State of Hawai`i
DEPARTMENT OF LAND AND NATURAL RESOURCES
Division of Forestry and Wildlife
Honolulu, Hawai`i 96813
May 26, 2009

Chairperson and Members
Natural Area Reserves System Commission
State of Hawai`i
Honolulu, Hawai`i

NARS Commission Members:

SUBJECT: REQUEST FOR APPROVAL OF SPECIAL USE PERMT TO: MR. RICHARD PENDER, Ph.D. CANDIDATE, UNIVERSITY OF HWAI`I MANOA, TO CONDUCT A STUDY OF THE REPRODUCTIVE BIOLOGY OF LISTED SPECIES *Cyanea superba* subsp. *superba* AND *Delissea subcordata* IN PAHOLE NATURAL AREA RESERVE, O`AHU AND *Clermontia drepanomorpha* IN PU`U O `UMI NATURAL AREA RESERVE HAWAI`I, AS WELL AS NON-LISTED *Clermontia* species.

BACKGROUND:
This is part of a larger study of Hawaiian lobeliads that seeks to provide insights into species, through: 1) floral biology/pollination syndrome study, 2) breeding system study, and 3) floral visitor analysis; it is being re-submitted to include collection of arthropods encountered visiting flowers to determine their identity and whether they are involved in the pollination.

STAFF ANALYSIS:
These listed species in Pahole and Pu`u O `Umi are being requested from these Reserves because they provide the most reliable source of plants that can be located with a minimum of disturbance. Treatments will use the same flowers, are non-destructive, and no flowers will be removed or damaged in any way. In making observations of pollinators, arthropods may be collected to determine species.

The applicant will be issued an Endangered Plant Permit prior to conducting this work, as well as a Protected Wildlife - Invertebrate Permit. Staff has reviewed this application and has made recommends approval of this application, subject to a current Endangered Plant Permit, notifying staff prior to field work, making arrangements for access to and across private lands.

RECOMMENDATION:
That the NARS Commission approve this proposed study and to forward it to the Board of Land and Natural Resources, or its authorized representative, for their review and further action.
Respectfully submitted,

BETSY H. GAGNE, NARS Commission Executive Secretary
Division of Forestry and Wildlife

Department of Land and Natural Resources
Division of Forestry and Wildlife

1151 Punchbowl St., Room 325; Honolulu, HI 96813
(808) 587-0063, (808) 587-0064 (Fax)

Application for NARS Special Use Permit

Name: Richard Pender
Title of Proposed Activity: Reproductive Biology of Hawaiian lobeliads

The following activities require a Special Use Permit under HAR §13-209-5. If your work in the Natural Area Reserve (NAR) will involve one or more of the following, please indicate with an 'X' below:

X remove, injure, or kill any form of plant or animal life, except game mammals and birds hunted according to department rules*
__ introduce any form of plant or animal life*
__ remove, damage, or disturb any geological or paleontological features or substances*
__ remove, damage or disturb any historic or prehistoric remains*
__ engage in any construction or improvement*
__ engage in any camping activity
__ establish a temporary or permanent residence
__ start or maintain a fire
__ litter, or to deposit refuse or any other substance
__ operate any motorized or nonmotorized land vehicle or air conveyance in any area (including roads and trails) not designated for its use
__ operate any motorized water vehicle of any shape or form in freshwater environments or marine waters, except as otherwise provided by DLNR's boating rules
__ enter into, place any vessel or material on, or otherwise disturb a lake or pond
__ engage in commercial activities, defined as "the use of or activity on state lands for which compensation is received by any person for goods or services or both rendered to customers or participants in that use or activity"
__ have or possess the following tools, equipments or implements: fishing gear or devices (in `Ahihi-Kina`u NAR), cutting or harvesting gear (in any NAR), and hunting gear or tools (except as permitted by the hunting rules of the department)
__ hike or conduct nature study **with a group larger than 10**
__ presence in an area closed pursuant to HAR §13-209-4.5 or after visiting hours established by §13-209-4.6
__ anchor any motorized or non-motorized water vehicle in the marine waters of `Ahihi-Kina`u NAR
__ other (please explain): _____

*** May require additional State or Federal permits. Applicants are responsible for identifying and securing all approvals that may be required.**

**** The NARS rules and recent rule amendments can be viewed on-line at http://www.state.hi.us/dlnr/dofaw/Unofficial%20compilation%20HAR%2013.209.pdf**

***** Please allow for a minimum permit processing time of three months*****

All permits will have the following standard conditions, pursuant to HAR § 13-209-5. Additional conditions may apply.

1) The permittee shall adhere to the specifications given in the permit application
2) Disturbance of vegetation and wildlife shall be avoided as much as possible
3) Precautions shall be taken to prevent introductions of plants or animals not naturally present in the area. The permittee is responsible for making sure that participants' clothing, equipment, and vehicles are free of seeds or dirt to lessen the chance of introducing any non-native plants or soil animals. Should an infestation develop attributable to the permittee, the permittee is responsible for eradication by methods specified by the department
4) Once approved, the permit is not transferable
5) Once approved, the permit does not exempt the permittee from complying with any other applicable rule or statute
6) The State of Hawaii shall be released and held harmless from any and all liability for injuries or death, or damage or loss of property however occurring during any activity related to the permit

I certify that the information contained in this application is true and correct.

Richard J. Pender

_____

Applicant's Signature

If approved, copies of the permit will be provided to:

- *Applicant*
- *NARS Commission Executive Secretary*
- *NARS Branch staff*
- *DLNR-DOCARE*

For internal use only:

Application received on: _____

Distributed to District staff for review on: _____

Approval ( ) recommended ( ) not recommended by NARS Commission or authorized representative on: _____ **( ) with the attached special conditions.**

( ) Approved **( ) Not Approved**

_____ _____

Chairperson, DLNR **Date**

Applicant Contact Information
You may either enter the information directly onto this form; if you need more space or need to attach additional pages, please indicate that there are attachments.

Name: Richard Pender

If you are applying on behalf of an organization, the organization and your title:

PhD candidate, University of Hawaii at Manoa.

Title of Proposed Activity: A study of the reproductive biology of *Cyanea superba* subsp. *superba* and *Delissea subcordata* at Pahole NAR, (Oahu) and *Clermontia* spp. At Pu'u O 'umi NAR (Island of Hawaii).

Primary contact person for this permit application: Richard Pender

Mailing Address:

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Honolulu, Hawaii 96822

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Email: pender@hawaii.edu

Supporting Information
Please provide the following information about your proposed activity that requires a special-use permit ("proposed special-use"). Failure to provide responses to the following questions may result in your application being rejected.

1. **What is the period of time for which the permit is requested (e.g., the date of a proposed single event or an ongoing research project from when to when)?**
   Please note: research permits are limited to one year in length, except where waived for permits to other governmental agencies where the board determines the waiver to be in the best interest of the State. Proposals for multi-year projects are advised of the need to apply for a new permit EACH year.

   1st June 2009 to 1st June 2010

2. **List the individual Natural Area Reserve(s) involved:**

   Pahole NAR, Oahu.

   Pu 'u O 'Umi NAR, Island of Hawaii.

3. **Attach a map that illustrates where in the Natural Area Reserve(s) you propose to conduct your special-use.** *The map should be legible and reproducible in black and white. The map should also be at the appropriate scale for the type of activity proposed and of sufficient detail to allow the Division to identify activity sites within 10 meters. For any activity off established trails, entry and exit routes should be marked.*

Pahole NAR. At Pahole I wish to use the out-plantings of *Cyanea superba* subsp. *superba* at two sites known as PAH-A (Pahole Gulch) and KAP-A and B (Kapuna Stream Site and One Acre Site). For *Delissea subcordata* the site is known as PAH-C (Pahole on the bench below the switchbacks).

Pu 'u O 'Umi NAR. At Pu 'u O Umi NAR I wish to work in the area immediately South of Puu Ahia within 100 meters of the fence line that separates the NAR from the adjacent farmland.

Please see the attached maps

4) Provide a thorough and detailed description of the proposed special use. *The description should be detailed enough so that those reviewing your application understand what you propose to do and the scope of your proposal. As part of your description, please include: a) a description of the planned method of transportation to and within the Natural Area Reserve, and b) if other people than you will participate in the proposed special-use, please note how many people, and whether they are volunteers, students, research assistants, paying customers, etc...*

For research proposals,
a) please explain your objectives, your methods, and why the proposed special-use is necessary to your research;
b) if the research is part of your undergraduate or graduate studies, please include the name and affiliation of your major professor;
c) if you are seeking permission to remove or introduce any form of plant or animal life, please list all species involved and specifically identify which are threatened, endangered, or candidate species.
d) if you are seeking permission for the collection of any specimens, please note type of specimen (species and parts collected, if less than entire specimen), quantities to be collected, storage methods, and ultimate disposition.

Failure to provide sufficient information may result in your application being returned for additional information or rejected. Please feel free to attach additional sheets as necessary.

a) please explain your objectives, your methods, and why the proposed special-use is necessary to your research;

Problem and objectives

The co-evolution of Hawaiian honeycreepers (Drepanidae) and lobeliads (Campanulaceae) has become a classic example of reciprocal natural selection within the popular scientific literature (Carlquist 1970, Frierson 1991, Buchmann and Nabhan 1996). The evolution of curved lobeliad corollas to accommodate nectar feeding honeycreeper bills is a truly appealing story. Despite this widely reported co-evolution, few studies have assessed whether each of these groups are in-fact co-evolved (*see* Lammers and Freeman 1986 for an exception), and what role each of these mutualists currently plays for the continued survival of the other. A clear understanding of whether these two parties have coevolved, whether they are still functioning reciprocally, and what the consequences are, if they are not, currently alludes us. Addressing these questions has been made even more difficult due to the increasing rarity of species within each group.

The first member of this potential mutualism, the honeycreepers, are an endemic, monophyletic adaptive radiation of 51 species, contained within the endemic subfamily Drepanidinae, within family Fringillidae (Pratt 2005). Included in this impressive radiation are 10 species known to obtain (or have obtained) their diet entirely or partially from nectar (Pratt 2005). Twelve published citings have been made of honeycreepers consuming nectar from lobeliad flowers, summarized in Table 1. The extinction of the majority of honeycreeper species is a tragic feature of this group. Habitat destruction, the introduction of a broad range of invasive predatory and competatory species and avian diseases are the major catalysts of honeycreeper extirpation (Pratt 2005, Stone and Stone 1989). As many as 32 species have been lost, almost half since European colonisation (Pratt 2005). Of the 16 species that remain, 14 are federally listed as endangered or threatened (USFWS 1999). Of these extant species, 6 ('i'wi, 'apapane, 'anianiau, Maui 'alauahio, Hawaii 'amakihi and Kauai 'amakihi) are known to fully or partially derive their diet from nectar sources, and therefore have the potential to pollinate lobeliads where they occur in sympatry (based on the diet regime presented in Pratt 2005).

Table 1. Published sightings of honeycreeper species feeding from or otherwise visiting lobeliad species. Adapted and updated from Cory (1984).

Reference	date	bird	plant	location
Berger 1981	July 1966	'i'iwi	*Clermontia arborescens*	Maui-Kula Forest
Berger 1981	August 1967	'i'iwi	*Trematolobelia macrostachys*	Maui-Kipahulu Valley
Bryan 1908	June 1907	black mamo	Lobeliad sp. *Drepanis funera*	Molokai-Head of *Waialua Valley 2" long purple flowers*
Munro 1960	November 1891	'akialoa *Hemignathus obscurus*	*Clermontia* sp. low growing	Big Island-Nawina
Perkins 1903	November 1891	black mamo	Lobeliad sp. Large flowers	Molokai
Perkins 1903	November 1891	'i'iwi	Lobeliad spp.	All islands
Perkins 1903	November 1891	'akialoa	Lobeliad spp. Large flowers	Kauai and Big Island Kona and Mauna Kea
Rothschild 1893-1900	November 1891	'akialoa	Lobeliad sp.	Lanai forests
Rothschild 1893-1900	November 1891	'akialoa	Lobeliad sp.	Big Island-Wailuku
Spieth 1966	July 1965	'i'iwi	*Clermontia arborescens*	Maui-Olinda trail
Lammers, Weller and Sakai (1987)	July 1985	Maui amakihi	*Clermontia arborescens*	Maui
Drake and Morden 2006.	July 2005	Kauai amakihi	*Clermontia fauriei*	Kauia
			Cyanea leptostegia	
		'i'iwi, Kauai amakihi	*Trematolobelia kauaiensis*	

Lobeliads, the plant component of this potential mutualism, are contained within the sub-family Lobelioideae of the family Campanulaceae. This group comprises 6 genera, of which 5 (*Brighamia*, *Cyanea*, *Clermontia*, *Trematolobelia* and *Delissea*) are endemic, while the last genus, *Lobelia,* is cosmopolitan in distribution (Lammers 1990). Collectively, lobeliads contain c. 126 taxa of woody trees, shrubs and herbaceous caudiciforms, all of which are endemic (Lammers 1990). This impressive diversity represents the largest plant adaptive radiation in the Hawaiian Islands, thought to be driven, in part, by honeycreeper selection pressures (Calquist 1970, Givnish et al. 1995). Lobeliads have faced a similar, perilous fate, to that of honeycreepers. Sadly, 25% of species are now extinct. A further 30% are in serious threat of extinction (Cory 1984). This decline in the populations of lobeliads has been drivin by human induced habitat distruction, introduced ungulates, and probably a loss of pollinators and seed dispersers (Stone and Stone 1989).

The increasing rarity of both lobeliads and honeycreepers has obvious implications for the survival of each potential mutualist. Honeycreepers will have lower nectar resources available from which to forage, leading the species to seek nectar from alternative sources, where available. Smith et al. (1995) reported that such a shift has occurred in i'iwi (*Vestiaria coccinea*), which now feed on ohia (*Metrosideros polymorpha*), rather than lobeliad species, leading to a reduction in beak length in living, compared to historically preserved specimens. The implications of lobeliads as foraging sources for other honeycreeper species is still unknown. From the plants perspective, an increasing scarcity of pollinating honeycreepers may reduce pollen transfer, resulting in pollen limitation (Burd 1994). Pollen limitation occurs if fewer seeds are formed than would have occurred under adequate pollen availability (Knight et al. 2005). Plants that are failing to be pollinated may still reproduce if the species is capable of self fertilization. However, successive selfing over generations may lead to inbreeding depression and the accumulation of deleterious mutations, which, in the absence of out crossing or mutation purging may impact the long-term survival of the population (Charlesworth and Charlesworth 1987, Ellstrand and Elam 1993, Frankham 2005). Given the rarity of honeycreepers in many extant Hawaiian plant communities, it is likely that both pollen limitation and inbreeding depression are taking place if alternative pollinators, such as introduced birds, are not dispersing pollen between plants.

Three previous studies have attempted to address if honeycreepers are still pollinating lobeliads (Cory 1984, Drake and Morden 2006, Gardener and Daehler 2006). Two of these studies (Cory 1984, Gardener and Daehler 2006) found that honeycreepers did not visit the lobeliad species in each case. However, honeycreepers were rare or largely absent in the sites where these studies were undertaken. The third study, that of Drake and Morden (2006), was carried out in the Ala Wai wetland on the Island of Kauai where honeycreepers are still present. The study showed that honeycreepers , in this case Kauai amakihi (*Hemignathus kauaiensis*) and 'i'iwi, visited species of *Trematolobelia*, *Cyanea* and *Clermontia*. Table 2 symmarizes the key findings of these studies. These findings indicate that where honeycreepers still occur in symapatry with lobeliads, they may function as pollinators. However, this assumption remains to be tested across multiple populations throughout the archipelago.

Table 2. Summary of pollination studies carried out on Hawaiian lobeliads

Study	Study species	Key results
Cory (1984) Study carried out on Mt Tantalus, Oahu	*Clermontia kakeana* *Cyanea angustifolia*	Visited by a single hawkmoth and an unidentified hymenoptoran. 136 hours of observations
Drake and Morden (2006). Undertook a study in montane habitats of Kauai	*Clermontia fauriei*	Visited by Kauai amakihi and Japanese white-eye (2,4 individuals respectively).16.5 hours
	Cyanea leptostegia	Visited by Kauai amakihi and Japanese white-eye (2, 24 individuals respectively). 31 hours
	Trematolobelia kauaiensis	Visited by 'i'iwi, Kauai amakihi (4, 52 individuals respectively). 37 hours
	Lobelia yuccoides	Did not flower during the study
	Lobelia villosa	Did not flower during the study
Gardener and Daehler	*Clermontia kakeana*	No visitors. 37 hours

(2006) Study conducted on Mt Tantalus, Honoulliuli and Pahole on the island of Oahu	*Cyanea pinnatifida*	*Hylaeus* bees were pollinators. Japanese white eyes were nectar robbers. 37 hours.
	Cyanea superba	*Hylaeus* bees were pollinators. 138 hours. Japanese white eyes were nectar robbers

The project proposed here aims to build on our current understanding of the reproductive biology of lobeliads, focussing on the genus *Clermontia. Clermontia* is a genus of 22 species and 9 subspecies of woody shrubs that occur in wet, montane forest habitats throughout the 6 main Hawaiian islands (Lammers 1990). This genus was chosen as it displays a diversity of floral morphology amoung the taxa, which may have evolved in response to avian pollinators (Lammers 1991). A further benefit is that many species of *Clermontia* have large populations with fewer extinctions compared to other lobeliad genera, making experimental studies feasible.

In addition, a seperate study is being undertaken with *Cyanea superba* subsp. *superba* and *Delissea subcordata* in collaboration with Army Environmental Resources, who are undertaking out-plantings of these taxa at Kahanahaiki Gulch on the Island of Oahu. This, and the study with *Clermontia* species, will use identical methodology, thereby allowing useful comparisons to be made.

The projects can be broken into three studies. The first study will assess the floral biology of each species. A suite of floral characters (including nectar and floral morphology) will be collected for each species. These characters will be compared to those that would be expected in bird pollinated plants. In addition, a molecular phylogeny will be constructed to understand the evolution (particularly floral evolution) and dispersal of *Clermontia* species. The second study will identify the breeding system of each species through the use of manipulative pollination experiments. The final study will identify potential pollinators of each *Clermontia* species studied, and those of *C. superba* subsp. *superba* and *D. subcordata.*

Project concept, as it specifically relates to the Pu'u O 'Umi Natural Area Reserve

The Kohala Mountain watershed represents an exceptional habitat for *Clermontia* species, the focal genus of this study. Five of the 22 species of *Clermontia* grow here. Four of these species occur nowhere else in the Hawaiian Islands. I wish to address if pollinators are visiting *Clermontia* spp in the higher elevation areas of the Natural Area Reserve. The reasons for undertaking the study at Pu'u O 'Umi Natural Area Reserve are as follows:

1. The data collected will provide much needed information regarding the pollination of *Clermontia* species in the NAR.
2. The data collected from Pu'u O 'Umi will be directly compared to that recorded at Hakalau National Wildlife Refuge and Hawaii Volcanoes National Park, providing the most comprehensive study of *Clermontia* pollination undertaken to date.
3. The upper regions of the NAR still have remaining populations of honeycreepers, therefore *Clermontia* species gowing at these upper elevations may still be being visited. Again, this will allow comparison of results obtained at Pu'u O 'Umi NAR to those from Hakalau National Wildlife Refuge and Hawaii Volcanoes National Park.

Project concept as it applies to the Pahole Natural Area Reserve

Out-plantings of the endangered *Delissea subcordata* and critically endangered *Cyanea superba* subsp. *superba* have been undertaken at Pahole Natural Area Reserve. The ultimate success of these restoration plantings despends on the successful reproduction of both species, allowing natural recruitment to take place. This study will test the reproductive success of these restoration plantings and will ultimately provide insights into the value of such undertakings.

The specific reasons for applying to use this site are:

1. I am presently undertaking pollination visitation and breeding system studies with *Delissea subcordata* and *Cyanea superba* subsp. *superba* at Kahanahaiki Gulch. Despite the ongoing success of this study, I am limited by the number of individual plants of both taxa on which I can undertake both observations and breeding system studies. By studying the outplantings at Pahole NAR, in addition to those at Kahanahaiki, the sample size of both taxa will be greatly increased, which will improve the overall quality of the study.

2. Similarly, by adding the plants at Pahole NAR to the study, it allows useful comparisons to be made between the pollination of outplantings of each taxon at each respective reserve.

3. Lastly, Pahole NAR represents the last recorded site at which *Cynea superba* subsp. *superba* grew. This being the case, this site may provide greater chances of natural pollinator interactions compared to other restoration sites in the Waianae Range.

Methods

Layout of experimental sites:

Pu ' u O 'Umi NAR

Populations of *Clermontia* species in the upper regions of Pu ' u O 'Umi NAR will be surveyed and mapped using a GPS (subject to the permit being granted). The survey area in which the plants occur will be no larger than 4.5 hectares. All flowering plants will be recorded and identification numbers allotted to each plant using flagging tape. This tape will be removed at the end of the study. The monitoring of floral visitors will be conducted on all flowering plants. Breeding system studies will be conducted on no more than 20 individual flowering plants.

Pahole NAR

At Pahole, flowering plants of *Delissea subcordata* and *Cyanea superba* subsp. *superba* will be identified, marked with flagging tape, and their locations recorded using a GPS. All location marking materials will be removed at the end of the study. All flowering plants will be monitored for visitor activity. Breeding system studies will be conducted on no more than 20 individual flowering plants of each species.

The field-based aspects of the study fall into three studies:

1. Floral biology/pollination syndrome study
2. Breeding system study, and;
3. Floral visitor analysis

1. Pollination syndromes and floral biology of each study species.

The following treatments will use the same 20 flowers

Corolla colour: Twenty flowers will be compared to Royal Horticulture Society colour charts. Colour of floral organs will be recorded and compared between *Clermontia* species. **No flowers will be removed or damaged for this treatment.** *Purpose*: By recording and comparing floral organ color between species, it will be possible to asses the selection pressures that have acted upon each taxon.

Floral measurements: A suite of measurements will be recorded from 20 flowers of each species. These measurements will include: corolla length, sepal length, petal length, stigma and anther length, corolla width in a number of places, sepal width, anther and stigma width and overall corolla curvature. **No flowers will be removed or damaged for this treatment.** *Purpose*: By measuring floral characters, I will be able to compare taxa and how they have evolved. These characters will be compared to a molecular phylogeny that is being undertaken as part of a seperate project to understand how these floral characters have evolved.

Nectar characteristics: Nectar will be sampled from 20 flowers. Samples will be taken from flowers at dawn and dusk for a set time period. Volume will be calculated. Concentration will be determined in the field using a handheld refactometer. Samples will be frozen and nectar sugar composition, ie the concentration of fructose, glucose and sucrose, will be identified using high performance liquid cromatography (HPLC) at the University of Hawaii, Manoa. **Flowers will not be damaged or removed for this treatment, however, the nectar will be removed.** *Purpose*: By sampling nectar, we are able to understand the evolutionary relationships that have occurred in the past. Bird pollinated plants have a nectar concentration and sugar content that is very specific, and I aim to identify the variation in these characteristics between *Clermontia* species.

Phenology: Twenty flowers will be monitored for their flowering period in each species. The duration of male and female phases will be calculated for each flower. **No flowers will be damaged or removed for this treatment**. *Purpose*: A basic understanding of phenology is needed to undertake any further research, as well as implement conservation and management measures.

Pollen viability: Twenty pollen samples will be taken from each species and combined with a solution of lactophenol blue, placed on a microscope slide, and grains counted for viability.The pollen is removed from the anthers by gently tapping the anther while encased in an eppendorf tube. **Flowers will not be damaged for this sampling procedure**. *Purpose*: As I am undertaking crosses between species, it is imperative that I know the pollen that I am using to cross has high viability. The results will also be of general interest in understanding the percentage of pollen in populations that is inviable.

Stigmatic receptivity: Ten stigmas will be tested for receptivity using a dilute solution of hydrogen peroxide applied to stigmas. The presence of bubbling on the stigma suggests peroxidase activity, indicating that enzymes are being produced for pollen germination. **This treatment will not damage the flowers.** *Purpose*: An understanding of stigmatic receptivity is imperative to undertake manipulative crosses between plants. It is also of general interest for an overall understanding of each species.

The following treatments require flowers or parts of flowers to be removed, in addition to the treatments addressed above.

Ovule number: Twenty fruits will be removed when mature, and fertilized and unfertilized ovules counted. **Counting the ovules will destroy the flowers**. *Purpose*: This treatment will be used to compare with the pollen limitation and mating system studies (described below), to assess the rates of fertilization that are occuring in each treatment.

Treatment	Method	Sample	Different	Will the

		size	or existing flowers	flowers be removed or damaged?
Floral measurements	Manual floral measurement of a suite of floral organs while the flower is in anthesis.	20	Existing	No
Nectar secretion rate	Nectar production recorded in the morning (7 - 9am) and evening (5 - 6 PM) in bagged vs open flowers	20	Existing	No
Sugar composition of nectar	High performance liquid chromatography will be used to analyse nectar. Analysis of nectar sugars following the methods of Freeman et al. (1984)	20	Existing	No
Nectar concentration	Nectar concentration recorded at the time of collection using a handheld refractometer	20	Existing	No
Corolla color	Floral organs compared to Royal Horticulture Society color charts	20	Existing	No
Phenology	Calculate average time of flowering	20	Existing	No
Pollen viability	Test of pollen viability using lactophenol blue	20	This is a non-invasive technique. No flower will be removed. Taken from the existing 20 flowers	No
Stigmatic receptivity	Stigmatic receptivity tested using hydrogen peroxide	10	Existing	No
Ovule number	Flowers removed and ovules counted	20	Additional to the above	Yes
Total number of flowers needed for this part of the study in each study species.....40. Twenty flowers will be removed. Twenty will not be removed or damaged in anyway.				

2. Mating system study

A study of mating systems will be carried out. Manipulative pollinations will be undertaken using 4 treatments. This will require 120 flowers for each study species. These will be obtained from 20 flowering plants per species. These flowers will not be damaged or removed from the plant, but the fruit that follow will be removed. The seeds that these fruit contain will be returned to the reserve for restoration plantings. The four treatments are as follows:

1. Control-flowers labelled and left untouched (tests natural seed set under normal field conditions) (30 flowers).
2. Test for selfing-flowers bagged with 5cm x 5cm material bags to exclude pollinators while in bud and left to flower (30 flowers).
3. Test for rate of out-crossing-flower emasculated (anthers removed) and unbagged (30 flowers).
4. Test for pollen limitation- flower emasculated (anthers removed) and bagged (as for selfing treatment), supplemental outcross pollen applied and flower rebagged (30 flowers).

The purpose of these crosses is to assess if out-cross pollen limitation is taking place in the populations of the respective taxa. The treatments will also confirm if each taxon is capable of self pollination.

By collecting and weighing each fruit, counting the seeds, and then germinating a subsample of the seeds from each treatment, I will test the vigour of seedlings of each treatment. The purpose of this is to test for genetic differences between the treatments, particularilily if selfing causes inbreeding depression compared to outcrossed seedlings, which is the expected result here.

Seeds of *Delissea subcordata* and *Cyanea superba* subsp. *superba* will be germinated at Army Environmantal Resources propagation facilities at Schoefield Barracks on the Island of Oahu. Seeds of *Clermontia* species from Pu 'u O 'Umi NAR will be germinated at UH Hilo and transferred to UH growing facilities at Hawaii Volcanoes National Park. Seedlings will be grown on for 1 year. After one year, the seedlings will be measured once before being returned for outplanting. The following measurements will be recorded:

1. Leaf length
2. Leaf width
3. Total number of leaves
4. Plant height
5. Stem diameter at base
6. General health of plant

3. Pollination biology study:

Floral Visitors

Invertabrate visitors: Three observation periods will be undertaken for invertabrate visitors to both species. These will be conducted at 25%, 50% and 75% flowering for the entire population (ie estimated total flowering within the population). The observation periods consist of three consequtive days (9 days in total), with observations occuring between dawn (5am) and dusk (6pm). The observations are undertaken by standing within 2 metres of the focal plant and recording insect visitors to the plants in alloted 10 minute time periods. I will rotate through the populations of each study species, recording insect visitation(s) to all flowering plants. The activity of the insects: whether they make anther or stigma contact, whether they forage on pollen, and the overall behaviour of the insect will be recorded during this period.
In instances where insects are visiting the flowers of each study species, I will record their visitation to the flowers and use a tag name to identify future visitations by these visitors. If more than 5 visitations are recorded for each insect identified, I propose collecting the insect for expert identification. No more than 10 individuals within a species will be collected. Any insects collected will be identified by Bishop Museum entomologists. All collections will be given to the Bishop Museum for permanent storage once the project is complete.

Vertabrate visitors: Three observation periods will be undertaken for vertabrate visitors to both species. Observation periods will be conducted at 25%, 50% and 75% flowering for the entire population (ie estimated total flowering within the population). The observation periods consist of three consequtive days (9 days in total), with observations occuring between dawn (5am) and dusk (6pm). The observations are undertaken by sitting no less than 15 metres from the focal plant(s). Using bicoulars, the visitors to these plants, and the duration of their visits, will be recorded. Anther and stigma contact with the head or body parts of vertabrate visitors will be recorded also, as will the behaviour and direction of entry and exit to and from the plant.

In addition, vertabrate visitors will be recorded using video cameras and digital video recorders (DVR's) throughout the flowering season of each taxon. Up to 200 hours of video footage will be recorded for each plant species. Vertabrate visitors will be identified to species level and their behaviour while visiting the plants recorded, as above.

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b) if the research is part of your undergraduate or graduate studies, please include the name and affiliation of your major professor;

I am a PhD candidate at the University of Hawaii at Manoa.

Dr. Clifford Morden
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Fax: (808) 956 3923
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c) If you are seeking permission to remove or introduce any form of plant or animal life, please list all species involved and specifically identify which are threatened, endangered, or candidate species.

Delissea subcordata: Endangered
Cyanea superba subsp. superba: Critically endangered
Clermontia parviflora: Secure
Clermontia kohalae: Secure
Clermontia drepanomorpha: Endangered
Clermontia waimeae: Secure
Clermontia calophylla: Secure

d) if you are seeking permission for the collection of any specimens, please note type of specimen (species and parts collected, if less than entire specimen), quantities to be collected, storage methods, and ultimate disposition.

Species to be collected:

Delissea subcordata
Cyanea superba subsp. superba
Clermontia parviflora
Clermontia kohalae
Clermontia drepanomorpha
Clermontia waimeae
Clermontia calophylla

Number of specimens to be collected:

1. Flowers: 20 flowers from each study species will be removed for the counting of ovules. These flowers will be stored in preserving alcohol and stored at UH Manoa until the ovule counting is undertaken. The counting of ovules will destroy the ovary of each flower. I do not plan to store the remaining parts of the flowers longterm, given that they will have the female reproductive parts removed.
2. Anthers: 60 anthers will be removed as part of the breeding system study. The anthers will be stored in preserving alcohol for the duration of the study and donated to Bishop Museum at the end of the study.
3. Fruit: 120 fruits per study species: Fruits will be collected as part of the breeding system study. The seeds will be extracted, germinated, and grown on for one year and returned to the respective reserves for out-planting.

4. Please answer the following questions about your proposed special use:

a. Can your proposed special use be conducted elsewhere? If not, why not?

Regarding Pahole NAR: Both study species have been out-planted at Kahanahaiki gulch to the west of Pahole NAR. However, at Kahanahaiki the populations of both taxa are relatively small. By adding plants of both taxa from Pahole into a broader study, a greater sample size will be obtained. The study will also provide valuable comparisons to be made between the two sites.

A further justification for the inclusion of Cyanea *superba* subsp. *superba* is the fact that Pahole is the last recorded natural site at which this taxon occurred. This being the case, this site may provide greater insights into natural pollinator interactions when compared with other restoration sites in the Waianae Range.

Regarding Pu 'u O 'Umi NAR: Four of the five *Clermontia* species in the Kohala Mountains occur no where else. The NAR represents the most pristine habitat in which these taxa and honeycreepers occur, thereby increasing the chances of observing pollinator interactions, allowing critical information to be obtained regarding the pollination of these species.

a. Is your proposed special-use consistent with the purpose and objectives of the Natural Area Reserves System (the purpose and objective of the NARS is to protect in perpetuity specific land and water areas which support communities, as relatively unmodified as possible, of the natural flora and fauna of Hawai`i)? If so, how?

Yes.

All seeds removed as part of the study will be returned to the respective sites for out planting.

The greatest care will be taken to minimize damage to the under-story of the forest. I will walk only along trails where possible. Where off-trail access must be undertaken, I will minimize the use of these trails. No cutting of trails will be undertaken, nor will any plants be cut or removed for access.

a. Is your proposed special-use consistent with the management plan developed for the individual Reserve(s) (*Management plans are available for review at* www.dofaw.net/nars *or by contacting the NARS office*)?

I have read both the Pu'u O 'Umi NAR management plan and the Pahole Environmental Assessment available at www.dofaw.net/nars and believe that my research is consistent with the conservation goals outlined in both documents.

b. Does your proposed special-use provide a benefit (direct or indirect) to the Natural Area Reserves System or to the individual Reserve(s) or both? (*For research, please note whether any studies have previously been made similar to the one proposed and how you will convey your research findings to the Department*).

Will this research provide a benefit? Yes.

Currently we have virtually no understanding of what is pollinating lobeliads, both within Pu'u O 'Umi and Paholae NAR, and throughout the archipelago. Given the precarious conservation status of the majority of honeycreeper and lobeliad species, it is an urgent necessity that we begin to learn how this plant-animal interaction is operating, partly to document this incredible co-evolution, and partly to provide managers with information that may be useful to ensure the longterm survival of the remaining mutualists.

Has similar research been conducted? Yes. The following three studies have been undertaken. All three tried to establish what, if anything, was visiting the flowers of the respective lobeliad species. Only one paper, that of Gardener and Daehler (2006) has been published.

Cory, C. (1984) Pollination biology of two species of Hawaiian Lobeliaceae (*Clermontia kakeana* and *Cyanea angustifolia*) and their presumed co-evolved relationship with native honeycreepers (Drepanididae). Unpublished masters thesis. California State University, Fullerton.

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What results will be provided: Reports will be submitted at the end of each field season. One publication will be submitted to a peer reviewed scientific journal. A poster or presentation will be presented at a Hawaiian and international conference.

C. Will the proposed special-use damage or threaten to damage the integrity or condition of the natural, geological, or cultural resources in the individual Natural Area Reserve(s) and adjacent area or region? If so, how? If not, why not?

Yes. There is a possibility that walking between plants observing pollinators may lead to the formation of trails in both NARS. I anticipate that no other significant damage will occur while undertaking this study at the respective NAR's.

d. Does the proposed special-use comply with the provisions and guidelines contained in HRS Chapter 205A, entitled 'Coastal Zone Management,' where applicable? ***HRS Chapter 205A can be accessed at: http://www.capitol.hawaii.gov/hrscurrent/Vol04_Ch0201-0257/HRS0205A/***

This is not applicable to my research.

e. Have you (the applicant) previously received a NARS Special Use Permit? If so, did you comply with the conditions of any previously approved permit (including providing a final report as requested)?

I have not previously received a NARS Special Use Permit.

f. Do you (the applicant) have any other current NARS special-use permits? If so, please list and state whether you are currently in compliance with the conditions of those permits.

I do not have any other NARS Special Use Permits currently. I am however applying for several T and E permits in conjunction with this permit application.

5. Is the proposed special-use expected to have an environmental impact on the Natural Area Reserve (s) or the surrounding area? If, so please elaborate. If not, why not? ***Please include discussion of any off-trail work, such as mist-netting, setting of traps, removal of vegetation, etc. and any measures planned to mitigate any short and long-term damage.***

Yes. I have to walk between plants within each reserve. This will likely create small trails between some of the study plants at each reserve. The greatest care will be taken to avoid damaging the forest understorey, however, some trail formation is unavoidable in a study of this nature.

Given the short duration of this study (three weeks of work at each site, plus several short follow-up visits), the damage created by trail formation will be short-lived, and I believe the benefits of the knowledge that we will obtain will out-way this short-term damage.