White Bluffs Bladderpod DNA Report Peer Review Documents

December 19, 2013

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Sequence Variation Among Physaria douglasii Isolates

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Executive Summary

We used DNA sequence data to investigate the taxonomic status of White Bluffs bladderpod, *Physaria douglasii*, ssp. *tuplashensis*. In the event that *P. douglasii* ssp. *tuplashensis* is indeed sufficiently divergent to warrant subspecies designation, this would be the result of independent evolution, and should be characterized by accumulation of genetic differences between *tuplashensis* and its *P. douglasii* sister taxon. Conversely, if *tuplashensis* individuals do not exhibit genetic differentiation when compared with other extant *P. douglasii* populations, this would be indicative of ongoing geneflow between populations, and would not support separate taxonomic status for the putative subspecies. We sequenced DNA from 4 loci, using DNA extracted from 15 *P. douglasii* individuals, including 3 that were identified as *P. douglasii* ssp *tuplashensis*. From this relatively small sample, we identified a number of genetic markers that differ between individuals. For two loci, ndhF and rbcL, there is generally little genetic variation, and what variation does occur does not in any way distinguish among samples, or indicate that the *tuplashensis* specimens are different from *P. douglasii* samples. ITS sequence data is somewhat more informative, placing 4 *douglasii* individuals in a weakly supported group, or clade, while clustering the t*uplashensis* samples with 4 *douglasii* specimens from Asotin, Chelan, and Yakima Counties. Finally, the data from the trnH-psbA locus shows a strongly supported clade of some of the *douglasii* samples, but does not distinguish the *tuplashensis* samples as a separate group. Individually and in aggregate, these data do not support a finding of *tuplashensis* as a separate taxon.

Methodology

Sample collection and DNA extraction
7 fresh samples were collected in the field, and rapidly desiccated to preserve resident DNA until the samples could be processed in the laboratory. An additional 8 samples were obtained from the University of Idaho Stillinger Herbarium. Samples were arbitrarily assigned numbers 1-15, and subsequent processing used only these numbers—processing and analysis was essentially blind, to ensure unbiased results.

Leaf tissue from all samples was pulverized, and total genomic DNA extracted from all samples using a standard DNA extraction protocol (Qiagen DNeasy Plant DNA Extraction Kit). An aliquot of DNA from each sample was visualized on an agarose gel to assess the condition and concentration of the extracted DNAs. While several of the older DNA samples showed signs of degradation, there was sufficient intact DNA in each sample to warrant their use for this study. Table 1 provides a list of all samples used in this study, and their provenance.

Sample number	**Taxon**	Provenance	Collection site and date collected
1	P. douglasii	Stillinger Herbarium ID071491	Nez Perce Co., ID, Limestone Point, near confluence of Snake and Grande Ronde Rivers. 1981
2	P. douglasii	Stillinger Herbarium ID071642	Wasco Co., OR. Columbia River Highway near Big Eddy. 1938
3	P. douglasii	Stillinger Herbarium ID071643	Grant Co., WA, Columbia River bank at Vernita Bridge, 30 mi SW of Othello. 1975
4	P. douglasii	Stillinger Herbarium ID071641	Yakima Co., WA, Columbia River, approx. ½ mi below Priest Rapids Dam. 1959
5	P. douglasii	Stillinger Herbarium ID071495	Chelan Co., WA, gravel bank 8 mi north of Wenatchee at mouth of Sakane Canyon. 1948
6	P. douglasii	Stillinger Herbarium ID071494	Kittitas Co., Columbia River south of Hanson Creek and North of Alkali Creek. 1991
7	P. douglasii	Stillinger Herbarium ID071493	Asotin Co., T7N R47E S20, southeast slopes of Lime Hill, approx. 8oo horizontal feet from Snake River, and approx. 1000 vertical feet above shoreline. 1999
8	*P. douglasii*	Stillinger Herbarium ID071492	Kittitas Co., WA, Priest Rapids Reservoir of Columbia River, Railroad Island west of Beverly at north end of Sentinel Gap. SW ¼ sec. 33, T16N, R23E. 1992
9	#1A, *P. douglasii*	S. Turner	6/25/2013
10	#2A, *P. douglasii*	S. Turner	6/28/2013 N46 33 32.54 W119 17 08.24
11	#3A, *P. douglasii*	S. Turner	6/28/2013 N46 33 32.54 W119 17 08.24
12	#5, *P. douglasii ssp. tuplashensis*	S. Turner	N46.34.872 W119.19.82 7/3/2013
13	#4, *P. douglasii ssp. tuplashensis*	S. Turner	N46.39.814 W11?? 7/3/2013
14	#7, *P. douglasii*	S. Turner	N46.38.602 W119.49.236 7/4/2013
15	#6, *P. douglasii ssp. tuplashensis*	S. Turner	N46.37.906 W119.49.36 7/3/2013

PCR and sequencing

We selected 5 loci, or regions of DNA, for PCR amplification and sequencing. These included: 1) Internal Transcribed Spacer (ITS) region; 2) ribulose-bisphosphate carboxylase gene (rbcL); 3) trnH-psbA intergenic spacer region (trnH); 4) maturase coding gene (matK); 5) NADH hydrogenase F (*ndhF*). Complete discussion and justification for the use of these loci is beyond the scope of this report, but some discussion is warranted; ITS, rbcL, trnH, and matK have properties that lend themselves to this kind of study. ITS is a nuclear gene, and present in high copy number, making it relatively easy to amplify in PCR, even if the source DNA is present in low concentration, or degraded. Because it is a nuclear locus, it also samples and reflects genetic variation from both parents. The other loci used in this study are located within the chloroplast genome. Chloroplasts are typically inherited from a single parent (usually the female parent in angiosperms), and because leaf tissue contains many chloroplasts, these loci are likewise present in high copy number, and hence relatively easy to amplify. Moreover, ITS, rbcL, trnH and matK regions have been shown to be highly variable in plants, and if genetic variation occurs, it is likely to be found in these regions. By way of example, matK and rbcL are the loci recommended for DNA barcoding for species identification (CBOL, http://www.barcoding.si.edu/plant_working_group.html). Consequently, these are the indicated loci for this type of study, because they maximize the likelihood of encountering useable, species-diagnostic genetic variation. Finally, we included *ndhF* because it has been extensively used to investigate phylogeny of other species in the genus *Physaria* (Beilstein et al., 2006). Comprehensive treatment of the relative merits of these loci, and their utility for species identification, can be found in the references provided at the end of this report.

PCR primer sequences and reaction conditions for rbcL, trnH, and matK were obtained from the Consortium for the Barcode of Life Plant Working Group website (CBOL, http://www.barcoding.si.edu/plant_working_group.html). Primer sequences and reaction conditions for *ndhF* amplification were as described in Beilstein et al., 2006. ITS primer sequences and reaction conditions were obtained from White et al., 1990.

PCR results

PCR reactions worked well for all samples when amplifying rbcL, trnH, and *ndhF*, generating strong bands, as visualized on an agarose gel. ITS was somewhat less successful, with good amplification from 10 samples. matK PCR failed for all *Physaria* samples, although a positive control using *Rosa* DNA as template was successful. We interpret this as evidence that the published matK primers and PCR conditions do not work for *Physaria* species. All PCR reactions were run in tandem with negative controls, using sterile water in place of template DNA, to test for contamination of reagents. Negative controls indicated no contamination of reagents.

Sequencing

PCR products were sequenced in both directions, using Big Dye v. 3.1 (Applied Biosystems). For each PCR product, complementary strands were aligned and inspected for errors and ambiguities, and a consensus sequence generated for use in analysis. Throughout the proofreading process, we adopted a very conservative approach, and any sequence data that was ambiguous was excluded from subsequent analysis. For the *ndhF* locus, we generated ca. 1100 contiguous bases of sequence data, 359 bases for trnH-psbA, 539 bases for rbcL, and 650 bases for ITS. 5 samples failed to amplify well for the ITS locus, and did not produce clean readable sequence data. A further 3 samples did produce clear ITS sequence, but not the full 650 bases obtained from other samples. Nevertheless, we were able to obtain full-length ITS sequence from 7 samples, including 2 putative *tuplashensis* examplars.

Phylogenetic analysis

Once the sequence data for each locus and sample had been assembled and aligned, it became clear that there was not sufficient variation to warrant assignment tests, or other statistical analyses that investigate population structure—there are too few variable markers, and too few samples for statistical validity. Instead, we carried out phylogenetic analysis, which uses sequence data to infer relatedness among individuals and groups. In this instance, we used PAUP (Phylogenetic Analysis Using Parsimony, vers. 4.0; Swofford, 2003) to construct phylogenies, so-called "gene-trees," for each of the sequenced regions. Among the ca. 1100 base-pairs (bp) analyzed for the *ndhF* locus, and ca. 530 bp analyzed for rbcL, there was overall very little variation, and the genetic markers that did vary did not do so in any discernable pattern, but were phylogenetically uninformative. Phylogenetic trees derived from these data are in the form of a polytomy, in other words, these data do not indicate any structure or closer degree of relatedness among the 15 taxa. For these loci, all 15 taxa were represented with full length sequences. Trees for *ndhF* and rbcL are shown in Figures 1 and 2, the aligned sequence data used for these analyses are included in the appendix. All trees shown here are unrooted.

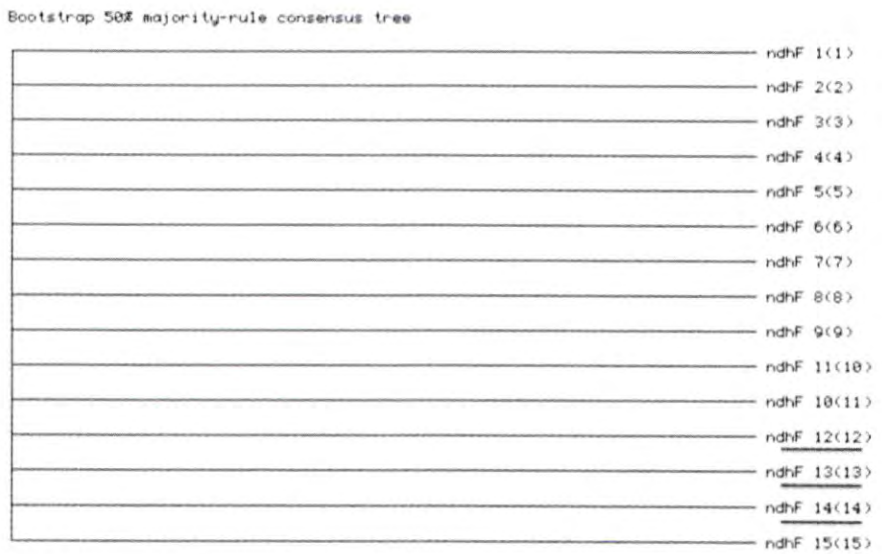

Figure 1. 50% majority rule concensus tree showing relatedness based on ndhF sequence data. *Tuplashensis* examplars underlined in red. Note complete absence of any clustering. Numbers in parentheses refer to order in which the taxon was added while calculating most parsimonious tree.

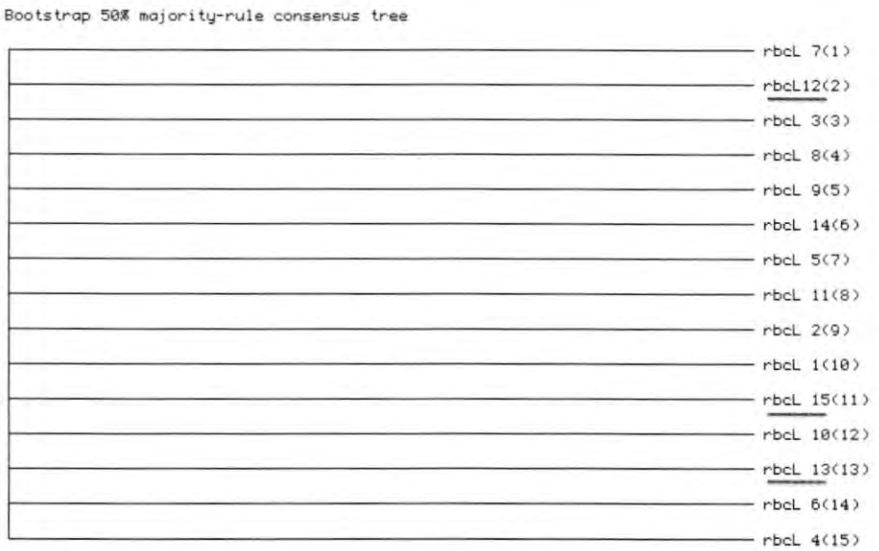

Figure 2. 50% majority rule concensus tree showing relatedness based on rbcL sequence data. *Tuplashensis* examplars underlined in red. Numbers in parentheses refer to order in which the taxon was added while calculating most parsimonious tree.

By contrast, the genetic variation evident in the ITS and trnH sequences does contain phylogenetic information. ITS sequence data places samples 6, 8, 9, and 11, all nominal P. douglasii samples, in a single clade distinct from other samples (see Table 1 and Figure 3), but does not resolve relationships among or between *tuplashensis* samples or the remaining P. douglasii exemplars. trnH, the shortest fragment sequenced in this study (359 bp), nevertheless showed the greatest amount of variation, and the greatest amount of phylogenetically informative information. Analysis of trnH sequence data shows a greater degree of relatedness among samples 2,3,4,5, 8, 9, and 11, all douglasii samples from multiple locations, which together comprise a strongly supported clade within the 15 samples tested. Samples 10 and 11, both *douglasii*, were likewise placed in their own, weakly supported clade. The 3 *tuplashensis* samples were not resolved, but rather appear in a polytomy, along with 3 *douglasii* samples. Figure 4 shows the phylogenetic tree derived from these data, and data matrices for both ITS and trnH appear in the appendix.

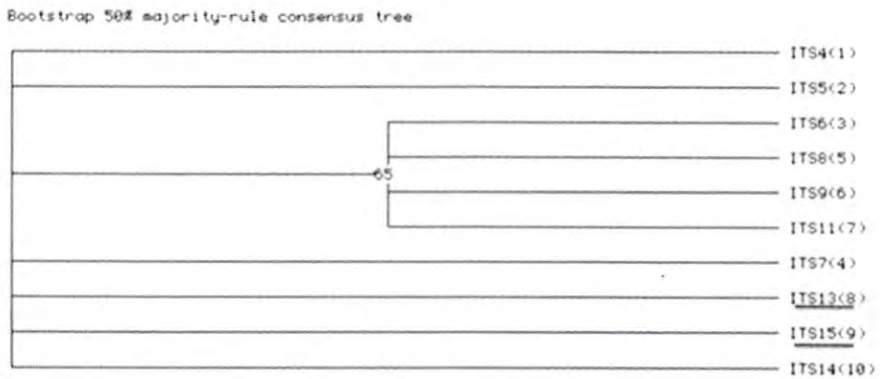

Figure 3. 50% majority rule concensus tree showing relatedness based on ITS sequence data. *Tuplashensis* examplars underlined in red. Number on node (65) is percentage of bootstrapping replicates that recover this topology. Numbers in parentheses refer to order in which the taxon was added while calculating most parsimonious tree.

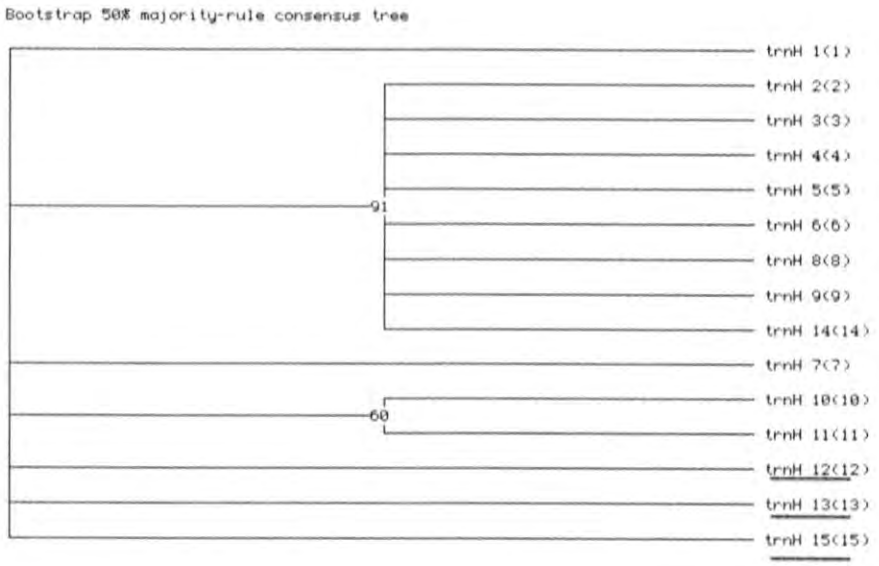

Figure 4. 50% majority rule concensus tree showing relatedness based on trnH sequence data. *Tuplashensis* examplars underlined in red. Numbers on nodes are percentage of bootstrapping replicates that support this topology. Numbers in parentheses refer to order in which the taxon was added while calculating most parsimonious tree.

Conclusions and recommendations

Based on the sequence data generated in this study, White Bluffs bladderpod is not genetically distinct, or distinguishable, from *P. douglasii* bladderpod populations or individuals. The nuclear and chloroplast regions used here are typically highly variable, and were specifically chosen to maximize the likelihood of detecting genetic differentiation of *tuplashensis*. In our study, this differentiation was not apparent. By way of comparison, we use ITS sequence to identify invasive and native watermilfoil species. Eurasian watermilfoil, *Myriphylum spicatum*, is an aggressively invasive aquatic plant that has infested fresh water bodies in most of the continental United States. The native watermilfoil, *Myriophylum sibiricum*, is morphologically quite similar, and hybridizes readily with the invasive Eurasian watermilfoil. Because of their ability to hybridize, and their morphological similarity, there is a great deal of discussion in the taxonomic community regarding the appropriate taxonomic status of these taxa—whether they should be considered distinct species, subspecies, or simply one species with genetically diverse geographic races. However, morphological

similarity notwithstanding, we find extensive and diagnostic genetic differences within the ITS region, and we use these markers to identify infestations of the invasive Eurasian taxon (Moody and Les, 2002, see also Figure 5). By extension, we might expect some degree of genetic differentiation among *Physara* samples, if White Bluff bladderpod is indeed a population distinct from *P. douglasii*. We find no evidence of such differentiation.

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gi|114434310_spicatum          TCGCGTTGCTCCCAAAGCCCACCCTTCAAGGATAAGGCGCTGCGGAAGCA
gi|24943231 spicatum           TCGCGTTGCTCCCAAAGCCCACCCTTCAAGGATAAGGCGCTGCGGAAGCA
gi|114434323 sibiricum         TCGCGTTGCTCCCAAAGCCCACCCTTCAAGGATATGGTGCTGCGGAAGCA
gi|24943230 sibiricum          TCGCGTTGCTCCCAAAGCCCACCCTTCAAGGATATGGTGCTGCGGAAGCA

gi|114434310_spicatum          GATATTGGCCTCCCGTGCCTGCGCACGGCTGGCCTAAATGCAAGCCTGGG
gi|24943231 spicatum           GATATTGGCCTCCCGTGCCTGCGCACGGCTGGCCTAAATGCAAGCCTGGG
gi|114434323 sibiricum         GATATTGGCCTCCCGTGCCTGTGCACGGATGGCCTAAATGCAAGCCTGGG
gi|24943230 sibiricum          GATATTGGCCTCCCGTGCCTGTGCACGGATGGCCTAAATGCAAGCCTGGG
```

Figure 5. Aligned GENBANK sequence with 2 exemplary ITS sequences from *M. spicatum* and 2 sequences from *M. sibiricum*. Sites that vary between species highlighted in red.

Recommendations

While the findings presented in this report do not indicate that *P. douglasii* ssp. *tuplashensis* is a distinct and independently evolving taxon, these results cannot be considered definitive or final. Our sampling was constrained by both the time available to collect and process samples, and the availability of *Physaria* samples. Ideally, this study would be expanded, to incorporate both more bladderpod samples from across the species' range, and more comprehensive sampling of the genomes of the *Physaria douglasii* specimens. The former will be important to establish a more representative sampling of bladderpod populations, necessary for rigorous statistical comparisons. The latter will be important to more reliably establish whether *tuplashensis* is indeed genetically different from other *douglasii* populations—the ca. 2500 total bases of DNA sequence analyzed in this study represent a miniscule fraction of the total *Physaria* genome. In order to more accurately infer population structure and patterns of gene flow in this taxon, it will be necessary to adopt alternate approaches that more comprehensively sample a larger fraction of the genome. Specifically, we recommend interested parties follow up with a study that incorporates either SNP (single nucleotide polymorphism) technology, or microsatellite DNA methods. These technologies do require an investment of time and resources to develop polymorphic markers. However, they also would provide much more comprehensive sampling of the genome, and hence would be much more likely to detect emergent divergent evolution of *tuplashensis* populations.

References

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From:	Thompson, Brad
To:	rcronn@fs.fed.us
Subject:	Peer review request for Physaria douglasii ssp. tuplashensis report
Date:	Tuesday, September 10, 2013 3:58:11 PM
Attachments:	Cronn_peer_review_9_9_2013.pdf White Bluffs Bladderpod report for peer review.pdf

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Dr. Cronn,

Thank you for agreeing to particpate in the peer review of the "Sequence Variation Among Physaria douglasii Isolates" report that US Fish and Wildlife Service received during the most recent public comment period for the proposed listing of White Bluffs bladderpod. Please see attached for 1) the cover letter that includes instructions and 2) the report: "Sequence Variation Among Physaria douglasii Isolates" for your review. Please provide me with your completed review by September 19, 2013.

Sincerely,
Brad Thompson

--
Brad Thompson, Ph.D
Listing and Recovery Division Manager
US Fish and Wildlife Service
Washington Fish and Wildlife Office
510 Desmond Dr. SE, Suite 102
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360-753-6046
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United States Department of the Interior

FISH AND WILDLIFE SERVICE

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510 Desmond Dr. SE, Suite 102
Lacey, Washington 98503

SEP 10 2013

Dr. Richard Cronn
USDA Forest Service
Pacific Northwest Research Station
3200 SW Jefferson Way
Corvallis, OR 97331-4401

Dear Dr. Cronn:

The U.S. Fish and Wildlife Service (Service) is soliciting independent scientific reviews of the attached report received during the reopening of the public comment period for the proposed rule to list Umtanum desert buckwheat and White Bluffs bladderpod under the Endangered Species Act and to designate critical habitat (May 15, 2012; 77 FR 28704). You have been identified as a potential Peer Reviewer for this report based on your knowledge and expertise in one or more of the following areas: genetic study design and methodologies; plant genetics; and plant taxonomy.

The purpose of seeking independent peer review is to ensure use of the best scientific and commercial information available, to maximize the quality, objectivity, utility, and integrity of the information upon which the proposed action is based, and to incorporate input by recognized experts into the rulemaking process. Please note that the Service is not seeking advice on policy or recommendations on the legal status of the species. Rather, the Service requests that Peer Reviewers focus their review on identifying and characterizing scientific uncertainties and ensuring the accuracy of the biological information presented in the document. Specifically, we ask Peer Reviewers to focus their comments on the technical aspects of the study design, analyses, results, and application to White Bluffs bladderpod (*Physaria douglasii* ssp. *tuplashensis*) taxonomic status.

The following questions are provided to aid, but not constrain, your review:

Study Design:

1) Was the study design and implementation appropriate to assess whether *P. douglasii* ssp. *tuplashensis* is indeed sufficiently divergent to warrant subspecies designation?

2) Was the number of *P. douglasii* and *P. douglasii* ssp. *tuplashensis* individual plants included in this study sufficient in number, geographic distribution, and time to accurately represent the populations of interest (i.e., locale and subspecies) in order to accurately conclude the taxonomic status of White Bluffs bladderpod? Were the samples collected from throughout the range of *P. douglasii* and *P. douglasii* ssp. *tuplashensis* to capture the distribution of genetic diversity within *P. douglasii* and *P. douglasii* ssp. *tuplashensis*? Were sample sizes and the spatiotemporal distribution of collections acceptable for accurately concluding taxonomic status for plants such as *P. douglasii* and *P. douglasii* ssp. *tuplashensis*?
3) What is the probability of making Type I and Type II errors based on the reported study design and statistical tests?

Application to White Bluffs bladderpod:

4) Were the genetics methods and regions of the DNA interrogated in this study the most appropriate for evaluating the taxonomic status of White Bluffs bladderpod?
5) Do the results of this study need to be peer-reviewed, published in a refereed journal, and replicated by others in order to accurately conclude the taxonomic status of the White Bluffs bladderpod?

Interpretation of Results:

6) Were the *P. douglasii* and *P. douglasii* ssp. *tuplashensis* genomes sufficiently explored with the loci chosen in this study to accurately determine the level of genetic similarity or difference required to determine taxonomic status of the White Bluffs bladderpod?
7) Based on the genetic techniques employed in this study, what is the accepted threshold for making a taxonomic subspecies determination for White Bluffs bladderpod? In responding to this question, please provide a quantitative threshold for the genetics metrics employed in this study or other genetics approaches relied upon in order to determine or change the taxonomic status of plant species or sub-species.
8) Are the study results and associated report conclusions sufficient to warrant changing the taxonomic status of the White Bluffs bladderpod? If so, what alternative taxonomic status do you recommend based upon your review of this study?

Thank you for agreeing to participate in this peer review. Please provide your individual, written response to us. We would also appreciate receiving a copy of your Curriculum Vitae for our records. Your completed review, including your name and affiliation, will be included in the administrative record of this rule and will be available to interested parties upon request. We will summarize and respond to the issues raised by all peer reviewers and use the information, as appropriate, in any final listing and critical habitat rules published by us.

Our final decision on the listing of Umtanum desert buckwheat and White Bluffs bladderpod as well as designating critical habitat for these two species is scheduled to be published in the Federal Register no later than November 22, 2013. So that we may fully consider your input and coordinate other peer review and public comments as we develop the final rules, we would

appreciate receiving your comments by September 19, 2013. If you are unable to adequately review the documents during this time period, please let us know when we may anticipate receiving your comments.

Please submit your comments and associated materials to:

U.S. Fish and Wildlife Service
Washington Fish and Wildlife Office
Attention: Brad Thompson
510 Desmond Drive, Suite 102
Lacey, Washington 98503

If you have any questions, please feel free to contact me at (360) 753-6046 or brad_thompson@fws.gov.

Sincerely,

Brad Thompson, Manager
Listing and Recovery Division
Washington Fish and Wildlife Office

From:	Thompson, Brad
To:	steve.okane@uni.edu
Subject:	Peer review request for Physaria douglasii ssp. tuplashensis report
Date:	Monday, September 09, 2013 3:46:05 PM
Attachments:	O"Kane_peer_review_9_9_2013.pdf White Bluffs Bladderpod report for peer review.pdf

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Dr. Stephen O'Kane
Biology Reserach Complex 59
University of Northern Iowa
Department of Biology
Cedar Falls, IA 50614-0421

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Brad Thompson, Manager
Listing and Recovery Division
Washington Fish and Wildlife Office

From:	Thompson, Brad
To:	Danica@authentechnologies.com
Subject:	Peer review request for Physaria douglasii ssp. tuplashensis report
Date:	Monday, September 09, 2013 10:48:24 AM
Attachments:	Harbaugh Reynaud_peer_review_9_9_2013.pdf White Bluffs Bladderpod report for peer review.pdf

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Dr. Harbaugh Reynaud,

Thank you for agreeing to particpate in the peer review of the "Sequence Variation Among Physaria douglasii Isolates" report that US Fish and Wildlife Service received during the most recent public comment period for the proposed listing of White Bluffs bladderpod. Please see attached for 1) the cover letter that includes instructions and 2) the report: "Sequence Variation Among Physaria douglasii Isolates" for your review. Please provide me with your completed review by September 19, 2013.

Sincerely,
Brad Thompson

--
Brad Thompson, Ph.D
Listing and Recovery Division Manager
US Fish and Wildlife Service
Washington Fish and Wildlife Office
510 Desmond Dr. SE, Suite 102
Lacey, WA 98503
360-753-6046
360-790-8187 (cell)

United States Department of the Interior

FISH AND WILDLIFE SERVICE

Washington Fish and Wildlife Office
510 Desmond Dr. SE, Suite 102
Lacey, Washington 98503

SEP - 9 2013

Dr. Danica Harbaugh Reynaud
AuthenTechnologies
2600 Hilltop Drive
Suite C346, Building B
Richmond, CA 94806

Dear Dr. Harbaugh Reynaud:

The U.S. Fish and Wildlife Service (Service) is soliciting independent scientific reviews of the attached report received during the reopening of the public comment period for the proposed rule to list Umtanum desert buckwheat and White Bluffs bladderpod under the Endangered Species Act and to designate critical habitat (May 15, 2012; 77 FR 28704). You have been identified as a potential Peer Reviewer for this report based on your knowledge and expertise in one or more of the following areas: genetic study design and methodologies; plant genetics; and plant taxonomy.

The purpose of seeking independent peer review is to ensure use of the best scientific and commercial information available, to maximize the quality, objectivity, utility, and integrity of the information upon which the proposed action is based, and to incorporate input by recognized experts into the rulemaking process. Please note that the Service is not seeking advice on policy or recommendations on the legal status of the species. Rather, the Service requests that Peer Reviewers focus their review on identifying and characterizing scientific uncertainties and ensuring the accuracy of the biological information presented in the document. Specifically, we ask Peer Reviewers to focus their comments on the technical aspects of the study design, analyses, results, and application to White Bluffs bladderpod (*Physaria douglasii* ssp. *tuplashensis*) taxonomic status.

The following questions are provided to aid, but not constrain, your review:

Study Design:

1) Was the study design and implementation appropriate to assess whether *P. douglasii* ssp. *tuplashensis* is indeed sufficiently divergent to warrant subspecies designation?

2) Was the number of *P. douglasii* and *P. douglasii* ssp. *tuplashensis* individual plants included in this study sufficient in number, geographic distribution, and time to accurately represent the populations of interest (i.e., locale and subspecies) in order to accurately conclude the taxonomic status of White Bluffs bladderpod? Were the samples collected from throughout the range of *P. douglasii* and *P. douglasii* ssp. *tuplashensis* to capture the distribution of genetic diversity within *P. douglasii* and *P. douglasii* ssp. *tuplashensis*? Were sample sizes and the spatiotemporal distribution of collections acceptable for accurately concluding taxonomic status for plants such as *P. douglasii* and *P. douglasii* ssp. *tuplashensis*?
3) What is the probability of making Type I and Type II errors based on the reported study design and statistical tests?

Application to White Bluffs bladderpod:

4) Were the genetics methods and regions of the DNA interrogated in this study the most appropriate for evaluating the taxonomic status of White Bluffs bladderpod?
5) Do the results of this study need to be peer-reviewed, published in a refereed journal, and replicated by others in order to accurately conclude the taxonomic status of the White Bluffs bladderpod?

Interpretation of Results:

6) Were the *P. douglasii* and *P. douglasii* ssp. *tuplashensis* genomes sufficiently explored with the loci chosen in this study to accurately determine the level of genetic similarity or difference required to determine taxonomic status of the White Bluffs bladderpod?
7) Based on the genetic techniques employed in this study, what is the accepted threshold for making a taxonomic subspecies determination for White Bluffs bladderpod? In responding to this question, please provide a quantitative threshold for the genetics metrics employed in this study or other genetics approaches relied upon in order to determine or change the taxonomic status of plant species or sub-species.
8) Are the study results and associated report conclusions sufficient to warrant changing the taxonomic status of the White Bluffs bladderpod? If so, what alternative taxonomic status do you recommend based upon your review of this study?

Thank you for agreeing to participate in this peer review. Please provide your individual, written response to us. We would also appreciate receiving a copy of your Curriculum Vitae for our records. Your completed review, including your name and affiliation, will be included in the administrative record of this rule and will be available to interested parties upon request. We will summarize and respond to the issues raised by all peer reviewers and use the information, as appropriate, in any final listing and critical habitat rules published by us.

Our final decision on the listing of Umtanum desert buckwheat and White Bluffs bladderpod as well as designating critical habitat for these two species is scheduled to be published in the Federal Register no later than November 22, 2013. So that we may fully consider your input and coordinate other peer review and public comments as we develop the final rules, we would

appreciate receiving your comments by September 19, 2013. If you are unable to adequately review the documents during this time period, please let us know when we may anticipate receiving your comments.

Please submit your comments and associated materials to:

U.S. Fish and Wildlife Service
Washington Fish and Wildlife Office
Attention: Brad Thompson
510 Desmond Drive, Suite 102
Lacey, Washington 98503

If you have any questions, please feel free to contact me at (360) 753-6046 or brad_thompson@fws.gov.

Sincerely,

Brad Thompson, Manager
Listing and Recovery Division
Washington Fish and Wildlife Office

From:	Thompson, Brad
To:	gallen@uvic.ca
Subject:	Peer review request for Physaria douglasii ssp. tuplashensis report
Date:	Monday, September 09, 2013 10:43:14 AM
Attachments:	Allen_peer_review_9_9_2013.pdf White Bluffs Bladderpod report for peer review.pdf

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Dr. Allen,

Thank you for agreeing to particpate in the peer review of the "Sequence Variation Among Physaria douglasii Isolates" report that US Fish and Wildlife Service received during the most recent public comment period for the proposed listing of White Bluffs bladderpod. Please see attached for 1) the cover letter that includes instructions and 2) the report: "Sequence Variation Among Physaria douglasii Isolates" for your review. Please provide me with your completed review by September 19, 2013.

Sincerely,
Brad Thompson

--
Brad Thompson, Ph.D
Listing and Recovery Division Manager
US Fish and Wildlife Service
Washington Fish and Wildlife Office
510 Desmond Dr. SE, Suite 102
Lacey, WA 98503
360-753-6046
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United States Department of the Interior

FISH AND WILDLIFE SERVICE

Washington Fish and Wildlife Office
510 Desmond Dr. SE, Suite 102
Lacey, Washington 98503

SEP - 9 2013

Dr. Geraldine Allen
Department of Biology
University of Victoria
Cunningham 202
3800 Finnerty Road
Victoria, BC V8P 5C2, Canada

Dear Dr. Allen:

The U.S. Fish and Wildlife Service (Service) is soliciting independent scientific reviews of the attached report received during the reopening of the public comment period for the proposed rule to list Umtanum desert buckwheat and White Bluffs bladderpod under the Endangered Species Act and to designate critical habitat (May 15, 2012; 77 FR 28704). You have been identified as a potential Peer Reviewer for this report based on your knowledge and expertise in one or more of the following areas: genetic study design and methodologies; plant genetics; and plant taxonomy.

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The following questions are provided to aid, but not constrain, your review:

Study Design:

1) Was the study design and implementation appropriate to assess whether *P. douglasii* ssp. *tuplashensis* is indeed sufficiently divergent to warrant subspecies designation?

2) Was the number of *P. douglasii* and *P. douglasii* ssp. *tuplashensis* individual plants included in this study sufficient in number, geographic distribution, and time to accurately represent the populations of interest (i.e., locale and subspecies) in order to accurately conclude the taxonomic status of White Bluffs bladderpod? Were the samples collected from throughout the range of *P. douglasii* and *P. douglasii* ssp. *tuplashensis* to capture the distribution of genetic diversity within *P. douglasii* and *P. douglasii* ssp. *tuplashensis*? Were sample sizes and the spatiotemporal distribution of collections acceptable for accurately concluding taxonomic status for plants such as *P. douglasii* and *P. douglasii* ssp. *tuplashensis*?
3) What is the probability of making Type I and Type II errors based on the reported study design and statistical tests?

Application to White Bluffs bladderpod:

4) Were the genetics methods and regions of the DNA interrogated in this study the most appropriate for evaluating the taxonomic status of White Bluffs bladderpod?
5) Do the results of this study need to be peer-reviewed, published in a refereed journal, and replicated by others in order to accurately conclude the taxonomic status of the White Bluffs bladderpod?

Interpretation of Results:

6) Were the *P. douglasii* and *P. douglasii* ssp. *tuplashensis* genomes sufficiently explored with the loci chosen in this study to accurately determine the level of genetic similarity or difference required to determine taxonomic status of the White Bluffs bladderpod?
7) Based on the genetic techniques employed in this study, what is the accepted threshold for making a taxonomic subspecies determination for White Bluffs bladderpod? In responding to this question, please provide a quantitative threshold for the genetics metrics employed in this study or other genetics approaches relied upon in order to determine or change the taxonomic status of plant species or sub-species.
8) Are the study results and associated report conclusions sufficient to warrant changing the taxonomic status of the White Bluffs bladderpod? If so, what alternative taxonomic status do you recommend based upon your review of this study?

Thank you for agreeing to participate in this peer review. Please provide your individual, written response to us. We would also appreciate receiving a copy of your Curriculum Vitae for our records. Your completed review, including your name and affiliation, will be included in the administrative record of this rule and will be available to interested parties upon request. We will summarize and respond to the issues raised by all peer reviewers and use the information, as appropriate, in any final listing and critical habitat rules published by us.

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appreciate receiving your comments by September 19, 2013. If you are unable to adequately review the documents during this time period, please let us know when we may anticipate receiving your comments.

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Washington Fish and Wildlife Office
Attention: Brad Thompson
510 Desmond Drive, Suite 102
Lacey, Washington 98503

If you have any questions, please feel free to contact me at (360) 753-6046 or brad_thompson@fws.gov.

Sincerely,

Brad Thompson

Brad Thompson, Manager
Listing and Recovery Division
Washington Fish and Wildlife Office

From:	Thompson, Brad
To:	mdw26@duke.edu
Subject:	Peer review request for Physaria douglasii ssp. tuplashensis report
Date:	Monday, September 09, 2013 10:39:00 AM
Attachments:	Windham_peer_review_9_9_2013.pdf White Bluffs Bladderpod report for peer review.pdf

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Dr. Windham

Thank you for agreeing to particpate in the peer review of the "Sequence Variation Among Physaria douglasii Isolates" report that US Fish and Wildlife Service received during the most recent public comment period for the proposed listing of White Bluffs bladderpod. Please see attached for 1) the cover letter that includes instructions and 2) the report: "Sequence Variation Among Physaria douglasii Isolates" for your review. Please provide me with your completed review by September 19, 2013.

Sincerely,
Brad Thompson

--
Brad Thompson, Ph.D
Listing and Recovery Division Manager
US Fish and Wildlife Service
Washington Fish and Wildlife Office
510 Desmond Dr. SE, Suite 102
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360-753-6046
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United States Department of the Interior

FISH AND WILDLIFE SERVICE

Washington Fish and Wildlife Office
510 Desmond Dr. SE, Suite 102
Lacey, Washington 98503

SEP - 9 2013

Dr. Michael D. Windham
Room 137
Biological Sciences Building
125 Science Drive
Duke University
Durham, NC 27708

Dear Dr. Windham:

The U.S. Fish and Wildlife Service (Service) is soliciting independent scientific reviews of the attached report received during the reopening of the public comment period for the proposed rule to list Umtanum desert buckwheat and White Bluffs bladderpod under the Endangered Species Act and to designate critical habitat (May 15, 2012; 77 FR 28704). You have been identified as a potential Peer Reviewer for this report based on your knowledge and expertise in one or more of the following areas: genetic study design and methodologies; plant genetics; and plant taxonomy.

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3) What is the probability of making Type I and Type II errors based on the reported study design and statistical tests?

Application to White Bluffs bladderpod:

4) Were the genetics methods and regions of the DNA interrogated in this study the most appropriate for evaluating the taxonomic status of White Bluffs bladderpod?
5) Do the results of this study need to be peer-reviewed, published in a refereed journal, and replicated by others in order to accurately conclude the taxonomic status of the White Bluffs bladderpod?

Interpretation of Results:

6) Were the *P. douglasii* and *P. douglasii* ssp. *tuplashensis* genomes sufficiently explored with the loci chosen in this study to accurately determine the level of genetic similarity or difference required to determine taxonomic status of the White Bluffs bladderpod?
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Lacey, Washington 98503

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Sincerely,

Brad Thompson, Manager
Listing and Recovery Division
Washington Fish and Wildlife Office

EVALUATION OF REPORT: "Sequence Variation Among *Physaria douglasii* Isolates", by Cort L. Anderson, University of Idaho

By Geraldine A. Allen
Department of Biology, University of Victoria

The objective of the above report was to provide genetic evidence to assist in determining the taxonomic status of the White Bluffs bladderpod, *Physaria douglasii* ssp*. tuplashensis*. This is a review of the report to determine if this objective was met.

Study Design

For the study, plant material (either recently collected or taken from herbarium specimens) was obtained from a number of localities, and each sample was sequenced for five DNA regions (four from the chloroplast genome and one from the nuclear genome). The general design of the study is appropriate for the question. However, the design is weak in two respects:

1. DNA regions used.
    a. Five DNA regions were sequenced (or sequencing was attempted) in each sample. Of these, three (the rbcL, matK, and ndhF genes) are not rapidly evolving genes, and would very likely show no differences between two subspecies of the same species. The rbcL gene in particular is best suited for assessing relationships among higher-level taxonomic groups (families and orders). No sequence data were obtained for the matK gene, but it is not surprising that little or no variation was found for the rbcL and ndhF genes. The remaining two regions, ITS and the trnH-psbA spacer, are both more suitable for studying variation within a species but may still underestimate the genetic variation present.
2. Sampling intensity.
    a. Only one sample was sequenced from each locality, thus no assessment of intrapopulation variability can be made.
    b. Fifteen localities were sampled in total (4 for *Physaria douglasii* ssp*. tuplashensis* and 11 for the typical subspecies). Practical considerations (e.g., time available for the study) can dictate sampling intensity. For the rare and highly localized ssp. *tuplashensis*, the number of localities is probably sufficient. For the widely distributed typical subspecies, the sampling is on the low side for its geographic range and the genetic variation is likely to be underestimated. For the purposes of this study, if there are sampling constraints, it is useful to sample the typical subspecies most intensively close to the range of ssp. *tuplashensis.*It is difficult to see from the locality table whether this was the case.

Because the study design is relatively weak, I would expect a high probability of Type II error (failure to detect differences between the subspecies, even though such

differences may be present). I am not able to estimate this precisely from the information available in the report.

Analysis and Interpretation of Results

The report gives very little detail about the methods of data analysis, except to state that the program PAUP was used. (I assume from the figures that these are Maximum Parsimony bootstrap analyses.) Phylogenetic analyses are not necessarily the most appropriate approach for assessing overlap between closely related taxa, and I found it difficult to assess how similar these subspecies are from the included figures. A network analysis method (e.g., with a program such as TCS), showing the numbers of mutations separating sequence variants, would be much more useful for showing the level of differentiation between sequences and the degree of overlap (sequence variants in common) between the taxa. The report mentions that individual sample sequences appear in an appendix. A sequence haplotype network could easily be produced from these sequences. Nevertheless, even if this was done, in my assessment the data are marginally sufficient for assessing the genetic differences between the two subspecies.

There is no absolute standard for determining how many DNA regions are needed to assess these differences; this depends on the amount of variation in each region. However, many published studies addressing a similar question might present sequence data from 3 to 5 variable regions, with 3-5 samples per population.

Application to White Bluffs bladderpod

The report does not provide sufficient information to make a recommendation on the taxonomic status of *Physaria douglasii* ssp*. tuplashensis.* This would, in any case, be based on other kinds of data as well, such as morphological and ecological differences.

The methods that were used in this report can provide useful data for assessing taxonomic status of taxa at this level, provided that enough DNA variation is found. Another alternative but often more powerful approach is to use methods that examine DNA banding patterns, such as AFLP. Although individual bands are scored only as presence-absence characters, the number of bands is usually large and can provide a very sensitive method for assessing overlap between taxa. This method usually involves a larger sample size, with 5-10 individual samples per locality, so it typically is not done using herbarium specimens. If further molecular work is done on these two subspecies, AFLP should be considered as a possible approach.

Geraldine Anne ALLEN

UNIVERSITY OF VICTORIA - CURRICULUM VITAE

Faculty: Science **Department: BIOLOGY**

EDUCATION and TRAINING

Degree	Institution	Year obtained
B.Sc.	University of British Columbia	1972
M.Sc.	University of British Columbia	1975
Ph.D.	Oregon State University	1981

POSITIONS HELD PRIOR to APPOINTMENT at UVic

1975-1977 Research Assistant, Botanical Garden, University of British Columbia

APPOINTMENTS at the UNIVERSITY of VICTORIA

Period	Rank	Academic unit
1981-86	Assistant Professor	Biology
1986-88	Assistant Professor w/Tenure	Biology
1988-	Associate Professor	Biology

MAJOR FIELD(S) of SCHOLARLY or PROFESSIONAL INTEREST

Plant systematics & evolution, phylogeography & biogeography, conservation biology

HONOURS and FELLOWSHIPS

Canadian Botanical Association, Mary E. Elliott Service Award, 2003

PUBLICATIONS and PRESENTATIONS

a. Articles published in refereed journals

1. Guppy [Allen], G. A. and B. A. Bohm. 1976. Flavonoids of five *Hieracium* species of British Columbia. Biochemical Systematics and Ecology 4: 231-234.
2. Guppy [Allen], G. A. 1976. The hawkweeds of British Columbia. Davidsonia 7: 13-17.
3. Beil, C.E., R.L. Taylor and G.A. Guppy [Allen]. 1976. The biogeoclimatic zones of British Columbia. Davidsonia 7: 45-55
4. Guppy [Allen], G. A. 1978. Species relationships of *Hieracium* (Asteraceae) in British Columbia. Can. J. Bot. 56: 3008-3019.

5. Semple, J.C., J.G. Chmielewski, K. Sankara Rao and G.A. Allen. 1983. The cytogeography of *Aster lanceolatus*. II. A preliminary survey of the range including *A. hesperius*. Can. J. Bot. 61: 434-441.
6. Allen, G. A., M. L. Dean and K. L. Chambers. 1983. Hybridization studies in the *Aster occidentalis* (Asteraceae) polyploid complex of western North America. Brittonia 35: 353-361.
7. Allen, G. A. 1984. Morphological and cytological variation in the western North American *Aster occidentalis* complex (Asteraceae). Syst. Bot. 9: 175-191.
8. Allen, G. A. 1985. The hybrid origin of *Aster ascendens* (Asteraceae). Amer. J. Bot. 72:268-277.
9. Allen, G. A. 1986. Amphiploid origin of two endemic races of *Aster* (Asteraceae) in southern California. Amer. J. Bot. 73: 330-335.
10. Allen, G..A. 1986. Flowering pattern and fruit production in the dioecious shrub *Oemleria cerasiformis* (Rosaceae). Can. J. Bot. 64: 1216-1220.
11. Allen, G. A. and J. A. Antos. 1988. Morphological and ecological variation across a hybrid zone between *Erythronium oregonum* and *E. revolutum* (Liliaceae). Madroño 35: 32-38.
12. Allen, G. A. 1988. Flavonoids of *Aster bracteolatus*. Biochemical Systematics and Ecology 16:139-141.
13. Allen, G. A. and J. A. Antos. 1988. Relative reproductive effort in males and females of the dioecious shrub *Oemleria cerasiformis*. Oecologia 76: 111-118.
14. Allen, G. A., V. S. Ford and L. D. Gottlieb. 1990. A new subspecies of *Clarkia concinna* (Onagraceae) from Marin County, California. Madroño 37: 305-310.
15. Antos, J. A. and G. A. Allen. 1990. Habitat relationships of the Pacific Coast shrub *Oemleria cerasiformis* (Rosaceae). Madroño 37: 249-260.
16. Antos, J. A. and G. A. Allen. 1990. A comparison of reproductive effort in the dioecious shrub *Oemleria cerasiformis* using nitrogen, energy and biomass as currencies. American Midland Naturalist 124: 254-262.
17. Shevock, J. R., J. A. Bartel and G. A. Allen. 1990. Distribution, ecology and taxonomy of *Erythronium* (Liliaceae) in the Sierra Nevada of California. Madroño 37: 261-273.
18. Allen, G. A., L. D. Gottlieb and V. S. Ford. 1991. Electrophoretic evidence for the independent origins of two self-pollinating subspecies of *Clarkia concinna* (Onagraceae). Canadian Journal of Botany 69: 2299-2301.
19. Shevock, J. R. and G. A. Allen. 1991. A new variety of *Erythronium citrinum* (Liliaceae) from the Scott Mountains of northwest California. Phytologia 71: 101-103.
20. Suttill, T. A. and G. A. Allen. 1992. Morphological and chromosomal variation in *Dodecatheon pulchellum* (Primulaceae). Canadian Journal of Botany 70:2476-2483.
21. Allen, G. A. and J. A. Antos. 1993. Sex ratio variation in the dioecious shrub *Oemleria cerasiformis*. American Naturalist 141:537-553.
22. Antos, J. A. and G. A. Allen. 1994. Biomass allocation among reproductive structures in the dioecious shrub *Oemleria cerasiformis* - a functional interpretation. Journal of Ecology 82: 21-29.
23. Allen, G. A. 1996. Mountain Plants of the Pacific by R. J. Taylor and G. W. Douglas (book review). Northwest Science 70: 287-288.

24. Allen, G. A., J. A. Antos, A. C. Worley, T. A. Suttill, and R. J. Hebda. 1996. Morphological and genetic variation in disjunct populations of the avalanche lily *Erythronium montanum*. Canadian Journal of Botany 74:403-412.
25. Shevock, J. R. and G. A. Allen. 1997. *Erythronium taylori* (Liliaceae), a new species from the central Sierra Nevada of California. Madroño 44: 359-363.
26. Miller, M. T. and G. A. Allen. 1997. Noteworthy collection from British Columbia: *Silene spaldingii*. Madroño 44: 281.
27. Allen, G. A. and C. L. Eccleston. 1998. Genetic resemblance of allotetraploid *Aster ascendens* to its diploid progenitors *A. falcatus* and *A. occidentalis*. Canadian Journal of Botany 76: 338-344.
28. Penny, J. L., G. W. Douglas and G. A. Allen. 1998. Status of the bearded owl-clover, *Triphysaria versicolor* ssp. *versicolor* (Scrophulariaceae), in Canada. Canadian Field-Naturalist 112: 481-485.
29. Nams, V., J. A. Antos and G. A. Allen. 1998. Seedling establishment in a patchy environment. Ecoscience 5 (1): 86-94.
30. Antos, J. A. and G. A. Allen. 1999. Patterns of reproductive effort in male and female shrubs of *Oemeria cerasiformis*: a six-year study. Journal of Ecology 87: 77-84.
31. Allen, G. A. 2001. Hybrid speciation in *Erythronium* (Liliaceae): a new allotetraploid species from Washington State. Systematic Botany 26(2): 263-272.
32. Loewen, D. C., G. A. Allen and J. A. Antos. 2001. Autecology of *Erythronium grandiflorum* in western Canada. Canadian Journal of Botany 79: 500-509.
33. Allen, G. A., D. E. Soltis and P. S. Soltis. 2003. Phylogeny and biogeography of *Erythronium* (Liliaceae) inferred from chloroplast matK and nuclear rDNA ITS sequences. Systematic Botany 28: 512-523.
34. Mitchell, M. G. E, J. A. Antos and G. A. Allen. 2004. Modules of reproduction in females of the dioecious shrub *Oemleria cerasiformis*. Canadian Journal of Botany 82: 393-400.
35. Miller, M. T., G. A. Allen and J. A. Antos. 2004. Dormancy and flowering in two mariposa lilies (*Calochortus*) with contrasting distribution patterns. Canadian Journal of Botany 82: 1790-1799.
36. Wheeler, E. J., R. Edward and G. A. Allen. 2005. Morphological and molecular evidence concerning the relationship of *Lupinus polyphyllus* and *L. wyethii* (Fabaceae). Madroño 52: 107-113.
37. Miller, M. T., J. A. Antos and G. A. Allen. 2007. Demographic differences between two sympatric lilies (*Calochortus*) with contrasting distributions, as revealed by matrix analysis. Plant Ecology 191: 265-278.
38. Karst, A., J. A. Antos and G. A. Allen. 2008. Flowering, fruit set and sex ratios in dioecious *Rubus chamaemorus* (Rosaceae) in Labrador. Botany-Botanique 86: 204-212.
39. Marr, K. L., G. A. Allen and R. J. Hebda. 2008. Refugia in the Cordilleran Ice Sheet of western North America: chloroplast DNA diversity in the Arctic-alpine plant *Oxyria digyna*. Journal of Biogeography 35(7): 1323-1334.
40. Allen, G. A. 2008. The origins of polyploids in western North American fawn-lilies (*Erythronium*). Botany-Botanique 86(8): 835-845. [invited paper]
41. Hawryzki, A. R., G. A. Allen and J. A. Antos. 2011. Prolonged dormancy in the geophyte *Allium amplectens* on Vancouver Island. Botany-Botanique 89(11): 737-744.

42. Allen, G. A., K. L. Marr, L. J. McCormick and R. J. Hebda. 2012. The impact of Pleistocene climate change on an ancient arctic-alpine plant: multiple lineages of disparate history in *Oxyria digyna*. Ecology and Evolution 2(3): 649-665, DOI 10.1002/ece3.213.
43. Miller, M. T., J. A. Antos and G. A. Allen. 2012. Demography of a dormancy-prone geophyte: influence of spatial scale on interpretation of dynamics. Plant Ecology 213: 569-579, DOI 10.1007/s11258-012-0022-8.
44. Marr, K. L., G. A. Allen, R. J. Hebda and L. J. McCormick. 2013. Phylogeographical patterns in the widespread arctic-alpine *Bistorta vivipara* (Polygonaceae) with emphasis on western North America. Journal of Biogeography 40(5): 847-856, DOI 10.1111/jbi.12042.

b. Books and chapters in books

Taxonomic treatments:

1. Allen, G. A. 1993. *Aster*. Pp. 205-209 in: J.C. Hickman (ed.), The Jepson Manual: Vascular Plants of California, Univ. California Press. [23 spp.]
2. Allen, G. A. 1993. *Chaetopappa*. P. 226 in:J.C. Hickman (ed.), The Jepson Manual: Vascular Plants of California, Univ. California Press. [1 sp.]
3. Allen, G. A. 1993. *Erythronium*. Pp. 1192-1194 in: J.C. Hickman (ed.), The Jepson Manual: Vascular Plants of California, Univ. California Press. [13 spp.]
4. Allen, G. A. 1993. *Townsendia*. P. 354 in: J.C. Hickman (ed.), The Jepson Manual: Vascular Plants of California, Univ. California Press. [4 spp.]
5. Allen, G. A. and K. R. Robertson. 2002. *Erythronium*. Pp. 153-164 in: Flora of North America Editorial Committee (eds.), *Flora of North America North of Mexico*. Vol. 26. Monocots. Oxford University Press, New York.
6. Allen, G.A. 2002. *Aster*. Pp. 120-121 in: Baldwin, B.G., S. Boyd, B. J. Ertter, R. W. Patterson, T. J. Rosatti, and D. H. Wilken (eds). The Jepson Desert Manual: Vascular Pants of Southeastern California. Univ. California Press, Berkeley.
7. Brouillet, L., J. C. Semple, G. A. Allen, K. L. Chambers and S. Sundburg. 2006. *Symphyotrichum*. Pp. 465-539 in: Flora of North America Editorial Committee (eds.), *Flora of North America North of Mexico*. Vol. 20. Asteraceae. Oxford University Press, New York.
8. Allen, G. A. 2006. *Eucephalus*. Pp. 39-42 in: Flora of North America Editorial Committee (eds.), *Flora of North America North of Mexico*. Vol. 20. Asteraceae. Oxford University Press, New York.
9. Keil, D. J. and G. A. Allen. 2012. *Chaetopappa.* In B. A. Baldwin, D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti & D. H. Wilken (eds), *The Jepson Manual, Vascular Plants of California*, 2nd edition, University of California Press, P 278
10. Allen, G. A. 2012. *Eucephalus*. In B. A. Baldwin, D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti & D. H. Wilken (eds.), *The Jepson Manual: Vascular Plants of California,* 2nd edition, University of California Press. Pp. 326-328.
11. Allen, G. A. 2012. *Symphyotrichum*. In B. A. Baldwin, D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti & D. H. Wilken (eds.), *The Jepson Manual: Vascular Plants of California*, 2nd edition, University of California Press.Pp. 428-431.
12. Allen, G. A. and J. Whitton. 2012. *Townsendia*. In B. A. Baldwin, D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti & D. H. Wilken (eds.), *The Jepson Manual: Vascular Plants of California*, 2nd edition, University of California Press. Pp. 436-437.

13. Allen, G. A. 2012. *Erythronium*. In B. A. Baldwin, D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti & D. H. Wilken (eds.), *The Jepson Manual: Vascular Plants of California*, 2nd edition, University of California Press. Pp. 1386-1387.

Other contributions to edited volumes:

1. Allen, G. A. and R. A. Cannings. 1985. Museum collections and life history studies. In Miller, E. H. (ed.), Museum Collections: Their Roles and Future in Biological Research. British Columbia Provincial Museum, Occasional Paper No. 25, pp. 169-208.
2. Allen, G. A. 1994. Speciation. In: S.L. Macey (ed.), Encyclopedia of Time. Garland Publishing, Hamden, Conn.
3. Allen, G. A. 1994. Coevolution. In: S.L. Macey (ed.), Encyclopedia of Time. Garland Publishing, Hamden, Conn.
4. Allen, G. A. 1994. Origin of Sex. In: S.L. Macey (ed.), Encyclopedia of Time. Garland Publishing, Hamden, Conn.
5. Allen, G. A. 1994. Eve's Genetic Legacy. In: S.L. Macey (ed.), Encyclopedia of Time. Garland Publishing, Hamden, Conn.

c. Other publications

1. Allen, G. A. 1992. Starch gel electrophoresis methods for surveying isozymes in *Alnus rubra* (red alder). Research Branch, B.C. Ministry of Forests.
2. Allen, G. A. and S. Ford. 1996. Genetic variation and reproductive ecology of *Rhododendron macrophyllum* (Pacific Rhododendron) in Skagit Valley and Manning Provincial Parks. Phase II. Contract report, Skagit Environmental Endowment Commission.
3. Penny, J. L., G. W. Douglas, and G. A. Allen. 1996. Status report on Bearded Owl-Clover (*Triphysaria versicolor* ssp. *versicolor*). Committee on Status of Endangered Wildlife in Canada (COSEWIC), Subcommittee for Vascular Plants, Mosses and Lichens.
4. Allen, G. A. 1996. Demographic and taxonomic studies of rare plant species. Final report, Forest Renewal B.C.
5. Allen, G. A. 1997. Ecological, genetic and taxonomic studies of rare plant species. Final report for 1996-97 research award, Forest Renewal B.C.
6. Allen, G. A. 1997. Ecological, genetic and taxonomic studies of rare plant species. Interim report for 1996-97, Forest Renewal B.C.
7. Allen, G. A. and B. Costanzo. 1997. Identification of introduced *Heracium* (hawkweed) specimens for Washington State Noxious Weed Control Board. [contract report].
8. Allen, G. A. 1998. Ecological, genetic and taxonomic studies of rare plant species. Interim report for 1997-98, Forest Renewal B.C.
9. Allen, G. A. 1999. Ecological, genetic and taxonomic studies of rare plant species. Interim report for 1998-99, Forest Renewal B.C.
10. Allen, G. A. 2000. Ecological, genetic and taxonomic studies of rare plant species. Interim report for 1999-2000, Forest Renewal B.C.
11. Allen, G. A. 2001. Ecological, genetic and taxonomic studies of rare plant species. Final five-year report, 1996-2001, Forest Renewal B.C.
12. Allen, G. A. 2002. Preliminary investigation of ISSR genetic markers in *Cirsium palustre* (marsh thistle) and *Cirsium arvense* (Canada thistle). Protection Branch, B.C. Ministry of Forests.

13. Allen, G. A. 2003. Preliminary investigation of chromosome numbers in weedy hawkweeds (*Hieracium*). Protection Branch, B.C. Ministry of Forests.
14. Marr, K. L. and G. A. Allen. 2005. Report on molecular variation in tamarack (*Larix laricina*) using two chloroplast spacer regions. Prince George Forest Region, B.C. Ministry of Forests.
15. Allen, G. A. 2007. Molecular Studies of *Erythronium elegans* and related species in western Oregon. Contract Report to USDI Bureau of Land Management, Salem, Oregon.
16. Allen, G. A. 2009. Taxonomic boundaries and molecular variation in *Eucephalus vialis* and related species. Contract report to Institute of Applied Ecology, Corvallis, Oregon.
17. Allen, G. A. 2009. Morphological and molecular variation in the endemic fawn-lily *Erythronium elegans* and its relationship to other Pacific Northwest fawn-lilies. Final contract Report to USDI Bureau of Land Management, Salem, Oregon.
18. Allen, G. A. 2012. Molecular variation in native and introduced clones of Common Reed Grass (*Phragmites australis*) in British Columbia. Contract report, Noxious Weed Program, BC Ministry of Forests.

d. Invited presentations at conferences or institutions

1. Allen, G. A. The hybrid origin of *Aster ascendens* and *A. bernardinus*. Department of Botany, University of British Columbia, December 1982 (**invited lecture**).
2. Allen, G. A. Reticulate evolution in *Aster*. British Columbia Provincial Museum, February 1984 (**invited lecture**).
3. Allen, G. A. Reticulate evolution in the *Aster occidentalis* complex. Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, May 1984 (**invited lecture**).
4. Allen, G.A. Multiple approaches to the study of rare and endangered plant species. AAAS Pacific Division Annual Meeting, Corvallis, Oregon, June 1988 (**invited lecture**).
5. Allen, G.A. Reticulate evolution in the *Aster occidentalis* complex. Rancho Santa Ana Botanic Garden, Claremont Graduate School, Claremont, California, February 1989 (**invited lecture**).
6. Allen, G.A. Polyploidy and speciation in *Aster*. Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, May 1990 (**invited lecture**).
7. Allen, G. A. Reproductive patterns and sex ratios in a north American dioecious shrub, *Oemleria cerasiformis*. Australian Systematic Botany Society, Canberra Chapter, December 1998 (**invited lecture**).
8. Allen, G. A. Genetic variation in plants: the consequences of Pleistocene glaciation. Department of Biological Science, St. Mary's University, Halifax, Nova Scotia, November 1999 (**invited lecture**).
9. Allen, G. A. The importance of hybridization in plant evolution. Dept of Biology, University of Victoria, February 2000 (departmental seminar).
10. Allen, G. A. After the ice: phylogeographic patterns in arctic-alpine plants of western Canada. Canadian Museum of Nature, Ottawa, ON, April 2008 (**invited talk**).
11. Allen, G. A. Phylogeographic patterns in alpine plants and the postglacial history of western North America. Université de Montréal, Institut de recherche en biologie végétale, Montréal, QC, May 2008 (**invited talk**).

12. Allen, G. A., K. L. Marr, H. J. Guest, S. Elwell and R. J. Hebda. Phylogeography of arctic-alpine plants and the glacial history of western North America. Botany 2008 (Botanical Society of America) Annual Meeting, Vancouver, BC, July 2008 (**invited symposium talk**).
13. Allen, G. A. Biogeography of arctic-alpine plants: DNA analyses and what we can learn from them. Botany BC Annual Meeting, Manning Park, BC (**invited talk**), July 2012.

e. Membership and service on international, national and provincial professional bodies and societies

1997-2002 Treasurer, Canadian Botanical Association
1999-2000 Member of Advisory Committee for Biodiversity through Taxonomy curriculum project, Open Learning Agency, B.C. Ministry of Advanced Education.
2002-2006 Systematics Section Chair, Canadian Botanical Association
2006-present Member, Conservation Section Advisory Group, Canadian Botanical Association

f. Conference organisational committees

2001 Organizer (with L. Brouillet and L. Hermanutz) of symposium "Phytogeography of Glaciated Landscapes" at the Canadian Botanical Association Annual Meeting, Kelowna, B.C., June 2001.
2005 Organizer (with M. Dale) of symposium "Ecology and Evolution of Arctic and Alpine Plants", Plant Canada Annual Meeting, Edmonton, AB, June 2005.

g. Grant proposals reviewed (1990-2012)

National Science Foundation, U.S.A. (9)
Natural Science and Engineering Research Council of Canada (16)
National Geographic Society (1)

h. Visiting scientists hosted

2002-present Dr. Ken Marr, Royal British Columbia Museum (phylogeography of Arctic-alpine plants in postglacial landscapes).

i. Reviews for journals, book reviews, published commentaries

1990-2012 reviews for journals: 1-8 per year for botanical, ecological and taxonomic journals

j. Other professional activities

1987-88 Visiting Scientist, Department of Genetics, University of California at Davis.
1990-91 Consultant on rare and endangered asters for Natural Heritage Section, Bureau of Wildlife, Idaho Department of Fish and Game.
1996-98 Hawkweed identification for Washington State Noxious Weed Control Board.
1998-99 Visiting Researcher, School of Botany and Zoology, The Australian National University, Canberra, Australia.
1999 Visiting Scientist, Department of Botany, Washington State University.
2000-2001 External Member, Collections Development Plan Committee, Canadian Museum of Nature.

2001-2004	Member, Rare Plants Recovery Action Group, Garry Oak Ecosystem Recovery Team (GOERT).
2002, 2003	Plant identification for Saanich Police Department
2002-2006	Regional reviewer, Flora North America Project
2003	Expert witness for Crown Prosecutor in arson case, Victoria.
2003-2004	Member, Rare Plants Recovery Implementation Group, Garry Oak Ecosystem Recovery Team (GOERT).
2004	Expert witness for Crown Prosecutor in arson case, B.C. Supreme Court.
2004-present	Member, Research Recovery Implementation Group, Garry Oak Ecosystem Recovery Team (GOERT).
2006-2008	Collaboration with Institute for Applied Ecology (Corvallis, Oregon, USA) on genetic studies and management strategies of rare asters.
2007-2008	Chercheur invitée, Institut de Recherche en Biologie Végétale, Université de Montréal.
2009-present	Consultation/advice to Bureau of Land Management (USA) and US Forest Service personnel on management of rare fawn-lilies (*Erythronium*) in w USA.
2009-present	Collaboration with C. Brochmann, National Centre for Biosystematics, University of Oslo, Norway (phylogeography of circumpolar plant species).
2012-present	Collaboration with R. Abbott, St. Andrews University, Scotland, and J.-Q. Liu, Lanzhou University, China (worldwide phylogeography & molecular ecology of mountain-sorrel).
2012-present	Collaboration with J. Wen and J.-Q. Zhang, Smithsonian Institution, USA (phylogeography of Asian and North American roseroots).

OUTREACH

February 1997	Keynote talk, Victoria Natural History Society (Annual General Meeting),native plants of British Columbia
November 1999	Presentation for Victoria Natural History Society, Native Plant Group (Australian plants)
March 2003	Presentation for Victoria Horticultural Society, Native Plant Study Group (evolution and diversity of fawn-lilies)
October 5, 2005	Radio interview by Camosun College campus radio station on the scientific basis for Evolution and Intelligent Design
February, 2006	Illustrated talk for Sooke Garden Club (gardening with native plants)
April, 2009	Interview by Campbell River Mirror on trilliums (article by Christine Scott, published May 8, 2009)
February 2010	Illustrated talk on worldwide diversity of fawn-lilies for Victoria Native Plant Group
July 2010	Interview with CHEK-TV (Victoria) on a local noxious weed, giant hogweed (aired July 23, 2010)
November 2010	Spearheaded drafting of position letter from UVic ecologists to BC Species At Risk Task Force (with Brian Starzomski, Envir. Studies)
May 2011	Group leader (vascular plants), Metchosin Bioblitz

May 2012	Group leader (vascular plants), Metchosin Bioblitz
April 2012	Interview with Oak Bay News (Victoria) on fawn-lilies and spring wildflowers (April 18, 2012)
October 2012	BC Flora Update (meeting of experts to advise the Conservation Data Centre on CDC rankings and taxonomic changes)
May 2013	Group leader (vascular plants), Metchosin Bioblitz

Review of "Sequence variation among *Physaria douglasii* isolates" for the US Fish and Wildlife Service

1. Was the study design and implementation appropriate to assess whether *P. douglasii* ssp. *tuplashensis* is sufficiently divergent to warrant subspecies designation?

   I think the study design wasn't relevant to addressing the subspecific validity of *P. douglasii.* ssp. *tuplashensis* for two important reasons:

   - First, molecular studies that focus on intraspecific variation and differentiation almost always use genetic markers that evolve at a rate that is relevant to population divergence. In contrast, this preliminary study used two classes of genetic markers -- chloroplast DNA and ribosomal RNA genes -- that are inappropriate for discriminating among or defining subspecies because they diverge at a pace that is almost always too slow to mark isolation and/or divergence due to geography or sexual isolation. These markers can be used to identify the movement of chloroplast types ("haplotypes" or "variants") on the landscape, but this is a separate question from subspecies validity. As noted by the author, these markers are commonly used to identify differences between higher-level taxa (e.g., species up to families).
   - Second, studies of intraspecific variation typically include sufficient numbers of individuals in order to get accurate estimates of the quantitative extent of divergence and differentiation between populations and intraspecific taxa. This is because 'infrataxa' are conspecific, and are expected to segregate for the same kinds of genetic variation. If infrataxa do differ, it will be in the *amount or apportionment of genetic diversity*; fixed differences are not expected. A total sample size of 15 is insufficient to estimate accurate frequencies for two taxa.

2. Was the number of individual plants included in this study sufficient in number to accurately conclude the taxonomic status of *P. d.* ssp. *tuplashensis*?
   - As noted above, the answer is no.

3. What is the probability of making Type I and Type II errors.
   - The authors of the study did not state an actual hypothesis, nor did they suggest statistical measures to validate a hypothesis.
   - Given the low variability of the genetic markers used, a 'false positive' determination that *P. d.* and *P. d.* ssp. *tuplashensis* are separate taxa (when they are really one taxon) is highly unlikely. Conversely, a 'false negative' determination that *P. d.* and *P. d.* ssp. *tuplashensis* are the same taxon (when they are really two taxa) is highly likely.

4. Were the genetics methods appropriate?
   - No. The DNA markers used (cpDNA sequences; rDNA sequences) are appropriate for questions concerning higher level taxa (species to families). Frequency-based estimates

need to be based on a co-dominant marker system like allozymes, microsatellites, or a very large number of single-nucleotide polymorphisms.

5. Do the results of this study need to be peer-reviewed, published or replicated?
    - No. The results of the study aren't relevant to the question of infrataxa in *P. douglasii.*

6. Were the *P. douglasii* and *P. d.* ssp*. tuplashensis* genomes sufficiently explored to accurately determine the level of genetic similarity or difference to determine the taxonomic status of White Bluffs bladderpod.
    - No. The question of the taxonomic status of White Bluffs bladderpod is separate from the methods used here. Determination of the taxonomic status of White Bluffs bladderpod will have to be made using other available information.

7. Based on the genetic techniques employed in this study, what is the accepted threshold for making taxonomic subspecies determination?
    - The markers used were inappropriate for delineating intraspecific taxa.

8. Are the study results and associated conclusions sufficient to warrant changing the status of White Bluffs bladderpod?
    - The information produced in this study is not relevant to the question of the taxonomic status of White Bluffs Bladderpod. I would encourage the USFWS to consider the validity of White Bluffs bladderpod using other available information, and to consider the information in this study as 'uninformative'.

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Curriculum Vitae for Richard C. Cronn

Expertise: Plant population genetics, transcriptomics, genomics.

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i. Professional Preparation

Drake University, Des Moines, IA. Biology B.A. 1986
University of Montana, Missoula, MT. Environmental Studies M.S. 1991
Iowa State University, Ames, IA Genetics Ph.D. (honors) 1997
Iowa State University, Ames, IA Botany Post-Doc. 1999-2001

ii. Professional Appointments

Supervisory Research Geneticist, Landscape and Watershed Management Program, USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR (2009 – present)

Courtesy Assistant Professor, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR (2002 – present)

Research Geneticist, Resource Management and Productivity Program, USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR (2001 – 2008)

Post-doctoral researcher, Evolutionary history of fiber quality genes in Upland Cotton. Botany Department, Iowa State University, Ames, IA (1998 – 2001)

Visiting Assistant Professor, Iowa State University, Plant Anatomy and Plant Taxonomy (1997 – 1998)

Graduate research assistant on genetic diversity and divergence in allotetraploid cotton species, Iowa State University, Ames, IA. (1993 – 1997)

Forestry/statistics Assistant, Leopold Wilderness Institute, US Forest Service, Missoula, MT (1992 – 1993)

Laboratory Research Assistant, polymerase biochemistry and retroviral chemotherapy, University of Montana, Missoula, MT (1988-1992)

iii. Five Relevant Publications

Jennings, T., B.J. Knaus, K. Alderman, P.E. Hennon, D.V. D'Amore, R. Cronn. 2013. Microsatellite primers for the Pacific Northwest conifer *Callitropsis nootkatensis* (Cupressaceae). *Applications in Plant Sciences* 1(9): 1300025.

Cronn, R., B. Knaus, A. Liston, P. Maughan, M. Parks, J. Syring, J. Udall. 2012. Targeted enrichment strategies for next-generation plant biology. *American Journal of Botany 99:291-311.*

Bajgain, P., B.A. Richardson, J.C. Price, R. Cronn, J.A. Udall. 2011. Transcriptome characterization and polymorphism detection between subspecies of big sagebrush (*Artemisia tridentata*). *BMC Genomics*, 12:370.

Knaus, B., R. Cronn, A. Liston, K. Pilgrim, M. K. Schwartz. 2011. Mitochondrial genome sequences illuminate maternal lineages of conservation concern in a rare carnivore. *BMC Ecology 11:10,* doi:10.1186/1472-6785-11-10.

Jennings, T., B.J. Knaus, T.D. Mullins, S.M. Haig, R. Cronn. 2011. Multiplexed microsatellite recovery using massively parallel sequencing. Molecular Ecology Resources 11(6):1060-1067.

iv. Five Additional Publications

Rai, H.S., K.E. Mock, B.A. Richardson, R.C. Cronn, K.J. Hayden, J.W. Wright, B.J. Knaus, P.E. Wolfe. 2013. Transcriptome characterization and detection of gene expression differences in Aspen (*Populus tremuloides*). *Tree Genetics and Genomes* (in press)

Alexander, A., D. Steel, B. Slikas, K. Hoekzema, C. Carraher, M. Parks, R. Cronn, C.S. Baker. 2013. Low diversity in the mitogenome of sperm whales revealed by next-generation sequencing. *Genome Biology and Evolution* 5(1):113-129.

Straub, S.C.K., M. Parks, K. Weitemier, M. Fishbein, R. Cronn, A. Liston. 2012. Navigating the tip of the genomic iceberg: Next-generation sequencing for plant systematics. *American Journal of Botany 99:349-364*.

Straub, S.C.K., M. Fishbein, T. Livshultz, Z. Foster, M. Parks, K. Weitemier, R.C. Cronn, A. Liston. 2011. Building a model: Developing genomic resources for common milkweed (*Asclepias syriaca*) with low coverage genome sequencing. *BMC Genomics 12:211,* doi:10.1186/1471-2164-12-211.

Cronn, R., A. Liston, M. Parks, D.S. Gernandt, R. Shen, and T. Mockler. 2008. Multiplex sequencing of plant chloroplast genomes using Solexa sequencing-by-synthesis technology. *Nucleic Acids Research* 36: e122. doi: 10.1093/nar/gkn502.

iv. Synergistic Activities

a. Graduate/undergraduate mentoring in research. I presently supervise or co-supervise one post-doctorate (Shannon Straub), and two M.S.-level laboratory assistants (Tara Jennings; Sanjuro Jogdeo), all of whom are conducting research into different aspects of plant genomics at Oregon State University. As a courtesy member of the Botany and Plant Pathology Department at OSU, I have also served as thesis advisor or co-advisor for three PhD students (Brian Knaus; John Syring; Ann Willyard), and am serving on (or have served on) 6 additional doctoral committees (Jason Alexander; Kelly Collins; Michael Dossett; Erika Hersch; Alija Mujic, Kevin Weitemeir). Since arriving at the USFS, I have directly mentored 20 undergraduate student research projects, and have provided additional laboratory training to 15 graduate students and three post-doctorates. Undergraduates working under my supervision (Katherine Alderman, Chris Edwards, Kathleen Farrell, Corrinne Grover, Tamara Haselkorn, Julie Ryburn) have served as co-authors on six peer-reviewed papers.

b. Graduate/undergraduate teaching in botany, plant genetics and genomics. Prior to starting my present position with the USFS, I taught courses in plant anatomy (Fall Semester, 1998) and plant systematics (Spring Semester, 1998) at Iowa State University. Since taking this position, I have given lectures on population genomics to Crop and Soil Science 599/699 ("Population genomics" for Crop Genetics and Breeding in 2005 & 2007; "Hapmap Tools" for Assocation Genetics and Breeding, 2009). I have also co-organized one day workshops entitled "Introduction to Next Generation Sequencing" for the national Botany 2010 and 2012 meetings.

c. Professional service. I have served on the editorial board for Systematic Biology (2005-2008), and American Journal of Botany (2012-2013).

Name	Institutional affiliation	Conflict type	PI
Alexander, Jason	Utah Valley State University	Ph.D. thesis advisee	Cronn
Alvarez, Inez	Iowa State Univ., IA	Co-author	Cronn
Bajgain, Prabin	Brigham Young Univ., UT	Co-author	Cronn
Bassil, Nahla	USDA Agricultural Research Service, OR	Co-author	Cronn
Beaman, Reed	University of Florida, FL	Collaborator	Cronn
Bromenshenk, Jerry	Univ. of Montana, MT	M.S. thesis advisor	Cronn
Brubaker, Curt	Bayer, Belgium	Co-author	Cronn
Buenrostro, Jason	Stanford Univ., CA	Co-author	Cronn
Campbell, Christopher	University Maine, ME	Collaborator	Cronn
Cellinese, Nico	University of Florida, FL	Collaborator	Cronn
del Castillo, Rafael	CIIDR Instituto Politecnico Nacional, MEX	Co-author	Cronn
Denver, Dee	Oregon State Univ., OR	Collaborator	Cronn
Dick, Cynthia	Santa Clara Univ., CA	Co-author	Cronn
Fishbein, Mark	Oklahoma State Univ., OK	Co-author	Cronn
Foster, Zachary	Oregon State Univ., OR	Co-author Cronn	
Gernandt , David	Universidad Aut. Estado Mexico, MEX	Collaborator	Cronn
Graham, Sean	University of British Columbia , CAN	Collaborator	Cronn
Haig, Susan	US Geological Survey, OR	Co-author	Cronn
Hersch, Erika	Michigan Technological Univ., MI	Ph.D. thesis advisee	Cronn
Horning, Matthew	USDA Forest Service, OR	Post-graduate sponsor	Cronn
Ickert-Bond, Steffi	University Alaska, AK	Collaborator	Cronn
Jennings, Tara	USDA Forest Service, OR	Co-author	Cronn
Johnson, G. Randy	USDA Forest Service , Wash. DC	Co-author	Cronn
Kelch, Dean	California Dept. Agriculture, CA	Collaborator	Cronn
Kolpak, Scott	Oregon State Univ., OR	Co-author	Cronn
Lambert, Scott	Bureau of Land Management, ID	Co-author	Cronn
Li, Jianhua	Hope College, MI	Collaborator	Cronn
Little, Damon	New York Botanic Garden, NY	Collaborator	Cronn
Livshultz, Tatyana	The Academy of Natural Sciences, PA	Co-author	Cronn
Mallory-Smith, Carol	Oregon State Univ., OR	Co-author	Cronn
Mathews, Sarah	Arnold Arboretum, MA	Collaborator	Cronn
Maughan, Peter (Jeff)	Brigham Young Univ., UT	Co-author	Cronn
Mockler, Todd	Danforth Center, St. Louis, Missouri	Co-author	Cronn
Mujic, Alia	Oregon State Univ., OR	Ph.D. thesis advisee	Cronn
Mullins, Tom	US Geological Survey, OR	Co-author	Cronn
Njuguna, Wambui	University of Oregon , OR	Co-author	Cronn
Parks, Matthew	Oregon State Univ., OR	Ph.D. thesis advisee	Cronn
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September 16, 2013

U.S. Fish and Wildlife Service
Washington Fish and Wildlife Office
Attn: Brad Thompson, Manager
510 Desmond Dr. SE, Suite 102
Lacey, Washington 98503

Dear Mr. Thompson,

I have reviewed the study by Cort L. Anderson titled "Sequence Variation Among *Physaria douglasii* Isolates." Following are my comments.

(1) The study design is not adequate to to test whether *P. douglasii* subsp. *douglasii* is a lineage separate from subsp. *tuplashensis* (see my responses (3) and (4) below).

(2) The number of samples and their distribution was adequate to test whether *P. douglasii* subsp. *douglasii* is a lineage separate from subsp. *tuplashensis* and is typical of this kind of study.

(3) Assessing the probability of Type I and Type II error is fraught with problems in most phylogenetic studies. This study used no statistical methods at all. At a minimum, bootstrap percentages should be reported on a 50% majority-rule *bootstrap* tree. This study provides only a 50% majority-rule tree of (presumably) most-parsimonious trees. Preferably, in a study of this nature one would provide a testable hypothesis (and appropriate null hypothesis) which is either accepted or rejected. In this case the hypothesis to be tested is "subsp. *tuplashensis* is a lineage separate from subsp. *douglasii.*" Furthermore, the reader is not even told how many most-parsimonious trees were found.

(4) The study employed a curious collection of molecular markers. A search of the literature would have shown that these markers (except *trn*H-*psb*A) do a very poor job of discerning among very closely related taxa of Brassicaceae, especially in *Physaria*. *Physaria* is a very recent lineage, hence, *very* rapidly evolving molecular markers must be used if any phylogenetic resolution is to be found. NADH hydrogenase F, *rbc*L, and *mat*K are protein coding genes and, therefore, are typically not used among very closely related taxa. In a situation like this, non-coding regions (intergenic spacers or introns of adequate length) must be used to capture enough variation. Nuclear ITS, which was employed, is often a good marker because it is not coding. However, in *Physaria* and the rest of the Brassicaceae it does a poor job of distinguishing among very closely related taxa. The chloroplast *trn*H-*psb*A that was used had some chance of capturing useful genetic variation (as shown in Figure 4) as it is an intergenic spacer. In my lab we use a minimum of three spacers and/or introns for these kinds of questions. When examining something as recently evolved as subsp. *tuplashensis* a similar strategy should be

employed.

(5) These results are not adequate for publication and additional research is needed.

(6) The genome was not adequately explored (see (4) above).

(7) Was an "accepted threshold for making a taxonomic supspecies determination" reached? This is a tricky question. For me and most (but certainly not all) systematics, we like our species-level taxon to be monophyletic, i.e. the species includes its common ancestor and *all* of the ancestor's descendent. We know that as a lineage splits the ancestral species will remain paraphyletic for some time, i.e. it will include its common ancestor but *not* all of the ancestor's descendents. When I encounter this situation I prefer to call the two entities subspecies (which I allow to be paraphyletic) rather than species. (Some researchers prefer varietal status.) The currently available data, however, do not support subspecific recognition of subsp. *tuplashensis* even given these criteria.

(8) The data and the conclusion are inadequate to answer the question at hand.

Personally, until more data are forthcoming I would leave the taxonomic status of *Physaria douglasii* subsp. *tuplashensis* as it is. The report, of course, in no way addresses whether or not subsp. *tuplashensis* should begin receiving legal protection. From what I know about this taxon it appears to be an ecologically distinct subpopulation of the larger species.

Sincerely,

Steve L. O'Kane, Jr., Ph.D.
Professor of Biology

STEVE L. O'KANE, JR.

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EDUCATION

Ph.D., Evolutionary and Population Biology, Washington University at St. Louis, 1993. Barbara A. Schaal and Peter H. Raven, coadvisors. Doctoral dissertation: "Molecular Systematics of Lopezieae (Onagraceae)."

M.S. , Botany, Colorado State University, 1984. Dieter H. Wilken and Ralph Dix, coadvisors. Masters thesis: "An Analysis of Some Rapid Field Techniques: Vegetation of the Miller Creek Drainages (Colorado)."

B.S., Range and Forest Management, Colorado State University, 1980.

Other: Course work in Biology and Teacher Education, University of Northern Colorado
Technical and Scientific Writing, Metropolitan State College, Denver.

POSITIONS HELD

Professor, University of Northern Iowa, Department of Biology, Cedar Falls, Iowa. Research in the taxonomy and molecular systematics of the Brassicaceae and in the floristics of western North America; curator of the Grant Herbarium; courses taught: Plant Systematics, Bioscientific Terminology, Ecology, Intermediate Ecology, Advanced Ecology, Advanced Systematics, Life: The Natural World, Life: The Natural World Lab, Diversity of Life, Biogeography and the Origins of Diversity, and Evolution, Ecology, Evolution & the Nature of Science. 3-07 through the present.

Associate Professor, University of Northern Iowa, Department of Biology, Cedar Falls, Iowa. 7-00 through 3-07.

Assistant Professor, University of Northern Iowa, Department of Biology, Cedar Falls, Iowa. 1-96 through 7-00.

Instructor, University of Montana, Flathead Lake Biological Station. Instructor of Terrestrial Plant Ecology. Summers 1995 and 1996.

Post-doctoral Researcher, Missouri Botanical Garden and **Research Associate**, Washington University at St. Louis. Phylogenetics and taxonomy of *Arabidopsis* (Brassicaceae) and related genera using nuclear and chloroplast DNA sequences and traditional morphological analyses. Ihsan Al-Shehbaz supervisor. 1-93 through 12-95.

Instructor, Washington University at St. Louis. Instructor of Problem-based Learning in Biology. Fall 1994 and 1995.

Graduate Student (Ph.D.), Washington University at St. Louis, Division of Biology and Biomedical Science, St., Louis, Missouri. Systematics and molecular phylogenetics of Central American *Lopezia* (Onagraceae) and Hawaiian *Bidens* (Asteraceae). Teaching Assistant for Plant Diversity. 8-87 through 12-93.

Botanist/inventory Coordinator, Colorado Natural Areas Inventory, Department of Natural Resources, Denver. Analysis of environmental documents; natural area design; ranking of taxa by rarity and endangerment; species status reports; population biology and monitoring studies; acquisition of data on rare plants from field surveys, herbaria, and literature; and data management. 1-85 through 7-87.

Co-instructor of Plant Ecology and **Laboratory Instructor** in General Biology. University of Northern Colorado, Department of Biology, Greeley. Fall 1984.

SEASONAL EMPLOYMENT:

RANGE TECHNICIAN. U.S. Forest Service. South Park District, Pike-San Isabel National Forest, Colorado. Mapped and implemented habitat-type inventories for 450 square miles of vegetation, supervised a technician, conducted range measurements, and revegetated riparian areas. 5-84 through 9-84.

TEACHING ASSISTANT. Colorado State University, Department of Botany, Fort Collins. Instructor of laboratory courses in Plant and Grass Systematics, Plant Classification, General Botany, and Poisonous Plants. 1-81 through 12-83.

BIOLOGICAL TECHNICIAN. U.S.D.A. Soil Conservation Service, Anchorage, Alaska. Compiled flora and vegetation for Haines Borough. Designed and began implementation of sampling regime for vegetation of the Delta Junction Bison range. Assisted on several soil surveys. 5-83 through 8-83.

BIOLOGICAL VOLUNTEER. U.S. Forest Service, Blanco District, White River National Forest, Colorado. Obtained data on plant communities and local flora of Miller Creek Drainages. 6-82 through 8-82.

BOTANIST. The Nature Conservancy, Denver, Colorado. Collected floristic and plant community data in an inventory of the Piceance Basin with an emphasis on rare taxa. Supervised a botanical technician. 5-82 through 6-82.

RANGE TECHNICIAN. U.S. Forest Service, Blanco District, White River National Forest, Colorado. 5-81 through 8-81.

BIOLOGICAL TECHNICIAN. U.S.D.A. Soil Conservation Service, Anchorage, Alaska. 6-80 through 8-80.

RANGE CONSERVATIONIST. U.S. Forest Service, Spanish Fork District, Uinta National Forest, Utah. This and the following were part of a CO-OP work/study from 5-78 through 5-80. 5-79 through 8-79.

RANGE CONSERVATIONIST. U.S. Forest Service, Spanish Fork District, Uinta National Forest, Utah. 5-78 through 10-78.

RANGE TECHNICIAN. U.S.D.I. Bureau of Land Management, Cañon City District, Colorado. 5-77 through 8-77.

Responsible for floristic lists, ecological measurements, vegetation and soil mapping, range and stream improvements, reforestation, hiring contractors and writing environmental reports in the last five positions.

AFFILIATIONS

- International Association for Plant Taxonomy
- Society of Systematic Biology
- Colorado Native Plant Society (honorary Lifetime Membership)

PUBLICATIONS

Heil, K.D., S.L. O'Kane, Jr., L.M. Reeves, and A. Clifford (eds.) 2013. Flora of the Four Corners Region: Vascular Plants of the San Juan River Drainage, Arizona, Colorado, New Mexico and Utah. Missouri Botanical Garden Press.

Heidel, B. and S.L. O'Kane, Jr. 2012. *Physaria* species of Wyoming: Part 2. *Physaria* key. *Castilleja* 31(3): 5-7.

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O'Kane, S.L., Jr. 2007. *Physaria scrotiformis* (Brassicaceae), a new high-elevation species from southwestern Colorado and new combinations in *Physaria*. *Novon* 17:376-382.

Grady, B. A. and O'Kane, S.L., Jr. 2007. New species and combinations in *Physaria* (Brassicaceae) from western North America. *Novon* 17:182-192.

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Bailey, C.D., M.A. Koch, M. Mayer, K. Mummenhoff, S.L.O'Kane, Jr., S.I. Warwick, M.D. Windham, and I.A. Al-Shehbaz. 2006. Toward a global phylogeny of the Brassicaceae. *Molecular Biology and Evolution* 23: 2142-2160.

O'Kane, S.L., Jr. 2006. *Lesquerella parvula* Greene (pygmy bladderpod): a technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available online at http://www.fs.fed.us/r2/projects/scp/assessments/lesquerellaparvula.pdf [peer reviewed].

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O'Kane, S.L., Jr. and I.A. Al-Shehbaz. 2004. The genus *Physaria* (Brassicaceae) in South America. *Novon* 14:196-204.

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Kitchen, D.J., D.D. Smith, J.E. Jurgenson and S.L. O'Kane, Jr. 2001. Genetic variation among remnant Iowa populations of rough blazing star (*Liatris aspera* Michx.). *Proceedings of the 17th North American Prairie Conference.*

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Al-Shehbaz, I. A. and S.L. O'Kane, Jr. 1999. *Ianhedgea* (Brassicaceae), a new generic name replacing the illegitimate *Microsisymbrium*. *Edinburgh Journal of Botany* 56:321-327.

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O'Kane, S.L., Jr. and B.A. Schaal. 1998. Phylogenetics of *Lopezia* (Onagraceae): Evidence from Chloroplast DNA Restriction Sites. *Systematic Botany* 23:5-20.

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Ko, S.C., S.L. O'Kane, Jr. and B.A. Schaal. 1993. Intraspecific taxonomy and comparisons of nrDNA ITS-2 sequences of *Arisaema ringens* (Araceae). *Rhodora* 95:254-260.

Schaal, B.A., S.L. O'Kane, Jr. and S.H. Rogstad. 1991. DNA Variation in Plant Populations. *Trends in Ecology and Evolution*. 6:329-333.

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O'Kane, S.L., Jr. 1988. Colorado's Rare Flora. *Great Basin Naturalist* 48:434-484.

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O'Kane, S.L., Jr., E.L. Hartman and M.L. Rottman. 1988. Noteworthy Collections (Colorado) of *Antennaria aromatica*. *Madroño* 35(1):72.

O'Kane, S.L., Jr., D.H. Wilken and R.L. Hartman. 1988. Noteworthy Collections (Colorado) of *Aralia racemosa, Astragalus humillimus, A. sericoleucus, Atriplex pleiantha, Crepis capillaris, Cryptantha weberi, Dithyrea wizlizenii, Ipomopsis congesta* ssp. *crebrifolia, Lomatium bicolor, Mentzelia densa, Neoparrya lithophila*, and *Rumex verticillatus*. *Madroño* 35(1):72-74.

O'Kane, S.L., Jr. and J.L. Anderson. 1987. *Penstemon debilis* (Scrophulariaceae): A New Species From Colorado Endemic to Oil Shale. *Brittonia* 39(4):412-416.

O'Kane, S.L., Jr. 1986. Colorado Natural Areas Inventory: Plant species of special concern. *Bulletin Colorado Native Plant Society* 10(1):3-5.

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Windham, M.D. and S.L. O'Kane, Jr. 2002. Taxonomic status and phylogenetic relationships of the Kodachrome Bladderpod (*Lesquerella tumulosa*). Final Report submitted to the Bureau of Land Management, 8 April. 26 pp.

Heil, K.D. and S.L. O'Kane, Jr. 2001. Rare plants of the San Juan and Rio Grande National Forests. Unpbl. rpt., San Juan National Forest, Colorado.

Galatowitsch, S., T. Naumann, S.L. O'Kane, Jr. and B.E. Neely. 1988. Inventory of Plant Species of Special Concern and the General Flora of Dinosaur National Monument. Annual Progress Report (1987). Unpbl. rpt., National Park Service, Denver, CO 24 pp. + appen.

O'Kane, S.L., Jr. 1987. A Floristic Reconnaissance of the San Luis Valley, Colorado. Unpbl. rpt., U.S.D.I. Bureau of Land Management. 57pp.

O'Kane, S.L., Jr. 1987, 1986, 1985. Plant Species of Special Concern for Colorado. Unpbl. rpt., Colorado Natural Areas Inventory, Denver, CO 80203. 15pp.

O'Kane, S.L., Jr. 1987. Status Report for *Phacelia submutica* Howell. Unpbl. rpt., U.S. Fish and Wildlife Service. 71pp.

O'Kane, S.L., Jr. 1987. Status Report for *Lesquerella parviflora* Rollins. Unpbl. rpt., U.S. Fish and Wildlife Service. 62pp.

O'Kane, S.L., Jr. 1987. Status Report for *Thalictrum heliophilum* Wilken et DeMott. Unpbl. rpt., U.S. Fish and Wildlife Service. 70pp.

O'Kane, S.L., Jr. 1987. Status Report for *Physaria obcordata* Rollins. Unpbl. rpt., U.S. Fish and Wildlife Service. 53pp.

O'Kane, S.L., Jr. and B.E. Neely. 1987. Inventory of Plant Species of Special Concern and the General Flora of Dinosaur National Monument--Progress Report. Unpbl. rpt., National Park Service, Denver, CO 80225. 41 pp.

Knight, P., A. Cully, B.C. Johnston and S.L. O'Kane, Jr. 1986. Status Report for *Phlox caryophylla*. Unpbl. rpt., U.S. Fish and Wildlife Service. 31pp.

O'Kane, S.L., Jr. 1986. The Vegetation and Additions to the Flora of Roxborough State Park, Colorado. Unpbl. rpt., Colo. Div. Parks and Outdoor Rec. 28pp.

O'Kane, S.L., Jr. 1986. Endangered Species Information System Workbooks. I: Species Distribution, and II: Species Biology of *Phacelia formosula* Osterhout. Prepared for the U.S. Fish and Wildlife Service, Washington, D.C.

O'Kane, S.L., Jr. 1985. Endangered Species Information System Workbooks. I: Species Distribution, and II: Species Biology of *Eriogonum pelinophilum* Reveal. Prepared for the U.S. Fish and Wildlife Service, Washington, D.C.

O'Kane, S.L., Jr. and D. Kautz. 1983. Vegetation Classification of Haines Borough, Alaska. Unpbl. rpt., U.S.D.A. Soil Conservation Service, Anchorage, Alaska.

CONTRIBUTED AND INVITED PAPERS AND POSTERS

O'Kane, S.L., Jr. 2012. Evolution and classification of *Physaria* (Brassicaceae). Botany 2012 meetings, Columbus, Ohio. [Invited]

O'Kane, S.L., Jr. 2012. Botanical Exploration in the Unexplored West: New Mexico. Brode Lecture, Whitman College, Walla Walla, Washington. [Invited]

O'Kane, S.L., Jr. 2012. Systematics of a Rapidly Evolving Group of Plants: *Physaria* (Brassicaceae). Department of Biology, Whitman College, Walla Walla, Washington. [Invited]

O'Kane, S.L., Jr. 2010. Bootlaces and Uncommon Places. Keynote address, Colorado native Plant Society Annual Meeting, Denver, Colorado. [Invited]

O'Kane, S.L., Jr. 2005. Rare plants of the San Juan River Drainage. Annual meeting of the Colorado Native Plant Society, Pagosa Springs, Colorado.

Heil, K.D., S. O'Kane and A. Clifford. 2004. Rare and endangered plants of the San Juan River drainage in Arizona, Colorado, New Mexico, and Utah. Fourth Southwestern Rare and Endangered Plant Conference, March 22-24, New Mexico State University, Las Cruces, New Mexico.

Clifford, A., K.D. Heil and S. O'Kane. 2004. Rare and endangered plants of the Navajo Nation, San Juan River drainage Arizona, New Mexico, and Utah. Fourth Southwestern Rare and Endangered Plant Conference, March 22-24, New Mexico State University, Las Cruces, New Mexico.

O'Kane, S.L., Jr. 2001. Implications of Holocene and Pleistocene glaciation-induced range fluctuations on the phylogeny of *Arabidopsis*. 11 November at the International Symposium on Genetic Diversity of Plants, Kaohsiung, Taiwan. [Invited]

O'Kane, S.L. 1998. The ins and outs of writing a flora. Invited presentation January 8 to the Boulder Chapter of the Colorado Native Plant Society. [Invited]

O'Kane, S.L. 1998. The species of *Lesquerella* and *Physaria* in Colorado. An invited series of two, full-day workshops presented to members of the Colorado Native Plant Society at the University of Colorado, Boulder January 10-11. [Invited]

O'Kane, S.L., Jr. 1997. A molecular systematic examination of *Lesquerella* and *Physaria* (Brassicaceae). Joint meeting of the American Society of Naturalists, the Society for Systematic Biology, and the Society for the Study of Evolution, Boulder, Colorado.

O'Kane, S.L. 1996. Biological collections as a source of DNA for research in systematics and conservation biology with special reference to herbarium collections. Presented at a symposium entitled "Univeristy research collections: their history, use and preservation" sponsored by the University of Iowa Collections Coalition, November 2, University of Iowa, Iowa City. [Invited]

O'Kane, S.L., I.A. Al-Shehbaz and B.A. Schaal. 1995. Phylogenetics of *Arabidopsis*: scope and content based on DNA sequences of nuclear rDNA internal transcribed spacers. American Institute of Biological Sciences meeting, San Diego, CA.

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O'Kane, S.L. 1995. Rare plants as a tool in understanding patterns of adaptation and evolution. Annual Meeting of the Colorado Native Plant Society, Fort Collins, CO. [Invited]

O'Kane, S.L., Jr., B.A. Schaal and P.H. Raven. 1992. Evolution in *Lopezia* (Onagraceae) as Indicated by rDNA Sequence and cpDNA Restriction Site Data. International Oranization of Plant Biosystematists (IOPB) Fifth International Symposium, St. Louis, MO.

O'Kane, S.L., Jr. and K. Helenurm. August 1991. Chloroplast DNA and the Adaptive Radiation of Hawaiian *Bidens*. Annual Meeting of the Society for the Study of Evolution, Hilo, Hawaii. [Invited]

Cully, A., D. House, P. Knight, S.L. O'Kane, Jr., and M. Olwell. 1986. Gathering base-line data on threatened and endangered plant species in New Mexico. Rare & Endangered Plants, California Native Plant Society Conference, Sacramento, CA, November.

O'Kane, S.L., Jr. October 1986. Colorado's Rare Plants and Their Habitats. Annual Meeting of the Colorado Native Plant Society, Fort Collins, CO. [Invited]

O'Kane, S.L., Jr. 1985. An Evaluation of Subjective Field Techniques in Plant Community Studies. Journal Colorado-Wyoming Academy of Sciences. 17(1):18 (abstract). Annual meeting of the Colorado/Wyoming Academy of Sciences, University of Denver.

GRANTS AND AWARDS

- Flora of New Mexico, New Mexico Bureau of Land Management 2007 ($10,000)
- Flora of New Mexico, T & E Foundation (New Mexico) 2007 ($5,000)
- Prem Sahai Distinguished Scholar, 2002-2003 ($25,000)
- Flora of the San Juan Basin (Colorado, New Mexico, Arizona, and Utah), San Juan College Foundation, 2000, 2001, 2002 ($8000 each year)
- Systematics of the *Lesquerella hitchcockii* Complex (Brassicaceae), University of Utah, 2001 ($2800)
- University of Northern Iowa Microcomputer Equipment Grant, 2000, ($1615)
- Genetics of the Western Prairie Fringed Orchid (with graduate student J. Hancock), Iowa Dept. Natural Resources & U.S. Fish & Wildlife Service, 1998-1999 ($10,000)
- University of Northern Iowa Microcomputer Equipment Grant (with Jean Gerrath), 1997 ($6756)
- University of Northern Iowa Microcomputer Equipment Grant, 1996 ($4,461)
- NSF Doctoral Dissertation Improvement Grant, 1991 ($15,900)
- Colorado Graduate Fellowship Grant, 1980 and 1984
- Gamma Sigma Delta National Honor Society of Agriculture
- Xi Sigma Pi National Honor Society of Forest Resource Management
- Population Biology of *Sclerocactus mesae-verdae*, U.S. Fish and Wildlife Service, 1986 and 1987 ($6000)
- Status Surveys for *Lesquerella parviflora, Thalictrum heliophilum, Phacelia submutica*, and *Physaria obcordata*, USFWS, 1986 ($8500)
- Floristic Inventory of the San Luis Valley, Colorado, Bureau of Land Management, 1986 ($4,000)
- Inventory of Plant Species of Special Concern and the General Flora of Dinosaur National Monument, U.S. Park Service, 1987 ($14,000)
- Recognition Award, City of Boulder (Colorado) Open Space Department, 1988
- Outstanding Team Achievement, Colorado State Parks, 1987-1988
- Washington University Graduate Fellowship, 1987-1993
- Morris B. Rettner Graduate Fellowship, 1989 ($2000)

From: Thompson, Brad
To: Danica Harbaugh Reynaud
Subject: Re: Peer review request for Physaria douglasii ssp. tuplashensis report
Date: Thursday, September 19, 2013 12:35:23 PM

Danica,

Thank you for your providing me with your peer review. I appreciate you taking the time to read and review the report. I may follow-up with you if I have questions about interpreting your comments in the review and therefore appreciate your offer to allow me to do so. In the meantime, please send me a copy of your CV as I will include it in our record along with your review.

Thanks,
Brad

On Thu, Sep 19, 2013 at 11:22 AM, Danica Harbaugh Reynaud <Danica@authentechnologies.com> wrote:

> Good morning Brad,
>
> Please find my review of the paper below; for many of these topics they are best discussed by phone, so please let me know if you would like to schedule a call.
>
> Thanks, Danica
>
> Overall, the study design was not appropriate for determination as to whether or not *P. douglassii* spp. *tuplashensis* is divergent enough to warrant taxonomic recognition. There are two main criticisms to this study. The first is that the genes selected were mostly inappropriate for a species or sub-specific level study. They selected several cpDNA genes that are used in DNA barcoding; however they are not useful for species-level discrimination, or especially at the subspecies level. The only appropriate genes for this project were ITS and *psbA-trnH;* however, they have very limited sampling, including only 2 samples of the target sub-species for the ITS region. Additional genes, such as ETS and *trnL-F* should have been used as well.
>
> The second criticism is the sampling; very few samples were included in this study. Additional samples from across the range of each subspecies should have been included. Also there was no indication that the samples of *P. douglassii* were the type variety- it is assumed, but it should have been stated.
>
> Even given more gene regions and more extensive sampling, we still might expect *not* to see genetic differentiation at the sub-specific level. It is common not to see any genetic differentiation at this level, or even at the species level for recently diverged lineages. I recommend this type of study as a *first step,* in case it yields differentiation. However to really assess genetic divergence and gene flow between these two subspecies, a microsatellite analysis would be required. Therefore, unless such a study is performed, I don't think that a decision on the recognition of the subspecies as a distinct taxon can be made. That said, the current study is very preliminary and I would not recommend that it be sent for peer-review. Additional sampling of plants and genes at least should be done prior

to this.

There is no generally accepted amount of genetic variation that plants must have to be designated as a species, subspecies, genus, family. All plants vary greatly in the amount of genetic variation that exists within and between species. In many ways it is an arbitrary distinction based on a botanists perspective, and a combination of data such as morphology and breeding, etc., along with genetics. I would say that if you are interested in the gene flow between the subspecies and the "main" species to understand if it has conservation value (i.e. it is isolated and may represent an emergent species), then I would follow up with a microsatellite analysis.

On Sep 9, 2013, at 10:48 AM, "Thompson, Brad" <brad_thompson@fws.gov> wrote:

> Dr. Harbaugh Reynaud,
>
> Thank you for agreeing to particpate in the peer review of the "Sequence Variation Among Physaria douglasii Isolates" report that US Fish and Wildlife Service received during the most recent public comment period for the proposed listing of White Bluffs bladderpod. Please see attached for 1) the cover letter that includes instructions and 2) the report: "Sequence Variation Among Physaria douglasii Isolates" for your review. Please provide me with your completed review by September 19, 2013.
>
> Sincerely,
> Brad Thompson
>
> --
> Brad Thompson, Ph.D
> Listing and Recovery Division Manager
> US Fish and Wildlife Service
> Washington Fish and Wildlife Office
> 510 Desmond Dr. SE, Suite 102
> Lacey, WA 98503
> 360-753-6046
> 360-790-8187 (cell)
> <Harbaugh Reynaud_peer_review_9_9_2013.pdf><White Bluffs Bladderpod report for peer review.pdf>

Danica Harbaugh Reynaud, Ph.D.
CEO & CSO

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Peer Review by M. Windham

Duke University

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NORTH CAROLINA
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DEPARTMENT OF BIOLOGY
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18 September 2013

Brad Thompson, Manager
Listing and Recovery Division
Washington Fish and Wildlife Office
510 Desmond Dr. SE, Suite 102
Lacey, Washington 98503

Dear Brad,

This letter represents my response to your recent request for an independent scientific review of the report received during the reopening of the public comment period for the proposed rule to list the White Bluffs bladderpod under the Endangered Species Act and to designate critical habitat (May 15,2012; 77 FR 28704). I've organized my comments in terms of the excellent set of questions you provided as part of your request.

Study Design:

Ql) Was the study design and implementation appropriate to assess whether P. douglasii ssp. tuplashensis is indeed sufficiently divergent to warrant subspecies designation?

The study design (i.e., drawing taxonomic conclusions from analyses that estimate overall genetic similarity or phylogenetic relationship) is standard and appropriate for this type of study. However, the implementation falls short of what is needed due to very small sample sizes and poor choice of genetic loci for analysis (see following sections).

Q2) Was the number of P. douglasii and P. douglasii ssp. tuplashensis individual plants included in this study sufficient in number, geographic distribution, and time to accurately represent the populations of interest (i.e., locale and subspecies) in order to accurately conclude the taxonomic status of White Bluffs bladderpod? Were the samples collected from throughout the range of P. douglasii and P. douglasii ssp. tuplashensis to capture the distribution of genetic diversity within P. douglasii and P. douglasii ssp. tuplashensis? Were sample sizes and the spatiotemporal distribution of collections acceptable for accurately concluding taxonomic status for plants such as P. douglasii and P. douglasii ssp. tuplashensis?

A sample of 15 individuals (12 of ssp. *douglasii* and 3 of ssp. *tuplashensis*) is insufficient to assess the taxonomic status of the plants involved, especially given the small number of relatively invariant genetic loci analyzed. These two factors make the geographic distribution of the sampling (which encompasses a reasonable amount of the range of ssp. *douglasii*) largely irrelevant.

Q3) What is the probability of making Type I and Type II errors based on the reported study design and statistical tests?

The application of statistical test theory to this case is not entirely appropriate; there is neither a clearly designated null hypothesis nor appropriate data from which to calculate probabilities. If the null hypothesis accepts ssp. douglasii and ssp. tuplashensis as being genetically distinct, the problems alluded to above (i.e., very small sample sizes and a focus on relatively invariant genetic loci) would yield a very high (but incalculable) probability of a Type I error; the probability of a Type II error in this case would be correspondingly low.

Application to White Bluffs bladderpod:

Q4) Were the genetic methods and regions of the DNA interrogated in this study the most appropriate for evaluating the taxonomic status of White Bluffs bladderpod?

Phenetic clustering based on genetic similarity measures might have been more appropriate than phylogenetic analysis considering the nature of the data, though I doubt the results would have been any more informative. Having chosen a phylogenetic methodology, the author should have added a closely related species of *Physaria* to the dataset to provide an outgroup for the generation of rooted trees. I'm not an expert in this area, but I believe this would be the best way to obtain a clear resolution of the relationship between ssp. *douglasii* and ssp. *tuplashensis*. Finally, it should have been apparent from the Brassicaceae literature that the four genetic loci chosen for analysis were not up to the task of assessing the taxonomic status of subspecies in *Physaria*. The two least informative loci (rbcL and ndhF) primarily are used to investigate intergeneric relationships in the mustard family and would not be expected to show much variation at the species (much less subspecies) level. The nuclear locus ITS has it's own set of problems, including a proclivity for concerted evolution when more than gene copy is present. This is especially problematic when trying to reconstruct relationships in a genus like *Physaria* where hybridization and polyploidy are rampant. The logic presented in the report that ITS should distinguish between "real taxa" in *Physaria* because it does so in the completely unrelated genus *Myriophyllum* is erroneous.

Q5) Do the results of this study need to be peer-reviewed, published in a refereed journal, and replicated by others in order to accurately conclude the taxonomic status of the White Bluffs bladderpod?

No. The results were inconclusive and replication *per se* will not help. Subsequent analyses need to significantly expand the number of individuals sampled and use an entirely different set of genetic loci. The author of the report does an excellent job of outlining such a study in the final "Recommendations" section.

Interpretation of Results :

Q6) Were the P. douglasii and P. douglasii ssp. tuplashensis genomes sufficiently explored with the loci chosen in this study to accurately determine the level of genetic similarity or difference required to determine taxonomic status of the White Bluffs bladderpod?

No. This study should have focused on other loci (such as chalcone synthase and *pistillata*) that have proven useful for studying species relationships in related genera of Brassicaceae. Even these might fail to provide the data needed to resolve the taxonomic status of ssp. *tuplashensis*. The best approach to the problem would entail the development of microsatellites or SNPs (see “Recommendations” section of report).

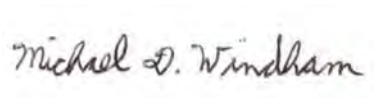
Q7) Based on the genetic techniques employed in this study, what is the accepted threshold for making a taxonomic subspecies determination for White Bluffs bladderpod? In responding to this question, please provide a quantitative threshold for the genetics metrics employed in this study or other genetics approaches relied upon in order to determine or change the taxonomic status of plant species or sub-species.

There is no standard genetic threshold for identifying species, much less subspecies. For example, the basic taxonomic units called species in the mustard family are much less divergent genetically than the taxa treated as species among ferns. Given the phylogenetic methodology employed by the author of this report, we would expect that 1) all samples of a valid taxonomic entity to be clustered together on one branch of a phylogenetic tree with no other taxa interspersed (i.e., form a monophyletic group) and 2) that this branch would have a bootstrap support value greater than 70% (i.e., be statistically significant). Because neither ssp. *douglasii* nor ssp. *tuplashensis* meet these requirements, they are not separable based on this dataset. But, as indicated earlier, this particular dataset is fatally flawed.

Q8) Are the study results and associated report conclusions sufficient to warrant changing the taxonomic status of the White Bluffs bladderpod? If so, what alternative taxonomic status do you recommend based upon your review of this study?

No. The fact that this study has a high probability for Type I error (i.e., unjustified rejection of the hypothesis that ssp. *tuplashensis* is distinct from ssp. *douglasii*) indicates that it should not be the impetus for taxonomic changes. Given our current understanding, subspecies represents an appropriate taxonomic status provided there is some morphological distinction between the taxa.

Sincerely,



Senior Research Scientist

Department of Biology • Duke University • 139 Biological Sciences • Durham, NC 27708-0338 USA

MICHAEL D. WINDHAM
Curator of the Vascular Plant Herbarium (DUKE), Department of Biology,
Duke University, Box 90338, Durham, NC 27708
Office: (919) 613-8732; FAX: (919) 660-7293; E-mail: mdw26@duke.edu

Professional Preparation

1988 Ph.D. Botany (with honors), University of Kansas, Lawrence, KS
1985 M.S. Biology (emphasis in Botany), Northern Arizona University, Flagstaff, AZ
1975 B.A. Anthropology (emphasis in Archeology), Northern Arizona Univ., Flagstaff, AZ

Professional Appointments

2007- Curator of Vascular Plant Herbarium, Department of Biology, Duke University
1989-2007 Curator of the Garrett Herbarium, Utah Museum of Natural History, Univ. of Utah
1989-2006 Adjunct Assistant Professor, Department of Biology, University of Utah
2000-2002 Assoc. Instructor, College of Arts and Sciences, Westminster College, Salt Lake City
1988-1989 Postdoctoral Associate, Flora North America Project, Missouri Botanical Garden

Grant Support

2008-2012 National Science Foundation, $193,212, "Collaborative Research: A model system revisited: Phylogenetic relationships and species limits among the diploid taxa of *Boechera* (Brassicaceae)."
2007-2012 National Science Foundation, $319,780, "Collaborative Research: The roles of polyploidy and apomixis in shaping the diversity and distribution of cheilanthoid ferns."
2007-2011 National Science Foundation, $467,691, "A plan to rehouse and reorganize the vascular plant herbarium and botanical library at Duke University (DUKE)."
1998-2003 Institute for Museum and Library Services, $32,116, "Stabilization and recuration of the Garrett Herbarium, Utah Museum of Natural History."
1998-2002 U.S. Bureau of Land Management, $4,950, "Biosystematic study of the Kodachrome bladderpod."
1997-1998 National Science Foundation, $10,000, "A proposal to rehouse the collections of the Garrett Herbarium, Utah Museum of Natural History."
1996-1998 U.S.D.A. Forest Service, $6,870, "Chromosome and enzyme analyses of rare *Draba* species in Utah National Forests."

Teaching

2008- Duke University
Courses taught: *Plant Systematics and Evolution* (Biol. 342L)
2008- Organization for Tropical Studies
Courses taught: *Tropical Ferns and Lycophytes*
2000-2002 Westminster College, Salt Lake City
Courses taught: *Environmental Biology Lab; Principles of Biology Labs (I and II); The Natural World*
1989-2003 University of Utah
Courses taught: *Plant Systematics; Plant Biology Lab; General Botany; General Biology; Graduate Seminar; Museum Science*

Service on Graduate Student Committees
Eric Green, Ph.D. student, Duke University
Martin Schilling, Ph.D. student, Utah State University
Jordan Metzger, Ph.D. candidate, University of Alaska
Catherine Rushworth, Ph.D. candidate, Duke University
Erin Sigel, Ph.D. candidate, Duke University
Amanda Grusz, Ph.D. candidate, Duke University
Carl Rothfels, Ph.D. 2012, Duke University
Therese Meyer, M.S. 2003, University of Utah
Mark Beilstein, M.S. 1999, University of Utah
Curtis Hansen, M.S. 1998, Brigham Young University
Loreen Allphin, Ph.D. 1996, University of Utah

Undergraduate Supervision
Anne Johnson, Duke University (2010-2011)
Grace Shih, Duke University (2008)
Abigail Moore, University of Utah (1999-2004), now a Ph.D. student in Integrative Biology, University of California at Berkeley, CA

Current Adjunct Appointments
2013- Adjunct Faculty, Utah State University, Logan, UT
2010- Adjunct Faculty, University of Alaska, Fairbanks, AK
2007- Adjunct Curator, Utah Museum of Natural History, University of Utah, UT

Professional Activities (last five years)
American Fern Society
Reviewer, *American Fern Journal*
President-elect (2008-2010)
President (2010-2012)
American Society of Plant Taxonomists
Reviewer, *Systematic Botany*
Botanical Society of America
Reviewer, *American Journal of Botany*
Chair of Pteridological Section and member of governing council (2007-present)
Field trip coordinator and leader for 2009 annual meetings
International Society for Plant Sciences
Reviewer, *International Journal of Plant Sciences*
Missouri Botanical Garden
Reviewer, *Novon* and *Annals of the Missouri Botanical Garden*
National Research Council Canada
Reviewer, *Botany/Botanique*
New York Botanical Garden
Reviewer, *Brittonia*
Taiwan Forestry Research Institute (Taipei, Taiwan)
Instructor, Cytogenetics Workshop

Manuscripts Submitted

Grusz, A. G., **M. D. Windham**, G. Yatskievych, R. L. Huiet, G. J. Gastony, and K. M. Pryer. Toward a monophyletic *Cheilanthes*: the resurrection and recircumscription of *Myriopteris* (Pteridaceae). *Systematic Botany* in review.

Rothfels, C. J., A. K. Johnson, P. H. Hovenkamp, D. L. Swofford, H. C. Roskam, C. R. Fraser-Jenkins, **M. D. Windham**, K. M. Pryer. A natural hybrid between two fern species that diverged over 50 million years ago. *Evolution* in review.

Sigel, E. M., **M. D. Windham**, A. R. Smith, R. J. Dyer, and K. M. Pryer. Rediscovery of *Polypodium calirhiza* (Polypodiaceae) in Mexico. *Brittonia* in review.

Manuscripts in Press

Al-Shehbaz, I. and **M. D. Windham**. 2013. *Draba*. *In* Heil, K., S. O'Kane, and L. Reeves, eds. *Flora of the Four Corners*. Missouri Botanical Garden Press.

Windham, M. D. and I. Al-Shehbaz. 2013. *Arabis*. *In* Heil, K., S. O'Kane, and L. Reeves, eds. *Flora of the Four Corners*. Missouri Botanical Garden Press.

Windham, M. D. and I. Al-Shehbaz. 2013. *Boechera*. *In* Heil, K., S. O'Kane, and L. Reeves, eds. *Flora of the Four Corners*. Missouri Botanical Garden Press.

Windham, M. D. and I. Al-Shehbaz. 2013. *Turritis*. *In* Heil, K., S. O'Kane, and L. Reeves, eds. *Flora of the Four Corners*. Missouri Botanical Garden Press.

Refereed Publications

Alexander, P. J., **M. D. Windham**, J. B. Beck, I. A. Al-Shehbaz, L. Allphin, and C. D. Bailey. 2013. Molecular phylogenetics and taxonomy of the genus *Boechera* and related genera (Brassicaceae: Boechereae). *Systematic Botany* 38: 192-209.

Arrigo, N., J. Therrien, C. L. Anderson, **M.D. Windham**, C. H. Haufler, and M. S. Barker. A total evidence approach to understanding phylogenetic relationships and ecological diversity in *Selaginella* subg. *Tetragonostachys*. *American Journal of Botany* 100: 1672-1682.

Rothfels, C. J., **M.D. Windham**, and K. M. Pryer, 2013. A plastid phylogeny of the cosmopolitan fern family Cystopteridaceae. *Systematic Botany* 38: 295-306.

Beck, J. B., J. Allison, K. M. Pryer, and **M.D. Windham.** 2012. Identifying multiple origins of polyploid taxa: a multilocus study of the hybrid cloak fern (*Astrolepis integerrima*; Pteridaceae). *American Journal of Botany* 99: 1857-1865.

Beck, J. B, P. J. Alexander, L. Allphin, I. A. Al-Shehbaz, C. Rushworth, C. D. Bailey, and **M. D. Windham**. 2012. Does hybridization drive the transition to asexuality in diploid *Boechera* (Brassicaceae)? *Evolution* 66: 985-995.

Johnson, A. K., C. J. Rothfels, **M. D. Windham**, and K. M. Pryer. 2012. Unique expression of a sporophytic character on the gametophytes of notholaenid ferns (Pteridaceae). *American Journal of Botany* 99: 1118-1124.

Li, F.-W., K. M. Pryer, and **M.D. Windham.** 2012. *Gaga*, a new fern genus segregated from *Cheilanthes* (Pteridaceae). *Systematic Botany* 37: 845-860.

Rothfels, C. J., E. M. Sigel, and **M. D. Windham**. 2012. *Cheilanthes feei* T. Moore (Pteridaceae) and *Dryopteris erythrosora* (D.C. Eaton) Kunze (Dryopteridaceae) new for the flora of North Carolina. American Fern Journal 102: 184-186.

Windham, M. D. and I. Al-Shehbaz. 2012. *Boechera*. *In* Baldwin, B. G., S. Boyd, D. J. Keil, R. Patterson, T. J. Rosatti, and D. Wilken, eds. *The Jepson Manual, 2nd Edition*. University of California Press, Berkeley, CA.

Beck, J. B., **M. D. Windham**, and K. M. Pryer. 2011. Do asexual lineages lead short evolutionary lives? A case study from the fern genus *Astrolepis*. *Evolution* 65: 3217-3229.

Brokaw, J., **M. D. Windham**, and L. Hufford. 2011. Chromosome counts and taxonomy of *Mentzelia thompsonii* (Loasaceae). *Madroño* 58: 50-56.

Li, F.-W., L.-Y. Kuo, C.J. Rothfels, A. Ebihara, W.-L. Chiou, **M.D. Windham**, and K.M. Pryer. 2011. *rbcL* and *matK* earn two thumbs up as the core DNA barcode for ferns. *PLoS One* 6(10): e26597. doi:10.1371/journal.pone.0026597.

Marcussen, T.,K. Blaxland, **M. D. Windham**, K. E. Haskins, and F. Armstrong. 2011. Establishing the phylogenetic origin, history and age of the narrow endemic *Viola guadalupensis* (Violaceae). *American Journal of Botany* 98: 1978-1988.

Sigel, E., **M. D. Windham**, L. Huiet, G. Yatskievych, and K. M. Pryer. 2011. Species relationships and farina evolution in the cheilanthoid fern genus *Argyrochosma* (Pteridaceae). *Systematic Botany* 36: 554-564.

Alexander, P. J., **M. D. Windham**, C. Donovan Bailey, G. Rajanikanth, and I. A. Al-Shehbaz. 2010. Molecular phylogenetics and taxonomy of the genus *Thysanocarpus* (Brassicaceae). *Systematic Botany* 35: 559-577.

Alexander, P. and **M. D. Windham**. 2010. *Thysanocarpus*. Pp. 739-741 *In* Flora of North America Editorial Committee, eds. *Flora of North America North of Mexico*. Oxford University Press.

Al-Shehbaz, I. and **M. D. Windham**. 2010. *Boechera*. Pp. 348-412 *In* Flora of North America Editorial Committee, eds. *Flora of North America North of Mexico*. Oxford University Press.

Al-Shehbaz, I., **M. D. Windham,** and R. Elven. 2010. *Draba*. Pp. 269-347 *In* Flora of North America Editorial Committee, eds. *Flora of North America North of Mexico*. Oxford University Press.

Beck, J. B., **M. D. Windham**, G. Yatskievych, and K. M. Pryer. 2010. A diploids-first approach to species delimitation and interpreting polyploid evolution in the fern genus *Astrolepis* (Pteridaceae). *Systematic Botany* 35: 223-234.

Pryer, K.M., E. Schuettpelz, L. Huiet, A.L. Grusz, C.J. Rothfels, T. Avent, D. Schwartz, and **M.D. Windham**. 2010. DNA barcoding exposes a case of mistaken identity in the fern horticultural trade. Molecular Ecology Resources 10: 979-985.

Grusz, A. G., **M. D. Windham**, and K. M. Pryer. 2009. Deciphering the origins of apomictic polyploids in the *Cheilanthes yavapensis* complex (Pteridaceae). *American Journal of Botany* 96: 1636-1645.

Windham, M. D., L. Huiet, E. Schettpelz, A. L. Grusz, C. J. Rothfels, J. B. Beck, G. Yatskievych, and K. M. Pryer. 2009. Using plastid DNA sequences to redraw generic boundaries and demystify species complexes in cheilanthoid ferns (Pteridaceae). *American Fern Journal* 99: 128-132.

Rothfels, C., **M. D. Windham**, A. Grusz, G. J. Gastony, and K. M. Pryer. 2008. Toward a monophyletic *Notholaena* (Pteridaceae): resolving patterns of evolutionary convergence in xeric-adapted ferns. *Taxon* 57: 712-724.

Schuettpelz, E., A. Grusz, **M. D. Windham**, and K. M. Pryer. 2008. The utility of nuclear *gapCp* in resolving polyploid fern origins. *Systematic Botany* 33: 621-629.

Yatskievych, G. and **M. D. Windham**. 2008. Vascular Plants of Arizona: Azollaceae (Mosquito Fern Family). *Canotia* 4: 31-34.

Yatskievych, G. and **M. D. Windham**. 2008. Vascular Plants of Arizona: Blechnaceae (Chain Fern Family). *Canotia* 4: 35-37.

Yatskievych, G. and **M. D. Windham**. 2008. Vascular Plants of Arizona: Dennstaedtiaceae (Bracken Family). *Canotia* 4: 38-40.

Yatskievych, G. and **M. D. Windham**. 2008. Vascular Plants of Arizona: Equisetaceae (Horsetail Family). *Canotia* 4: 41-45.

Yatskievych, G. and **M. D. Windham**. 2008. Vascular Plants of Arizona: Salviniaceae (Floating Fern Family). *Canotia* 4: 46-49.

Al-Shehbaz, I. and **M. D. Windham**. 2007. New or noteworthy North American *Draba* (Brassicaceae). *Harvard Papers in Botany* 12: 409-419.

Windham, M. D. and I. Al-Shehbaz. 2007. New and noteworthy species of *Boechera* (Brassicaceae). III. Additional sexual diploids and apomictic hybrids. *Harvard Papers in Botany* 12: 235-257.

Schuettpelz, E., H. Schneider, L. Huiet, **M. D. Windham**, and K. M. Pryer. 2007. A molecular phylogeny of the fern family Pteridaceae: assessing overall relationships and the affinities of previously unsampled genera. *Molecular Phylogenetics and Evolution* 44: 1172-1185.

Windham, M. D. and I. Al-Shehbaz. 2007. New and noteworthy species of *Boechera* (Brassicaceae). II. Apomictic hybrids. *Harvard Papers in Botany* 11: 257-274.

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Invited Seminars

Windham, M. D., J. Beck, F.-W. Li, C. Rushworth, P. Alexander, L. Allphin, C. D. Bailey, and I. Al-Shehbaz, 2012. Easy come, easy go: diversification and extinction via hybridization in the genus *Boechera* (Brassicaceae). Contribution to symposium entitled "Principles of crucifer evolution" presented at annual BSA meetings, Columbus, OH. http://2012.botanyconference.org/engine/search/index.php?func=detail&aid=802

Windham, M. D., J. Beck, F.-W. Li, P. Alexander, L. Allphin, C. D. Bailey, C. Call, C. Rushworth, and I. Al-Shehbaz, 2012. On the importance of being earnest (about taxonomy): species concepts and conservation in the genus *Boechera* (Brassicaceae). Brigham Young University, Provo, UT.

Windham, M. D., J. Beck, P. Alexander, L. Allphin, C. D. Bailey, C. Call, F.-W. Li, C. Rushworth, and I. Al-Shehbaz, 2011. Retooling systematics to investigate hybrid/polyploid complexes: lessons from the genus *Boechera* (Brassicaceae). Rancho Santa Ana Botanical Garden, Claremont, CA.

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Windham, M. D. 2010. A peek inside the black box: investigating factors affecting long-distance dispersal and sporophyte establishment in seed-free vascular plants. Contribution to symposium entitled “Dispersal in Fungi and Plants” presented at annual Canadian Botanical Association meetings, Ottawa, ON.

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Windham, M. D. 1982. Dendrochronological studies of prehistoric cultural evolution in the southwestern United States. Interdepartmental seminar (Anthropology/Biology) at Northern Arizona University, Flagstaff, AZ.

Contributed Papers (Published Abstracts)

Call, C., L. Allphin, **M.D. Windham**, J. Beck, F.-W. Li, P. Alexander, C. D. Bailey, and I. Al-Shehbaz. 2013. Ecological and genetic diversity across two divergent species of *Boechera* (Brassicaceae) in biogeographic space. Paper presented at annual BSA meetings, New Orleans, LA. http://www.botanyconference.org/engine/search/index.php?func=detail&aid=944

Hildebrand, T., I. Al-Shehbaz, and **M.D. Windham**. 2013. A new mustard discovered from the West Desert Region of the North American Great Basin. Paper presented at annual BSA meetings, New Orleans, LA. http://www.botanyconference.org/engine/search/index.php?func=detail&aid=647

Johnson, A. K., A. Grusz, J. Beck, K. Pryer, and **M. D. Windham**. 2013. So, if they are evolutionary dead-ends, why are apomictic cheilanthoid ferns more widely distributed than their sexual diploid progenitors?. Paper presented at annual BSA meetings, New Orleans, LA. http://www.botanyconference.org/engine/search/index.php?func=detail&aid=803

Alexander, P. J., J. Beck, F.-W. Li, **M. D. Windham**, L. Allphin, I. Al-Shehbaz, and C. D. Bailey. 2012. Patterns of evolutionary divergence and homoploid hybridization in *Boechera* (Brassicaceae). Paper presented at annual BSA meetings, Columbus, OH. http://2012.botanyconference.org/engine/search/index.php?func=detail&aid=764

Beck, J. B., J. Allison, K. M. Pryer, and **M.D. Windham.** Identifying multiple origins of polyploid taxa: a multilocus study of the hybrid cloak fern (*Astrolepis integerrima*; Pteridaceae). Paper presented at annual BSA meetings, Columbus, OH. http://2012.botanyconference.org/engine/search/index.php?func=detail&aid=293

Grusz, A. G., **M. D. Windham**, and K. M. Pryer. 2012. Using next generation sequencing to develop microsatellite markers in ferns. Paper presented at annual BSA meetings, Columbus, OH. http://2012.botanyconference.org/engine/search/index.php?func=detail&aid=946

Huiet, L., K. M. Pryer**,** C. H. Haufler, and **M. D. Windham**. 2012. Simplifying the complex: an integrative analysis using cytogenetics, morphology, and DNA sequence data to resolve relationships within the *Pellaea wrightiana* complex (Pteridaceae). Paper presented at annual BSA meetings, Columbus, OH. http://2012.botanyconference.org/engine/search/index.php?func=detail&aid=651

Sigel, E., **M. D. Windham,** C. H. Haufler, and K. M. Pryer. 2012. Reassessing phylogenetic relationships in the *Polypodium vulgare* complex (Polypodiaceae) using nuclear *gapCp* sequence data. Paper presented at annual BSA meetings, Columbus, OH. http://2012.botanyconference.org/engine/search/index.php?func=detail&aid=890

Beck, J., P. Alexander, L. Allphin, C. Rushworth, C. D. Bailey, and **M. D. Windham**. 2011. Does hybridization drive the transition to asexuality in diploid *Boechera*? Paper presented at annual BSA meetings, St. Louis, MO.

Hooper, E., G. Yatskievych, L. Huiet, **M. D. Windham**, and K. Pryer. 2011. Into or out of Africa? What do molecular data reveal about the identity and biogeographic origin of Aleuritopteris farinosa (Forssk.) Fee (Pteridaceae)? Paper presented at annual BSA meetings, St. Louis, MO.

Johnson, A. K., C. Rothfels, A. Grusz, E. Sigel, **M. D. Windham**, and K. Pryer. 2011. Sporophytes and gametophytes of notholaenid ferns (Pteridaceae) show correlated presence/absence of farina. Poster presented at annual BSA meetings, St. Louis, MO.

Li, F.-W., L.-Y. Kuo, C. Rothfels, A. Ebihara, W.-L. Chiou, **M. D. Windham**, and K. Pryer. 2011. *rbcL* and *matK* earn a thumbs up as the core DNA barcode for ferns. Paper presented at International Botanical Congress in Melbourne, Australia and annual BSA meetings, St. Louis, MO.

Windham, M. D., J. Beck, P. Alexander, L. Allphin, C. D. Bailey, C. Call, F.-W. Li, C. Rushworth, and I. Al-Shehbaz, 2011. Is the glass half empty or half full?: The current state of *Boechera* (Brassicaeae) systematics. Paper presented at annual BSA meetings, St. Louis, MO.

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Sigel, E. M., **M. D. Windham**, and K. M. Pryer. 2010. Using spore data to infer ploidy and reproductive mode in land plants. Paper presented at annual BSA meetings, Providence, RI.

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Beck, J., **M. D. Windham**, and K. M. Pryer. 2009. Tempo and mode of evolution in the star-scaled cloak ferns (*Astrolepis*). Paper presented at annual BSA meetings, Snowbird, UT.

Grusz, A. G., **M. D. Windham**, and K. M. Pryer. 2009. A *Cheilanthes* by any other name: Evolutionary complexity in the New World myriopterid clade (Pteridaceae). Paper presented at annual BSA meetings, Snowbird, UT.

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Research Interests

Speciation and evolution in vascular plants, especially the evolutionary consequences of hybridization, polyploidy and apomixis; chromosomal and molecular biogeography

Professional Affiliations

American Fern Society, American Society of Plant Taxonomists, Botanical Society of America, California Botanical Society, International Association for Plant Taxonomy

New Taxa Described

2013

Yosemitea P.J. Alexander & Windham (new genus)

2012

Gaga Pryer, Fay-Wei Li & Windham (new genus)
Gaga germanotta Fay-Wei Li & Windham
Gaga monstraparva Fay-Wei Li & Windham

2010

Astrolepis obscura J. Beck & Windham

2007

Boechera elkoensis Windham & Al-Shehbaz
Boechera peirsonii Windham & Al-Shehbaz
Boechera tularensis Windham & Al-Shehbaz
Draba abajoensis Windham & Al-Shehbaz
Draba malpighiacea Windham & Al-Shehbaz
Draba santaquinensis Windham & Allphin

2006

Boechera breweri (S. Wats.) Al-Shehbaz subsp. *shastaensis* Windham & Al-Shehbaz
Boechera evadens Windham & Al-Shehbaz
Boechera lincolnensis Windham & Al-Shehbaz
Boechera quebecensis Windham & Al-Shehbaz
Boechera rollinsiorum Windham & Al-Shehbaz
Boechera serpenticola Windham & Al-Shehbaz
Boechera shevockii Windham & Al-Shehbaz
Boechera texana Windham & Al-Shehbaz
Boechera ultraalsa Windham & Al-Shehbaz
Boechera villosa Windham & Al-Shehbaz

2005

Polypodium x aztecum Windham & Yatsk.

2004

Woodsia cystopteroides Windham & Mickel

2001

Pellaea ribae A. Mend. & Windham

1993

Cheilanthes yavapensis T. Reeves ex Windham
Notholaena californica D.C. Eaton subsp. *leucophylla* Windham
Notholaena grayi Davenp. subsp. *sonorensis* Windham
Pellaea gastonyi Windham
Pellaea lyngholmii Windham
Pellaea ternifolia (Cav.) Link subsp. *arizonica* Windham
Pellaea ternifolia (Cav.) Link subsp. *villosa* Windham
Polypodium saximontanum Windham
Woodsia cochisensis Windham
Woodsia neomexicana Windham
Woodsia obtusa (Sprengel) Torrey subsp. *occidentalis* Windham
Woodsia phillipsii Windham

Woodsia scopulina D.C. Eaton subsp. *laurentiana* Windham
1992
Astrolepis D.M. Benham & Windham (new genus)
1991
Cystopteris utahensis Windham & Haufler
Polypodium appalachianum Haufler & Windham
1990
Pentagramma Yatsk., Windham & E. Wollenw. (new genus)

New Nomenclatural Combinations Published
2013
Borodinia burkii (Porter) P.J. Alexander & Windham
Borodinia canadensis (L.) P.J. Alexander & Windham
Borodinia dentata (Raf.) P.J. Alexander & Windham
Borodinia laevigata (Muhl. ex Willd.) P.J. Alexander & Windham
Borodinia missouriensis (Greene) P.J. Alexander & Windham
Borodinia perstellata (E. Braun) P.J. Alexander & Windham
Borodinia serotina (Steele) P.J. Alexander & Windham
Yosemitea repanda (S. Wats.) P.J. Alexander & Windham
2012
Gaga angustifolia (Kunth) Fay-Wei Li & Windham
Gaga apiacea (Mickel) Fay-Wei Li & Windham
Gaga arizonica (Maxon) Fay-Wei Li & Windham
Gaga chaerophylla (M. Martens & Galeotti) Fay-Wei Li & Windham
Gaga complanata (A.R. Sm.) Fay-Wei Li & Windham
Gaga cuneata (Link) Fay-Wei Li & Windham
Gaga decomposita (M. Martens & Galeotti) Fay-Wei Li & Windham
Gaga decurrens (Mickel) Fay-Wei Li & Windham
Gaga harrisii (Maxon) Fay-Wei Li & Windham
Gaga hirsuta (Link) Fay-Wei Li & Windham
Gaga hintoniorum (Mendenh. & Nesom) Fay-Wei Li & Windham
Gaga kaulfussii (Kunze) Fay-Wei Li & Windham
Gaga lerstenii (Mickel & Beitel) Fay-Wei Li & Windham
Gaga marginata (Kunth) Fay-Wei Li & Windham
Gaga membranacea (Davenp.) Fay-Wei Li & Windham
Gaga pellaeopsis (Mickel) Fay-Wei Li & Windham
Gaga purpusii (T. Reeves) Fay-Wei Li & Windham
2010
Astrolepis deltoidea (Baker) J. Beck & Windham
2007
Boechera acutina (Greene) Windham & Al-Shehbaz
Boechera burkii (Porter) Windham & Al-Shehbaz
Boechera calderi (G.A. Mulligan) Windham & Al-Shehbaz
Boechera californica (Rollins) Windham & Al-Shehbaz
Boechera cascadensis Windham & Al-Shehbaz
Boechera consanguinea (Greene) Windham & Al-Shehbaz

Boechera covillei (Greene) Windham & Al-Shehbaz
Boechera depauperata (A. Nelson & P.B. Kennedy) Windham & Al-Shehbaz
Boechera drepanoloba (Greene) Windham & Al-Shehbaz
Boechera duchesnensis (Rollins) Windham, Al-Shehbaz & Allphin
Boechera gracilenta (Greene) Windham & Al-Shehbaz
Boechera grahamii (Lehmann) Windham & Al-Shehbaz
Boechera harrisonii (S.L. Welsh) Windham & Al-Shehbaz
Boechera horizontalis (Greene) Windham & Al-Shehbaz
Boechera languida (Rollins) Windham & Al-Shehbaz
Boechera macounii (S. Wats.) Windham & Al-Shehbaz
Boechera paddoensis (Rollins) Windham & Al-Shehbaz
Boechera pauciflora (Nutt.) Windham & Al-Shehbaz
Boechera pinetorum (Tidestrom) Windham & Al-Shehbaz
Boechera porphyrea (Wooton & Standley) Windham, Al-Shehbaz & P. Alexander
Boechera pratincola (Greene) Windham & Al-Shehbaz
Boechera rubicundula (Jepson) Windham & Al-Shehbaz
Boechera saximontana (Rollins) Windham & Al-Shehbaz
Boechera serotina (Steele) Windham & Al-Shehbaz
Boechera xylopoda Windham & Al-Shehbaz
Draba bifurcata (C.L. Hitchc.) Al-Shehbaz &Windham
Draba serpentina (Tiehm & P. Holmgren) Al-Shehbaz &Windham

2006

Boechera arcuata (Nutt.) Windham & Al-Shehbaz
Boechera atrorubens (Suksd. ex Greene) Windham & Al-Shehbaz
Boechera fernaldiana (Rollins) W.A. Weber subsp. *vivariensis* (S.L. Welsh) Windham & Al-Shehbaz
Boechera formosa (Greene) Windham & Al-Shehbaz
Boechera howellii (S. Wats.) Windham & Al-Shehbaz
Boechera nevadensis (Tidestrom) Windham & Al-Shehbaz
Boechera paupercula (Greene) Windham & Al-Shehbaz
Boechera pendulocarpa (A. Nelson) Windham & Al-Shehbaz
Boechera polyantha (Greene) Windham & Al-Shehbaz
Boechera spatifolia (Rydb.) Windham & Al-Shehbaz

2003

Draba burkei (C.L. Hitchc.) Windham & Beilstein
Draba pedicellata (Rollins & Price) Windham
Pellaea villosa (Windham) Windham & Yatsk.

1993

Argyrochosma limitanea (Maxon) Windham subsp. *mexicana* (Maxon) Windham
Pellaea glabella Mett. ex Kuhn subsp. *missouriensis* (Gastony) Windham
Pellaea glabella Mett. ex Kuhn subsp. *occidentalis* (E.E. Nelson) Windham
Pellaea mucronata (D.C. Eaton) D.C. Eaton subsp. *californica* (Lemmon) Windham
Pleopeltis guttata (Maxon) E.G. Andrews & Windham

Pleopeltis polypodioides (L.) E.G. Andrews & Windham
Pleopeltis polypodioides (L.) E.G. Andrews & Windham var. *acicularis* (Weath.) E.G. Andrews & Windham
Pleopeltis polypodioides (L.) E.G. Andrews & Windham var. *michauxiana* (Weath.) E.G. Andrews & Windham
Pleopeltis riograndensis (Wendt) E.G. Andrews & Windham
Pleopeltis thyssanolepis (A. Braun ex Klotzsch) E.G. Andrews & Windham
Woodsia oregana D.C. Eaton subsp. *cathcartiana* (B.L. Rob.) Windham
Woodsia scopulina D.C. Eaton subsp. *appalachiana* (T.M.C. Taylor) Windham

1992

Astrolepis beitelii (Mickel) D.M. Benham & Windham
Astrolepis cochisensis (Goodd.) D.M. Benham & Windham
Astrolepis crassifolia (Houlston & T. Moore) D.M. Benham & Windham
Astrolepis integerrima (Hook.) D.M. Benham & Windham
Astrolepis sinuata (Lag. ex Sw.) D.M. Benham & Windham

1990

Pentagramma pallida (Weath.) Yatsk., Windham & E. Wollenw.
Pentagramma triangularis (Kaulf.) Yatsk., Windham & E. Wollenw.
Pentagramma triangularis (Kaulf.) Yatsk., Windham & E. Wollenw. subsp. *maxonii* (Weath.) Yatsk., Windham & E. Wollenw.
Pentagramma triangularis (Kaulf.) Yatsk., Windham & E. Wollenw. subsp. *semipallida* (J.T. Howell) Yatsk., Windham & E. Wollenw.
Pentagramma triangularis (Kaulf.) Yatsk., Windham & E. Wollenw. subsp. *viscosa* (Nutt. ex D.C. Eaton) Yatsk., Windham & E. Wollenw.

1987

Argyrochosma (J. Smith) Windham
Argyrochosma chilensis (Fée & Remy) Windham
Argyrochosma dealbata (Pursh) Windham
Argyrochosma delicatula (Maxon & Weath.) Windham
Argyrochosma fendleri (Kunze) Windham
Argyrochosma formosa (Liebm.) Windham
Argyrochosma incana (C. Presl) Windham
Argyrochosma jonesii (Maxon) Windham
Argyrochosma limitanea (Maxon) Windham
Argyrochosma lumholtzii (Maxon & Weath.) Windham
Argyrochosma microphylla (Mett. ex Kuhn) Windham
Argyrochosma nivea (Poir.) Windham
Argyrochosma pallens (Weath.) Windham
Argyrochosma palmeri (Baker) Windham
Argyrochosma peninsularis (Maxon & Weath.) Windham
Argyrochosma pilifera (R. Tryon) Windham
Argyrochosma stuebeliana (Hieron.) Windham

COMMENT (5) RE: BLADDERPOD GENETIC STUDY

AND U.S. FISH AND WILDLIFE SERVICE RESPONSE

EXCERPTED FROM RULE PUBLISHED IN FEDERAL REGISTER

DECEMBER 20, 2013

(5) *Comment*: One commenter provided us a recent unpublished genetic analysis titled "Sequence Variation Among *Physaria douglasii* Isolates," authored by C.L. Anderson, Ph.D., with the University of Idaho Laboratory for Evolutionary, Ecological and Conservation Genetics. A number of commenters asserted that this new analysis establishes that the White Bluffs bladderpod is not a distinct subspecies of *Physaria douglasii*.

Our Response: The genetic analysis (Anderson 2013) used DNA sequence data to investigate the taxonomic status of the White Bluffs bladderpod and concluded that the findings presented in the report did not indicate that *Physaria douglasii* ssp. *tuplashensis* is a distinct subspecies. However, the report states that these results cannot be considered definitive or final due to sampling constraints (Anderson 2013, p. 9). The Service had the genetic analysis report (Anderson 2013) externally peer reviewed given its potential significance to our final listing determination. We requested peer review from five subject and related field experts and received comments from all five reviewers. Their unanimous, independent conclusion was that this analysis was insufficient to warrant a change to the current taxonomic status of White Bluffs bladderpod. All five peer reviewers indicated that this study was inconclusive as to the taxonomic status of *tuplashensis*. Peer reviewers stated that the genetic markers selected for this study were insufficient for determining differences between closely related taxa in the genus *Physaria*. In addition, all peer reviewers stated that too few samples were collected to adequately characterize genetic diversity and compare *tuplashensis* and *douglasii* under the study

design. We find the peer reviewers' critiques of Anderson 2013 to be well-reasoned. Anderson examined only three samples of White Bluffs bladderpod, and he acknowledged that there were "too few samples for statistical validity." Therefore, the Service, consistent with the currently accepted taxonomic status, affirms its previous determination that the White Bluffs bladderpod is a distinct subspecies.