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2 DR. JEANNE ROBERTSON (Orcid ID : 0000-0003-2642-6280)

3 DR. DAVID S PILLIOD (Orcid ID : 0000-0003-4207-3518)

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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^{1,7*}, MELANIE A. MURPHY^{2*}, CHRISTOPHER A.
14 PEARL³, MICHAEL J. ADAMS³, MÓNICA I. PÁEZ-VACAS^{4,7}, SUSAN M. HAIG³,
15 DAVID S. PILLIOD⁵, ANDREW STORFER⁶, and W. CHRIS FUNK⁷

16

17 **Co-first authors*

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

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

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

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

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

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

30 ⁷*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

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37

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.**

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 logistically 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 ΔK 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.*
218 *luteiventris*, including variables related to site productivity, temperature, moisture,
219 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 site, c) ((Murphy, Dezzani, Pilliod & Storfer 2010). We calculated Nei's chord
230 distance (D_a), estimated in MICROSATELLITE ANALYZER (Dieringer & Schloetterer 2003)
231 and used $(1 - D_a)$, i.e., "flow" as a measure of connectivity in gravity models. We
232 selected D_a for these analysis as: 1) it is highly correlated with proportion of shared
233 alleles (D_{ps}) used in the justification of gravity models for landscape genetics (Murphy *et*
234 *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).

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.

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).

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).

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

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695

696 **DATA ACCESSIBILITY**

697 Microsatellite data is available in Dryad® digital repository with

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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.

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 ₀	H _E	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

			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
			KI_Pen	4	613156	4759834	1378	NAD83/10	7	0.40	0.50	0.913
			KI_Devine	5	608332	4757450	1377	NAD83/10	17	0.48	0.55	0.085
			KI_MilCros	6	608742	4756182	1379	NAD83/10	23	0.50	0.54	<0.001
			KI_Corral	7	603306	4754733	1379	NAD83/10	7	0.52	0.49	1.00
			KI_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

			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
Littlethom	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);

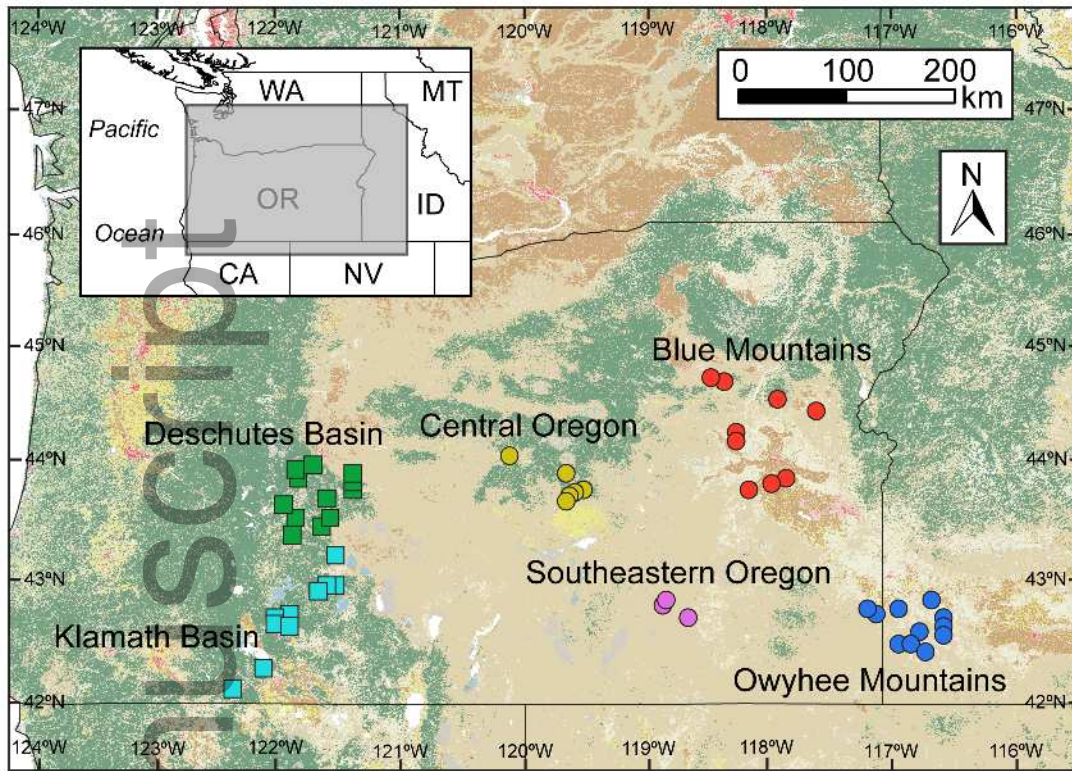
Resistance (c)	Temperature	Heat load Index	hli	Measure of solar intercept (McCune & Keon 2002)	NED	GaGM	productivity is higher in mountains and lower in desert (Pilliod et al. 2002)
		Frost free period	ffp	Measure of the number of days above freezing	MFSL	-	Longer growing season should result in higher 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

	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

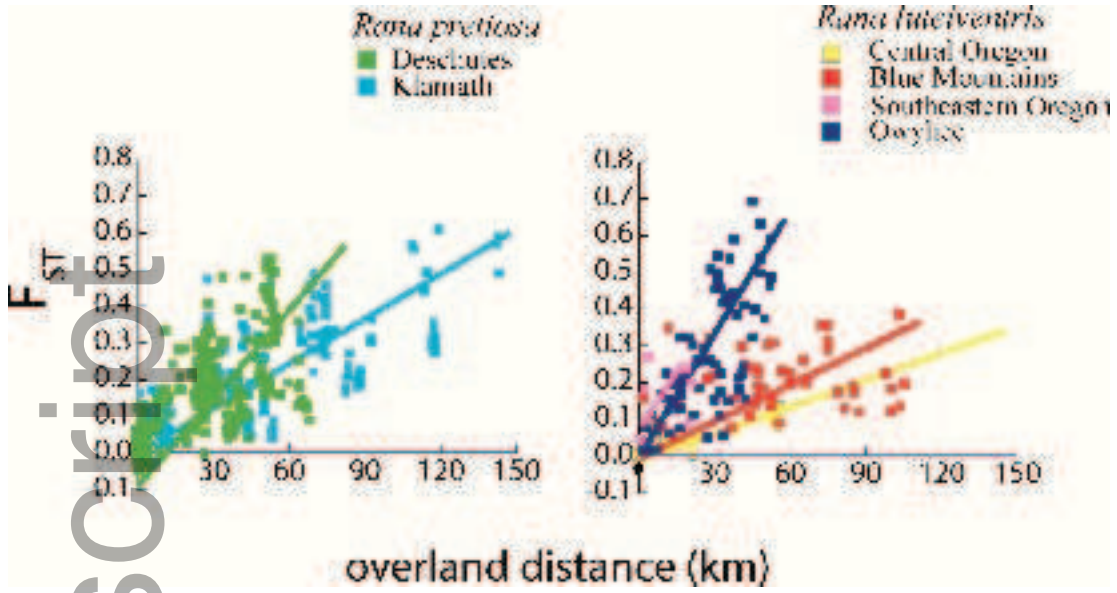
	position	rsp27	between valleys and mountains. The slope position of site relative to mean elevation for that window. Localized pooling of water is represented by rsp3 (3 X 3 cell window) while placement of a site within a drainage is represented by rsp27 (27 X 27 cell window)			valleys in mountainous 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)
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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).

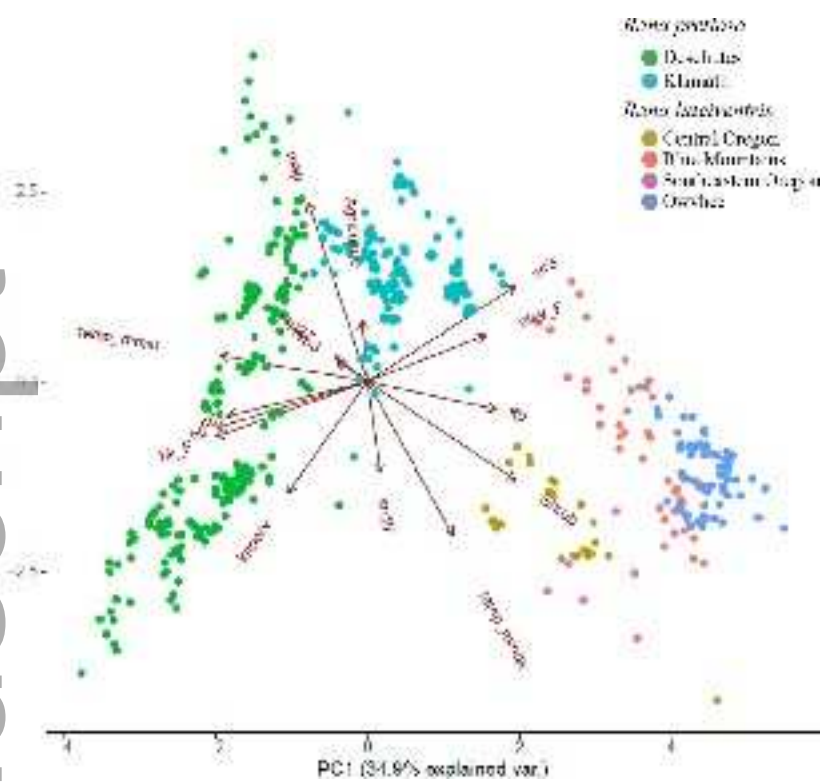


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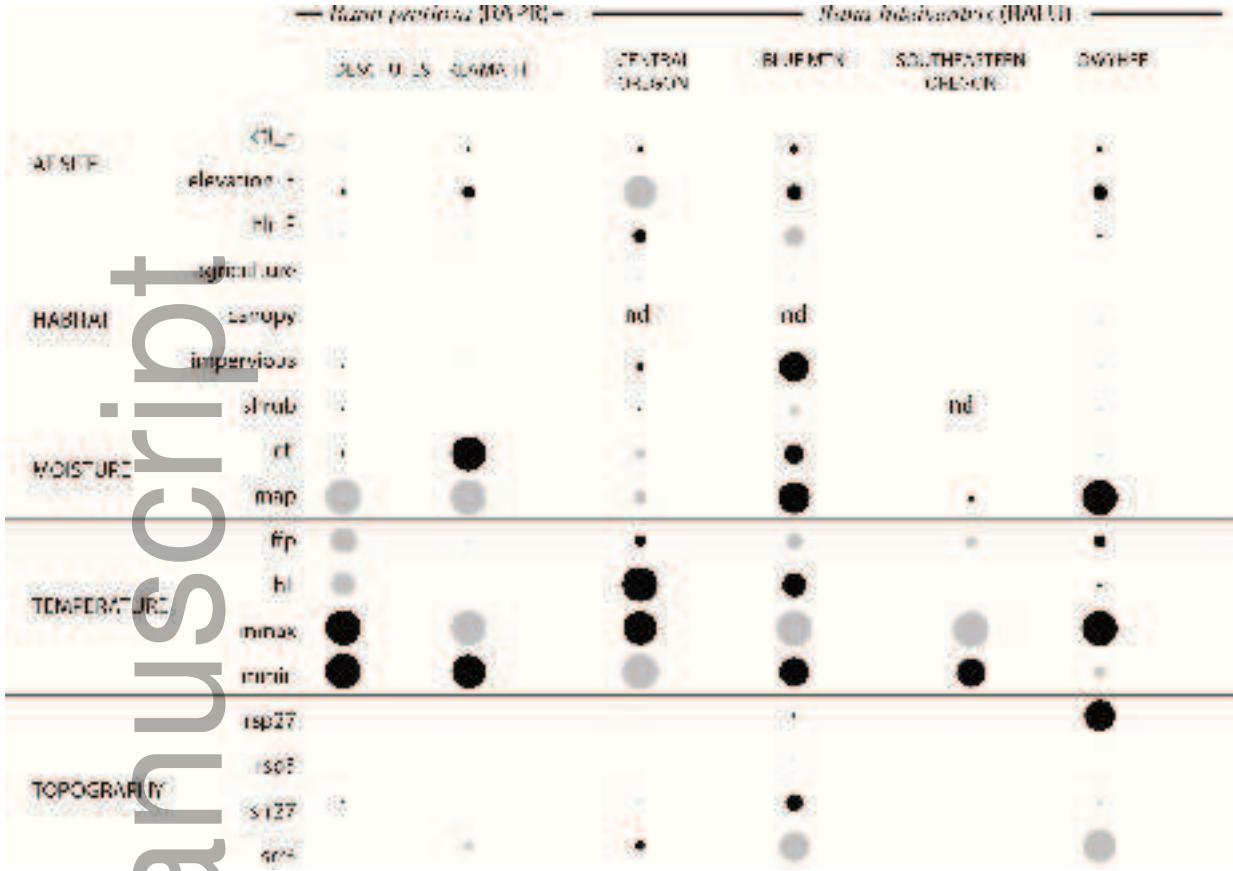


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PC2 (17.9% explained var.)



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