

Proposal Reviews

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

California State University, Chico

Research and Restoration Technical Panel Review

Sacramento Regional Review

External Scientific Review

#1

#2

#3

Environmental Compliance

Budget

Research and Restoration Technical Panel Review:

CALFED Bay-Delta 2002 ERP PSP Research and Restoration Technical Panel Review Form

Proposal Number: 34

Applicant Organization: California State University, Chico

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

Review:

Please provide an overall evaluation summary rating:

Superior: outstanding in all respects;

Above Average: Quality proposal, medium or high regional value, and no significant administrative concerns;

Adequate: No serious deficiencies, no significant regional impediments, and no significant administrative concerns;

Not Recommended: Serious deficiencies, significant regional impediments or significant administrative concerns.

Overall Evaluation Summary Rating	Provide a brief explanation of your summary rating
-Superior	Unfortunately, the results of the proposed study are unlikely to directly add to our knowledge of the above questions for <i>J. hindsii</i> and <i>P. racemosa</i> , and the application of this knowledge to riparian restoration was not made clear.
-Above average	
-Adequate	
XNot recommended	

1. **Goals and Justification.** Does the proposal present a clear statement of goals, objectives and hypotheses? Does the proposal present a clear justification and conceptual model for the project?

The goals of the project are clearly stated: 1) to identify native and non-native genotypes of *Juglans* and *Platanus* species in Northern California riparian zones. 2) identify the extent and direction of hybridization between native and non-native genotypes of *Juglans* and *Platanus*. There is a clear justification for the author's proposal to quantify the extent to which hybridization is occurring, and its impact on the population genetics of *J. hindsii*. The author provides less support for the claim that *P. racemosa* is threatened by hybridization with *P. X acerifolia*.

2. **Likelihood of Success (Approach, Feasibility, Capabilities and Performance Measures).** Is the project likely to succeed based on the approach, feasibility and project team capabilities? Are the proposed performance measures adequate for measuring the project's success?

There are two principal concerns with the approach: 1) the spatial scope and rational for population sampling. 2) the proposed genetic methodology. Of these two, concerns about the genetic methodology are more serious; the genetic methodology is undocumented and untested. There is no way to judge whether this method is feasible.

3. **Outcomes and Products.** Will the project advance the state of scientific knowledge in general and/or make an important contribution to the state of knowledge of the Bay-Delta Watershed? For restoration proposals, is the project likely to contribute to ecosystem restoration or species recoveries in a significant way? Will the project produce products useful to decision-makers and scientists?

Due to sampling problems enumerated by one reviewer, there is some question as to whether a regional assessment of hybridization will be produced, even supposing the genetic methods are feasible. Also, as the trees are wind pollinated, how would their genetic integrity be preserved if hybridization is ongoing and widespread?

4. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed?

The budget is reasonable and adequate for the proposed work

5. **Regional Review.** How did the regional panel(s) rank the proposal (High, Medium, Low)? Did the regional panel(s) identify significant benefits (regional priorities, linkages with other activities, local involvement) or impediments (local constraints, conflicts with other activities, lack of local involvement) to this proposal? What were they?

The Sacramento Regional Panel gave this proposal a Low priority. The relevance of extent of hybridization to restoration efforts was not clear. Would hybrids be more invasive than the pure native genotypes, especially on restoration sites? There was no involvement with local institutions/people

6. **Administrative Review.** Were there significant concerns about the proposal with regard to the prior performance, environmental compliance and budget administrative reviews? What were they?

No

Miscellaneous comments:

None

Sacramento Regional Review:

Proposal Number: 34

Applicant Organization: California State University, Chico

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

Overall Ranking: ☒Low ☐Medium ☐High

Provide a brief summary explanation of the committee's ranking:

Although identifying the degree of hybridization of native black walnuts and sycamores with non native species is of interest, its relevance to restoration efforts in the Sacramento region was not made clear in the proposal.

1. Is the project feasible based on local constraints?

☒Yes ☐No

How?

The project applicant has obtained permission to collect specimens, and has all of the necessary laboratory equipment and expertise to conduct the study.

2. Does the project pursue the restoration priorities applicable to the region as outlined in the PSP?

☐Yes ☒No

How?

Our no is qualified. Although the study will peripherally address regional priority 5, implementing actions to prevent, control, and reduce impacts of non-native invasive plants, the proposal did not clearly identify how determining to what extent California black walnuts and sycamores have hybridized with non natives will be applicable to restoration efforts. Will the project identify native seed sources?

3. Is the project adequately linked with other restoration activities in the region, such as ongoing implementation projects and regional planning efforts?

☐Yes ☒No

How?

The proposed project is not directly linked to any particular restoration project identified in the proposal.

4. Does the project adequately involve local people and institutions?

-Yes ☒No

How?

The applicant does not appear to have coordinated with local people and institutions who might use the results of the project to direct their restoration efforts.

Other Comments:

x

External Scientific: #1

Research and Restoration External Scientific Review Form

Proposal Number: **34**

Applicant Organization: **California State University, Chico**

Proposal Title: **Hybridization between native and non-native plant species in the riparian ecosystem**

Conflict of Interest Statements:

I have no financial interest in this proposal.

XCorrect

-Incorrect

In the blank below please explain any connection to proposal, to applicant, co-applicant or subcontractor or to submitting institution (write "none" if no connection):

none

Review:

Please provide an overall evaluation summary rating:

Excellent: outstanding in all respects;

Good: quality but some deficiencies;

Poor: serious deficiencies.

Overall Evaluation Summary Rating	Provide a brief explanation of your summary rating
-Excellent	The questions may be valuable to determine the extinction risks posed by hybridization to an at-risk species, costs are reasonable, and personnel qualified, however, there are serious questions with the molecular method which make the feasibility doubtful.
XGood	
-Poor	

1. **Goals.** Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the concept timely and important?

The goal of the proposed research is to determine the amount of hybridization that has occurred between native tree species, Juglans and Platanus, and ornamental or horticultural species in these genera. Hybrid trees could cause a decline in the genetic integrity of the native species, one of which is an "at risk" species, and potentially alter the structure of the riparian ecosystems where they occur. This is a preliminary investigation into the genetic backgrounds of the species. The research has been clearly outlined and is internally consistent.

2. **Justification.** Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full-scale implementation project justified?

The study does address a gap in our knowledge; the conceptual model - that opportunities for hybridization have arisen on widespread scales - is clear, and the underlaying basis for the proposed research.

3. **Approach.** Is the approach well designed and appropriate for meeting the objectives of the project? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology or approaches? Will the information ultimately be useful to decision-makers?

The sampling technique is to obtain leaf material from allopatric populations of each putative non-native hybrid partner and from allopatric or "pure" stands of the natives to find DNA markers which distinguish all the species. The molecular fingerprint of putative hybrids can then be examined and will yield useful information on the question. This part of the methodology is sound and has been used with success in other investigations of hybridization. The actual molecular method is a serious problem to be address in the following section.

4. **Feasibility.** Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives?

The molecular approach - to examine species specific restriction fragments (id'd from known sequence data) of 2 sites of the nuclear ITS gene - is not referenced and, as near as I can determine, has virtually never been used. The key question is not that species specific fragments will be generated, but rather how many polymorphic fragments can be obtained by this method. If hybridization is as pervasive as suspected, introgression (hybrids backcrossing with parental species and with each other) may well be highly advanced. To detect highly introgressed individuals would require 10+ species specific markers from each species. Other studies using this method on hybrids, or preliminary studies on this system need to be in place to consider this method feasible. Finally, RAPDs have been successfully used to look at backcrossing in Juglans - why was this method not considered? In addition, I cannot see the value of phylogenetic analysis of hybrids.

5. **Project-Specific Performance Measures.** Does the project include appropriate performance measures to measure success relative to the project's goals and objectives? Is there enough detail as to how the performance measures will be quantified? For restoration projects, are monitoring plans explicit and detailed enough to determine if performance measures will be adequately assessed?

Details are sketchy, but adequate.

6. **Products.** Are products of value likely from the project? Specifically for restoration projects, are products of value also likely from the monitoring component? Are interpretative outcomes likely from the project?

They expect two publications, and 3 poster/paper presentations.

7. **Capabilities.** What is the track record of applicants in terms of past projects? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

They have the necessary facilities and equipments, and Dr. Schierenbeck is well qualified to perform the study,

8. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed?

yes

Miscellaneous comments:

External Scientific: #2

Research and Restoration External Scientific Review Form

Proposal Number: **34**

Applicant Organization: **California State University, Chico**

Proposal Title: **Hybridization between native and non-native plant species in the riparian ecosystem**

Conflict of Interest Statements:

I have no financial interest in this proposal.

XCorrect

-Incorrect

In the blank below please explain any connection to proposal, to applicant, co-applicant or subcontractor or to submitting institution (write "none" if no connection):

None

Review:

Please provide an overall evaluation summary rating:

Excellent: outstanding in all respects;

Good: quality but some deficiencies;

Poor: serious deficiencies.

Overall Evaluation Summary Rating	Provide a brief explanation of your summary rating
-Excellent	Hybridization between natives and their non-native relatives can be an important conservation concern, particularly if the native species is rare or the extent of hybridization is large. Additionally, hybrids can threaten the integrity and functioning of entire native ecosystems. Consequently, understanding the extent of hybridization, the factors that promote hybridization, and where in the landscape hybridization occurs are important questions.
-Good	Unfortunately, the results of the proposed study are unlikely to directly add to our knowledge of the above questions for <i>J. hindsii</i> and <i>P. racemosa</i> . First, there are questions about whether the proposed molecular genetic methodology will allow for the detection of distinct native, non-native, and hybrid genotypes. Even if the methodology is successful however, the project is unlikely to provide a detailed picture of the extent and spatial pattern of hybridization in the northern California landscape.
XPoor	I rate the proposal as poor because of these two concerns

1. **Goals.** Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the concept timely and important?

The goals of the project are clearly stated: 1) to identify native and non-native genotypes of *Juglans* and *Platanus* species in Northern California riparian zones. 2) identify the extent and direction of hybridization between native and non-native genotypes of *Juglans* and *Platanus*.

Broadly, these questions are timely and important. There is growing evidence that non-native plant species commonly hybridize with native taxa. In some cases hybrid genotypes can have dramatically different characteristics than either of the parents, and an equally dramatic impact on native communities and ecosystems. Understanding the dynamics of hybridizations as well as the ecological traits of hybrid genotypes can be very important for the management and control of certain invasions. The most salient of such cases occur when a non-native species threatens a rare or restricted native species with genetic assimilation or when hybrid genotypes are markedly more invasive than parents. I discuss in more detail below the extent to which there is evidence that the study systems proposed by the author likely represent such cases.

2. **Justification.** Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full-scale implementation project justified?

The author provides good support for the claim that *J. hindsii* is distinct from southern populations of *J. californica* and thus represents a relatively restricted species. The author further demonstrates that hybridization is readily observed between *J. hindsii* and a number of other introduced walnut taxa, suggesting that the genetic integrity of *J. hindsii* may be eroding. There is, therefore, a clear justification for the author's proposal to quantify the extent to which hybridization is occurring, and its impact on the population genetics of *J. hindsii*.

The author provides less support for the claim that *P. racemosa* is threatened by hybridization with *P. X acerifolia*. *Platanus racemosa* is a common and widespread species. The author provides only anecdotal and suggestive evidence that the two species hybridize through artificial crosses or in nature. Although *Platanus* is wind pollinated, the author provides no evidence that *P. racemosa* and *P. X acerifolia* commonly come in close enough proximity to each other to foster cross-pollination.

In neither case does the author provide evidence suggesting that hybrids are ecologically distinct from parents or that they threaten to alter riparian ecosystem structure or function. Although the author clearly states that such ecological assessments are not a component of the current proposal, the author suggests that the present proposal is a required first step for future work in this regard. Yet, the author presents no evidence suggesting that such future studies are warranted. Clearly, lack of evidence does not necessarily mean that a problem does not exist or might not exist in the future. However, obvious ecological differences that would have elicited the concern of land managers do not seem to exist.

3. **Approach.** Is the approach well designed and appropriate for meeting the objectives of the project? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology or approaches? Will the information ultimately be useful to decision-makers?

I have two principal concerns with the approach outlined by the author: 1) the spatial scope and rational for population sampling. 2) the proposed genetic methodology. I outline these concerns below:

a) The author states that the data generated by the proposed work will "allow the determination of rates of gene flow and regional incidence of hybridization." This is an important goal, because as the author points out in sections B1-B2 (pgs. 6-7), understanding the regional distribution of native and hybrid genotypes and the location and dynamics of hybrid zones can be important information for ecosystem management. For both *Juglans* and *Platanus*, however, the author provides only a vague description of the spatial pattern of the proposed population sampling, and more importantly only a vague conceptual description of the factors that could influence hybrid formation.

For example, how will the samples of riparian black walnut populations be spatially arranged? One hypothesis might be that hybridization is more extensive in populations that are closer to urban areas or agriculture plantings. This pattern might correspond spatially with a cline of increasing hybridization from high in the watershed to low in the watershed. The author does not provide any detail about how samples will be arranged along the riparian corridor.

Also, if natives, non-natives, and hybrids do exhibit ecological differences, they might assort themselves spatially at a given location (e.g. at different heights along the bank...or perhaps even outside the riparian corridor altogether). The author does not state that sampling will be stratified by any environmental factors. If such spatial structure does exist it could bias estimates of hybridization frequency.

Similarly, the author proposes to sample suspected *Platanus* hybrids from four natural populations and one restoration site. Even if hybrid individuals are confirmed at these sites, what will that tell us about the frequency of hybridization across the wide range of *P. racemosa* in California, or even simply within the Bay-Delta region? Since the sites were chosen because they contain suspected hybrids, it is unclear that they fully represent the range of natural *P. racemosa* populations. Are these populations exceptional because they contain putative hybrids? Are they near non-native cultivars? Do they consist of relatively recent recruits? The author states that the samples taken from these populations consist of saplings. What is the frequency of natives, non-natives, and hybrids in the adult members of the populations?

Perhaps the most direct way to test whether the genetic integrity of *P. racemosa* is threatened would be to measure the proportion of *P. racemosa* offspring that are hybrid. The author proposes to sample old growth *P. racemosa* in order to ensure samples of pure *P. racemosa*. Are these old established trees siring hybrids? The author does not propose to test this.

Related to this, the frequency of hybrid individuals may have a demographic structure. For example, if hybridization is increasing, younger hybrids would be more common than older hybrids. The author does not clarify whether both established trees and juveniles will be sampled at each site.

b). My second concern is with the proposed DNA methodology. The author proposes to amplify two nrDNA regions (ITS-1 and ITS-2), then digest them with restriction enzymes to produce a series of restriction polymorphisms. These restriction polymorphisms will be analyzed to determine species specific markers that can be used to classify individuals as native, non-native, or hybrid. This methodology is not a standard one for the detection of hybrids. The more typical approach is to use random amplified polymorphic DNA (RAPD). One of the advantages of the RAPD approach is that it commonly generates numerous polymorphisms

which greatly aids the discrimination of individual genotypes. While analysis of sequence variation in ITS regions is commonly used in population and systematic studies (including those documenting hybridization), it is unclear whether restriction digests will provide sufficient species-specific polymorphic markers. The authors do not provide any citations demonstrating the applicability of this technique.

4. **Feasibility.** Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives?

The genetic sampling of populations is technically feasible given the schedule of the proposed work. In fact, the geographic spread and detail of sampling is surprisingly limited given the goal of the proposal to determine the "rates of gene flow and regional incidence of hybridization".

It is unclear whether the proposed molecular genetic methodology is feasible. While it is possible that the proposed methodology will succeed, I cannot find other studies in the literature where a similar approach has been used.

5. **Project-Specific Performance Measures.** Does the project include appropriate performance measures to measure success relative to the project's goals and objectives? Is there enough detail as to how the performance measures will be quantified? For restoration projects, are monitoring plans explicit and detailed enough to determine if performance measures will be adequately assessed?

Within the context of the proposed methodology there are logical performance measures and appropriate quantification. As I discuss above and in section 6, it is unclear from the proposal whether the data obtained from the proposed work will allow the author to answer the project's main objectives.

6. **Products.** Are products of value likely from the project? Specifically for restoration projects, are products of value also likely from the monitoring component? Are interpretative outcomes likely from the project?

Assuming that the genetic methodology is feasible, the proposed project is likely to produce:
a) a description of whether *Juglans hindsii* and *Platanus racemosa* can hybridize with non-native congeners, and which taxa are involved in hybridization events. b) a limited, and potentially biased map of where hybridization takes place.

It will not produce a regional assessment of the extent of hybridization, a detailed map of where hybridization occurs and what sort of environmental factors are associated with hybridization, or an assessment of how hybridization will likely influence the long term population genetics of *Juglans hindsii* and *Platanus racemosa* populations. In my opinion, these questions are the most pertinent to the management of the two species in Northern California. These types of data are also important baselines for the future research outlined by the author.

7. **Capabilities.** What is the track record of applicants in terms of past projects? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

I do not have direct knowledge of the track record of the author with respect to previous projects. However Kristina Schierenbeck is a well known researcher in the field of plant population genetics. She has a good record of peer-reviewed contributions in a number of areas pertinent to the current proposal.

8. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed?

The budget is reasonable and adequate for the proposed work.

Miscellaneous comments:

External Scientific: #3

Research and Restoration External Scientific Review Form

Proposal Number: **34**

Applicant Organization: **California State University, Chico**

Proposal Title: **Hybridization between native and non-native plant species in the riparian ecosystem**

Conflict of Interest Statements:

I have no financial interest in this proposal.

XCorrect

-Incorrect

In the blank below please explain any connection to proposal, to applicant, co-applicant or subcontractor or to submitting institution (write "none" if no connection):

None

Review:

Please provide an overall evaluation summary rating:

Excellent: outstanding in all respects;

Good: quality but some deficiencies;

Poor: serious deficiencies.

Overall Evaluation Summary Rating	Provide a brief explanation of your summary rating
-Excellent	This would be a MUCH more promising and supportable project if it included some ecological relevance to the planning, implementing and monitoring of CALFED restoration.
-Good	
XPoor	

1. **Goals.** Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the concept timely and important?

Hybridization can be a complicating factor in planning, implementing and (especially) monitoring estuarine restoration, aka *Spartina alterniflora* and *S. foliosa* in the Bay. This proposal addresses potential hybridization of two riparian canopy species, *Platanus racemosa* (California sycamore) and *Juglans hindsii* (North California black walnut). The goal is rather implicit, that hybridization alters the complexity of important vegetation assemblages, and is of special concern with rare native species. The objectives are designed to identify the scope of that hybridization. By itself, from a conservation biology perspective, this is an interesting and worthwhile study, but whether it is timely within the context of CALFEDs science and restoration priorities is less obvious.

2. **Justification.** Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full-scale implementation project justified?

Although this study should provide important, new information about the degree of hybridization in important riparian canopy species, its applicability to CALFED is minimally justified. There is no conceptual model and there is not real indication how the study relates to the role of the native species, non-native species and hybrids in the restoration of riparian ecosystems.

3. **Approach.** Is the approach well designed and appropriate for meeting the objectives of the project? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology or approaches? Will the information ultimately be useful to decision-makers?

From the standpoint of effectively determining the level of hybridization of California sycamore and North California black walnut, the approach appears scientifically sound. It appears to address a significant gap in the knowledge about the genetic interrelationships among riparian woody plants of importance in the CALFED region.

4. **Feasibility.** Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives?

The study appears to be quite feasible.

5. **Project-Specific Performance Measures.** Does the project include appropriate performance measures to measure success relative to the project's goals and objectives? Is there enough detail as to how the performance measures will be quantified? For restoration projects, are monitoring plans explicit and detailed enough to determine if performance measures will be adequately assessed?

Performance measures are based purely on meeting a schedule/timeline, and as such are inadequate indicators of scientific progress.

6. **Products.** Are products of value likely from the project? Specifically for restoration projects, are products of value also likely from the monitoring component? Are interpretative outcomes likely from the project?

Project products are entirely academic, involving reports and peer-reviewed journal publications. However, interpretive outcomes relative to CALFED restoration and science may not be forthcoming?

7. **Capabilities.** What is the track record of applicants in terms of past projects? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Investigator and institutional expertise and background appear to be more than sufficient to accomplish the study objectives.

8. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed?

Estimated costs (\$149,846) appear to be quite reasonable for what is being proposed.

Miscellaneous comments:

A good example of good science that is very poorly linked to the needs of CALFED.

Environmental Compliance:

Proposal Number: 34

Applicant Organization: California State University, Chico

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

1. Are the legal or regulatory issues that affect the proposal identified adequately in the proposal?

☒Yes -No

If no, please explain:

2. Does the project's timeline and budget reflect adequate planning to address legal and regulatory issues that affect the proposal?

☒Yes -No

If no, please explain:

3. Do the legal and regulatory issues that affect the proposal significantly impair the project's feasibility?

-Yes ☒No

If yes, please explain:

Other Comments:

Budget:

Proposal Number: 34

Applicant Organization: California State University, Chico

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

1. Does the proposal include a detailed budget for each year of requested support?

-Yes **X**No

If no, please explain:

Requesting 3 years (16) on Project Information Sheet, but have based tasks and budget on 2 years. Two-year budget Grand Total equals Total Federal Funds requested (17a). However, under narrative for Work Schedule (8) under Project Description, project spans 2001-2004 2-1/2 to 3 years)?

2. Does the proposal include a detailed budget for each task identified?

-Yes **X**No

If no, please explain:

See notes under Q#1. Tasks not identified in proposal other than in Budget Summary.

3. Does the proposal clearly state the type of expenses encompassed in indirect rates or overhead costs?

-Yes **X**No

If no, please explain:

Noted it is a Federally negotiated rate not covering fringe benefits in Budget Justification, assuming only salaries and wages and all other costs covered by University?

4. Are appropriate project management costs clearly identified?

XYes -No

If no, please explain:

5. Do the total funds requested (Form I, Question 17A) equal the combined total annual costs in the budget summary?

XYes -No

If no, please explain (for example, are costs to be reimbursed by cost share funds included in the budget summary).

6. Does the budget justification adequately explain major expenses?

☒Yes ☐No

If no, please explain:

7. Are there other budget issues that warrant consideration?

☐Yes ☒No

If yes, please explain:

Other Comments: