Hybridization between native and non-native plant species in the riparian ecosystem

Project Information

1. **Proposal Title:**

   Hybridization between native and non-native plant species in the riparian ecosystem

2. **Proposal applicants:**

   Kristina Schierenbeck, California State University, Chico

3. **Corresponding Contact Person:**

   Kristina Schierenbeck
   Calif. State Univ. Chico
   Dept. of Biological Sciences Chico, CA 95929-0515
   530 898-6410
   kschierenbeck@csuchico.edu

4. **Project Keywords:**

   At-risk species, plants
   Genetic Conservation and Engineering
   Nonnative Invasive Species

5. **Type of project:**

   Research

6. **Does the project involve land acquisition, either in fee or through a conservation easement?**

   No

7. **Topic Area**:

   Non-Native Invasive Species

8. **Type of applicant:**

   University

9. **Location - GIS coordinates:**

Latitude:	40.0
Longitude:	-121.5
Datum:	

Describe project location using information such as water bodies, river miles, road intersections, landmarks, and size in acres.

in riparian corridors along Sacramento River and its tributaries

10. **Location - Ecozone:**

    3.3 Chico Landing to Colusa, 4.1 Clear Creek, 7.3 Mill Creek, 7.4 Deer Creek, 7.5 Big Chico Creek, 7.6 Butte Creek, 8.1 Feather River, 9.2 Lower American River, 10.1 Cache Creek, 10.2 Putah Creek

11. **Location - County:**

    Butte, Colusa, Glenn, Plumas, Tehama, Yolo

12. **Location - City:**

    Does your project fall within a city jurisdiction?

    Yes

    If yes, please list the city: Chico

13. **Location - Tribal Lands:**

    Does your project fall on or adjacent to tribal lands?

    No

14. **Location - Congressional District:**

    second

15. **Location:**

    California State Senate District Number: 1

    California Assembly District Number: 3

16. **How many years of funding are you requesting?**

    3

17. **Requested Funds:**
    a) Are your overhead rates different depending on whether funds are state or federal?

       Yes

       If yes, list the different overhead rates and total requested funds:

State Overhead Rate: 20 TDC

Total State Funds: 143490

Federal Overhead Rate: 45 S&W

Total Federal Funds: 149846

b) Do you have cost share partners <u>already identified</u>?

No

c) Do you have <u>potential</u> cost share partners?

No

d) Are you specifically seeking non-federal cost share funds through this solicitation?

No

If the total non-federal cost share funds requested above does not match the total state funds requested in 17a, please explain the difference:

18. **Is this proposal for next-phase funding of an ongoing project funded by CALFED?**

No

Have you previously received funding from CALFED for other projects not listed above?

No

19. **Is this proposal for next-phase funding of an ongoing project funded by CVPIA?**

No

Have you previously received funding from CVPIA for other projects not listed above?

No

20. **Is this proposal for next-phase funding of an ongoing project funded by an entity other than CALFED or CVPIA?**

No

Please list suggested reviewers for your proposal. (optional)

Ingrid Parker UC Santa Cruz

Linnea Hansen U. S. Forest Service

Carla D'Antonio UC Berkeley

Norm Ellstrand UC Riverside

21. **Comments:**

Environmental Compliance Checklist

<u>Hybridization between native and non-native plant species in the riparian ecosystem</u>

1. **CEQA or NEPA Compliance**
   a) Will this project require compliance with CEQA?

      No
   b) Will this project require compliance with NEPA?

      No
   c) If neither CEQA or NEPA compliance is required, please explain why compliance is not required for the actions in this proposal.

      There will be no environmental impact as a result of this project

2. **If the project will require CEQA and/or NEPA compliance, identify the lead agency(ies).** *If not applicable, put "None".*

   <u>CEQA Lead Agency:</u> California Department of Fish and Game
   <u>NEPA Lead Agency (or co-lead:)</u>
   <u>NEPA Co-Lead Agency (if applicable):</u>

3. **Please check which type of CEQA/NEPA documentation is anticipated.**

   CEQA
   -Categorical Exemption
   -Negative Declaration or Mitigated Negative Declaration
   -EIR
   Xnone

   NEPA
   -Categorical Exclusion
   -Environmental Assessment/FONSI
   -EIS
   Xnone

   If you anticipate relying on either the Categorical Exemption or Categorical Exclusion for this project, please specifically identify the exemption and/or exclusion that you believe covers this project.

4. **CEQA/NEPA Process**
   a) Is the CEQA/NEPA process complete?

      Not Applicable

   b) If the CEQA/NEPA document has been completed, please list document name(s):

5. **Environmental Permitting and Approvals** (*If a permit is not required, leave both Required? and Obtained? check boxes blank.*)

LOCAL PERMITS AND APPROVALS

Conditional use permit

Variance

Subdivision Map Act

Grading Permit

General Plan Amendment

Specific Plan Approval

Rezone

Williamson Act Contract Cancellation

Other

STATE PERMITS AND APPROVALS

Scientific Collecting Permit   Required, Obtained

CESA Compliance: 2081

CESA Compliance: NCCP

1601/03

CWA 401 certification

Coastal Development Permit

Reclamation Board Approval

Notification of DPC or BCDC

Other

FEDERAL PERMITS AND APPROVALS

ESA Compliance Section 7 Consultation

ESA Compliance Section 10 Permit

Rivers and Harbors Act

CWA 404

Other

PERMISSION TO ACCESS PROPERTY

Permission to access city, county or other local agency land. Agency Name: City of Chico	Required, Obtained
Permission to access state land. Agency Name: California Department of Fish and Game	Required, Obtained
Permission to access federal land. Agency Name: United States Fish and Wildlife Service	Required, Obtained
Permission to access private land. Landowner Name:	

6. **Comments.**

Collections for this project requires only that we collected one to two leaves from very large trees. There will be very limited impact as a result of this activity.

Land Use Checklist

<u>Hybridization between native and non-native plant species in the riparian ecosystem</u>

1. **Does the project involve land acquisition, either in fee or through a conservation easement?**

   No

2. **Will the applicant require access across public or private property that the applicant does not own to accomplish the activities in the proposal?**

   Yes

3. **Do the actions in the proposal involve physical changes in the land use?**

   No

   If you answered no to #3, explain what type of actions are involved in the proposal (i.e., research only, planning only).

   Research only, one to two leaves collected from large trees.

4. **Comments.**

Conflict of Interest Checklist

Hybridization between native and non-native plant species in the riparian ecosystem

Please list below the full names and organizations of all individuals in the following categories:

- Applicants listed in the proposal who wrote the proposal, will be performing the tasks listed in the proposal or who will benefit financially if the proposal is funded.
- Subcontractors listed in the proposal who will perform some tasks listed in the proposal and will benefit financially if the proposal is funded.
- Individuals not listed in the proposal who helped with proposal development, for example by reviewing drafts, or by providing critical suggestions or ideas contained within the proposal.

The information provided on this form will be used to select appropriate and unbiased reviewers for your proposal.

Applicant(s):

Kristina Schierenbeck, California State University, Chico

Subcontractor(s):

Are specific subcontractors identified in this proposal? No

Helped with proposal development:

Are there persons who helped with proposal development?

No

Comments:

Budget Summary

Hybridization between native and non-native plant species in the riparian ecosystem

Please provide a detailed budget for each year of requested funds, indicating on the form whether the indirect costs are based on the Federal overhead rate, State overhead rate, or are independent of fund source.

Federal Funds

Year 1												
Task No.	**Task Description**	**Direct Labor Hours**	**Salary *(per year)***	**Benefits *(per year)***	**Travel**	**Supplies & Expendables**	**Services or Consultants**	**Equipment**	**Other Direct Costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
1	Project coordination, training and assistance in data collection, writing final reports	160	6557	787	500	0	0	0	0	7844.0	2951	10795.00
2	Plant and date collection, data analysis in field and laboratory, wriring final reports	16320	26880	1843	500	21000				50223.0	12096	62319.00
3	Conference Presentation				1500	0				1500.0		1500.00
		16480	33437.00	2630.00	2500.00	21000.00	0.00	0.00	0.00	59567.00	15047.00	74614.00

Year 2												
Task No.	**Task Description**	**Direct Labor Hours**	**Salary *(per year)***	**Benefits *(per year)***	**Travel**	**Supplies & Expendables**	**Services or Consultants**	**Equipment**	**Other Direct Costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
1	Project coordination/oversight/reporting	160	6951	834	500					8285.0	3128	11413.00
2	Field research - 2 graduate students	16320	26880	1843	1000	21000				50723.0	12096	62819.00
3	Conference presentation				1000					1000.0		1000.00
		16480	33831.00	2677.00	2500.00	21000.00	0.00	0.00	0.00	60008.00	15224.00	75232.00

Year 3												
Task No.	**Task Description**	**Direct Labor Hours**	**Salary *(per year)***	**Benefits *(per year)***	**Travel**	**Supplies & Expendables**	**Services or Consultants**	**Equipment**	**Other Direct Costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Grand Total=149846.00

Comments.

Budget Justification

Hybridization between native and non-native plant species in the riparian ecosystem

Direct Labor Hours. Provide estimated hours proposed for each individual.

Schierenbeck 160 hours 2 Graduate students 640 hours each during the academic year 2 Graduate students 480 hours each during the summer

Salary. Provide estimated rate of compensation proposed for each individual.

Schierenbeck Annual salary divided by 9 months = 1 summer month @ $6,557 Graduate students @ $12/hour

Benefits. Provide the overall benefit rate applicable to each category of employee proposed in the project.

Schierenbeck Summer benefit rate = 12% Graduate students rates = 3% during the academic year and 12% during the summer

Travel. Provide purpose and estimate costs for all non-local travel.

Schierenbeck and graduate students will present research at one conference per year. Site to be determined. All other travel costs are for travel to field sites.

Supplies & Expendables. Indicate separately the amounts proposed for office, laboratory, computing, and field supplies.

Enzymes, PCR reagents and miscellaneous lab chemicals and disposables

Services or Consultants. Identify the specific tasks for which these services would be used. Estimate amount of time required and the hourly or daily rate.

None

Equipment. Identify non-expendable personal property having a useful life of more than one (1) year and an acquisition cost of more than $5,000 per unit. If fabrication of equipment is proposed, list parts and materials required for each, and show costs separately from the other items.

None

Project Management. Describe the specific costs associated with insuring accomplishment of a specific project, such as inspection of work in progress, validation of costs, report preparation, giving presentatons, reponse to project specific questions and necessary costs directly associated with specific project oversight.

Fifty percent of Schierenbeck's time will be devoted to training students in plant identification and data collection each year and for project management. 37.5% of her time will be used for writing final reports. 12.5% will be used for conference preparation and attendance.

Other Direct Costs. Provide any other direct costs not already covered.

None

Indirect Costs. Explain what is encompassed in the overhead rate (indirect costs). Overhead should include costs associated with general office requirements such as rent, phones, furniture, general office staff, etc., generally distributed by a predetermined percentage (or surcharge) of specific costs.

The CSU, Chico Research Foundation's federally-negotiated indirect cost rate is 45% of salaries and wages. The current rate was negotiated with the U.S. Department of Health and Humans Services and will be in effect until 06/30/04. It does not cover fringe benefits.

Executive Summary

Hybridization between native and non-native plant species in the riparian ecosystem

The protection and restoration of the remaining California riparian ecosystems depends on a thorough understanding of species, community, and ecosystem complexity, made more difficult by the persistence of non-native plant species. Two important canopy species throughout riparian ecosystems in California are Platanus racemosa (California sycamore) and Juglans hindsii (Northern California black walnut). We propose as an initial step of a long-term series of genetic and ecological experiments, to identify native genotypes of Platanus racemosa and Juglans hindsii and determine the extent these species are hybridizing with their respective non-native congeners. Although Platanus racemosa and Juglans hindsii are quite different taxa, both are important components of riparian forests within the Sacramento Valley riparian Ecozone. We will analyze two regions (ITS-1 and ITS-2) of nuclear ribosomal RNA (nrDNA) among the native and non-native taxa using genetic markers (ITS) from each of the putative parents to reach the following objectives: 1.) characterize the native and non-native genotypes for Juglans and Platanus species currently occurring in and around Northern California riparian forests. 2.) identify the extent and direction of hybridization that has occurred in putative Juglans hindsii in riparian forests of Northern California. 3.) identify the extent and direction of hybridization that has occurred in putative Platanus racemosa in riparian forests of Northern California.

Proposal

California State University, Chico

Hybridization between native and non-native plant species in the riparian ecosystem

Kristina Schierenbeck, California State University, Chico

Hybridization between Native and Non-native Plant Species in the Riparian Ecosystem

Kristina A. Schierenbeck, Ph.D.
California State University, Chico
Department of Biological Sciences
Chico, CA 95929-0515

A. Project Description: Project Goals and Scope of Work

Less than five percent of the riparian forests present in California prior to European settlement remain, and of these forests, more than half are degraded and in poor condition (Barbour et al 1993). Riparian trees serve to reinforce riverbanks, provide greater stability to stream channels, act as windbreaks, and reduce evaporation, transpiration and wind damage to other riparian species (Thompson 1961). The degradation of riparian ecosystems is exacerbated by the invasion of non-native vegetation, preventing the successful establishment and persistence of native species and communities, and disrupting ecosystem processes (McIntrye et al. 1988, Griffin et al. 1989, Thebaud and DeBussche 1991, Huenneke and Thompson 1995).

The protection and restoration of the remaining California riparian ecosystems depends on a thorough understanding of species, community, and ecosystem complexity, made more difficult by the persistence of non-native plant species. Two important canopy species throughout riparian ecosystems in California are *Platanus racemosa* (California sycamore) and *Juglans hindsii* (Northern California black walnut) (Holland and Keil 1995). Fossil records date ancestors of *Juglans hindsii* and *Platanus racemosa* back to the late Tertiary fossil period 20 million years ago (Chaney et al., 1944).

The Nature Conservancy has noted that seedlings used from wild-collected seed from *Platanus racemosa* for restoration of riparian woodlands may be the product of hybridization with the non-native *Platanus X acerifolia* (London plane tree) (Tom Griggs, personal communication). The taxonomy of *Juglans hindsii* has been a point of confusion for decades and has been complicated by its propensity to hybridize with a number of agricultural walnut species. Both *Platanus* spp. and *Juglans* spp. are wind pollinated and because their non-native congeners are planted frequently in California as shade or agricultural trees, non-native pollen is likely creating new hybrids. Virtually nothing is known about the population structure of either of these important riparian tree species, to what extent their genetic integrity is maintained, and of the comparative invasive properties of their hybrid offspring.

1. Problem: We propose, as an initial step of a long-term series of genetic and ecological experiments, to identify native genotypes of *Platanus racemosa* and *Juglans hindsii* and determine the extent these species are hybridizing with their respective non-native congeners. Although *Platanus racemosa* and *Juglans hindsii* are quite different taxa, both are important components of riparian forests within the Sacramento Valley riparian Ecozone. We will analyze two regions (ITS-1 and ITS-2) of nuclear ribosomal RNA (nrDNA) among the native and non-native taxa using genetic markers (ITS) from each of the putative parents to reach the following ***objectives:***

1.) characterize the native and non-native genotypes for *Juglans* and *Platanus* species currently occurring in and around Northern California riparian forests.

2.) identify the extent and direction of hybridization that has occurred in putative *Juglans hindsii* in riparian forests of Northern California.

3.) identify the extent and direction of hybridization that has occurred in putative *Platanus racemosa* in riparian forests of Northern California.

Additionally, these data will allow the determination of rates of gene flow and regional incidence of hybridization. Further, these data are crucial to set the stage for ecological experimentation to (funding **not** requested in this grant) 1.) determine fitness differences between the genotypes, 2.) estimate rates of invasion of non-native genotypes, 3.) determine differential ecosystem effects of the native and non-native genotypes.

2. Justification

Juglans hindsii (Northern California black walnut)

Juglans hindsii is listed as a rare and endangered species endemic to California (Skinner 1994), however the taxonomic status of the *J. hindsii* has not been clear for over 100 years. Between 1908 and 1923, Jepson used either *Juglans californica* var. *hindsii* or *J. hindsii* to refer to this species (Howell 1973). Wilken (1993) stated the northern and southern California Black Walnuts were conspecific, and thus *J. californica* var. *hindsii* and *J. californica* var. *californica*, respectively, are often the accepted taxa (Hickman 1993). Fjellstrom and Parfitt (1994) and McGranahan et al. (1988), however, used genetic data to conclude that *J. hindsii* was distinct from *J. californica*. Stanford et al. (2000) used chloroplast and ribosomal DNA sequencing to study the biogeography of the Juglandaceae and also distinguished *J. hindsii* from *J. californica*. Throughout this proposal we refer to the Northern California black walnut as *J. hindsii*.

Although Jepson did not designate a holotype for *J. hindsii*, Howell (1973) designated a lectotype choosing a specimen that Jepson had collected in Napa County, California in 1903. Grifffin & Critchfield (1972) mapped three stands in Contra Costa County, one in Solano County, and 13 questionable stands in seven counties. Skinner & Pavlik (1994) considered three stands native, two of which are extant. Thomsen (1963) reviewed the paleobotanical literature of Axelrod and others and concluded that *J. hindsii* was native to California with origins distinct from *J. californica*. She argued that it was part of the Arcto-tertiary flora that had been restricted to a few locations in the central valley of California as the climate had changed from subtropical to Mediterranean during the last 20 million years.

The commercial success of *J. regia* (Persian or English walnut), and the predominant use of *J. hindsii* hybrids as *J. regia* root stock has added to the taxonomic confusion of wild black walnuts. *Juglans regia* has been grown commercially in California since the 1850's and four additional species of black walnuts not native to northern California have been used for root stock: *J. nigra*, *J. californica, J. microcarpa* and *J. major* (D. Potter, personal communication). The Paradox hybrid (*J. regia* X *J. hindsii)* is used as rootstock in over 95% of the *J. regia* orchards (Champion 1967). Abandoned and neglected orchards are taken over by the hearty rootstock, hybrid nuts are carried down stream and hybrid pollen is cast to the wind.

Juglans nigra or the eastern black walnut introduces further confusion. In the 1800's many settlers planted *J. nigra* for its lumber, nut and shade values. John Bidwell was an early proponent of *J. nigra* and planted them throughout Chico and sold them to Northern

California clients from his nursery. Black walnuts are widely naturalized in riparian habitats of Northern California and are common along the streets of many Central Valley towns (Champion 1967). The taxonomic status of these black walnuts is in question, due to widespread introductions of other black walnut species since the 1800's that readily hybridize with *J. hindsii*. All black walnut hybrids are fertile (Fuller 1978), and thus can backcross to produce a variety of hybrids. F1 hybrids are easy to distinguish, but this is not so for successive crosses and backcrosses (personal observation). Chico has over 10,000 black walnuts (Champion 1967) and two local botanists (R. Schlising and W. Dempsey, personal communication) have noted intermediate forms, with respect to leaves and fruits, and consider many of these black walnuts as hybrids.

Platanus racemosa (California Sycamore)

The range of *Platanus racemosa* extends throughout California and down the coastal side of the Peninsular ranges into Baja, California. The distribution of *P. racemosa* occurs along the Transverse and Peninsular ranges, along the southwestern side along the Sierra Nevada and along the Sacramento and San Joaquin rivers of the Central Valley, where it ascends the main tributaries to low elevations in the Sierra Nevada foothills (Schoenherr 1992). It is a common tree throughout limited remaining riparian ecosystems in California.

Platanus X acerifolia (London plane tree) originated at the Oxford Botanic Garden in England about 1670 (Henry and Flood, 1919) as a result of hybridization between *P. orientalis* (oriental plane tree) and *P. occidentalis* (American sycamore). *Platanus X acerifolia* is a heterogeneous cross of the parent trees and thus shows extreme variability in form and growth rate. Because it is more resistant to sycamore anthracnose, it is widely used instead of native sycamores as an ornamental tree (Oswald 1994).

It has been noted that in both crosses between *P. occidentalis* and *P. racemosa* and crosses between *P. orientalis* and *P. racemosa* viable seeds are produced (Santamour 1971). The chromosome number in all four species (including *P. x acerifolia*) is $2n = 42$ (Santamour 1969, 1971).

3. Approach

Collection of Samples: For significant statistical analysis each population collected consists of twenty individuals and are located at least ten miles apart to avoid the possibility of pollination between populations.

Sample Collection of *Juglans*

<u>Northern California Black Walnut samples:</u> Fresh leaves of old growth *J. hindsii* will be collected from the two remaining native stands in Contra Costa and Napa counties. Dry plant material of *J. hindsii* will be collected from herbarium specimens, including the lectotype, at University of California in Berkeley. These samples will provide, as much as is feasible, pure *J. hindsii*.

<u>Riparian walnut stands:</u> Fresh leaves of black walnut (of varying composition) will be collected from eight riparian populations along the Sacramento, Consumnes, and American Rivers and Big Chico Creek. These samples will provide us with a measure of gene flow occurring in naturalized stands.

Black Walnut samples: Fresh leaves of *J. nigra* will also be collected from three sites yet to be determined east of the Mississippi River. Vouchers of the fresh plant material will be deposited in the herbarium at California State University, Chico.

Agricultural Species samples: Fresh leaves from at least (as available) 10 samples of the species that hybridize with *J. hindsii* (*J. nigra*, *J. regia*, *J. californica*, *J. microcarpa*, and *J. major*) will be obtained from the National Center Germplasm Center near Davis California. Collection data and parentage information is available for all samples at the germplasm center.

Sample Collection of *Platanus*

California sycamore samples: Eight populations of old growth *P. racemosa* isolated from urban areas will be collected. Care will be taken to gather leaves from trees existing before the introduction of *P. x acerifolia* to Northern California. Collections will be made from the following locations: on the Sacramento river (north to south): Jelly's Ferry above Red Bluff, Woodson Bridge near Corning, Princeston Ferry and Garden Hwy above Sacramento; and the Sacramento river tributaries (north to south), Battle creek near Anderson, Pine creek above Chico, Big Chico creek in Chico, Yuba river near Smartville.

Eastern sycamore samples: Four populations have been provided from collaborators in Virginia (north to south): Whistle creek near Lexington, Buffalo creek near Lexington, New River near Pembroke and Sinking creek near Maybrooke.

Oriental plane tree samples: Four populations have been provided from collaborators in Europe (north to south): Vannes (Dept. of Morbihan, Brittany), Rennes (Dept. of Ille et Vilaine), Brittany), Saint Leger (Dept. of Charentes Maritimes), and Balarac (Dept. of Herault).

Suspected hybrid samples: Four populations of *Platanus spp*. taken from saplings displaying hybrid leaf morphology were collected (north to south): Mill Creek at hwy 99, Deer creek at hwy 99, Butte creek below Chico, and Feather river in Marysville. In addition, samples of seedlings planted by the Nature Conservancy at Phelan Island (near the Sacramento river) will be assessed for the percentage of the young growth that are hybrids.

DNA Methodology

DNA from each of the leaves from all populations will be extracted using Bio 101 Fast Prep Kit. The DNA will be treated with primers to amplify the ITS-1 and ITS-2 regions within the 18S-26S nrDNA gene family (White et al. 1990). The 18S-26S nrDNA gene family has proven to be a valuable tool for phylogeny reconstruction in plants. Ribosomal DNA is arranged in tandem repeats in one or a few chromosomal loci and consist of a single transcribed region containing the external transcribed spacer (ETS), the 18-18S gene, an internal transcribed spacer (ITS-1), the 5.8S gene, a second internal transcribed spacer (ITS-2), and the 26S gene (Hamby and Zimmer 1992).

We have successful amplification from a number of samples for both *Platanus* and *Juglans* for the nuclear ITS-1 and ITS-2 regions of the respective study species. ITS sequences for both *Platanus* and *Juglans* are available (GenBank, NCBI) and have used to identify restriction sites to separate the native species and the species putatively hybridizing with the native species. The ITS-1 and ITS-2 sites will be amplified using polymerase chain reaction (PCR) for all samples and subsequently cut with restriction enzymes. The resulting PCR/restriction digestion product will be run on an agarose gel using electrophoresis, dyed with ethidium bromide, then photographed.

In addition, three samples from each study taxon will be sequenced and submitted to GenBank (NCBI) for access by other researchers.

Data Analysis

Restriction site polymorphisms and length variations will be scored as binary markers. Phylogenetic analyses of haplotypes will be used using Wagner parsimony of PAUP (Swofford 2000), and internal consistencies analyzed by bootstrap analysis. The frequency of species-specific markers will be assessed and compared with marker presence in the hybrid individuals. Population and taxon level chi-square tests will be executed to determine the expected versus actual frequency of different genotypes. Depending on the number of markers in species, and variation within species, patterns in gene flow between the native and non-native species will be examined. The geographic distribution among the different genotypes will be assessed using GeoDis (Posada et al. 1999) which uses nested clade analysis among populations to analyze patterns of intra- and interspecific gene flow.

We will measure the measure genetic diversity between parent and composite wild populations, and between distinct wild populations, using average heterozygosity, proportions of polymorphic genes, F-statistics and most-parsimonious trees.

4. Feasibility

This work is being and will continue to be conducted by myself (K. Schierenbeck) and my graduate students (Paul Kirk and Darhl Whitlock) during the 2002/2003 and 2003/2004 academic years. The DNA analyses will be conducted in Dr. Schierenbeck's laboratory within the biology department at CSU Chico. The lab has the gel apparatuses, power packs, centrifuges, refrigerators, ultracold freezers, and MJ Research thermocycler to conduct this research.

Dr. Schierenbeck has extensive experience with the use of the PCR technique through numerous publications and presentations and is very familiar with its strengths and shortcomings. The funds requested here are for chemical supplies, tips and tubes in the lab to complete the DNA analyses; the enzymes necessary to complete this work are requested here.

Research on this project thus far has been provided by a combination of funds from the California Native Plant Society, the College of Sciences and the Department of Biological Sciences. Further funding is absolutely necessary to meet the goals outlined in this proposal.

5. Performance Measure

Completed by Spring/Summer 2002: Plant sample collection, DNA extraction
Completed by end of Summer 2003: DNA amplifications, restriction digestions, gel electrophoresis.
Completed by end of 2003: Data analysis
Completed by Spring/Summer 2004: Final report writing/Conference presentations.

Reports of progress on the above goals will be provided to the reviewers as requested.

6. Data handling and Storage

All data collected as a part of the proposed work will be stored at California State University Chico in a number of locations as follows: hard copy in the laboratory, computer copies in the offices and homes of the research participants (Schierenbeck, Kirk, and Whitlock). All collection, genetic, and analytical data will be available as requested to kschierenbeck@csuchico.edu.

7. Expected Products/Outcomes

Abstracts will be submitted for paper or poster presentations at the annual meetings of the American Society of Botanists, California Botanical Society, and the Society for the Study of Evolution in 2003. Two publications are expected to result from this (one for *Platanus* and one for *Juglans*) will be submitted to scientific journals (e.g. American Journal of Botany). A final and formal report will be provided to the funding agency, and other governmental and non-governmental agencies at the completion of this project. Local and regional presentations will also be made as a form of public outreach and information.

All data will be available to local agencies (California Dept. of Fish and Game, U.S. Dept. of Fish and Wildlife, etc.) and non-government organizations as requested during the duration and following the completion of the project.

8. Work Schedule

Fall 2001 through Spring/Summer 2002: Plant sample collection, DNA extraction
Fall 2002 through Summer 2003: DNA amplifications, restriction digestions, gel electrophoresis.
Fall/early winter 2003: Data analysis
Late winter through Spring/Summer 2004: Final report writing/Conference presentations.

B. Applicability to CALFED ERP and Science Program Goals and Implementation Plan and CVPIA Priorities

1. ERP, Science Program and CVPIA Priorities

The goals of the Ecosystem Restoration Program (ERP) include (as outlined in the Proposal Solication Package): “reversing downward population trends of native species not listed, maintain or rehabilitate ecosystem processes that favor native members of those communities, protect and/or restore functional habitat types, ecological process, and most importantly in regard to this proposed project, prevent the establishment of additional non-native species.” This proposal clearly will aid in reversing the downward population trends of native species not listed (*Platanus racemosa*) and an “at-risk” species (*Juglans hindsii*). The data collected as a part of this study will help prevent the establishment of additional non-native species and their hybrid offspring.

The ERP emphasizes the need to halt the introduction of new species that can negate the effects of ecosystem restoration efforts. The project we propose, to document the extent of hybridization between native and non-native species, is the first crucial step in determining the ecological impact of the non-native species and their hybrids.

Determining the level and direction of gene flow between *Platanus racemosa*, *Juglans hindsii* and their respective native congeners is directly related to the CALFED Science program goals in relations to Ecosystem restoration. Specifically, this project will address some of these goals. Understanding the level of gene flow between dominant species will allow us to:

1.) "Allow a comparison of the relative effectiveness of different restoration strategies". Because wild seed are collected and planted for vegetative restoration, many of the plantings have hybrid morphotypes. Once the respective genotypes are identified and levels of gene flow between native and non-native species are identified, further experimentation will be pursued to determine potentially different ecosystem functions between the native and non-native genotypes. It may be necessary to develop laboratory screening techniques for plants to be used in revegetation efforts.

2.) Determine the extent of the hybridization. Because the relative occurrences of native and non-native genotypes will be identified, we will be able to calculate the rate at which new hybrids may be forming.

3.) Address landscape scale issues. The introduction and establishment of non-native species that are riparian dominants are landscape issues. Reintroduction of native species are important at the regional scale. Rates of hybridization may be different throughout the riparian ecozone, depending on the distribution of the native and non-native genotypes.

4.) Extend the scientific basis of regulatory activies. Currently there are no safeguards or scientific basis for the identification of non-native genotypes that are used in restoration efforts. The data we gather will provide an easy method for the identification of certain genotypes.

2. Relationship to Other Ecosystem Projects

The study proposed here can be directly related to a number of the strategic goals of the ERP and subsequently, other ecosystem projects. Strategic goal 1 is to "maintain the abundance and distribution of at-risk species and contribute to the recovery of the at-risk species". *Juglans hindsii* is listed as an "at-risk species". Stragetic goal 2 states that "better knowledge of ecosystem processes and biotic communities can not occur without understanding the genetic composition and population structure of the dominant native species. Protection and restoration of functional habitat (strategic goal 4) can not occur without the enhancement and/or maintenance of native biotic communities. Most directly, strategic goal 5 applies which is to "prevent the establishment of additional non-native species and reduce the negative biological impacts of established non-native species".

In direct agreement with the goals outlined by the implementation plan, the work proposed here will, "increase our understanding of the invasion process and the role of established non-native species in ecosystems in the CALFED program through research and monitoring" and "limit the spread or, when possible and appropriate, eliminate populations of non-native species invasive species throught management." Specifically, for *Juglans hindsii*, the results of this research will be used to form restoration, protection, and management strategies for contributing to species recovery.

The management zones that are a direct part of this study are: Sacramento Region Ecological Management Zones, including the North Sacramento Valley, Sacramento River, Feather River, and American River Basin.

3. Requests for Next-Phase Funding Not applicable.

4. Previous Recipients of CALFED Program or CVPIA funding. Not applicable.

5. System-Wide Ecosystem Benefits

Protection and restoration of functional habitat can not occur without the enhancement and/or maintenance of native biotic communities. Interspecific gene transfer is a strong ecological and evolutionary force (Anderson 1949, Grant 1963, Heiser 1973). The importance of the ecological roles of *Juglans hindsii* and *Platanus racemosa* cannot be fully understood until we know to what extent these native species genetically still exist. Current riparian forests furnish cover and food sources for land and arboreal animals; among mid-level riparian trees, *Juglans* and *Platanus* are of potential value to birds because of their large size and substantial dead wood (Bock and Bock 1981). Hybrid *Juglans* and *Platanus* hybrids have demonstrated resistance to fungal pathogens and may not develop dead wood and cavities that provide important habitat for the ringtail cat and wood duck.

6. Additional Information for Proposals containing land acquistion. Not applicable.

C. Qualifications

Kristina A. Schierenbeck will serve as the principle investigator and will be ultimately responsible for the completion of all work.

a. Professional Preparation

Post-doctoral associate with Drs. Diana and Milton Lieberman, University of North Dakota. Population change and genetic diversity. January, 1993 to December, 1993.

Ph.D. Botany. Washington State University, December 1992. Comparative ecological and genetic studies between a native (*Lonicera sempervirens* L.) and an introduced congener (*L. japonica* Thunb.)

M. A. Ecology and Systematics. San Francisco State University, May 1988. Evolutionary relationships among *Arctostaphylos mewukka* and associated species.

B. S. Biology (Botany). San Francisco State University, May 1985.

b. Appointments

Current Position (September, 2001 – present): Associate Professor (Botany); Herbarium Director. Department of Biological Sciences, California State University, Chico, CA

August, 1998 – August, 2001. Assistant Professor; Herbarium Director. Department of Biological Sciences, California State University, Chico

January, 1994 – June, 1998. Assistant Professor, Department of Biology, California State University, Fresno

c. Publications, five most closely related to the proposed project:

Ellstrand, N. C. and **K. A. Schierenbeck.** 2000. Hybridization as stimulus for the evolution of invasive success in exotic plants. *Proceedings of the National Academy of Sciences* 97:7043-7050.

Gallagher, K. G., **K. A. Schierenbeck**, and C. M. D'Antonio. 1997. Hybridization and introgression in *Carpobrotus* spp. (Aizoaceae) in California. II. Allozyme evidence. *American Journal of Botany* 84:905-911.

Schierenbeck, K. A., M. Skupski, D. Lieberman and M. Lieberman. 1997. Population structure and genetic diversity in four tropical tree species in Costa Rica. *Molecular Ecology* 6:137-144.

Schierenbeck, K. A., J. L. Hamrick and R. N. Mack. 1995. Allozyme variability between a native and an introduced species of *Lonicera*. *Heredity* 75:1-9.
Schierenbeck, K. A., G. L. Stebbins, and R. W. Patterson. 1992. Morphological and cytological evidence for polyphyletic allopolyploidy in *Arctostaphylos mewukka*. *Plant Systematics and Evolution* 179:187-206.

Five other significant publications:
Albert, M., C. M. D'Antonio and **K. A. Schierenbeck**. 1997. Hybridization and introgression in *Carpobrotus* spp. (Aizoaceae) in California. I. Morphological evidence. *American Journal of Botany* 84:896-904.
Schierenbeck, K. A. 1995. The threat to the California flora from invasive species: problems and possible solutions. *Madroño* 42:168-174.
Schierenbeck, K. A., R. N. Mack and R. R. Sharitz. 1994. Effects of herbivory on growth and biomass allocation in native and introduced species of *Lonicera*. *Ecology* 75:1661-1672.
Schierenbeck, K. A. and D. B. Jensen. 1994. Vegetation of the Upper Raider and Hornback Creek basins, South Warner Mountains: Northwestern limit of *Abies concolor* var. *lowiana*. *Madroño* 41:53-64.
Schierenbeck, K. A. and J. D. Marshall. 1993. Photosynthesis in a closed canopy and an open field for introduced and native species of *Lonicera*. *American Journal of Botany* 80:1292-1299.

d. Synergistic Activities
Editor-in-chief, *Madroño*. 1998-2001.
Judge, Chico Science Fair. March, 1999.
Editorial Board, *Madroño*. 1994-1998.
Judge, Central California Science and Engineering Fair. March, 1995.
California Science Implementation Network. July, 1996 - 1998. Teach workshops on scientific principles to K-8 teachers.
Faculty advisor to Minority Undergraduate Student Enrichment (MUSE) program. 1995.
Faculty advisor to Ronald E. McNair program. 1996.
Event Coordinator, Science Olympiad Fresno Regional Tournament. 1996.

e. Collaborators and Other Affiliations
(i) Collaborators. Norman Ellstrand
(ii) Graduate and Post Doctoral Advisors:
Robert W. Patterson, M.A. Advisor. San Francisco State University.
Richard N. Mack, Ph.D. Advisor. Washington State University
Rebecca Sharitz, co-Ph.D. Advisor. University of Georgia, Savannah River Ecology Lab.
Diana and Milton Lieberman, Post-doc sponsors. University of Georgia and University of North Dakota.
(iii) Thesis Advisor:
Former students:
Sherry Ellberg, Ph.D. student, University of Missouri – Columbia.
Kelly Gallagher, Ph.D. Candidate, New Mexico State University
Geoffrey Gray, U.S. Fish and Wildlife Service
Frances Phipps, Lab technician, CSU Chico
V. Vaughan Symonds, Ph.D. Candidate, University of Texas
Natasha Sherman, Research Assistant, University of Maryland.
Ann Willyard, Independent consulting botanist
Current Graduate Students:
Gavin Blosser, Darhl Whitlock, Anil Kapoor, Paul Kirk, Stephanie Lopez, David Parks

D. Cost

1. Budget (detail of lab work only)

Taq polymerase		
Approx. 400 samples x 6 reactions at $2.50 ea.	(*Juglans*)	$6000
Approx. 400 samples x 6 reactions at $2.50 ea.	(*Platanus*)	$6000
Restriction Enzymes		$7000
PCR reagents and miscellaneous lab chemicals and supplies		$2000
	Total	$21,000

2. Cost sharing. Not applicable.

E. Local involvement

Local land managers are aware of this project are fully supportive, e.g. Joe Silviera, U.S. Fish and Wildlife Service. Following the completion of this project we will give presentations to local government and non-government organizations such as the California Native Plant Society, California Dept. of Fish and Game, Butte Environmental Council, Butte County Farm Advisory, and U.S. Fish and Wildlife Service..

F. Compliance with Standard Terms and Conditions

We agree with to comply with the standard State and Federal contract terms described in Attachments D and E of ERP.

G. Literature Cited

Anderson, E. 1949. Introgressive hybridization. John Wiley, New York, NY.

Baker, H.G. 1965. Characteristics and modes for origins of weeds. *In* H. G. Baker and G.L. Stebbins (eds.) The genetic colonizing species, 147-172. Academic Press, New York, NY.

Barbour, M., B. Pavlik, F. Drysdale, and S. Lindstrom. 1993 California's Changing Landscape. California Native Plant Society, Berkeley, CA.

Bennett, B. D. and J. Grace. 1990. Shade tolerance and its effect on segregation of two species of Louisiana irises and their hybrids. American Journal of Botany 77:100-107.

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