage from each site, as well as salvage and storage methods.

Chapter 4. Success Criteria and Monitoring Methods. This chapter shall present the proposed success criteria and monitoring methods. The success criteria shall be quantitative, specific, and *a priori* (e.g. establishment of self-sustaining populations evaluated by showing an increase from x transplants to x individuals, with such a rate of increase maintained over an x year period and following exposure to significant environmental variability). It is expected that the period for demonstrated success will need be lengthy given the ecology of Yadon's piperia (i.e. something on the order of 10-15 years). The methods shall include specific measures for monitoring survival, reproduction, recruitment, threats (including herbivory and disease), and management activities.

Chapter 5. Adaptive Management Approach. This chapter shall describe an approach for implementing an adaptive management program based on the data gathered during the monitoring effort. This shall include measures to be implemented in the event of failure of transplantation and enhancement measures over time to fully replace the Yadon's piperia populations removed and/or degraded as a result in DMF/PDP implementation.

Phase 2 – Site Selection and Final TEAM PLAN

Task 2.1 Select Transplantation Receiver Sites and Enhancement Sites

Based on the results of the draft TEAM PLAN, the comments received during the environmental review process, and the County determinations as to mitigation adopted and project conditions relative to Yadon's piperia, the County's consultant and PBC shall identify and prioritize the actual transplantation and enhancement sites proposed to meet the mitigation adopted for the project. PBC shall submit its proposed site in the form of a site selection report identifying each of the sites and the general activities proposed for each site. The site selection report shall be provided to the County and resource agencies (DFG and USFWS) for their review. If the County and resource agencies determine that these transplantation receiver sites and enhancement sites are appropriate and meet the mitigation adopted/project conditions, the qualified third party shall then be directed to implement Task 2.2 (described below).

Task 2.2 Finalize TEAM PLAN

The Draft TEAM PLAN shall be updated by a qualified third party with the specific transplantation design proposed for each transplantation receiver site and the specific enhancement measures proposed for each enhancement site. As an example, for each transplantation site, the final plan shall include recommendations for the number of tubers each site shall receive based on area of prime habitat in each site, proposed spacing of tubers, and optimal distribution of tubers at each proposed receiver sites. For each enhancement site, the final plan shall include recommendations for proposed enhancement methods (e.g., deer exclusionary fencing and other measures). All measures shall be identified on a site-specific basis for each site approved by the County as a mitigation site pursuant to review of the site selection report required by Task 2.1. An annual schedule of activities shall be identified for each approved mitigation site. Monitoring and reporting schedules shall also be identified. The final TEAM PLAN shall be provided to the County and resource agencies (DFG and USFWS) for their review. The County shall review and approve the final TEAM PLAN prior to issuance of any building or grading permit for any site wherein Yadon's piperia shall be adversely affected by DMF/PDP development. PBC shall be responsible to implement all measures included in the approved final plan.

Phase 3. Implementation

The Final TEAM PLAN, including transplantation, enhancement, monitoring, and adaptive management shall be implemented over a minimum of 15-years. Transplantation and enhancement shall be conducted at the same time that the DMF/PDP is being developed. Annual monitoring shall be required until success criteria are met (or population lost) and then every 3 years thereafter, for a minimum of 15 years after transplantation. Monitoring shall be conducted by a qualified third-party. As noted above, the expected period of demonstrated success to be adopted in the success criteria will be lengthy given the ecology of the target species (i.e., on the order of 10-15 years). The monitoring results, progress towards success criteria, and management recommendations shall be prepared by the qualified third party and presented in an annual report to the County and resource agencies. PBC shall be responsible to implement recommended changes in the implementation of the plan identified in County's review of the monitoring reports. If the success criteria are not met additional actions to meet the criteria, such as an expansion of the transplantation/enhancement sites or the dedication of additional areas, shall be implemented. This Phase will not conclude until the success criteria are met which may take more than 15 years.

Phase 4. Long-Term Management

A long-term management plan shall begin to be prepared no later than the beginning of Phase 3 Implementation and shall be completed no later than the completion of Phase 3. This plan shall be based on the understanding developed through the transplantation, enhancement, and monitoring efforts. The plan shall identify recommended measures for long-term maintenance of the transplanted and enhanced populations as well as the other piperia populations within preservation areas associated with the DMF/PDP. This plan shall be submitted for review by the County, USFWS, and CDFG, and County approval. The plan shall also identify the applicant's funding commitment to implement those maintenance recommendations in perpetuity. This plan shall be adaptive to allow for changing circumstances.

APPENDIX B SOILS STUDY METHODOLOGIES

Soil Assessments in the Del Monte Forest: Soils and *Piperia yadonii* Distribution

David B. Kelley, Consulting Plant and Soil Scientist

<u>Objective</u>: Characterize the soils of the Del Monte Forest and their relationship to *Piperia yadonii* distribution.

Recent work on the soils of the Del Monte Forest has proceeded apace with habitat and vegetation community characterization being undertaken by Erin Avery and Paul Kephart. The work has included review of previous work on soils of the forest and acquisition of data from a series of hand-dug auger pits (near permanent plots) and backhoe excavations in designated areas of the forest. The most intensive examination has been of the profiles exposed in 26 backhoe pits. Those examinations, generally speaking, included, as possible, descriptions and dimensions of soil properties and qualities including the following:

- soil horizons
- soil textures by horizon
- degree of disturbance
- root distribution and size
- ped structure
- parent materials for the soils
- dimensions (thickness of horizons, depth to constraining layers, etc.)
- various other soil features and inclusions (redoximorphic features, krotovina, root traces, accumulation zones of organic matter, organic horizons, color, stones, and others)

Other information on slope, aspect, and landform for each plot (and for *Piperia* locations) is being collected.

Soil samples were secured at several sites. These samples were taken from each horizon identified in the profile examination. They will be sent to a soils laboratory for analysis of

- pH
- texture
- nutrients (including N, P, K, Ca)
- toxicities (salts, metals, etc.)
- organic matter
- electrical conductivity
- SAR

  - CEC

These data will be used to

- help identify those features that might be considered constraints to plant growth (including root penetration problems, soil drainage, nutrient deficiencies or ion toxicities, and other processes).
- assess fidelity of soils as they are found in the forest to map units described and mapped in the USDA SCS Monterey County Soil Survey Report
- investigate each mapped soil unit in the parts of the forest where impacts, preservation, mitigation, or other project-related activities might take place
- provide supporting information for eco-characterization of randomized and permanent plots
- help identify/assess/quantify topsoil resources for potential salvaging or translocation
- assess quantity of organic materials (duff and decomposed duff) atop soil surface

These assessments will allow researchers to begin to identify and characterize soils where *Piperia* grows and does not grow.

<u>Backhoe Excavations by Area to Date</u>

B one pit along trail between Congress and 17 Mile drive; no Piya or plot

C one pit near Congress Road; no Piya or plot

F1 one pit near Congress Road, 30 feet from permanent plot

H two pits near Spruance Road—one near permanent plot and one in topographic low along horse trail in mesic area with hydrophytes

I1 one pit in mid-forest along trail; one exposure in slope cut/trench at new construction site (fire station and PBCSD facility) along Forest Lake Road

J1 one pit near permanent plot along Spyglass Woods Drive

J2 one pit near permanent plot along Spyglass Woods Drive

K two pits between Spyglass Hill fairway and Stevenson Drive—one near permanent plot; second upslope on knoll southwest of plot

L one pit in Indian Village area, between *Potentilla* exclosure and fairway

N1 three pits along Drake Road: one pit near permanent plot; one pit on edge in swale near permanent plot; and one pit midway between plot and Stevenson Road

O one pit near trail off Drake Court

PQR eight pits: one near permanent plot near Fire Road PC20; one on higher terrace along Fire Road PC20; one in dry meadow near Spruance Road; one near dry meadow, in forest, near Spruance Road; three pits along Fire Roads PC21 and PC22 on flanks of Pescadero canyon above 17 Mile Drive

V1 one pit near permanent plot off Drake Court

Equestrian Center one pit northwest of northwest boundary of existing Center, near Stevenson Road

Huckleberry Hill one pit near permanent plot at intersection Fire Roads HH04 and HH07

Auger Pits at Fixed Plots (Preliminary Assessments) to date:

- **F1**
- **H1**
- **I2**
- **J2**
- **K1**
- **N**
- **O**
- **PQR**

The soil-plant-landform relationships in the Del Monte Forest are complex, but might be considered well-ordered. The soils have different parent materials (generally, they are forming in sands, granite, and sandstones) and different landscape residence time (probably from the very recent late Holocene to Mid-Pleistocene, a span of hundreds of thousands of years). Some buried soil features on the oldest soil landscapes (in the Narlon series, for example) are highly developed (indicating long-term soil-forming processes or maritime consolidation and development of redoximorphic features associated with the depositional hydrologic regime before uplift as a terrace deposit) with massive relictual redoximorphic features (duripans, nodules, or concretions of iron silicates, for example). Some soil features are of recent vintage, reflecting little time for soil genesis (for example, the Baywood sands appear to be relatively young, very deep soils with moderate or weak development).

The soil landscape is ordered by landscape position (on different terraces or on different slope positions, for example), by parent material, and by hydrologic regime. In general, morphologic features of the soils reflect these factors. The response of plant communities to substrate is a function of these factors as well, though the ordering of plant communities is not as apparent (to me).

It is too early in the current investigations to begin to draw conclusions about the relationships of the various soils with the distribution of *Piperia* or other plants. However, it is apparent that the soils supporting strong populations of *Piperia*, with a couple of exceptions, have permeability and penetration constraints in the upper 12 to 20 inches; they are generally sandy (in the upper part of the profile in which the *Piperia* corms are found), and they are widespread through the forest.

Soils of the random plots where habitat data are being collected will be characterized with hand-dug auger pits as time permits.

APPENDIX C PRELIMINARY HABITAT ASSESSMENT

Introduction

To facilitate site assessment (Task 1.1) and design of both the transplantation and enhancement projects in the TEAM Plan (Task 1.2), a quantitative sampling study was conducted to determine suitable habitat conditions for Yadon's piperia (*Piperia yadonii*). In order to complete the study within the timeframe of the TEAM Plan, two important constraints were placed on the study: 1) data were collected outside the growing season for *P. yadonii*, and 2) only 20 randomly allocated replicates (plots) were sampled. In a joint meeting held on Thursday November 20 between County of Monterey officials, the County's consultants, and Pebble Beach Company's consultants, consensus was reached that the available data would be analyzed to determine whether they indicate suitable habitat characteristics and, if not, to inform future studies to obtain this essential information.

Data collection and entry was completed on November 25, 2003 and data analyses occurred November 29 and 30, 2003. The purpose of this report is to summarize results of the preliminary analyses and make recommendations for future studies.

Methods and Results

This study uses multivariate statistical analyses to determine whether aspects of plant community structure and composition influence habitat suitability for *P. yadonii*. It consisted of two main steps: 1) data collection, and 2) statistical analyses.

Data Collection

The following habitat characteristics were quantified in twenty (20) 10 m x 10 m (100 $m^{2)}$ plots randomly located in the Del Monte Forest.

1. *P. yadonii* presence: Plots were classified as having *P. yadonii* present or not based on their location relative to the previous known distribution of *P. yadonii* (Allen 1996)

2. Slope: visually estimated to the nearest 5%

3. Aspect: determined using a hand-held compass.

4. Canopy cover: mean based on four measurements (one in each corner of the plot) using a spherical densiometer,

5. Litter Depth: measured to the nearest 0.25" in a random location within the plot

6. Plant cover: absolute cover of all plant species, as well as the cover of litter, bare soil, rock (any stone over 7mm) estimated visually using cover classes[4] Because trees and

[4] 1 (<1%); 2 (1-5%); 3 (5-15%); 4 (15-25%); 5 (25-50%); 6 (50-75%); 7 (75-85%); 8 (85-95%); 9 (95-99%); 10 (>99%)

shrubs were measured both in terms of their canopy (number 4) and their overall percent cover, two separate 'species-classes' were created for each tree and shrub, with seedlings and saplings measured in terms of their absolute cover, and adult trees measured in terms of their canopy cover. As a result, there are more 'species classes' analyzed than there were actual species.

Data Analysis

Analyses of the data occurred in three phases: 1) description, 2) ordination, and 3) group definition.

Description

Species Frequency Distributions

Analyses were conducted to describe the variability in plant community structure amongst the plots. The 100 m^2 plots were very diverse, with mean species class richness (number of species-classes) of 20.4 and a range from 11 to 29. Of the total 92 species classes sampled in the study, nearly half only occurred in 1 or 2 plots, while only 12 species classes occurred in 10 or more of the plots (i.e. ≥50%; Figure 1). Though the ratio of common to rare species in this study is observed in many community studies, the overall low number of plots sampled renders it difficult to infer much about *P. yadonii* habitat suitability from the rare species (i.e. those found in fewer than 3 plots), despite the fact that many are not truly rare and may be important indicators of habitat variability.

Species Area Curves

Examination of the species area curve which relates the number of species observed to the number of samples obtained indicates that the 20 samples currently being analyzed were insufficient to capture the species composition in the potential transplantation and enhancement sites (Figure 2). If additional samples were adding new species to the overall dataset at a low rate, as one would expect if sampling was sufficient to include most of the species in the system, then a plateau would be observed in the plot of species richness against the number of samples. Instead, the lack of substantial plateau in the figure suggests that not only would increased samples increase the resolution of the data set for the current 'rare species' but would also likely result in additional species in the Monterey Pine forest being included in the analysis.

Outlier Analysis

Many of the subsequent multivariate analyses incorporate an algorithm to measure the distance of the plots based on their differences in species composition. This distance can be used to determine whether there are any outliers within the data set, where an outlier is a plot that is very different from the other plots and can have disproportionately large effects on the results. Plot RSHH2 was flagged as an outlier because its distance from the other plots was more than 2 standard deviations away from the mean distance between all plots. This is presumably due to its high cover of *Pinus muricata.* In order to decide how this plot should be treated in future analyses, one would want to know whether this sample is truly aberrant in some way (data entry error, odd sampling due to plot configuration) or whether the plant community from which it was sampled is simply underrepresented in the current small data set.

Ordination

Given the more than 90 species-class attributes in the data set, individual plots can vary greatly in species composition. The purpose of ordination is to synthesize the variability between plots in n-dimensional species space, where n is the number of species-classes, into just a few variables. These synthetic variables can be used as the axes of a graph plotting the samples such that the distance between plots illustrates their difference in species composition. Species can similarly be plotted against these axes such that the difference between species is proportional to the extent to which they co-occur within the plots.

Nonmetric multidimensional scaling (NMS) is one of the most common and reliable methods of ordination and was used to ordinate the 20 plots. Preliminary analyses indicate that 3 statistically significant axis cumulatively represent 86% of the variation in species composition between the plots. This indicates that the ordination is robust and captures an exceptionally high amount of the variation that exists. When the individual sample plots are coded according to whether *P. yadonii* is present or absent (Figure 3a-c), it is clear that plots with *P. yadonii* have higher axis 1 scores than those without (though Plot RSHH2 is a large outlier). This axis is strongly negatively associated with bare ground, *Arctostaphylos tomentosa, Carex bracteatus, Cupressus goviniana, Pinus muricata, and Pteridium aquilinium* var. *pubescens* and strongly positively correlated with *Achillea millefolium, Briza maxima, Elymus glaucus, Holcus lanatus, Mimulus aurantiacus,* and canopy cover from *Pinus radiata*. The other two axes do not separate plots with *P. yadonii* from those without. While they are not useful for identifying potential indicators of *P. yadonii* habitat, they represent a lot of the variance in species composition in the Monterey pine forest and can provide some understanding or factors that *may not* be important in determining transplantation and enhancement success.

Group Identification and Description

Cluster analysis

Cluster analysis is a process by which individual plots are joined in dendrogram or tree successively based on their similarity in species composition. The resulting dendrogram provides a hierarchical representation that can be examined to ask the questions: *Do plots with P. yadonii differ in species composition from those without?* Examination of the dendrogram from the cluster analysis of the 20 samples indicates that 6 of the plots with *P. yadonii* are more similar to each other than plots without *P. yadonii*; however, the other two *P. yadonii* plots are more similar to non-*P. yadonii* plots. Examination of the plots indicates that perhaps some caution must be used when examining these data, however, as they may reflect a site difference rather than a difference in *P. yadonii* distribution *across* sites, as one would hope to determine if unoccupied sites are to be deemed suitable. The six adjacent *P. yadonii* plots are all in N, O, and V while the non *P. yadonii* plots are in F, G, H, and I. The same caution in interpreting results applies to the ordination results discussed above.

Indicator Species Analysis

Indicator species analysis is a procedure used to identify species (or other attributes), which might indicate the presence of suitable *P. yadonii* habitat based on analysis of species composition in plots with and without *P. yadonii* present. For each species, an indicator value is calculated by multiplying the relative frequency of that species in each group (*P. yadonii* present vs. *P. yadonii* absent) by the relative abundance of that species in each group. Species that are relatively more frequent and more abundant in a given group have a high indicator value for that group. Though ISA can be a powerful tool, rare species (those found in just a few samples) cannot be indicators. Due to the low overall sampling in this study, most species were rare. Based on the current sampling, however, ISA indicates that plots without *P. yadonii* are indicated by *Arctostaphylos tomentosa, Toxicodendron diversilobum,* and *Vaccinium ovatum*. Plots with *P. yadonii* present are significantly indicated by *Solidago spathulata* ssp. *spathulata* and *Holcus lanatus*.

Discussion

With over 80 plant species occurring in the mere 2000 m^2 sampled in this study, the Monterey Pine forest community is very diverse. Though alpha diversity (i.e. number of species within an area) is high, averaging nearly 20 species per 100 m^2, beta diversity, or the diversity *between* areas, strongly contributes to the overall (or gamma) diversity. Within the areas sampled in this study, there is a large degree of heterogeneity in plant species composition. This variation may be due to a combination of edaphic factors (incl. soils, hydrology, etc.), disturbance history (fire etc.), and land use history (logging etc.); however, the current data do not allow for hypotheses about such causal relationships.

Once variability in plant community composition within the area of interest was identified, the goal of a study to inform transplantation and enhancement project design is to identify the factors that may contribute to the variability in the distribution and abundance of *P. yadonii*. These potential factors can be determined by identifying indicators associated with the presence of *P. yadonii*. Ideally, a data set would contain plots with and without *P. yadonii*, and the plots with *P. yadonii* would have a varying abundance of *P. yadonii*. Unfortunately, the present study was limited to 20 samples collected outside of the growing season, such that previous data on the distribution of *P. yadonii* had to be used to categorize plots based on *P. yadonii* presence (unless flower stalks were visible). These may or may not be accurate to the scale of the 100 m^2 plot sampled. That is, while previous mapping efforts have identified the presence of *P. yadonii* in polygons within a site, a randomly allocated 10 m x 10m plot may or may not have included extant *P. yadonii* individuals. In addition, the present study could not accurately quantify the abundance of *P. yadonii* (i.e. cover). These constraints substantially reduce the ability of the present study to accurately quantify *P. yadonii* habitat and determine indicators.

Preliminary analyses (NMS, Indicator species analysis, and Cluster analysis) indicating that plots with *P. yadonii* differ from those without piperia suggest there may be important differences between the two habitats. Piperia appears to be preferentially found in areas with *S. spathulata* spp. *spathulata, H. lanatus* and *B. maxima,* and restricted from areas with *A. tomentosa, V. ovatum, T. diversilobum.* However, because the majority of the plots with *P. yadonii* occurred in different sites (K, N, O, V) than those without *P. yadonii* (F, G, I, J), these results may simply suggest that sites differ in plant community composition. While these site-level differences may be important determinants of habitat quality for *P. yadonii*, it is also possible that the small number of samples just happened to land in areas without *P. yadonii* at one subset of sites and in areas with piperia in the other, suggesting systematic differences that may not occur.

Recommendations

By distilling the large amount of variability that exists within the Monterey pine forest into a few synthetic variables to facilitate determination of whether areas with and without *P. yadonii* differ in important traits that can influence plant persistence, multivariate statistical analyses of environmental factors can provide a powerful tool in the design of the transplantation and enhancement projects of the TEAM Plan. Preliminary analyses to facilitate site assessment (task 1.1) of the Plan indicated that considerable variability in species composition across the diverse Monterey Pine forest habitat exists and that this variation may provide invaluable indications about habitat characteristics associated with *P. yadonii* that my influence population success in transplantation and enhancement efforts. However, the limitations of the current dataset due to the short time frame for the study render it impossible to draw conclusions about *P. yadonii* habitat based on the present study.

Future efforts to determine site availability and design transplantation and enhancement would benefit greatly from a study similar to the one conducted but with the following characteristics:

1. Greater sample size

2. Data on *P. yadonii* distribution, abundance, and reproductive success based on observations of aboveground plants

3. Data on the distribution and abundance of ephemeral species in the Monterey Pine Forest system

4. Data on abiotic factors that likely influence *P. yadonii* distribution and abundance (e.g. soil factors, hydrology, etc.)

<u>Sample Size</u>

More replicate samples can reduced the noise in the current data set and allow more powerful assessment of the factors that may influence *P. yadonii* distribution. If sites differ predictably in plant community composition, yet each site has areas with and without *P. yadonii*, then stratifying sampling by site can improve the ability of the study to identify the factors that influence *P. yadonii* distribution both within and between different sites in the forest. A total of 80-100 plots would likely provide a robust study, though at a minimum 60 is recommended.

<u>*P. yadonii* Distribution and Abundance</u>

As described above, data on *P. yadonii* distribution and abundance are essential to providing a clearer picture of the factors that might influence the success of transplantation and enhancement projects. Relying on past distributions that are up to 7 years old and the persistence of remaining flower stalks may be misleading. Plots categorized as having *P. yadonii* may be just outside of the distribution due to important small-scale change(s) in habitat characteristics, or even alterations in habitat suitability during the past 7 years. Many more plants produce leaves each year than produce flower (stalks); thus, including information about aboveground individuals will enhance ability to determine habitat characteristics. As a result, data should be collected during the period over which aboveground leaves are observed and should include not only presence or absence, but also relative abundance (% cover), and density. Should resources (including time) allow, data should be collected on the proportion of individuals that flower and reproductive output (e.g. inflorescence length). Such data allow assessment of the factors that influence reproduction, which is important to the design of successful transplantation and enhancement projects.

Ephemeral Plant Species

Collecting data during the spring when *P. yadonii* leaves are visible will also allow inclusion of abundance data on ephemeral plant species in the Monterey pine forest communities as well. Ephemeral plants may exhibit distribution and abundance patterns indicative of *P. yadonii* habitat suitability. Though the present study incorporated 'forensic botany' whereby attempts were made to include abundance data on ephemeral plants species that have persistent dead material in the fall, many species were undoubtedly excluded from this study.

Abiotic Factors

A predictive understanding of the factors that influence *P. yadonii* as well as the different associations of plants within the Monterey pine forest would be facilitated by integrating data on abiotic factors into the multivariate analyses. Biologists have hypothesized that the distribution of *P. yadonii* may be influenced by a variety of soil characteristics including hydrologic factors. A future study should examine correlations of piperia distribution and abundance with soil characteristics thought to influence piperia by incorporating soils data in the analyses. Optimally, the relevant soils data would be collected in each sample plot. This would be relatively straightforward for soil nutrient availability, soil moisture, and soil texture. In addition, perhaps hydrology could be examined at the plot level through the use of hydrometers and peziometers. However, some relevant soil characteristics, including the presence of a clay hardpan layer that constrains permeability, would be difficult to assess in each replicate due to the effort required. In addition, doing so may not be necessary, as some factors do not vary at small spatial scales (i.e. within a site). A preliminary report by David Kelley based on his analysis of soils in the Monterey Pine forest though soil pits indicated that the soils are ordered by landscape position, parent material, and hydrologic regime. This information, along with subsequent analyses from Mr. Kelley may be used to incorporate larger scale soil variability into a future study *without* sampling each plot but instead, based on site or sub-site characterizations.

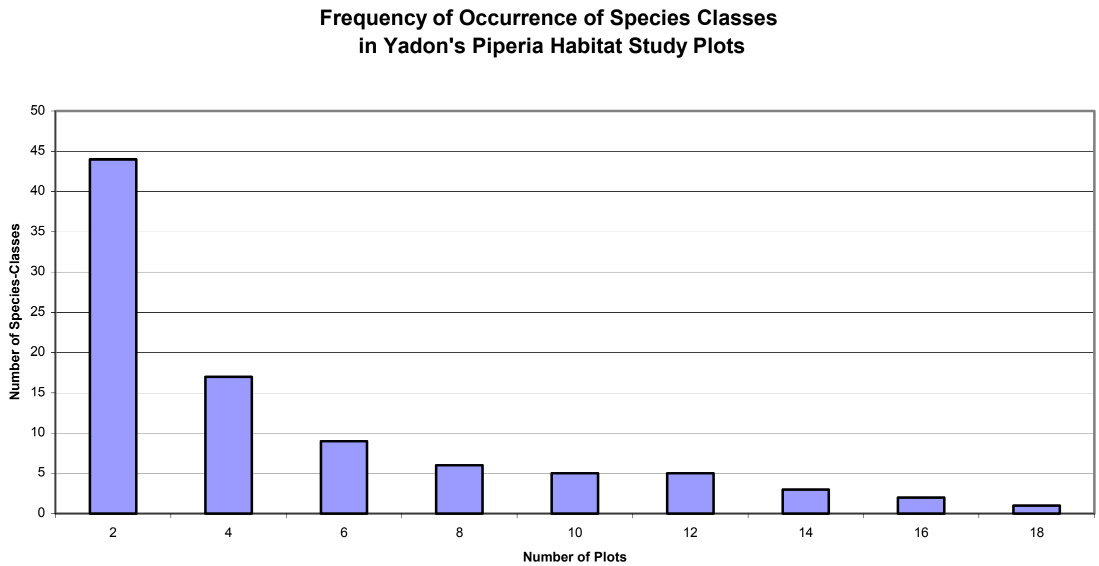


Figure 1: Number of species-classes that were found in each number of the 20 sample plots.

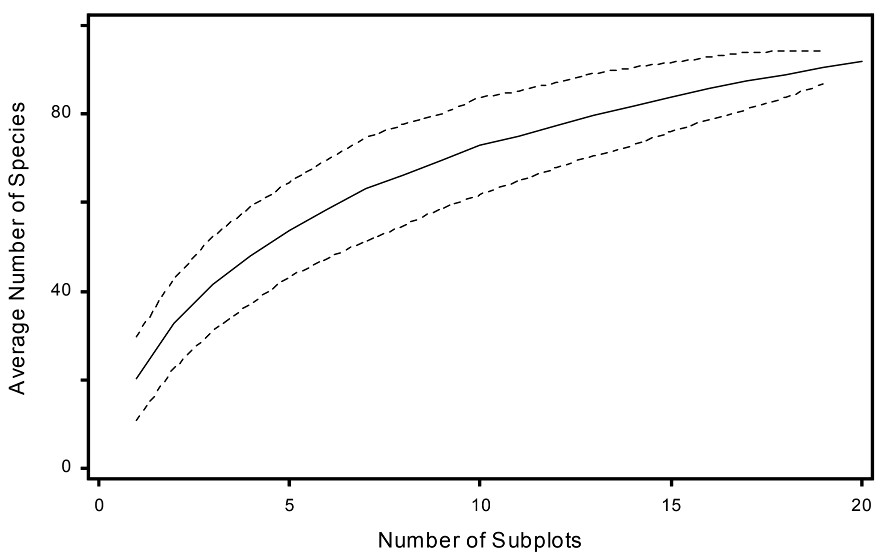


Figure 2: Species Area Curve showing the number of species increasing continuously across the 20 plots sampled. Bold line is the number of species; dashed lines are the 95% confidence intervals.

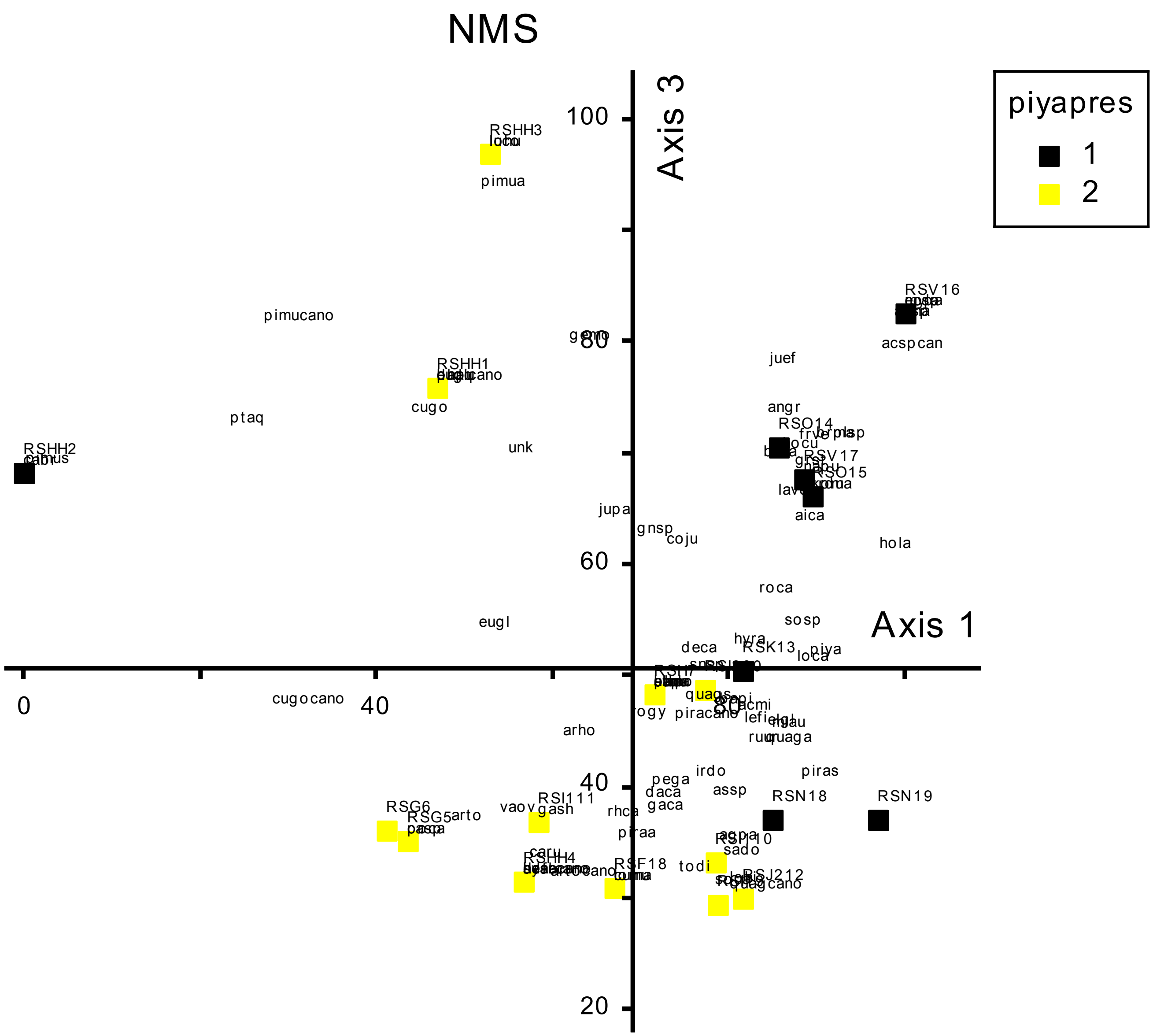
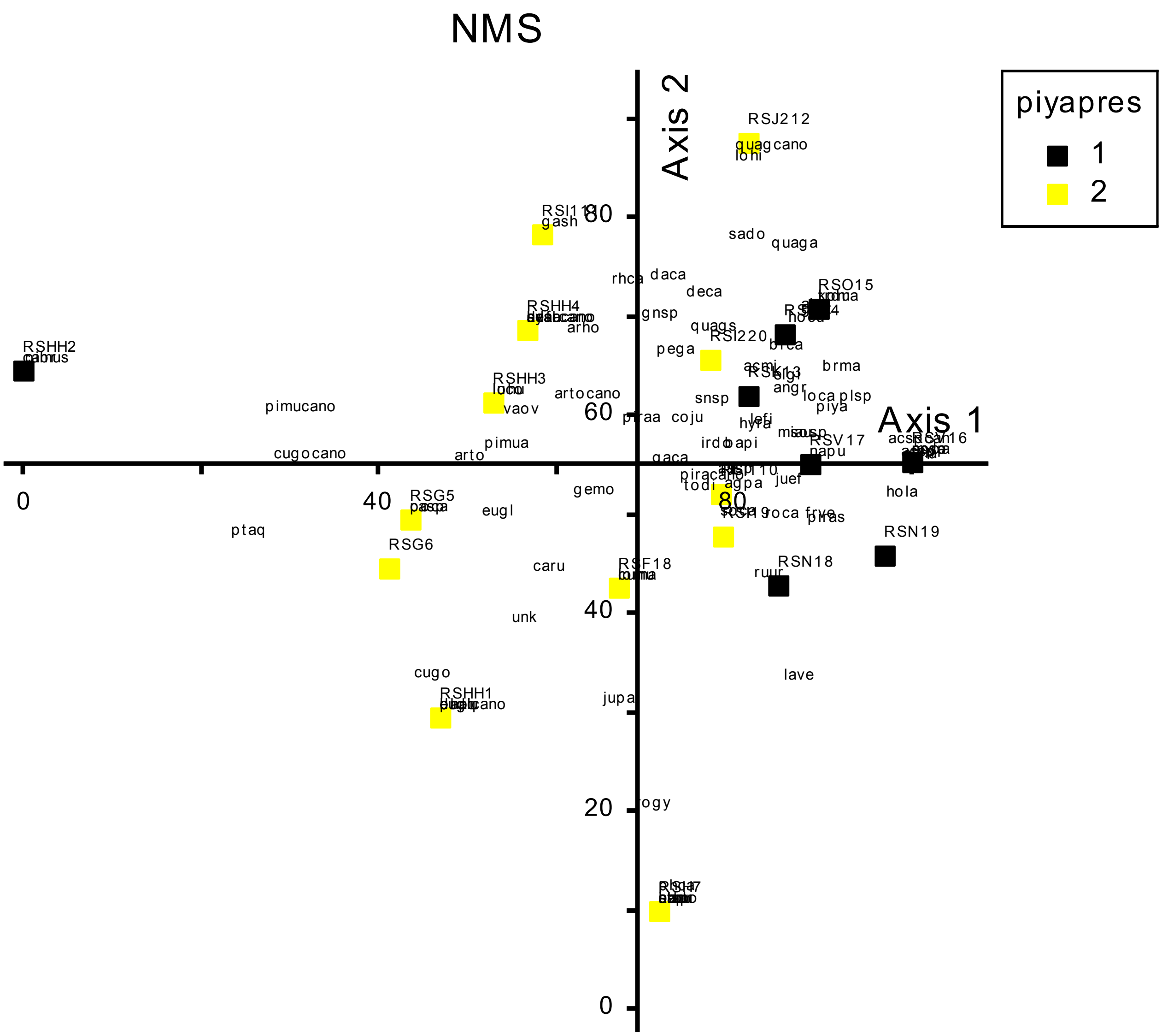


Figure 3: Ordination diagram of sites and species, with sites coded according to whether *P. yadonii* is present (black) or absent (yellow/gray). Species are listed with their four letter codes (suffix "cano' refers to canopy of species). a) axis 1 vs 3, b) axis 1 vs 2, and c) axis 2 vs 3.

Figure 3b


NMS
Axis 1
Axis 2
piyapres
1
2
RSJ212
RSI118
RSHH4
RSHH2
RSHH3
RSO15
RSI220
RSV17
RSN19
RSN18
RSG5
RSG6
RSF18
RSHH1
pimucano
cugocano
ptaq
artocano
pimua
arto
gemo
eugl
caru
unk
cugo
jupa
rogy
lave
hola
brma
piya
napu
sado
quaga
daca
deca
rhca
gnsp
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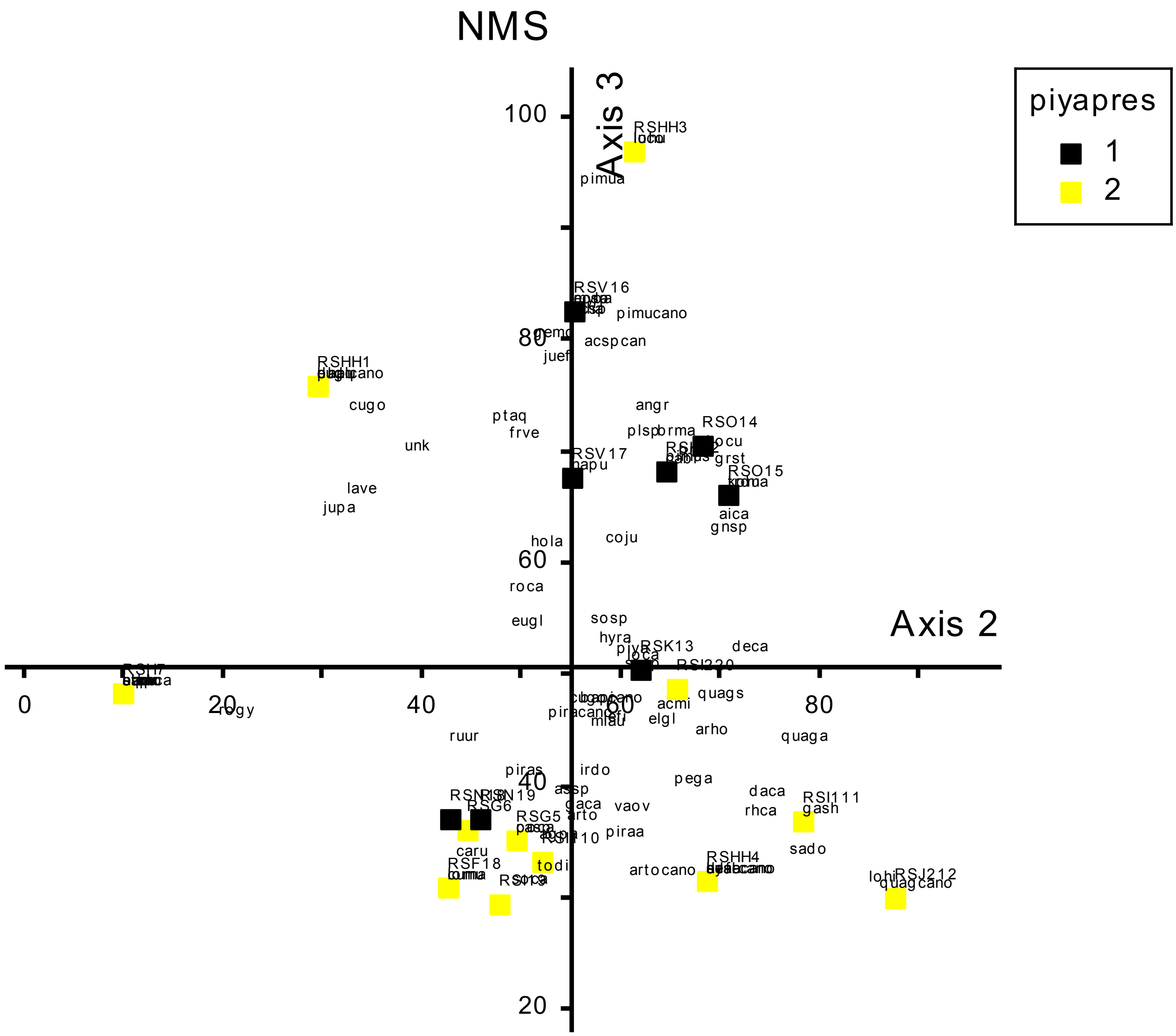
juef
ruur
prnas
vaov
gash
arho
lohi
quagcano

Figure 3c



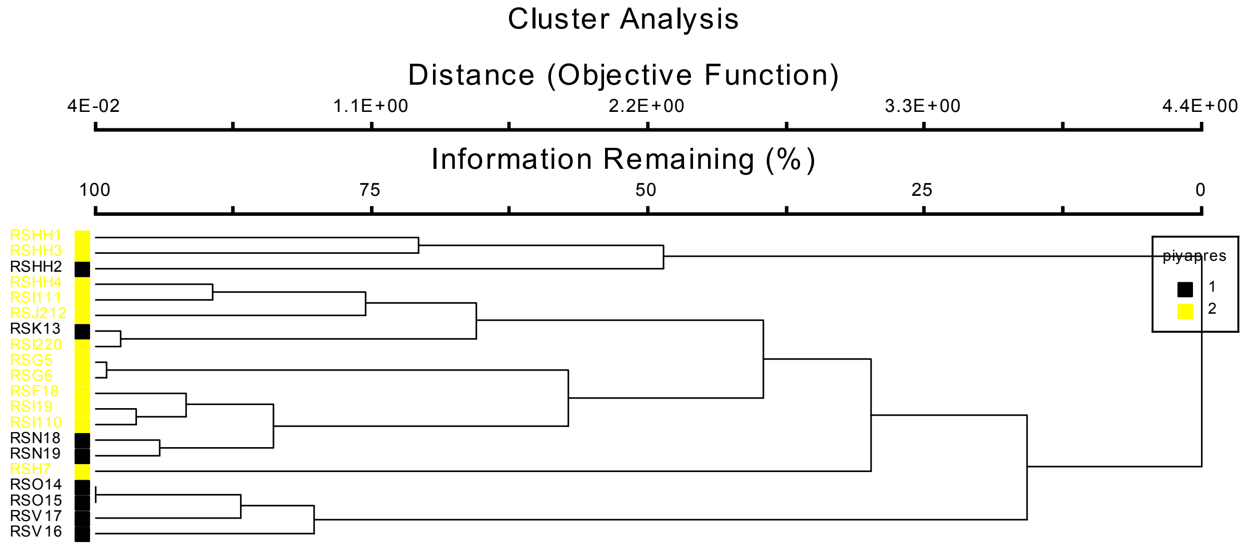


Figure 4: **Dendrogram of sample sites based on hierarchical cluster analysis showing plots categorized based on *P. yadonii* presence. Black plots have *P. yadonii*, yellow/gray plots do not.**