

1  
2 DR. JEANNE ROBERTSON (Orcid ID : 0000-0003-2642-6280)  
3 DR. DAVID S PILLIOD (Orcid ID : 0000-0003-4207-3518)

4  
5  
6 Article type : Original Article  
7  
8

## 9 **Regional variation in drivers of connectivity for two frog 10 species (*Rana pretiosa* and *R. luteiventris*) from the U.S. Pacific 11 Northwest**

12  
13 JEANNE M. ROBERTSON<sup>1,7\*</sup>, MELANIE A. MURPHY<sup>2\*</sup>, CHRISTOPHER A.  
14 PEARL<sup>3</sup>, MICHAEL J. ADAMS<sup>3</sup>, MÓNICA I. PÁEZ-VACAS<sup>4,7</sup>, SUSAN M. HAIG<sup>3</sup>,  
15 DAVID S. PILLIOD<sup>5</sup>, ANDREW STORFER<sup>6</sup>, and W. CHRIS FUNK<sup>7</sup>

16  
17 \*Co-first authors

18 <sup>1</sup>*Department of Biology, California State University, 18111 Nordhoff Street, Northridge,  
19 CA 91330, USA*

20 <sup>2</sup>*Department of Ecosystem Science and Management, Program in Ecology, 1000 East  
21 University Avenue, University of Wyoming, Laramie, WY 82071, USA*

22 <sup>3</sup>*U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, 3200  
23 Jefferson Way, Corvallis, OR 97331, USA*

24 <sup>4</sup>*Centro de Investigación de la Biodiversidad y Cambio Climático, Universidad  
25 Tecnológica Indoamérica, Quito, Ecuador*

26 <sup>5</sup>*U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, 970 Lusk  
27 Street, Boise, ID 83706, USA*

This is the author manuscript accepted for publication and has undergone full peer review  
but has not been through the copyediting, typesetting, pagination and proofreading process,  
which may lead to differences between this version and the [Version of Record](#). Please cite  
this article as [doi:10.1111/mec.14798](https://doi.org/10.1111/mec.14798)

This article is protected by copyright. All rights reserved

28     <sup>6</sup>School of Biological Sciences, Washington State University, P.O. Box 644236, Pullman,  
29     WA 99164, USA

30     <sup>7</sup>Department of Biology, Graduate Degree Program in Ecology, Colorado State  
31     University, 1878 Campus Delivery, Fort Collins, CO 80523, USA

32  
33     Correspondence; Jeanne M. Robertson, Fax: +1 818-677-2034; E-mail:  
34     Jeanne.Robertson@csun.edu

35  
36     **Keywords:** comparative landscape genetics, gene flow, climate, species traits, Ranidae

38     Running title: Species traits predict connectivity in frogs**Abstract (250 word limit)**

39     **Comparative landscape genetics has uncovered high levels of variability in which**  
40     **landscape factors affect connectivity among species and regions. However, the**  
41     **relative importance of species traits vs. environmental variation for predicting**  
42     **landscape patterns of connectivity is unresolved. We provide evidence from a**  
43     **landscape genetics study of two sister taxa of frogs, the Oregon spotted frog (*Rana***  
44     ***pretiosa*) and the Columbia spotted frog (*R. luteiventris*) in Oregon and Idaho, USA.**  
45     ***Rana pretiosa* is relatively more dependent on moisture for dispersal than *R.***  
46     ***luteiventris*, so if species traits influence connectivity, we predicted that connectivity**  
47     **among *R. pretiosa* populations would be more positively associated with moisture**  
48     **than *R. luteiventris*. However, if environmental differences are important drivers of**  
49     **gene flow, we predicted that connectivity would be more positively related to**  
50     **moisture in arid regions. We tested these predictions using eight microsatellite loci**  
51     **and gravity models in two *R. pretiosa* regions and four *R. luteiventris* regions (*n* =**  
52     **1,168 frogs). In *R. pretiosa*, but not *R. luteiventris*, connectivity was positively**  
53     **related to mean annual precipitation, supporting our first prediction. In contrast,**  
54     **connectivity was not more positively related to moisture in more arid regions.**  
55     **Various temperature metrics were important predictors for both species and in all**  
56     **regions, but the directionality of their effects varied. Therefore, the pattern of**  
57     **variation in drivers of connectivity was consistent with predictions based on species**  
58     **traits rather than on environmental variation.**

59

## 60 1 INTRODUCTION

61 Connectivity is essential for long-term persistence of populations and metapopulations  
62 (Crooks & Sanjayan 2006). Functional connectivity is the degree to which individuals  
63 move through a landscape, which is affected by landscape features and environmental  
64 heterogeneity (Tischendorf & Fahrig 2000). One way connectivity can increase  
65 population persistence is through the demographic contribution of immigrants to  
66 population growth rate, termed demographic rescue (Brown & Kodricbrown 1977).  
67 Population persistence can also be increased by connectivity that leads to genetic rescue,  
68 defined as the increase in population fitness (population size or growth rates) by the  
69 immigration of new alleles (Tallmon, Luikart & Waples 2004; Whiteley, Fitzpatrick,  
70 Funk & Tallmon 2015).

71 Landscape genetics tests which landscape and environmental factors best explain  
72 genetic connectivity among populations or individuals (Balkenhol, Cushman, Storfer &  
73 Waits 2016; Manel, Schwartz, Luikart & Taberlet 2003; Storfer *et al.* 2007; Storfer *et al.*  
74 2010) and highlight which landscape features need to be maintained or enhanced to  
75 facilitate connectivity for different species. Moreover, when connectivity is affected by  
76 temperature or precipitation, landscape genetics can suggest organismal responses to  
77 climate change. For example, future increases in temperature may decrease connectivity  
78 for species in which gene flow is negatively related to temperature. Similarly, decreases  
79 in water availability could decrease connectivity for species in which gene flow is  
80 positively related to moisture (Goldberg & Waits 2010; Pilliod *et al.* 2015).

81 In general, comparative landscape genetic studies have found that the factors  
82 affecting connectivity vary considerably, even among species that occupy similar  
83 landscapes and have similar ecological niches (Aparicio, Hampe, Fernandez-Carrillo &  
84 Albaladejo 2012; Dudaniec *et al.* 2016; Engler *et al.* 2014; Frantz *et al.* 2012; Goldberg  
85 & Waits 2010; Poelchau & Hamrick 2012; Richardson 2012; Storfer *et al.* 2007;  
86 Whiteley, McGarigal & Schwartz 2014; Wultsch, Waits & Kelly 2016). Thus, the  
87 relative influence of landscape factors on gene flow is context dependent. Does this mean  
88 that a landscape genetic study will be necessary for every species and region for which an  
89 understanding of connectivity is desired? This would be a daunting prospect for managers

90 charged with conservation of biodiversity, because obtaining these data for the thousands  
91 of threatened species in the world would be logically unfeasible. Alternatively, it  
92 would be useful if the factors that affect connectivity could be predicted by life history  
93 traits, as cross-species predictions may then be made. For example, if connectivity is  
94 positively related to forest cover for most forest obligate species, then perhaps a similar  
95 relationship for other forest species for which no landscape genetic data have been  
96 collected could be assumed (see Goldberg & Waits 2010). Landscape genetics can help  
97 test the influence of species traits on the environmental factors that govern connectivity  
98 among populations.

99 We use a landscape genetics approach to address this question in two closely related  
100 species of frogs—the Oregon spotted frog, *Rana pretiosa* (Baird & Girard 1853) and  
101 parts of the Great Basin and Northern clades of the Columbia spotted frog, *Rana*  
102 *luteiventris* (Thompson 1913)—for which maintaining connectivity is considered a  
103 conservation priority (Pilliod *et al.* 2015). We predicted that drivers of functional  
104 connectivity would vary among regions, given differences in species traits, as well as  
105 dissimilarities in environmental characteristics among regions. In particular, we expected  
106 the pronounced gradient of decreasing precipitation going from west to east would shape  
107 connectivity for these wetland breeding amphibians.

108 We predicted that metrics of moisture availability should be stronger predictors of  
109 connectivity in *R. pretiosa* due to the relatively greater dependence on moisture for  
110 population connectivity (Pearl, Adams & Leuthold 2009; Watson, McAllister & Pierce  
111 2003), compared to *R. luteiventris*, for which overland dispersal has been documented  
112 (Goldberg & Waits 2010; Pilliod, Peterson & Ritson 2002). When considering regional  
113 differences in environmental conditions, we expected that connectivity would be  
114 positively related to precipitation or moisture gradients (Pilliod *et al.* 2015). For example,  
115 we expected the desert sites in eastern Oregon and southwestern Idaho to have lower  
116 connectivity than the Cascades where moisture is abundant. We also expected  
117 connectivity to be negatively related to temperature because high maximum temperatures  
118 may limit dispersal due to threat of desiccation. Thus, overall, we expected that  
119 connectivity would be positively related to moisture and negatively related to temperature

120 in both species; however, the relative importance of these metrics will also depend on the  
121 importance of species traits versus environmental variation in driving connectivity.

122

## 123 **2 METHODS AND MATERIALS**

### 124 **2.1 Study species**

125 We estimated connectivity for *R. pretiosa* from two regions (Klamath and Deschutes) and  
126 *R. luteiventris* from four regions (central Oregon, Blue Mountains in northeastern  
127 Oregon, southeastern Oregon, and Owyhee in southwestern Idaho). The two sister  
128 species, *R. pretiosa* and *R. luteiventris*, breed and lay eggs in ponds, lakes, wetlands, and  
129 backwaters of rivers and metamorphose in late summer or early fall (Funk *et al.* 2008;  
130 Green, Sharbel, Kearsley & Kaiser 1996; Green *et al.* 1997). *Rana luteiventris* disperse  
131 relatively soon after metamorphosis or subsequently as juveniles prior to becoming  
132 sexually mature, with most dispersal occurring in their first summer (Reaser & Pilliod  
133 2005). Timing and life stages of *R. pretiosa* dispersal are less understood than for *R.*  
134 *luteiventris*. *Rana luteiventris* is known to travel long distances (estimated seasonal  
135 migration ~400 – 1000m, isolated reports of 5 – 6.5 km) and cross upland terrain (Bull &  
136 Hayes 2001; Engle 2001; Funk *et al.* 2005a; Pilliod *et al.* 2002; Reaser 1996). In contrast,  
137 *R. pretiosa* typically has smaller home ranges, smaller seasonal migration distances (200  
138 – 500m, with isolated reports of 1km) and very rarely strays from wetlands (Blouin,  
139 Phillipsen & Monsen 2010; Chelgren, Pearl, Adams & Bowerman 2008; McAllister *et al.*  
140 2004; Pearl & Hayes 2005; Watson *et al.* 2003).

141 There are other important differences between these species. *Rana pretiosa* is  
142 relatively more aquatic than *R. luteiventris* (Blouin *et al.* 2010; Funk *et al.* 2005a; Funk,  
143 Greene, Corn & Allendorf 2005b) and has eyes that are more dorsally oriented. This eye  
144 orientation is hypothesized to be adaptive for floating at the surface of the water (Green  
145 *et al.* 1997). The range of Great Basin populations of *R. luteiventris* (described below) is  
146 much drier than that of *R. pretiosa*. Thus, *R. pretiosa* may be more reliant on  
147 precipitation, due to their closer association with moisture, but Great Basin *R. luteiventris*  
148 may be more dependent on moisture due to their arid habitats.

149 Both *R. pretiosa* and Great Basin populations of *R. luteiventris* are of conservation  
150 concern. *Rana pretiosa* was historically found in southwestern British Columbia, the

151 Puget Trough of Washington, the Willamette Valley of Oregon, and the Cascade  
152 Mountains of Washington, Oregon, and California. Extant *R. pretiosa* are concentrated in  
153 the upper Deschutes River and Klamath River basins on the eastern flank of the Oregon  
154 Cascades, and in portions of western Washington and southwestern British Columbia  
155 (Pearl & Hayes 2005). It was recently listed as Threatened under the U.S. Endangered  
156 Species Act due to loss and alteration of its marsh habitats (USFWS 2014). The historic  
157 range of *R. luteiventris* stretches from the southern Yukon, Canada, to Nevada and Utah  
158 and includes three major clades (Funk *et al.* 2008). Great Basin populations occupy  
159 springs, ponds, and streams in southeastern Oregon, southwestern Idaho, and Nevada  
160 (Arkle & Pilliod 2015). The Great Basin clade has experienced localized declines and is  
161 considered a taxon of conservation concern in Oregon, Nevada, and Idaho, although not  
162 warranted for listing under the Endangered Species Act as of 2015 (USFWS 2015).

163

## 164 **2.2 Field sampling**

165 We collected tissue from 1,168 frogs from 77 sites across the six regions, with an average  
166 of 12 sites per region (Fig. 1, Table 1). We analyzed *R. pretiosa* samples from the  
167 Deschutes (24 sites) and Klamath (19 sites). The *R. luteiventris* in our analysis were  
168 collected from the northern (18 sites) and the Great Basin (16 sites) clades. The average,  
169 minimum and maximum distances within each region are provided in Table S1. Tissue  
170 samples consisted of toe clips and buccal swabs from adults (Goldberg, Kaplan &  
171 Schwable 2003) or tail clips from tadpoles (Murphy, Dezzani, Pilliod & Storfer 2010).  
172 We collected samples during spring and summer (April through September) in 2007–  
173 2012.

174

## 175 **2.3 Laboratory methods**

176 We extracted genomic DNA from both tail and toe clips and buccal swabs using the  
177 Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) and amplified every  
178 individual of both species at eight microsatellite loci in two multiplex reactions (Murphy,  
179 Dezzani, Pilliod & Storfer 2010). We never included full siblings (larvae sampled from  
180 the same clutch and from different years). We followed the PCR protocol and all  
181 laboratory steps that are detailed in Murphy *et al.* (2010) including: using negative

182 controls (reagents only) at both extraction and PCR stages, including samples with  
183 known genotypes in every run, and random sampling of 10% of the samples for  
184 reamplification to assess genotyping error. PCR products were visualized on an  
185 ABI3730xL Data Analyzer (Applied Biosystems) using the GeneScan LIZ500 (Applied  
186 Biosystems) size standard. Genotypes were binned and visually verified using  
187 GENEMARKER (SoftGenetics 2010).

188

## 189 **2.4 Data analysis**

### 190 **2.4.1 Population structure**

191 We tested for possible null alleles in MICROCHECKER (van Oosterhout, Weetman &  
192 Hutchinson 2006) and divergence from Hardy-Weinberg (HW) proportions and linkage  
193 disequilibrium (LD) in GENEPOP VER4.0.10 (Rousset 2008). We estimated pairwise  $F_{ST}$   
194 between all sites within and among regions and assessed for significance in ARLEQUIN  
195 VER3.5 (Excoffier & Lischer 2010). We inferred the number of genetic clusters ( $K$ ) for  
196 each species using the program STRUCTURE VER2.3.3 (Pritchard, Stephens & Donnelly  
197 2000). STRUCTURE implements a Bayesian clustering algorithm to infer the best-  
198 supported number of clusters ( $K$ ) in the sample and the proportion of each individual's  
199 genome assigned to each cluster ( $q_k$ ) based on Hardy-Weinberg proportions and gametic  
200 phase disequilibrium, and then assigns each individual to one or more clusters (model  
201 conditions: burn-in 100,000 iterations; 3,000,000 iterations post burn-in; admixture  
202 model; correlated allele frequencies;  $K = 1-15$ ; 10 replicates for each  $K$ ). We selected  $K$   
203 based on the  $\Delta K$  method (Evanno, Regnaut & Goudet 2005) and the posterior probability  
204 of  $K$  given the data (Pritchard *et al.* 2000).

205 We estimated the relationship between overland distance and genetic distance and  
206 predicted that the slope would be steeper for *R. pretiosa*, due to the relatively more  
207 restricted overland movement in *R. pretiosa* compared to *R. luteiventris*. We tested for  
208 isolation-by-distance (IBD) using a mantel test with  $F_{ST}$  and distance, implemented in R  
209 VER2.4.1 (999 repetitions) using the ader4 package. We calculated the  $r^2$  and slope for  
210 each study region using a linear model.

211

### 212 **2.4.2 Characterizing environmental variation among regions**

213 We performed a PCA on the at site and between site landscape characteristics to test  
214 whether regions vary in ecological and abiotic factors, and thus demonstrating that we  
215 modeled functional connectivity in distinct ecological regions (Legendre & Legendre  
216 2012; prcomp in stats package (variables centered and scaled) in program R). Our PCA  
217 included 15 landscape variables hypothesized to affect connectivity in *R. pretiosa* and *R.  
luteiventris*, including variables related to site productivity, temperature, moisture,  
218 topography, and habitat (see Table 2).

220

### 221 **2.4.3 Functional connectivity**

222 We used singly-constrained gravity models based on a saturated network to test the  
223 relative importance of different landscape variables on connectivity separately for each of  
224 our six regions. Gravity models employ a network-based approach for testing which  
225 landscape factors best predict functional connectivity (Fotheringham & O'Kelly 1989).  
226 Gravity models integrate three components to model functional connectivity: spatial  
227 proximity, or distance between sites ( $w$ ), the productivity/attraction of individuals from/to  
228 each site (at site,  $v$ ), and the resistance of intervening habitat between sites to flow  
229 (between cite,  $c$ ) ((Murphy, Dezzani, Pilliod & Storfer 2010). We calculated Nei's chord  
230 distance (Da), estimated in MICROSATELLITE ANALYZER (Dieringer & Schloetterer 2003)  
231 and used  $(1 - Da)$ , i.e., “flow” as a measure of connectivity in gravity models. We  
232 selected Da for these analysis as: 1) it is highly correlated with proportion of shared  
233 alleles (Dps) used in the justification of gravity models for landscape genetics (Murphy *et  
al.* 2010), 2) is more familiar to population geneticists, and 3) does not make equilibrium  
235 assumptions.

236 We implemented singly-constrained gravity models in R in package GENETIT (Evans  
237 and Murphy 2015). A complete list of all variables tested in gravity models, the  
238 ecological justification for each, and data sources are shown in Table 2. We included the  
239 geographic distance between sites ( $w$ ) in each gravity model, as a measure of spatial  
240 proximity is required as part of the gravity form. We assessed at site characteristics ( $v$ )  
241 that could influence the production of potential migrants that would contribute to gene  
242 flow (singly-constrained gravity model, production constraint), including: compound  
243 topographic index (cti\_F) elevation (elev\_F), and solar exposure (heat load index, hli\_F).

244 Between sites (*c*), we included several variables thought to limit or promote connectivity  
245 (mean temperature, moisture, topography, and habitat condition). To characterize  
246 variables between sites, we sampled the network edge every 30 m and calculated the  
247 mean value (Murphy *et al.* 2010; Evans and Murphy 2015).

248 We linearized the equation for the gravity models by taking the natural log of the  
249 response variable (1-Da) and all predictor variables (Table 2) and then estimated the  
250 singly-constrained gravity model using a mixed effects model ((Murphy et al. 2010,  
251 implemented in GENETIT. For a detailed discussion of gravity models and  
252 implementation in landscape genetics, see Murphy *et al.* (2010) and GENETIT for details  
253 of specific functions in R (Evans and Murphy 2015).

254 In order for analyses to be comparable across regions, we fit all possible variable  
255 combinations up to three parameters plus geographic distance. The total possible number  
256 of gravity models containing up to three predictor variables is 3213 models. However, the  
257 actual number of gravity models implemented in our analyses varied by region because  
258 models that contained strongly co-varying variables (Pearson's  $r > 0.7$ ) were dropped  
259 from analyses. Also, the small number of sites in southeastern Oregon precluded analyses  
260 of more than one variable (plus distance) per model. Thus, our analyses are based on the  
261 following number of gravity models per region: Deschutes (3213); Klamath (3213);  
262 central Oregon (2516); Blue Mountains (2516); southeastern Oregon (17); Owyhee  
263 (3204). Overall parameter weights for each variable were summed across all gravity  
264 models, allowing us to evaluate the overall effect of each variable on functional  
265 connectivity. Parameter weights for each variable were calculated for each region as the  
266 sum of the Akaike weights for each model that included the given variable (Burnham &  
267 Anderson 1998). We used parameter weights to assess the relative importance of each  
268 variable for functional connectivity. We used beta estimates from univariate models to  
269 infer a given variable's directional effect on connectivity (whether it facilitated [+] or  
270 impeded [-] connectivity; Table S2).

271 One possible explanation for variation among regions in the models selected is that  
272 the predictor variables chosen are those with the highest level of variance in the given  
273 region, rather than that the predictor variable is biologically more important (Short Bull *et*

274     *al.* 2011). To test this possibility, we calculated the coefficient of variation (CV) for each  
275     landscape variable in each region.

276

## 277     **3     RESULTS**

### 278     **3.1     Population structure**

279     No loci showed evidence of linkage disequilibrium (LD), and in general, loci conformed  
280     to Hardy-Weinberg (HW) proportions (Table 1). Genotyping error was low (< 0.04%).  
281     We detected very high levels of genetic subdivision ( $F_{ST}$ ) between the two *R. pretiosa*  
282     regions ( $F_{ST} = 0.539$ ) and among the four *R. luteiventris* regions ( $F_{ST}$  mean [range] =  
283     0.405 [0.197–0.610]).  $F_{ST}$  values were variable among sites within each *R. pretiosa*  
284     region (Klamath = 0.218 [0.00 – 0.609]; Deschutes = 0.165 [0.00 – 0.531]) and within  
285     each *R. luteiventris* region (central Oregon = 0.052 [0.00 – 0.151]; Blue Mountains =  
286     0.207 [0.077 – 0.385]; southeastern Oregon = 0.173 [0.048 – 0.272]; Owyhee = 0.288  
287     [0.012 – 0.690]). The delineation of six genetic units was strongly supported by regional  
288      $F_{ST}$  estimates and Bayesian assignment test in STRUCTURE (Pritchard *et al.* 2000),  
289     validated by delta K (Evanno *et al.* 2005). STRUCTURE identified three distinct regions of  
290     *R. pretiosa* and 3 regions of *R. luteiventris* (Southeastern Oregon/Blue Mountains,  
291     Owyhee, Central; Fig. S1). For *R. pretiosa*, Klamath was divided into two genetic demes  
292     when K=6. However, we selected to consider Klamath as a single region for the  
293     landscape genetic analyses because the Klamath is distinct biogeographic region with  
294     continuously distributed sites. Moreover, our plot of IBD (genetic vs. geographic  
295     distance) did not detect any distinct genetic groups within the Klamath region (Fig. S2),  
296     suggesting genetic subdivision is not strong within this region. We further delineated  
297     Southeastern Oregon from Blue Mountains (despite assignment to the same deme) based  
298     on three factors: sites from these two regions are geographically isolated with no suitable  
299     intervening frog habitat; sites occur in distinct habitat types (Southeastern Oregon is in  
300     the high desert and Blue Mountains is coniferous mountain range); high, significant,  
301     pairwise  $F_{ST}$  estimates between the two regions (range = 0.065-0.137 average = 0.105; all  
302     p-value < 0.001).

303         Patterns of IBD varied by region (Fig. 2, Fig. S2). The two desert sites for *R.*  
304         *luteiventris* (southeastern OR and Owyhee, Fig. 1) had the steepest IBD (slopes). The two

305 more mountainous *R. luteiventris* regions (Blue Mountains and central OR) had the  
306 shallowest IBD. Finally, *R. pretiosa* showed an intermediate degree of IBD, with a  
307 slightly steeper slope for Deschutes populations (relatively greater isolation of those  
308 sites).

309

### 310 **3.2 Patterns of environmental variation among regions**

311 Our PCA identified environmental differences among our study regions, primarily in  
312 variables related to precipitation and temperature (Fig. 3). The Deschutes and Klamath  
313 basins, where *R. pretiosa* is found, were wetter (as indicated by positive loadings for  
314 compound topographic index [cti] and mean annual precipitation [map]) and had higher  
315 mean minimum temperatures than the four regions with *R. luteiventris* (central Oregon,  
316 Blue Mountains, southeastern Oregon, and Owyhee). Conversely, the regions occupied  
317 by *R. luteiventris* had higher mean maximum temperatures and longer frost free periods,  
318 despite occurring at higher elevation than *R. pretiosa* sites. Overall, the PCA serves to  
319 demonstrate that we model functional connectivity in distinct, ecological regions.

320

### 321 **3.3 Functional connectivity**

322 Three main results emerged from our gravity models. First, connectivity was more  
323 positively related to moisture (as measured by mean annual precipitation [map]) for *R.*  
324 *pretiosa* than for *R. luteiventris* (Fig. 4). Further, connectivity was negatively related to  
325 mean annual precipitation in two *R. luteiventris* regions, the Blue Mountains and  
326 Owyhee. Second, the directionality of the relationships between connectivity and  
327 temperature (whether temperature facilitated or impeded connectivity) varied among  
328 regions and were not aligned with our general expectations based on temperature  
329 variation among regions. For example, temperature (as measured by mean maximum  
330 temperature [mmax] and mean minimum temperature [mmin]) was identified as an  
331 important predictor of connectivity for both species and in all six regions (Fig. 4). In  
332 general, and as expected, some variables had higher variance than others, but there was  
333 no apparent relationship between parameter weight and CV (Figs. S3, S4, Table S2).

334

## 335 **4 DISCUSSION**

336 We found support for species traits having a stronger influence than environmental  
337 variation in determining connectivity in our system. Precipitation was more important for  
338 the more aquatic species (*R. pretiosa*) compared to the species living in a drier landscape.  
339 This suggests that understanding differences in species traits can help predict the relative  
340 importance of landscape factors to connectivity. These results also concur with previous  
341 research that shows, while both species are aquatic and require water for survival and  
342 reproduction, *R. pretiosa* is highly dependent on wet habitat for dispersal (Green *et al.*  
343 1997; Watson *et al.* 2003) and that connectivity among *R. luteiventris* sites is not  
344 predicted by stream distance, but by overland topographic distance (Goldberg & Waits  
345 2010; Murphy *et al.* 2010). Additionally, the PCA results supported our *a priori*  
346 expectation that *R. pretiosa* occupies wetter sites with less extreme temperatures (higher  
347 mean minimum temperature, but lower mean maximum temperature) than *R. luteiventris*.  
348 We found little support for our environmental variation hypothesis because connectivity  
349 was not positively related to moisture availability in the hot, dry conditions of the high  
350 desert, perhaps because *R. luteiventris* is adapted to relatively dry conditions in desert  
351 sites (Pilliod *et al.* 2015).

352 Our findings add to evidence that show that species traits can help predict which  
353 factors will affect connectivity (Dudaniec *et al.* 2016; Engler *et al.* 2014; Frantz *et al.*  
354 2012; Goldberg & Waits 2010; Richardson 2012; Selkoe *et al.* 2010; Whiteley *et al.*  
355 2014; Wultsch *et al.* 2016). For example, differences in landscape genetic patterns were  
356 attributed to ecological (species) traits in a comparative landscape genetics study of two  
357 co-occurring amphibians in northern Idaho, the long-toed salamander (*Ambystoma*  
358 *macrodactylum*) and the Columbia spotted frog (*R. luteiventris*). Goldberg and Waits  
359 (2010) found that moisture gradients were more important for population connectivity for  
360 *A. macrodactylum*, whereas gene flow was facilitated by land cover with low physical  
361 structure (e.g., agriculture and clear cut areas) for *R. luteiventris*. Another example of the  
362 importance of species traits was observed for three woodland marsupials in the same  
363 landscape in Queensland, Australia. In this study, genetic connectivity was largely  
364 associated with foliage protective cover for two glider species (sugar glider and squirrel  
365 glider), whereas connectivity was facilitated by understory and fallen timber for the  
366 yellow-footed antechinus, a ground-dwelling marsupial (Dudaniec *et al.* 2016).

We found high variability among regions in the factors affecting connectivity and in the directionality of their effects on connectivity. That is, no two regions had the same highly weighted variables (Fig. 4), which may not be surprising due to local adaptation and/or that we tested a large number of models. However, this finding is still consistent with other studies showing pronounced variation in landscape patterns of connectivity among regions, even within the same species in similar landscapes (Funk *et al.* 2005a; Short Bull *et al.* 2011; Trumbo, Spear, Baumsteiger & Storfer 2013). For example, Short Bull *et al.* (2011) tested 36 alternative landscape genetic models for black bears (*Ursus americanus*) in 12 landscapes with similar basic elements, but differences in features such as forest fragmentation, altitude, and roads. They found that the landscape features that affected connectivity differed significantly among these landscapes, partly due to differences in the level of variability in these features. While CV was not obviously related to parameter weight in our study, it is possible that overall differences between regions (overall range of parameters and range of conditions in a given region) combined with biological differences between species would result in differences in factors driving functional connectivity by region.

383

#### 384 **4.1 Variable effect of temperature on connectivity**

385 Temperature was important for connectivity in all regions, yet, the directionality of  
386 temperature effects differed among regions (Fig. 4). Extreme values of temperature may  
387 influence connectivity, especially if the temperature crosses a biologically-relevant  
388 threshold. For instance, consider mean maximum temperature (mmax) and mean  
389 minimum temperature (mmin). Deschutes is warmer than other regions, which may  
390 explain why high mmax values impede connectivity for this *R. pretiosa* region. Hot  
391 temperatures may, however, be less detrimental to *R. luteiventris* due to differences in  
392 desiccation risk between species. This relationship was observed in the Blue Mountains  
393 and southeastern Oregon: both show that mmax facilitates connectivity and both show a  
394 large range in maximum temperatures (supplemental material, Fig. S3-S4). Temperature  
395 extremes could also be correlated with snow melt or other ecological processes critical  
396 for connectivity.

397 If functional connectivity is at least partially controlled by temperature, then the  
398 potential effects of climate change on connectivity are a conservation concern for these  
399 threatened populations of frogs (Grobler, Mafumo & Minter 2003; Hangartner, Laurila &  
400 Raesaenen 2011; Pilliod *et al.* 2015; Rasanen, Laurila & Merila 2003). Some climate  
401 models predict that, on average, continental air temperatures will rise 0.24 °C per decade,  
402 annual precipitation will increase 1-2% per decade, and extreme climatic events, such as  
403 droughts and floods, will become more common (Burrows *et al.* 2011; Mote & Salathé  
404 2010). For *R. luteiventris*, projected climate change is predicted to have large, negative  
405 effects on Great Basin populations of *R. luteiventris* due to the loss of suitable climate  
406 and habitat, thereby exacerbating the genetic consequences of small and isolated  
407 populations (Pilliod *et al.* 2015).

408 There is general agreement that the overall effects of climate change will vary across  
409 the range of a species (Araujo, Thuiller & Pearson 2006; Avolio, Beaulieu & Smith 2013;  
410 Blair, Jimenez Arcos, Mendez de la Cruz & Murphy 2013; Pauls, Nowak, Balint &  
411 Pfenninger 2013), and this appears to be the case for our target species. In our study, frog  
412 connectivity was associated with paths with low temperatures in two regions (central  
413 Oregon and the Owyhee) but with high temperatures in the Blue Mountains and  
414 southeastern Oregon for *R. luteiventris*. For *R. pretiosa* extreme temperatures (both high  
415 and low temperatures) reduced connectivity in Deschutes, whereas connectivity was  
416 facilitated by high maximum temperatures in Klamath. Thus, for all regions, shifts in  
417 temperature and aquatic wetland availability is likely to disrupt connectivity patterns,  
418 with the important caveat that changes to climate will alter both temperature as well as  
419 seasonal water availability and breeding site connectivity (Pilliod *et al.* 2015).

## 420

### 421 **4.2 Effects of other landscape factors on connectivity**

422 At site elevation (elev\_F) was positively related to connectivity in central Oregon (Fig.  
423 4), suggesting that high elevation sites were important sources of immigrants. In contrast,  
424 Owyhee sites showed a decline in genetic diversity at high elevations, indicating isolation  
425 of high elevation sites in central Idaho (Fig. 4). Several other landscape factors not  
426 related to climate were included in our models to control for their effects rather than test  
427 predictions. In some cases, these landscape variables explained significant variation in

428 genetic distance. The only habitat variable that explained significant variation in  
429 connectivity was the presence of impervious surfaces (imper), such as roads and  
430 development, which impede population connectivity in the Blue Mountains. This finding  
431 was consistent with research that showed that roads commonly act as impediments to  
432 amphibian dispersal (e.g., Goldberg & Waits 2010; Murphy *et al.* 2010). The only  
433 topographic variable related to connectivity was fine-scale surface relief ratio (ssr3),  
434 which was positively related to connectivity for both the Blue Mountains and Owyhee.  
435 Fine-scale topographic complexity may facilitate movement by providing microclimatic  
436 refuges from heat/cold or desiccation, and could prove to be important in light of  
437 projected changes in climate. Finally, the presence of predatory fish limit functional  
438 connectivity in populations of *R. luteiventris* in subalpine ecosystems (Murphy *et al.*  
439 2010). However, our models did not include the presence of predatory fish because we  
440 did not have data on the presence of predatory fish (or wetland depth as a surrogate) for  
441 all regions.

442

### 443 **4.3 Conservation implications**

444 Our study has several conservation implications. First, our finding that gene flow is  
445 positively correlated with mean annual precipitation in *R. pretiosa* in both the Deschutes  
446 River and Klamath River basins supports previous work indicating that this species is  
447 highly dependent on areas with high precipitation for dispersal. As such, connectivity  
448 among sites may be reduced by landscape changes that reduce moisture availability. A  
449 previous census study for Klamath and Deschutes populations indicated that Klamath  
450 populations were at greater risk of local extirpation due to the lower number of egg  
451 masses and greater distance among breeding sites (Pearl *et al.* 2009). However, our  
452 estimates of isolation by distance do not necessarily support this conclusion: Deschutes  
453 and Klamath populations are relatively equal in terms of overall genetic isolation (with  
454 Deschutes slightly more isolated; Fig. 2), suggesting that long term persistence in both of  
455 these major drainages will rely heavily on aquatic systems for population connectivity.

456       Although mean annual precipitation was associated with lower connectivity in  
457 two *R. luteiventris* regions (Blue Mountain and Owyhee), this does not imply that  
458 moisture is not important for this species. Future reductions in moisture outside the

459 current range of variability could reduce habitat availability and connectivity (Pilliod *et*  
460 *al.* 2015). Our measures of IBD show that *R. luteiventris* populations in Southeastern  
461 Oregon and the Owyhee have relatively greater isolation at smaller spatial scales  
462 (steepest slopes), indicating overall reduced connectivity; however, the results for  
463 Southeastern Oregon should be interpreted with caution, given the few number of sites  
464 studied in that region. In contrast, the two more mountainous *R. luteiventris* regions (Blue  
465 Mountains and central OR) had the shallowest IBD, consistent with greater connectivity  
466 among sites, even though connectivity among Blue Mountain populations is impeded, in  
467 small part, by impervious surfaces. The finding that Blue Mountain populations are  
468 relatively less isolated is consistent with egg mass census data which show these  
469 populations to be relatively large (Pearl, Adams & Wente 2007).

470 In sum, we detected variability in the landscape factors that affect connectivity. The  
471 variation in the directionality of their effects suggests that information on connectivity  
472 from one region may be a poor surrogate for inferring connectivity in a related species or  
473 different region. That is, if it is deemed important to have an accurate understanding of  
474 the landscape factors affecting connectivity for a species of conservation concern, the  
475 emerging consensus from comparative landscape genetics studies is that species- and  
476 region-specific studies are necessary because of the high level of variability in  
477 connectivity in different regions and species.

478

## 479 **ACKNOWLEDGEMENTS**

480 This research was funded by the U.S. Bureau of Land Management, the U.S. Fish and  
481 Wildlife Service, the Sunriver Nature Center, the Oregon Community Foundation,  
482 National Institute of Food and Agriculture – SAES – University of Wyoming, and project  
483 WYO-5360-14. We thank J. Evans for use of the Geomorphometrics toolbox (Beta test  
484 version) and L. Chan for helpful comments on earlier drafts of this manuscript. The  
485 research was approved by the Institutional Animal Care and Use Committee at Colorado  
486 State University (09-152A-01). Any use of trade, firm, or product names is for  
487 descriptive purposes only and does not imply endorsement by the U.S. government. This  
488 is product number XXX of the U.S. Geological Survey’s Amphibian Research and  
489 Monitoring Initiative.

490 **References**

- 491 Aparicio A., Hampe A., Fernandez-Carrillo L., Albaladejo R. G. (2012) Fragmentation  
492 and comparative genetic structure of four mediterranean woody species: complex  
493 interactions between life history traits and the landscape context. *Diversity and*  
494 *Distributions* 18(3), 226-235.
- 495 Araujo M. B., Thuiller W., Pearson R. G. (2006) Climate warming and the decline of  
496 amphibians and reptiles in Europe. *Journal of Biogeography* 33(10), 1712-1728.
- 497 Avolio M. L., Beaulieu J. M., Smith M. D. (2013) Genetic diversity of a dominant C-4  
498 grass is altered with increased precipitation variability. *Oecologia* 171(2), 571-  
499 581.
- 500 Baird S. F., Girard C. (1853) No title. *Proceedings of the Academy of Natural Sciences of*  
501 *Philadelphia* 6(378-379).
- 502 Balkenhol N., Cushman S. A., Storfer A. T., Waits L. P. (2016) *Landscape Genetics:*  
503 *Concepts, Methods, Applications* Wiley Blackwell, Oxford.
- 504 Blair C., Jimenez Arcos V. H., Mendez de la Cruz F. R., Murphy R. W. (2013)  
505 Landscape genetics of leaf-toed geckos in the tropical dry forest of northern  
506 Mexico. *PLoS ONE* 8(2), e57433-e57433.
- 507 Blouin M. S., Phillipsen I. C., Monsen K. J. (2010) Population structure and conservation  
508 genetics of the Oregon spotted frog, *Rana pretiosa*. *Conservation Genetics* 11(6),  
509 2179-2194.
- 510 Brown J. H., Kodricbrown A. (1977) Turnover rates in insular biogeography - effect of  
511 immigration on extinction. *Ecology* 58(2), 445-449.
- 512 Bull E. L., Hayes M. P. (2001) Post-breeding season movements of Columbia spotted  
513 frogs (*Rana luteiventris*) in northeastern Oregon. *Western North American*  
514 *Naturalist* 61(1), 119-123.
- 515 Burnham K. P., Anderson D. R. (1998) *Model selection and multimodel inference: a*  
516 *practical information-theoretic approach* Springer New York, New York, USA.
- 517 Burrows M. T., Schoeman D. S., Buckley L. B., *et al.* (2011) The Pace of Shifting  
518 Climate in Marine and Terrestrial Ecosystems. *Science (Washington D C)*  
519 334(6056), 652-655.

- 520 Chelgren N. D., Pearl C. A., Adams M. J., Bowerman J. (2008) Demography and  
521 Movement in a Relocated Population of Oregon Spotted Frogs (*Rana pretiosa*):  
522 Influence of Season and Gender. *Copeia*(4), 742-751.
- 523 Crooks K. R., Sanjayan M. (2006) *Connectivity Conservation: Maintaining Connections*  
524 for Nature Cambridge University Press, Cambridge.
- 525 Delaney K. S., Riley S. P. D., Fisher R. N. (2010) A Rapid, Strong, and Convergent  
526 Genetic Response to Urban Habitat Fragmentation in Four Divergent and  
527 Widespread Vertebrates. *PLoS ONE* 5(9),  
528 <https://doi.org/10.1371/journal.pone.0012767>.
- 529 Dieringer D., Schloetterer C. (2003) MICROSATELLITE ANALYSER (MSA): A  
530 platform independent analysis tool for large microsatellite data sets. *Molecular  
531 Ecology Notes* 3(1), 167-169.
- 532 Dudaniec R. Y., Wilmer J. W., Hanson J. O., *et al.* (2016) Dealing with uncertainty in  
533 landscape genetic resistance models: a case of three co-occurring marsupials.  
534 *Molecular Ecology* 25(2), 470-486.
- 535 Engle J. C. (2001) *Population Biology and Natural History of Columbia Spotted Frogs*  
536 (*Rana Luteiventris*) *in the Owyhee Uplands of Southwest Idaho: Implications for*  
537 *Monitoring and Management*, Boise State University.
- 538 Engler J. O., Balkenhol N., Filz K. J., Habel J. C., Rodder D. (2014) Comparative  
539 landscape genetics of three closely related sympatric hesperid butterflies with  
540 diverging ecological traits. *PLoS ONE* 9(9).
- 541 Evans, J.S., Murphy M.A. (2015). GeNetIt. R package version 0.1-0.
- 542 Evanno G., Regnaut S., Goudet J. (2005) Detecting the number of clusters of individuals  
543 using the software STRUCTURE: a simulation study. *Molecular Ecology* 14(8),  
544 2611-2620.
- 545 Excoffier L., Lischer H. E. (2010) Arlequin suite ver 3.5: A new series of programs to  
546 perform population genetics analyses under Linux and Windows. *Molecular  
547 Ecology Resources* 10564-567.
- 548 Fishburn K. A., Carswell W. J. J. (2017) US Topo—Topographic maps for the Nation  
549 (ed. 2017-3045 USGSFS), p. 2.

- 550 Fotheringham A. S., O'Kelly M. E. (1989) *Spatial interaction models: formulation and*  
551 *applications.* Kluwer Academic, Dordrecht.
- 552 Frantz A. C., Bertouille S., Eloy M. C., *et al.* (2012) Comparative landscape genetic  
553 analyses show a Belgian motorway to be a gene flow barrier for red deer (*Cervus*  
554 *elaphus*), but not wild boars (*Sus scrofa*). *Molecular Ecology* 21(14), 3445-3457.
- 555 Fry J., Xian G., Jin S., *et al.* (2011) Completion of the 2006 National Land Cover  
556 Database for the Conterminous United States. *Photogrammetric Engineering &*  
557 *Remote Sensing* 77(9), 858-864.
- 558 Funk W. C., Blouin M. S., Corn P. S., *et al.* (2005a) Population structure of Columbia  
559 spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular*  
560 *Ecology* 14(2), 483-496.
- 561 Funk W. C., Greene A. E., Corn P. S., Allendorf F. W. (2005b) High dispersal in a frog  
562 species suggests that it is vulnerable to habitat fragmentation. *Biology Letters*  
563 1(1), 13-16.
- 564 Funk W. C., Pearl C. A., Draheim H. M., *et al.* (2008) Range-wide phylogeographic  
565 analysis of the spotted frog complex (*Rana luteiventris* and *Rana pretiosa*) in  
566 northwestern North America. *Molecular Phylogenetics and Evolution* 49(1), 198-  
567 210.
- 568 Gessler P. E., Moore I. D., McKenzie N. J., Ryan P. J. (1995) Soil-landscape modeling  
569 and spatial prediction of soil attributes. *International Journal of Geographical*  
570 *Information Systems* 9(4), 421-432.
- 571 Goldberg C. S., Kaplan M. E., Schwable C. R. (2003) From the frog's mouth: buccal  
572 swabs for collection of DNA from amphibians. *Herpetological Review* 34:220-  
573 221.
- 574 Goldberg C. S., Waits L. P. (2010) Comparative landscape genetics of two pond-breeding  
575 amphibian species in a highly modified agricultural landscape. *Molecular Ecology*  
576 19(17), 3650-3663.
- 577 Green D., Sharbel T., Kearsley J., Kaiser H. (1996) Postglacial range fluctuation, genetic  
578 subdivision and speciation in the western North American spotted frog complex,  
579 *Rana pretiosa*. *Evolution* 50:374-390.

- 580 Green D. M., Kaiser H., Sharbel T. F., Kearsley J., McAllister K. R. (1997) Cryptic  
581 species of spotted frogs, *Rana pretiosa* complex, in western North America.  
582 *Copeia*(1), 1-8.
- 583 Grobler J. P., Mafumo H. B., Minter L. R. (2003) Genetic differentiation among five  
584 populations of the South African ghost frog, *Heleophryne natalensis*. *Biochemical*  
585 *Systematics and Ecology* 31(9), 1023-1032.
- 586 Gryz J., Krauze D. (2008) Mortality of vertebrates on a road crossing the Biebrza Valley  
587 (NE Poland). *European Journal of Wildlife Research* 54(4), 709-714.
- 588 Hangartner S., Laurila A., Raesaenen K. (2011) Adaptive divergence of the moor frog  
589 (*Rana arvalis*) along an acidification gradient. *BMC Evolutionary Biology* 11366-  
590 Article No.: 366.
- 591 Legendre P., Legendre L. (2012) *Numerical Ecology* Elsevier, Amsterdam.
- 592 Manel S., Schwartz M. K., Luikart G., Taberlet P. (2003) Landscape genetics: Combining  
593 landscape ecology and population genetics. *Trends Ecol Evolut* 18(4), 189-197.
- 594 McAllister K. R., Watson J. W., Risenhoover K., McBride T., Adams M. J. (2004)  
595 Marking and radiotelemetry of oregon spotted frogs (*Rana pretiosa*).  
596 *Northwestern Naturalist* 85(1), 20-25.
- 597 McCune B., Keon D. (2002) Equations for potential annual direct incident radiation and  
598 heat load. *Journal of Vegetation Science* 13(4), 603-606.
- 599 Moore I. D., Gessler P. E., Nielsen E. E., Petersen G. A. (1993) Terrain attributes:  
600 estimation methods and scale effects. In: *Modeling Change in Environmental*  
601 *Systems* (eds. Jakeman AJ, Beck MB, McAleer M), pp. 189-214. Wiley, London.
- 602 Mote P. W., Salathé E. P. (2010) Future climate in the Pacific Northwest. In: *The*  
603 *Washington climate change impacts assessment: evaluating Washington's future*  
604 *in a changing climate, climate impacts group*. University of Washington, Seattle,  
605 Washington.
- 606 Munger J. C., Gerber M., Madrid K., *et al.* (1998) U.S. national wetland inventory  
607 classifications as predictors of the occurrence of Columbia spotted frogs (*Rana*  
608 *luteiventris*) and Pacific treefrogs (*Hyla regilla*). *Conservation Biology* 12(2),  
609 320-330.

- 610 Murphy M. A., Dezzani R., Pilliod D. S., Storfer A. (2010) Landscape genetics of high  
611 mountain frog metapopulations. *Molecular Ecology* 19(17), 3634-3649.
- 612 Palo J. U., O'Hara R. B., Laugen A. T., *et al.* (2003) Latitudinal divergence of common  
613 frog (*Rana temporaria*) life history traits by natural selection: Evidence from a  
614 comparison of molecular and quantitative genetic data. *Molecular Ecology* 12(7),  
615 1963-1978.
- 616 Pauls S. U., Nowak C., Balint M., Pfenninger M. (2013) The impact of global climate  
617 change on genetic diversity within populations and species. *Molecular Ecology*  
618 22(4), 925-946.
- 619 Pearl C. A., Adams M. J., Leuthold N. (2009) Breeding habitat and local population size  
620 of the Oregon spotted frog (*Rana pretiosa*) in Oregon, USA. *Northwest Naturalist*  
621 90:136-147.
- 622 Pearl C. A., Adams M. J., Wente W. H. (2007) Characteristics of Columbia spotted frog  
623 (*Rana luteiventris*) oviposition sites in northeastern Oregon, USA. *Western North*  
624 *American Naturalist* 67(1), 86-91.
- 625 Pearl C. A., Hayes M. P. (2005) *Rana pretiosa*, Oregon spotted frog. In: *Amphibian*  
626 *Declines: The Conservation Status of United States Species* (ed. Lannoo MJ), pp.  
627 577-580. University of California Press, Berkeley, CA.
- 628 Pilliod D. S., Arkle R. S., Robertson J. M., Murphy M. A., Funk W. C. (2015) Effects of  
629 changing climate on aquatic habitat and connectivity for remnant populations of a  
630 wide-ranging frog species in an arid landscape. *Ecology and Evolution* 5(18),  
631 3979-3994.
- 632 Pilliod D. S., Peterson C. R., Ritson P. I. (2002) Seasonal migration of Columbia spotted  
633 frogs (*Rana luteiventris*) among complementary resources in a high mountain  
634 basin. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 80(11),  
635 1849-1862.
- 636 Poelchau M. F., Hamrick J. L. (2012) Differential effects of landscape-level  
637 environmental features on genetic structure in three codistributed tree species in  
638 Central America. *Molecular Ecology* 21(20), 4970-4982.
- 639 Pritchard J. K., Stephens M., Donnelly P. (2000) Inference of population structure using  
640 multilocus genotype data. *Genetics* 155(2), 945-959.

- 641 Rasanen K., Laurila A., Merila J. (2003) Geographic variation in acid stress tolerance of  
642 the moor frog, *Rana arvalis*. I. Local adaptation. *Evolution* 57(2), 352-362.
- 643 Reaser J. K. (1996) *Rana pretiosa* (spotted frogs). Vagility. *Herpetol Rev* 27(4), 196-197.
- 644 Reaser J. K., Pilliod D. S. (2005) *Rana luteiventris*, Columbia spotted frog. In:  
645 *Amphibian Declines: The Conservation Status of United States Species* (ed.  
646 Lannoo MJ). University of California Press, Berkeley, CA.
- 647 Rehfeldt G. E., Crookston N. L., Warwell M. V., Evans J. S. (2006) Empirical analyses  
648 of plant-climate relationships for the western United States. *International Journal  
649 of Plant Sciences* 167(6), 1123-1150.
- 650 Richardson J. L. (2012) Divergent landscape effects on population connectivity in two  
651 co-occurring amphibian species. *Molecular Ecology* 21(18), 4437-4451.
- 652 Rousset F. (2008) Genepop'007: a complete reimplementation of the Genepop software  
653 for Windows and Linux. *Molecular Ecology Resources* 8103-106.
- 654 Selkoe K. A., Watson J. R., White C., *et al.* (2010) Taking the chaos out of genetic  
655 patchiness: seascape genetics reveals ecological and oceanographic drivers of  
656 genetic patterns in three temperate reef species. *Molecular Ecology* 19(17), 3708-  
657 3726.
- 658 Short Bull R. A., Cushman S. A., Mace R., *et al.* (2011) Why replication is important in  
659 landscape genetics: American black bear in the Rocky Mountains. *Molecular  
660 Ecology* 20(6), 1092-1107.
- 661 SoftGenetics (2010) GeneMarker. State College, PA.
- 662 Storfer A., Murphy M. A., Evans J. S., *et al.* (2007) Putting the 'landscape' in landscape  
663 genetics. *Heredity* 98(3), 128-142.
- 664 Storfer A., Murphy M. A., Spear S. F., Holderegger R., Waits L. P. (2010) Landscape  
665 genetics: where are we now? *Molecular Ecology* 19(17), 3496-3514.
- 666 Tallmon D. A., Luikart G., Waples R. S. (2004) The alluring simplicity and complex  
667 reality of genetic rescue. *Trends in Ecology & Evolution* 19(9), 489-496.
- 668 Thompson H. B. (1913) Description of a new subspecies of *Rana pretiosa* from Nevada.  
669 *Proceedings of the Biological Society of Washington* 2653-55.
- 670 Tischendorf L., Fahrig L. (2000) How should we measure landscape connectivity?  
671 *Landscape Ecology* 15(7), 633-641.

- 672 Trumbo D. R., Spear S. F., Baumsteiger J., Storfer A. (2013) Rangewide landscape  
673 genetics of an endemic Pacific northwestern salamander. *Molecular Ecology*  
674 22(5), 1250-1266.
- 675 USFWS (2014) Endangered and Threatened Wildlife and Plants; Threatened Status for  
676 Oregon Spotted Frog (ed. Fish and Wildlife Service I).
- 677 van Oosterhout C., Weetman D., Hutchinson W. F. (2006) Estimation and adjustment of  
678 microsatellite null alleles in nonequilibrium populations. *Molecular Ecology*  
679 Notes 6(1), 255-256.
- 680 Vogelmann J. E., Howard S. M., Yang L., *et al.* (2001) Completion of the 1990's  
681 National Land Cover Data Set for the conterminous United States.  
682 *Photogrammetric Engineering and Remote Sensing* 67:650-652.
- 683 Watson J. W., McAllister K. R., Pierce D. J. (2003) Home ranges, movements, and  
684 habitat selection of Oregon Spotted Frogs (*Rana pretiosa*). *Journal of*  
685 *Herpetology* 37(2), 292-300.
- 686 Whiteley A. R., Fitzpatrick S. W., Funk W. C., Tallmon D. A. (2015) Genetic rescue to  
687 the rescue. *Trends in Ecology & Evolution* 30(1), 42-49.
- 688 Whiteley A. R., McGarigal K., Schwartz M. K. (2014) Pronounced differences in genetic  
689 structure despite overall ecological similarity for two *Ambystoma* salamanders in  
690 the same landscape. *Conservation Genetics* 15(3), 573-591.
- 691 Wultsch C., Waits L. P., Kelly M. J. (2016) A Comparative Analysis of Genetic Diversity  
692 and Structure in Jaguars (*Panthera onca*), Pumas (*Puma concolor*), and Ocelots  
693 (*Leopardus pardalis*) in Fragmented Landscapes of a Critical Mesoamerican  
694 Linkage Zone. *PLoS ONE* 11(3).
- 695

## 696 DATA ACCESSIBILITY

697 Microsatellite data is available in Dryad® digital repository with  
698 doi:10.5061/dryad.5n18790

- 699
- 700
- 701 **AUTHOR CONTRIBUTIONS**

702 J.M.R., M.A.M, and W.C.F.: Research concept and design; C.A.P., M.J.A., W.C.F., and  
703 S.M.H.: Sample collection; M.I.P., J.M.R., and M.A.M.: Microsatellite genotyping;  
704 J.M.R., M.A.M., and M.I.P.: Data analysis; J.M.R., W.C.F., M.J.A. and M.A.M.: Writing  
705 the article; All authors: Critical revision and final approval of the article.

706

707 **Supporting information**

708 Additional supporting information may be found in the online version of this article.

Author Manuscript

**Table 1** Sampling site information for *R. pretiosa* (RAPR) and *R. luteiventris* (RALU) across six study regions

Species	State	Region/abbreviation	Site name	#	UTM easting	UTM northing	Elev (m)	Datum/zone	N	H <sub>0</sub>	H <sub>E</sub>	HW
RAPR	OR	Deschutes/DES	Hosmer	1	597653	4868236	1514	NAD27/10	10	0.26	0.57	<0.001
			Marsh/Mud	2	586790	4865165	1478	NAD27/10	7	0.43	0.42	0.898
			Muskrat	3	588352	4857034	1485	NAD83/10	16	0.26	0.41	<0.001
			SR_Duck	4	624650	4860910	1267	NAD83/10	10	0.34	0.40	0.261
			SR_NCPond	5	624651	4860342	1267	NAD83/10	21	0.33	0.34	0.398
			SR_17th Fair	6	624954	4859506	1268	NAD83/10	6	0.33	0.31	1.00
			SR_9th	7	624821	4858594	1268	NAD83/10	19	0.30	0.31	0.948
			SR_Vista	8	624258	4858287	1269	NAD83/10	21	0.38	0.39	0.612
			CR_LWest	9	624859	4856685	1269	NAD83/10	12	0.30	0.35	0.018
			CR_Least	10	624876	4856725	1269	NAD83/10	13	0.23	0.50	<0.001
			CR_Fair	11	624650	4855810	1272	NAD83/10	5	0.40	0.50	0.501
			CR_NoDriv	12	625006	4856556	1269	NAD83/10	6	0.38	0.40	0.294
			CR_07_Bull	13	624846	4856193	1269	NAD83/10	5	0.23	0.35	0.103
			CR_09_Bull	14	624846	4856193	1269	NAD83/10	23	0.37	0.42	0.750
			DilmanEES, Mid	15	607617	4839136	1312	NAD83/10	12	0.43	0.49	0.480
			DilmanPond1,7	16	607730	4839382	1307	NAD83/10	11	0.45	0.39	0.941
			Dilman_Pond5	17	607466	4839111	1312	NAD83/10	10	0.17	0.07	0.423
			Dilman_Pond6	18	607837	4839300	1308	NAD83/10	10	0.43	0.42	0.472
			CAS	19	622965	4847791	1276	NAD27/10	10	0.28	0.57	<0.001
			LitDesch_BLM	20	608387	4819324	1321	NAD83/10	7	0.37	0.43	0.435
			LitDesch_100	21	602927	4812165	1366	NAD83/10	20	0.29	0.26	1.00
			GoldLake	22	577652	4832065	1468	NAD27/10	11	0.31	0.34	0.203
			ScottyBig	23	586581	4822053	1429	NAD83/10	11	0.26	0.30	0.864

# Author Manuscript

			BigMarsh	24	584987	4805166	1443	NAD27/10	23	0.31	0.34	0.072
RAPR	OR	Klamath/KLA	Jack_USFS	1	612785	4788015	1615	NAD83/10	21	0.36	0.39	0.251
			Jack_09_Jame	2	613757	4786847	1604	NAD83/10	15	0.33	0.38	0.087
			Jack_10_Jame	3	613746	4786837	1604	NAD83/10	12	0.34	0.38	0.406
			Kl_Pen	4	613156	4759834	1378	NAD83/10	7	0.40	0.50	0.913
			Kl_Devine	5	608332	4757450	1377	NAD83/10	17	0.48	0.55	0.085
			Kl_MilCros	6	608742	4756182	1379	NAD83/10	23	0.50	0.54	<0.001
			Kl_Corral	7	603306	4754733	1379	NAD83/10	7	0.52	0.49	1.00
			Kl_SouthGage	8	603002	4753787	1379	NAD83/10	7	0.458	0.574	0.549
			Dixon	9	582327	4730764	1280	NAD83/10	14	0.67	0.59	0.648
			Wood_Hawk2	10	583919	4720672	1264	NAD83/10	12	0.42	0.43	0.044
			Wood_Hawk1	11	584188	4719770	1263	NAD83/10	10	0.41	0.44	0.721
			Wood	12	584663	4718912	1263	NAD27/10	12	0.36	0.47	<0.001
			Seven10	13	575424	4729249	1283	NAD83/10	7	0.47	0.63	0.035
			Cr_Found2007	14	575148	4723339	1266	NAD83/10	26	0.40	0.40	0.114
			Cr_mys	15	575443	4723125	1266	NAD83/10	14	0.34	0.42	0.018
			Buck_NW	16	566028	4680198	1507	NAD83/10	22	0.46	0.44	0.447
			Buck_West	17	566046	4680068	1507	NAD83/10	20	0.64	0.62	0.267
			Buck_07	18	566192	4679710	1507	NAD27/10	14	0.51	0.47	0.380
			Parsnip	19	545426	4661799	1264	NAD83/10	18	0.27	0.45	0.371
RALU	OR	Central /CEN	Camp	1	729715	4882105	1138	NAD27/10	7	0.42	0.64	0.008
			CR45SICR	2	283890	4862990	1504	NAD83/11	22	0.56	0.61	0.006
			Claw	3	295637	4848890	1464	NAD83/11	11	0.47	0.53	<0.001
			9RNASICR	4	288643	4846768	1387	NAD83/11	29	0.46	0.50	<0.001
			RNASICR	5	288632	4846733	1388	NAD83/11	18	0.52	0.57	<0.001

RALU	OR	Blue Mountains /BLU	SICR	6	287524	4842107	1363	NAD83/11	19	0.62	0.61	<0.001
			4150SICR	7	287480	4842037	1363	NAD83/11	7	0.57	0.65	0.094
			Nicoll	8	284473	4840128	1364	NAD83/11	27	0.65	0.64	<0.001
RALU	OR	Blue Mountains /BLU	Slab	1	383444	4948950	1594	NAD83/11	8	0.44	0.50	0.233
			Burnt	2	392792	4946872	1293	NAD27/11	8	0.78	0.70	1.000
			Pine	3	427045	4932116	1371	NAD27/11	10	0.65	0.56	0.990
			Casp	4	427112	4929719	1250	NAD83/11	6	0.39	0.69	<0.001
			Mormon	5	453056	4918793	1433	NAD83/11	12	0.54	0.52	0.974
			LTMAR_06-08	6	399383	4899496	1453	NAD83/11	28	0.30	0.34	0.058
			Bear	7	398936	4890957	1533	NAD83/11	29	0.42	0.41	0.002
			Cotton	8	431687	4856779	1365	NAD83/11	9	0.41	0.48	0.322
			Calf	9	421260	4850293	900	NAD83/11	17	0.47	0.52	0.012
			KingsHib	10	405774	4847041	1036	NAD27/11	33	0.52	0.48	0.024
RALU	OR	Southeastern/SEO	Brid_15	1	348245	4745368	1275	NAD83/11	23	0.76	0.69	0.036
			Mud	2	348319	4743846	1281	NAD27/11	21	0.81	0.73	0.439
			Page	3	347312	4740840	1294	NAD83/11	19	0.67	0.63	0.006
			LittleFish1	4	363501	4729648	2184	NAD83/11	23	0.62	0.59	0.377
RALU	ID,OR	Owyhee/OWY	Castro1	1	489011	4734746	1462	NAD83/11	20	0.28	0.28	0.010
			Cobur	2	492339	4732198	1451	NAD83/11	11	0.30	0.26	0.883
			Rail	3	507740	4738225	1634	NAD83/11	14	0.16	0.16	1.00
			Meadow	4	530561	4741793	1576	NAD83/11	25	0.40	0.46	<0.001
			Rock	5	538192	4726553	1696	NAD83/11	19	0.37	0.43	0.008
			Samnoble3	6	538362	4719204	1772	NAD83/11	30	0.38	0.40	<0.001
			Polecreek	7	537251	4714636	1710	NAD83/11	12	0.44	0.46	0.7466

Stoneman	8	521219	4713102	1607	NAD83/11	30	0.43	0.35	0.130
Cottonwood	9	508275	4707146	1720	NAD83/11	21	0.43	0.40	0.052
Pleasant	10	511019	4706993	1712	NAD83/11	6	0.50	0.42	0.987
Littlelhom	11	516388	4703257	1611	NAD83/11	10	0.33	0.37	0.779
Castle	12	525311	4697038	1496	NAD83/11	7	0.45	0.54	0.336

Sampling site number (#), Geographic locality data (UTM, elevation in meters [elev], map datum, and zone), sample size (N), observed ( $H_0$ ) and expected ( $H_E$ ) heterozygosity, and p-value for tests for Hardy-Weinberg proportions (HW) for each site.

**Table 2** Variables used for testing candidate gravity models to explain functional connectivity in *Rana pretiosa* and *R. luteiventris*

Parameter	Process	Variable	Code	Description	Source	Calculation	Ecological Justification
Distance (w)	IBD	Geographic distance	dist	Terrestrial-stage frogs move overland	NED	Distance between sites	Distance is an important limiting factor for connectivity (Funk et al. 2005a; Funk et al. 2005b; Murphy et al. 2010; Pilliod et al. 2015)
Production/attraction (v)	Productivity	Compound topographic index	cti	A steady-state measure of wetness based on topography (upslope potential contribution of moisture and ability to hold moisture (Gessler, Moore, McKenzie & Ryan 1995; Moore, Gessler, Nielsen & Petersen 1993))	NED	GaGM	Wetter breeding sites have greater productivity (Munger et al. 1998; Pearl et al. 2007)
	Elevation	elev		Elevation in meters	NED	DEM	Site productivity is higher at low elevations in mountainous regions and site productivity is lower at low elevation in deserts (Funk et al. 2005b)
	Heat load index	hli		Measure of solar intercept (McCune & Keon 2002)	NED	GaGM	Solar radiation controls water temp and primary productivity (breeding);

# Author Manuscript

Resistance (c)	Temperature Index	Heat load Index	hli	Measure of solar intercept (McCune & Keon 2002)	NED	GaGM	productivity is higher in mountains and lower in desert (Pilliod et al. 2002)
	Frost free period		ffp	Measure of the number of days above freezing	MFSL	-	Longer growing season should result in higher dispersal among sites (Palo et al. 2003)
	Mean max temperature		mmax	Mean annual maximum air temperature .	MFSL	-	Warmer matrix habitat may impede movement in desert sites (frogs) (Pilliod et al. 2015)
	Mean min temperature		mmin	Mean annual minimum air temperature.	MFSL	-	Warmer matrix habitat may impede movement in desert sites (frogs) (Pilliod et al. 2015)
Moisture	Compound topographic		cti	A steady-state measure of wetness based on topography	DEM	GaGM	Amphibians rely on moisture gradients for

# Author Manuscript

	index		(upslope potential contribution of moisture and ability to hold moisture (Gessler et al. 1995; Moore et al. 1993)			dispersal; wetter intervening habitat should increase connectivity, particularly in <i>R. pretiosa</i> (Pilliod et al. 2015; Pilliod et al. 2002)
	Mean Annual precipitation	map	Mean annual precipitation	MFSL	-	More precipitation during growing season should result in higher dispersal among sites, particularly in <i>R. pretiosa</i> (Pilliod et al. 2015)
Topography	Surface relief ratio	srr3, srr27	Topographic complexity impedes movement (30 m cell windows). Fine scale topographic complexity is represented by srr3 (3 X 3 cell window for calculation while course-scale complexity is represented by srr27 (27 X 27 cell window)	NED	GaGM	Fine scale topographic complexity made impede dispersal due to energetic costs. Course scale topographic complexity acts as major barrier to dispersal (e.g., ridges) (Funk et al. 2005a; Murphy et al. 2010)
Slope	rsp3,	Relative slope position		NED	GaGM	Site productivity higher in

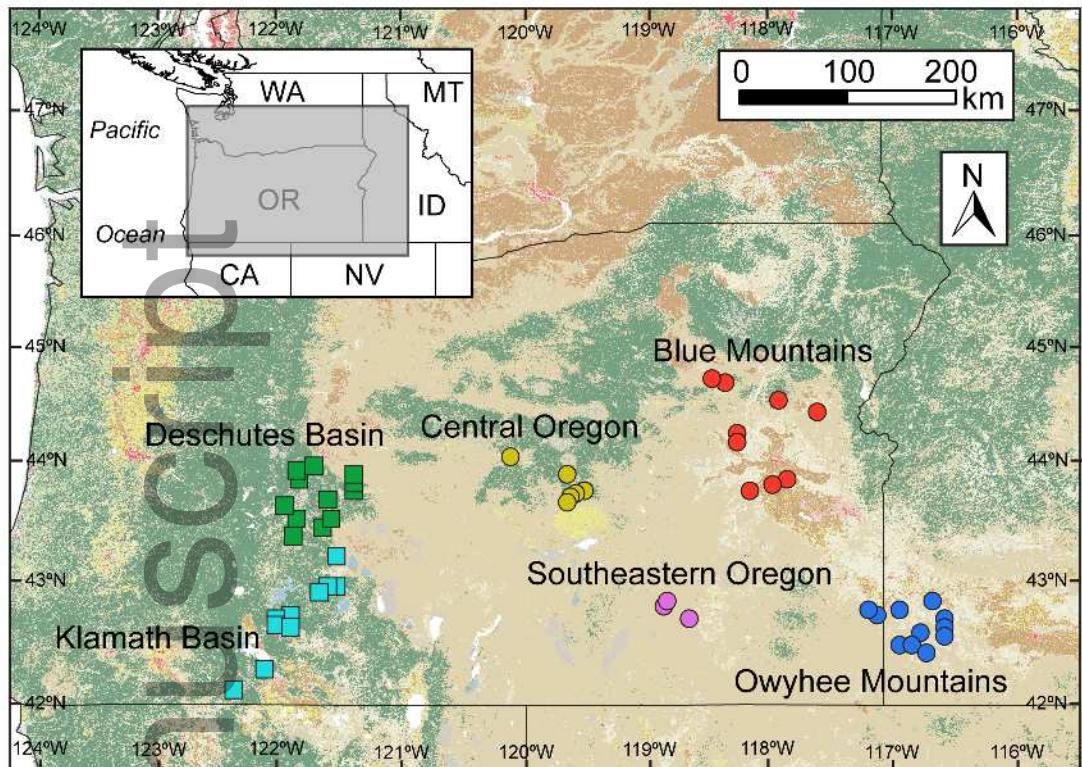
# Author Manuscript

position	rsp27	between valleys and mountains. The slope position of site relative to mean elevation for that window. Localized pooling of water is represented by rsp3 (3 X 3 cell window) while placement of a site within a drainage is represented by rsp27 (27 X 27 cell window)		valleys in mountainous regions and site productivity lower in valleys in deserts (Funk et al. 2005b)
Habitat	Agriculture (81/82)	Crop	Percent of land cover that includes pasture/hay and cultivated crops	NLCD 2006 - Habitat permeability: agricultural land (pasture/hay, cultivated crops) facilitates dispersal (Goldberg & Waits 2010)
	Shrub (52)	Shrub	Percent of land cover that includes shrub/scrub.	NLCD 2006 - Shrub (woody vegetation < 6 m) impedes dispersal (Goldberg & Waits 2010)
	Impervious surface	imper	Percent of land cover that includes roads, rocks, impervious gravel.	NLCD 2006 - Roads and other impermeable surfaces impede dispersal (Delaney, Riley & Fisher 2010;

Canopy	canopy	Percent of land cover the includes forest canopy	NLCD 2006	-	Goldberg & Waits 2010; Gryz & Krauze 2008; Murphy et al. 2010) Forest has minimal permeability for dispersal (Goldberg & Waits 2010)
--------	--------	--	-----------	---	---

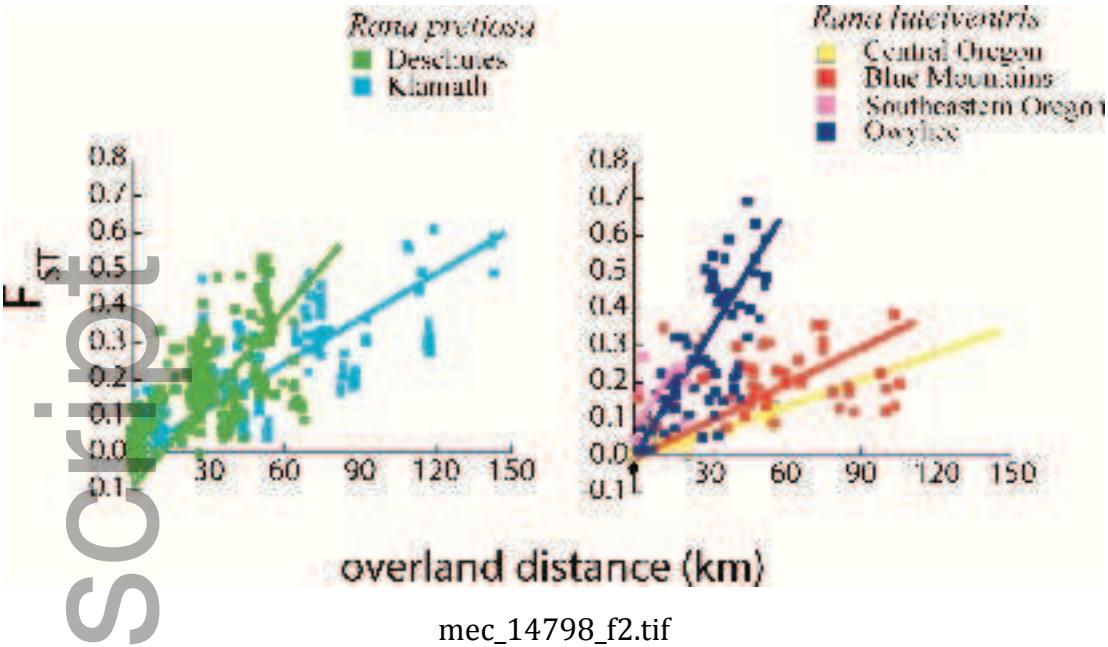
---

Parameter: the parameter estimated in the gravity equation (distance [w], production/attraction [v], resistance [c]). Process: the landscape process that each variable measures: isolation by distance (IBD), productivity, temperature, moisture, topography, habitat. Variable: The independent variable name. Code: variable code or abbreviation. Source: source of data containing the variable or used to derive the variable. Data source abbreviations as follows: Moscow Forestry Sciences Laboratory (MFSL; 1 km resolution upscaled to 30 m resolution, 30 m resolution, 30-year normal climate model based on 1961-1990 (Rehfeldt, Crookston, Warwell & Evans 2006), National Land Cover Data (NLCD) (Fry et al. 2011); National Elevation Dataset (NED; 30 m resolution (Fishburn & Carswell 2017). Calculation: a description for implementation of metrics derived from the original source dat (-): Geomorphology and Gradient Metrics (GaGM) toolbox in ArcGIS V10, Digital Elevation Model (DEM).



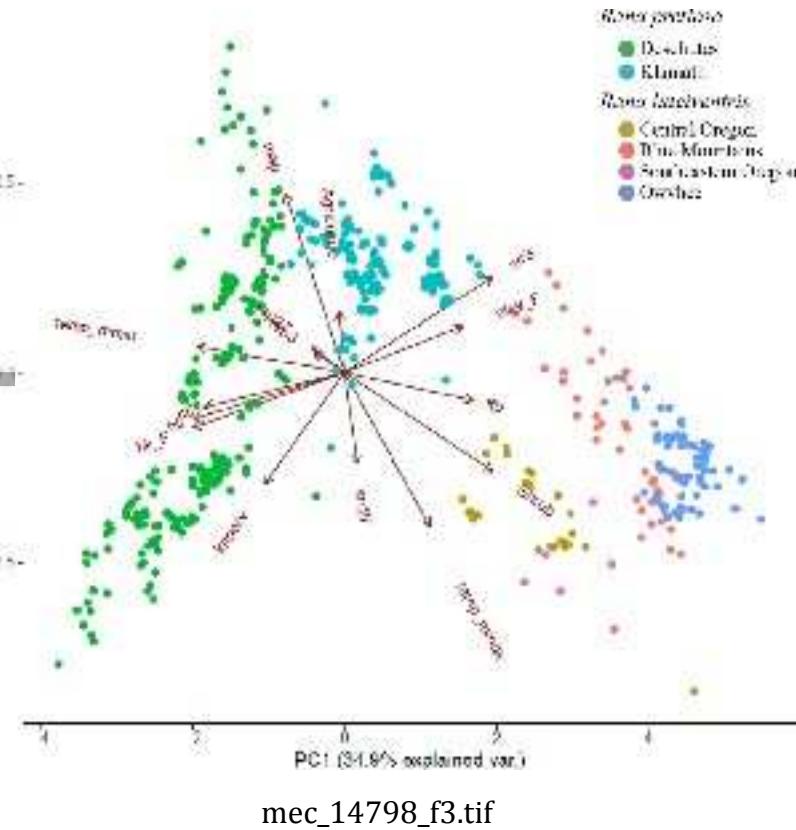
mec\_14798\_f1.tif

Author Manuscript

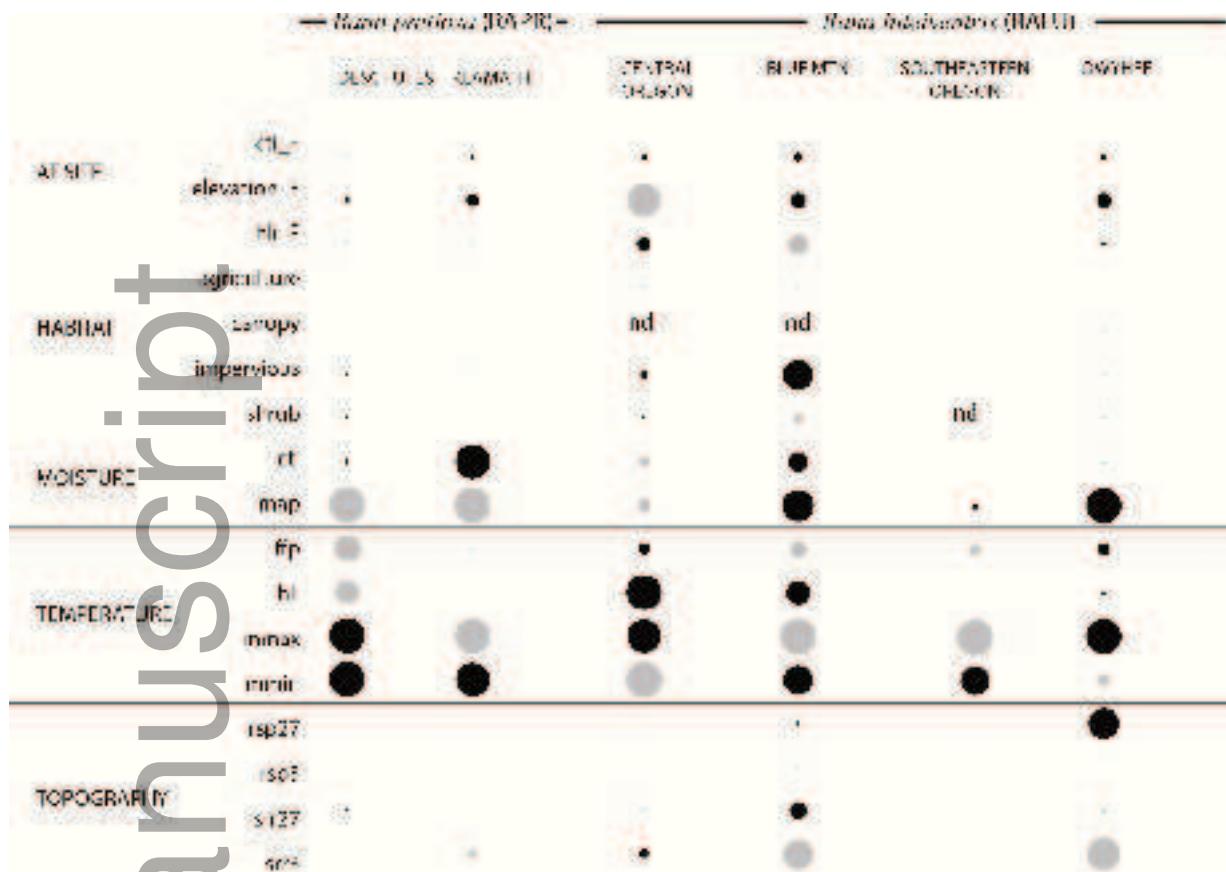


Author Manuscript

# Author Manuscript



mec\_14798\_f3.tif



mec\_14798\_f4.tif

Author Manuscript