

# COMPARATIVE ANALYSES OF EFFECTIVE POPULATION SIZE WITHIN AND AMONG SPECIES: RANID FROGS AS A CASE STUDY

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It has recently become practicable to estimate the effective sizes ( $N_e$ ) of multiple populations within species. Such efforts are valuable for estimating  $N_e$  in evolutionary modeling and conservation planning. We used microsatellite loci to estimate  $N_e$  of 90 populations of four ranid frog species (20–26 populations per species, mean  $n$  per population = 29). Our objectives were to determine typical values of  $N_e$  for populations of each species, compare  $N_e$  estimates among the species, and test for correlations between several geographic variables and  $N_e$  within species. We used single-sample linkage disequilibrium (LD), approximate Bayesian computation (ABC), and sibship assignment (SA) methods to estimate contemporary  $N_e$  for each population. Three of the species—*Rana pretiosa*, *R. luteiventris*, and *R. cascadae*— have consistently small effective population sizes (<50).  $N_e$  in *Lithobates pipiens* spans a wider range, with some values in the hundreds or thousands. There is a strong east-to-west trend of decreasing  $N_e$  in *L. pipiens*. The smaller effective sizes of western populations of this species may be related to habitat fragmentation and population bottlenecks.

**Key words:** amphibians, population genetics, genetic diversity, Ranidae, conservation genetics.

A key parameter in the theory and application of population genetics is the effective population size ( $N_e$ ): the number of breeding individuals in a conceptual, ideal population that would lose genetic diversity at the same rate as the real population being studied (Wright 1931; Charlesworth 2009). How a population responds to evolutionary forces depends on  $N_e$ , rather than the actual number of individuals in the population ( $N$ , the census population size). Although direct estimates of  $N_e$  can be calculated from

demographic data, such data are often prohibitively difficult to obtain (Wang 2005). Given this limitation, and the importance of  $N_e$  in population and conservation genetics, it is not surprising that considerable effort has been put into developing methods of using molecular genetic data to obtain indirect estimates of  $N_e$ .

With advances in these methods and with the increasing accessibility of multilocus genotype data, it has recently become practicable to estimate the effective sizes of many populations

of the same species (Luikart et al. 2010; Waples and Do, 2010). Such an effort is highly worthwhile for a couple of reasons. First, by gathering estimates from multiple populations, investigators might identify a reasonably narrow range of typical  $N_e$  values for a species, an “educated guess” for the value of this parameter in any given population. This information could be used to approximate  $N_e$  in evolutionary modeling of the species. For example, an expected  $N_e$  could be used as input for simulations of evolutionary processes or as a Bayesian prior in analyses used to infer other population genetic parameters, such as migration rates or selection coefficients. Expected  $N_e$  values for a species would also be useful in conservation and management, in situations in which an estimate of  $N_e$  for a population is desired but genetic and demographic data are unavailable. In such cases, it would be helpful to know if populations of the focal species typically have, for example,  $N_e$  estimates less than 100. Furthermore, when estimates of both  $N_e$  and  $N$  (census size) can be obtained for multiple populations, it may be possible to identify the typical range of  $N_e/N$  for the species. If so, estimates of  $N$  for the species might then be used as proxies for  $N_e$ , when the former are easier to obtain than the latter.

Second, a comparative analysis of  $N_e$  estimates from multiple populations across more than one species can help generate hypotheses about what particular biological factors influence  $N_e$  within species. Consistent differences in  $N_e$  among species might correspond to differences in habitat, dispersal capabilities, or breeding behaviors. Hypotheses generated in a comparative analysis could then be tested in subsequent studies. Similarly, the factors that influence  $N_e$  within species can also be investigated by evaluating correlations between environmental variables and  $N_e$  for multiple populations of a species.

Despite the fact that such multipopulation, empirical investigations of  $N_e$  can lead to valuable insights, surprisingly few examples of this approach exist (Fraser et al. 2007; Beebee 2009). In this study, we employed an unusually large dataset of 90 populations to conduct a comparative analysis of  $N_e$  within and among four species of North American frogs in the family Ranidae: the Oregon spotted frog (*Rana pretiosa*), the Columbia spotted frog (*R. luteiventris*), the Cascades frog (*R. cascadae*), and the northern leopard frog (*Lithobates [Rana] pipiens*). Although the focus of our discussion is on these species, the methods we used should be applicable in many other study systems. Thus, this study demonstrates the general value of gathering and analyzing  $N_e$  estimates from multiple populations and species, in addition to furthering our knowledge of  $N_e$  in ranid frogs.

We described the population structures of the four frog species in previous studies that did not explicitly assess  $N_e$  (Blouin et al. 2010; Funk et al. 2005; 2008; Mosen and Blouin 2003; 2004; Hoffman and Blouin 2004a; b). Research on other species suggests that amphibian populations tend to have small local  $N_e$ ,

on the order of a few 10s to no more than a few thousand (e.g., Eastale 1985; Funk et al. 1999; Jehle et al. 2001; Brede and Beebee 2006; Schmeller and Merila 2007; Beebee 2009). Still, much remains unknown about  $N_e$  in amphibians, as trends in  $N_e$  estimates across multiple populations have not been analyzed for the vast majority of species.

We used the single-sample linkage disequilibrium (LD; Hill 1981) method and two newer methods—an approximate Bayesian computation (ABC) method (Tallmon et al. 2008) and a method based on sibship assignment (SA; Wang, 2009b)—to estimate contemporary  $N_e$  for each population. Estimates from these methods should generally reflect  $N_e$  over a contemporary time scale (Waples 2005). We assessed the correlation among estimates from the three methods within each species. We also compared these single-sample estimates with estimates from the temporal method (Waples 1989) for two of the *R. pretiosa* populations and five of the *L. pipiens* populations.

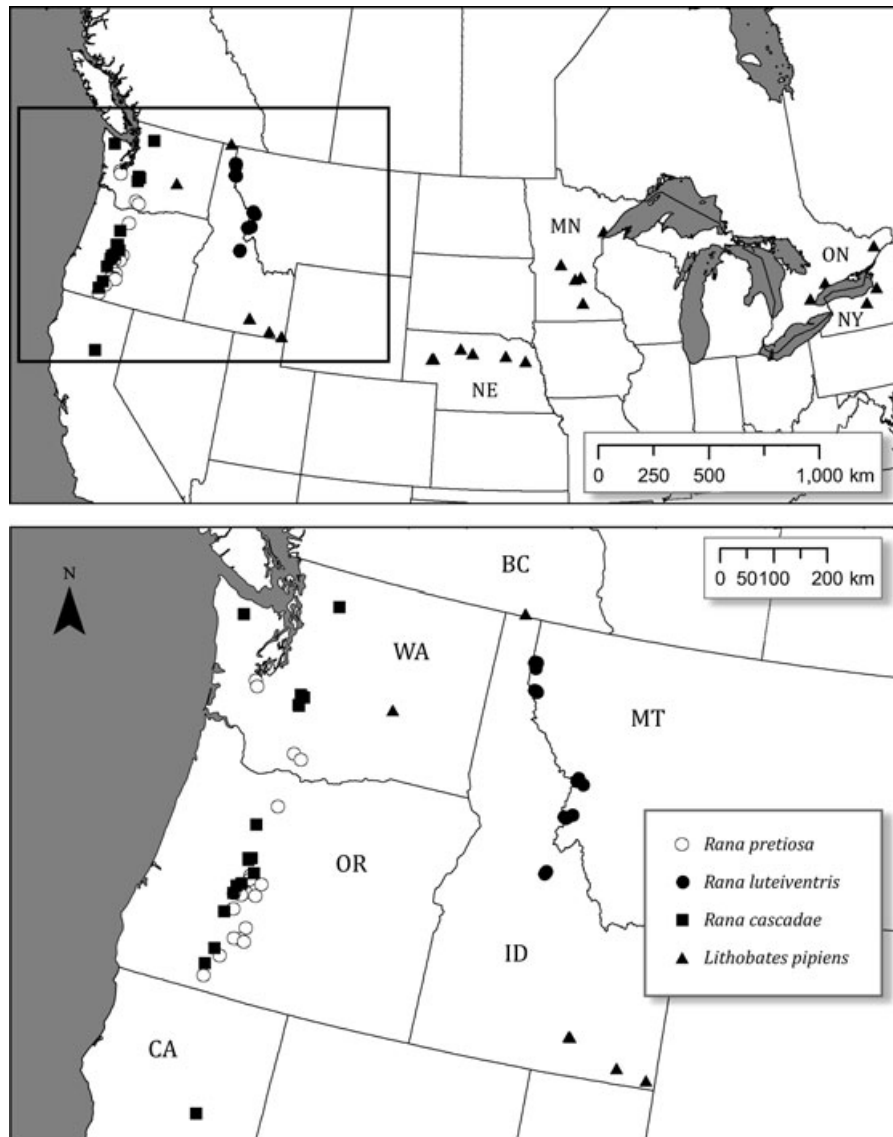
Our main objectives for this study were to: (1) determine the typical  $N_e$  values for each species, (2) compare  $N_e$  estimates among the species and offer hypotheses to explain the differences, and (3) test for correlations between each of three geographic variables (latitude, longitude, and elevation) and  $N_e$  estimates within each species. Patterns in  $N_e$  estimates at these levels of comparison may reflect important population genetic processes in these frogs, knowledge of which would be valuable to the study of amphibian evolutionary biology and to the conservation and management of these organisms.

## Materials and Methods

### STUDY SPECIES

Frogs in the family Ranidae are widely distributed at the global scale, with 28 species occurring in the United States and Canada (Collins and Taggart 2009). *Rana pretiosa*, *R. luteiventris*, and *R. cascadae* are distributed across the northwestern region of North America. *Rana pretiosa* and *R. cascadae* have relatively small ranges that overlap in Oregon and Washington; *R. luteiventris* has a larger range that extends from Utah to Alaska. Most populations of these “northwestern” species are found in lakes and ponds in mountain environments (Jones et al. 2005). *Rana pretiosa* and the Great Basin populations of *R. luteiventris* are candidates for listing under the U.S. Endangered Species Act (ESA; U.S. Fish and Wildlife Service 2005; U.S. Fish and Wildlife Service 2004), and *R. cascadae* is a Species of Concern at the federal level. Each of the three species has some level of protection at the state or provincial level. *Rana pretiosa* is listed as endangered in Canada (Seburn and Seburn 2000).

The natural range of *L. pipiens* is one of the largest for a North American amphibian, spanning much of the continent



**Figure 1.** Collection localities for populations of the four frog species used in this study. The upper panel depicts the localities across North America. The inset black rectangle in the upper panel outlines the extent of the lower panel, which shows the northwestern localities in more detail. Abbreviations are shown for states/provinces in which samples were collected.

(Stebbins 2003). The primary habitat of *L. pipiens* is valley wetlands and forests (Rorabaugh 2005). Western populations of this species are currently under review for listing under the ESA (Federal Register 2009).

#### Sampling and molecular methods

We collected population samples and obtained genetic data from the four frog species as described in previous studies (Fig. 1, Appendix 1): Twenty-one *R. pretiosa* populations were sampled from across the species' range in Oregon and Washington (Blouin et al. 2010); 26 *R. luteiventris* populations were sampled in the mountains of Montana and Idaho (Funk et al. 2005); 20 *R. cascadae* populations were sampled at a similar geographic scale,

across the Cascades Mountains of Oregon and Washington (Monsen and Blouin 2003); and 23 *L. pipiens* populations were sampled in the Canadian provinces of British Columbia and Ontario and in the states of Idaho, Nebraska, and Minnesota (Hoffman and Blouin 2004b; Hoffman et al. 2006). Two *R. pretiosa* populations and 5 *L. pipiens* populations are each represented by two temporally spaced samples (Appendix 1).

Toe clips were taken from adult frogs that were collected opportunistically within wetland habitats. Each of our samples probably includes multiple cohorts, in unknown proportions. The four frog species should have similar age-structures in their populations, with frogs reaching sexually maturity at similar ages. Populations of these species are likely isolated by low gene flow,

**Table 1.** Notation used.

$H_e$	Expected heterozygosity
$AR$	Allelic richness
$N_e$	Effective population size
$N$	Census population size
$\hat{N}_e$	Estimate of effective population size
LD	Linkage disequilibrium
ABC	Approximate Bayesian computation
TM	Temporal moments method of Waples (1989)
$\hat{N}_{e(LD)}$	Estimate of effective population size from the linkage disequilibrium method
$\hat{N}_{e(ABC,2000)}$	Estimate of effective population size from the approximate Bayesian computation method, with an upper prior of 2000
$\hat{N}_{e(ABC,10000)}$	Estimate of effective population size from the approximate Bayesian computation method, with an upper prior of 10,000
$\hat{N}_{e(SA)}$	Estimate of effective population size from the sibship assignment method
$\hat{N}_{e(TM)}$	Estimate of effective population size from the temporal moments method

as indicated by their strong genetic differentiation at small spatial scales (Blouin et al. 2010; Funk et al. 2005; 2008; Monsen and Blouin 2003; 2004; Hoffman and Blouin 2004a and 2004b). Thus, we assume that data obtained from each population sample are largely independent, even among neighboring populations.

For DNA extraction, microsatellite amplification, and genotyping methods see Blouin et al. (2010) for *R. pretiosa*, Funk et al. (2005) for *R. luteiventris*, Monsen and Blouin (2003) for *R. cascadae*, and Hoffman et al. (2003) for *L. pipiens*. The microsatellite loci used for the four species are listed in Appendix 2.

### Estimation of effective population sizes

We obtained estimates of effective population size ( $\hat{N}_e$ ) for each population using the linkage disequilibrium (LD) method (Hill 1981), the ABC method implemented in the program ONeSAMP (Tallmon et al. 2008), and Wang's (2009b) SA method. Two *R. pretiosa* populations were analyzed using Waples' (1989) temporal moment (TM) version of the temporal method (Nei and Tajima 1981). We used previously published TM estimates for five of the *L. pipiens* populations (Hoffman et al. 2004). See Table 1 for the notation used in this article.

The LD method is based on the principle that nonrandom associations between neutral alleles at independent loci can be generated by genetic drift. In theory, the amount of linkage (i.e., gametic) disequilibrium in randomly mating, isolated populations is entirely a function of drift and can be used to calculate  $\hat{N}_e$  (Hill 1981). This method provides an estimate of contemporary, local  $N_e$  in the previous generation, although LD generated over

several generations can influence the estimate (Waples 2005). In addition to the assumptions of random mating and isolation, the LD method assumes selective neutrality of the genetic markers, no genetic substructure within the population, and nonoverlapping generations. The other methods in this study generally share these assumptions. We calculated  $\hat{N}_e$  via the LD method using the program LDNe (Waples and Do 2008), which incorporates Waples' (2006) correction for the downward bias in  $\hat{N}_e$  that is introduced when the sample size is smaller than the true effective size. We ran LDNe under the random-mating model and report  $\hat{N}_{e(LD)}$  based on calculations that excluded rare alleles with frequencies less than 0.02 when sample size ( $S$ ) was greater than 25, following the recommendations of Waples and Do (2010). When  $S \leq 25$ , we adjusted the critical allele frequency ( $P_{crit}$ ) to  $1/2S < P_{crit} < 1/S$ . Negative values for  $\hat{N}_e$  from the LD method are interpreted as infinity (Waples and Do 2010). We obtained confidence intervals using the jackknife option in LDNe, which performed better than the traditional parametric method in the simulation study of Waples and Do (2008).

ONeSAMP uses an ABC procedure to obtain  $\hat{N}_e$  by comparing eight summary statistics calculated for each of 50,000 simulated populations to statistics from the real population under consideration (Tallmon et al. 2008). Each of the summary statistics (including a measure of linkage disequilibrium) is a function of  $N_e$ . ONeSAMP requires the specification of upper and lower bounds on the uniform prior distribution for  $N_e$ . For each population, we performed analyses under two different prior ranges: 2–2000 and 2–10,000. The ONeSAMP input cannot include monomorphic loci or individuals missing data at more than one locus (see Supporting Information for reduced sample sizes). The full dataset for each population was used for the LD and SA methods. For two populations of each species, we ran four replicate runs of ONeSAMP with identical starting parameters (priors 2–10,000). Although this small number of replicates did not allow for a thorough evaluation, the results gave some indication of the variability across independent runs of ONeSAMP.

The SA method is implemented in the program COLONY (Wang 2009b). This method derives an estimate of  $N_e$  from the estimated frequencies of full or half siblings in a population, which are obtained from an analysis of SA. The SA method works best for a random sample of individuals belonging to a single cohort. Our samples consist of adult frogs that likely represent several cohorts. The SA method should still be appropriate in this situation, if we assume that our samples do not include any parent–offspring pairs or other close relationships across cohorts (J. Wang, pers. comm.). We ran COLONY using the full likelihood option, with medium length runs.

For *R. pretiosa* populations RP1 and RP10, we collected samples in both 1999 ( $n = 28$  for both RP1-A and RP10-A) and 2006 ( $n = 33$  and 32 for RP1-B and RP10-B, respectively).

This sampling allowed us to apply the TM method of  $N_e$  estimation for these populations. This method derives an estimate of  $N_e$  from the variance of neutral allele frequencies between samples taken at two different times (Waples 1989).  $N_e$  estimated by the temporal method ( $\hat{N}_{e(TM)}$ ) applies to the time between the sampling events (Waples 2005). An estimate of the number of generations between the two temporal samples ( $t$ ) is needed to apply the temporal method. Based on skeletochronology data from the RP10 population, males and females first breed at 2 and 3 years, respectively (M.S. Blouin, unpubl. data). We combined this information with data on age-specific mortality for *R. pretiosa* (Licht 1969; Licht 1974) to estimate the generation time in this species as approximately 3.1 years (see Supporting Information). Thus, we assumed that  $t = 2.25$  generations between the 1999 and 2006 samples. To estimate  $N_e$  with the temporal method (Nei and Tajima 1981), we used Waples' (1989) moment-based approach. We calculated  $\hat{N}_{e(TM)}$  using equations (9) and (12) from Waples (1989) and calculated confidence intervals using his equation (16). Because the temporal samples were taken nondestructively from adults, Waples' Plan I sampling design was appropriate (Waples 1989). This approach requires an estimate of the census size for the population at the time of the first sample. We calculated estimates separately using both  $N = 500$  and  $N = 2000$  because egg mass counts and mark–recapture work suggest that the RP1 and RP10 population sizes are each between 500 and 2000 individuals (K. McAllister and J. Bowerman, pers. comm.).

### Genetic diversity

We calculated the average expected heterozygosity ( $H_e$ ) and allelic richness ( $AR$ ) for each population using the programs FSTAT (Goudet 1995) and POPULATIONS (Langella 1999), respectively. We estimated  $AR$  via rarefaction at a common sample size of 15. To compare these measures of genetic diversity across populations and species, we excluded some populations due to small sample sizes and/or missing data. Thus, in our genetic diversity comparison, we used only 20 *R. pretiosa* populations and 16 *R. cascadae* populations. For those populations that had more than one temporal sample (RP1, RP10, LP1–LP4), we used the more recent sample (e.g., RP1-B) in our comparisons.

### Statistical analysis

We tested for differences in  $\hat{N}_e$ ,  $H_e$ , and  $AR$ , among the four species using the nonparametric Kruskal–Wallis test. When this test had a significant outcome, we conducted post-hoc analyses, using the Mann–Whitney U procedure to test for differences between each species pair. We tested for correlations among  $N_e$  estimates from the LD, ABC, and SA methods ( $\hat{N}_{e(LD)}$ ,  $\hat{N}_{e(ABC)}$ ,  $\hat{N}_{e(SA)}$ ) within species.  $\hat{N}_{e(LD)}$  values were negative for some populations (see results). As a way to correct for skewness of the  $\hat{N}_{e(LD)}$  distribution introduced by negative values in our tests of

correlation between  $N_e$  estimators, we transformed each  $N_e$  estimate by taking its inverse, so that negative values were still properly ranked as smaller than the smallest positive values (R. Waples, pers. comm.).

To investigate relationships between geographic variables and  $\hat{N}_e$  within species, we tested for correlations between  $\hat{N}_e$  and each of three variables: latitude, longitude, and elevation. We log-transformed data, where appropriate, and tested the assumption of normality for variables using the Shapiro–Wilk test. Standard Pearson correlation ( $r$ ) was used when both variables were normally distributed; Spearman rank correlation ( $\rho$ ) was used in all other cases. We used  $\hat{N}_{e(ABC, 2000)}$  in all of these statistical tests, because the ABC method appeared to give the most reliable estimates and our a priori expectation is that the effective sizes of these frog populations should be much smaller than 2000.

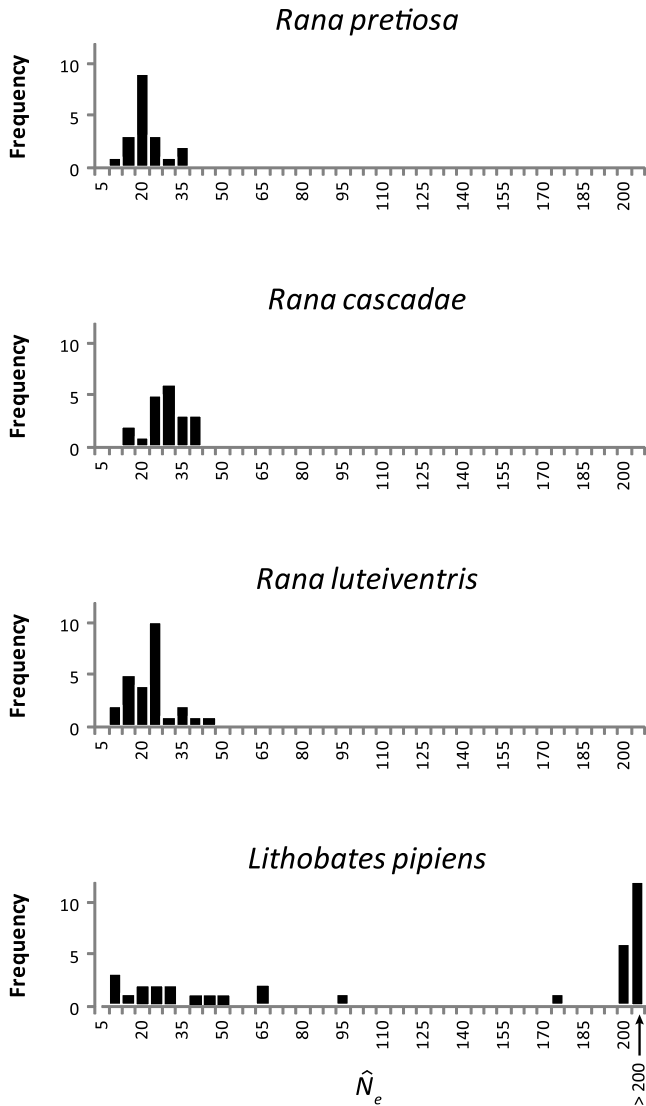
In cases in which two of the geographic variables were significantly correlated with each other and were each correlated with  $\hat{N}_e$ , we performed a linear regression analysis of residuals in an attempt to determine which geographic variable was driving the relationship. To do this, we regressed the genetic parameter on the first geographic variable and then used the residuals of this regression as the dependent variable of a second regression, using the second geographic variable as the independent variable in this case. We then repeated this procedure, switching the two geographic variables. If the regression of the residuals versus one of the two correlated geographic variables was significant but not the other, we took this as evidence that the first variable was driving the relationship. For example, in a case in which longitude and elevation are correlated, we would conclude that there is evidence that  $N_e$  is influenced by elevation only if the regression of the residuals from [ $\hat{N}_e$  versus longitude] versus elevation was significant, but the regression of the residuals from [ $\hat{N}_e$  versus elevation] versus longitude was not significant.

To account for multiple comparisons, we adjusted the significance levels in the Mann–Whitney U correlation tests and regression analyses using a Bonferroni correction at the 0.05 significance level. All of the statistical analyses were performed using R (R Core Development Team 2009).

## Results

### SINGLE SAMPLE ESTIMATES OF EFFECTIVE POPULATION SIZE—LD, ABC, AND SA METHODS

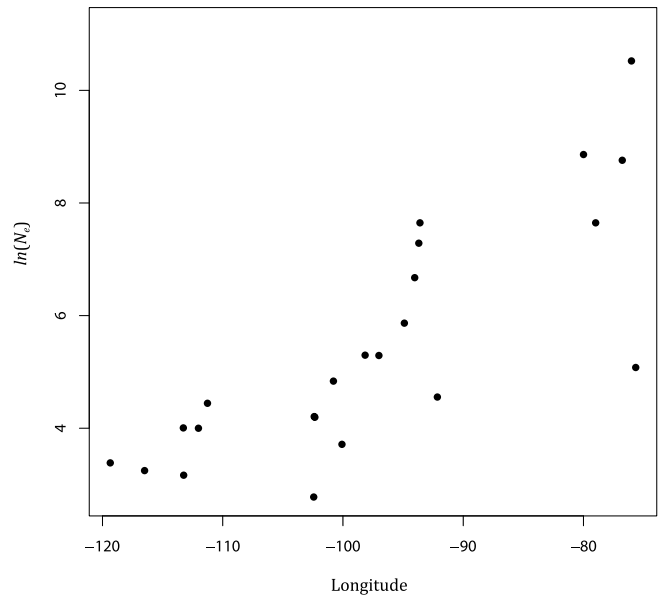
Estimates of  $N_e$  from the LD, ABC, and SA methods are presented in Appendix 3.  $\hat{N}_e$  from all three methods was less than 50 for most populations of the northwestern frogs (Fig. 2; harmonic means:  $\hat{N}_{e(LD)} = 21$ ;  $\hat{N}_{e(ABC, 2000)} = 22$ ;  $\hat{N}_{e(SA)} = 26$ ). Estimates for *L. pipiens* spanned a wider range and were mostly larger (harmonic means:  $\hat{N}_{e(LD)} = 42$ ;  $\hat{N}_{e(ABC, 2000)} = 86$ ;  $\hat{N}_{e(SA)} = 49$ ). No significant differences in  $\hat{N}_e$  were found between any pair of



**Figure 2.** Estimates of effective population size ( $\hat{N}_e$ ) for the four frog species. Histograms showing  $\hat{N}_e$  values obtained using the ABC method with upper priors of 2000. There are no significant differences in  $\hat{N}_e$  among the northwestern species, for which all populations have  $\hat{N}_e < 50$ . *Lithobates pipiens* has significantly higher median  $\hat{N}_e$  than any of the other species, with  $\hat{N}_e > 200$  for some populations. See text for results of statistical tests.

northwestern species, but  $\hat{N}_e$  is significantly larger in *L. pipiens* than in the other species (Table 2).

Effective population sizes within *R. pretiosa* and *R. cascadae* were not significantly correlated with latitude, longitude, or elevation (Table 3). However,  $\hat{N}_e$  is correlated with both longitude and elevation within *R. luteiventris* and *L. pipiens*.  $\hat{N}_e$  decreases from west to east in *R. luteiventris* but increases in *L. pipiens* (Fig. 3).  $\hat{N}_e$  decreases with elevation in both species and increases with latitude in *R. luteiventris*. In *R. luteiventris*, latitude and elevation were correlated and each was correlated with  $\hat{N}_e$  (Table



**Figure 3.** Plot of  $\hat{N}_e$  from the ABC method versus longitude for populations of *Lithobates pipiens*. A log transformation was applied to  $\hat{N}_e$  to improve the linear fit. There is a strong west-to-east pattern of increasing effective population size in this species. Measures of genetic diversity ( $H_e$  and  $AR$ ) for these populations follow similar patterns.

3). The regression analyses of residuals was unable to reveal which of these variables was driving the correlation with  $\hat{N}_e$  (i.e., neither regression was significant). In *L. pipiens*, results of the regression analyses suggest that, although longitude and elevation are correlated in this species, longitude appears to be the important variable with respect to  $\hat{N}_e$ . The regression of the residuals from [ $\hat{N}_e$  versus longitude] versus elevation was not significant ( $R^2 = -0.03$ ,  $P = 0.52$ ), whereas the regression of the residuals from [ $\hat{N}_e$  versus elevation] versus longitude was significant ( $R^2 = 0.20$ ,  $P = 0.02$ ), although only before applying the Bonferroni correction.

LD method point estimates were infinity (i.e., had negative estimates) for 17 population samples, distributed across all species; upper confidence limits were infinity for all but 31 samples (Appendix 3). Estimates of infinity are returned when the signal in the genetic data can be attributed entirely to sampling error, rather than genetic drift, which is the case for a very large population or when the population sample contains too little information (Waples and Do 2010).

Across the four species, none of the single-sample  $N_e$  estimators was consistently higher or lower than the others. However, values of  $\hat{N}_{e(ABC)}$  and  $\hat{N}_{e(SA)}$  were less variable than  $\hat{N}_{e(LD)}$  among populations within species (Fig. 4). Results of the correlation tests between estimators within each species are presented in Table 4. The strongest, most consistent correlations were between  $\hat{N}_{e(ABC)}$  and  $\hat{N}_{e(SA)}$ , which were positive and statistically

**Table 2.** Results of statistical tests of differences between species in effective population size  $\hat{N}_e$  (from the ABC method with upper prior of 2000) and measures of genetic diversity ( $H_e$  and  $AR$ ). Statistics and their associated  $P$ -values are given for the Kruskal–Wallis test of difference in the median values among all species and the Mann–Whitney U test of pairwise differences between each pair of species. For the latter, the test statistic ( $U$ ) is given in the lower half of the matrix and the  $P$ -value is given in the upper half. Test statistics that are significant are in bold.

	Kruskal–Wallis		Mann–Whitney U				
	$\chi^2$	$P$		<i>R. pretiosa</i>	<i>R. luteiventris</i>	<i>R. cascadae</i>	<i>L. pipiens</i>
$\hat{N}_e$	<b>33.5</b>	<0.0001	<i>R. pretiosa</i>	-	0.213	0.398	<0.0001
			<i>R. luteiventris</i>	203	-	0.058	<0.0001
			<i>R. cascadae</i>	168	174	-	<0.0001
			<i>L. pipiens</i>	<b>89</b>	<b>627</b>	<b>452</b>	-
$H_e$	<b>55.0</b>	<0.0001	<i>R. pretiosa</i>	-	0.0002	<0.0001	<0.0001
			<i>R. luteiventris</i>	<b>107</b>	-	0.047	<0.0001
			<i>R. cascadae</i>	<b>17</b>	131	-	<0.0001
			<i>L. pipiens</i>	<b>18</b>	<b>634</b>	<b>378</b>	-
$AR$	<b>53.6</b>	<0.0001	<i>R. pretiosa</i>	-	0.001	<0.0001	<0.0001
			<i>R. luteiventris</i>	<b>454</b>	-	0.051	<0.0001
			<i>R. cascadae</i>	<b>18</b>	132	-	<0.0001
			<i>L. pipiens</i>	<b>24</b>	<b>637</b>	<b>380</b>	-

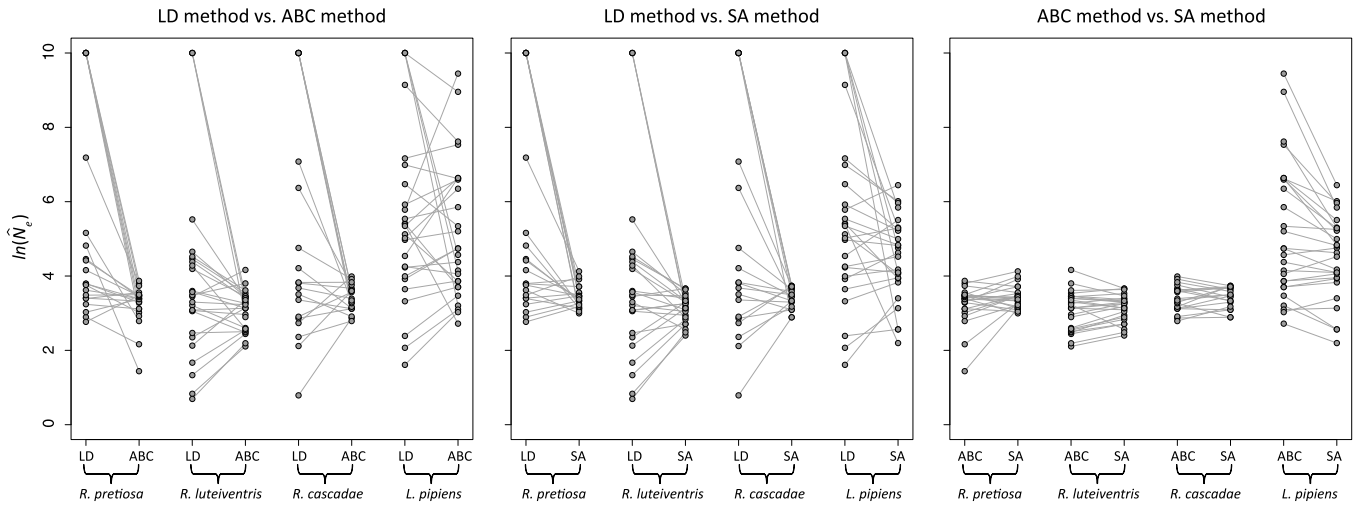
significant in *R. luteiventris* and *L. pipiens*, and nearly significant in *R. cascadae*.

Most of the point estimates and confidence limits from the ABC method increased or decreased somewhat when the upper prior was raised from 2000 to 10,000 (Appendix 3). The mean change in  $\hat{N}_{e(ABC)}$  that occurred when the prior was raised to 10,000 was 21 (SD = 39) for *R. pretiosa*, 6 (SD = 20) for *R. luteiventris*, -6 (SD = 13) for *R. cascadae*, and 1194 (SD = 4,365) for *L. pipiens*. The greater mean change for *L. pipiens* is likely due to the fact that some of the populations sampled for this species are large. The signal from genetic drift in a large

population can be weak relative to noise in the data, making it difficult to generate an accurate estimate of effective size (Waples and Yakota, 2007). The theoretical coefficient of variation of  $\hat{N}_e$  increases with true  $N_e$  for the LD and TM methods (Waples and Do, 2010) and may do so for the ABC method. Thus, we expect greater uncertainty and variability in our estimates for the larger populations. Broadly speaking, estimates obtained with the relatively uninformative upper prior of 10,000 were not dramatically different from those obtained with the upper prior of 2000. Because we have a priori reasons to expect these frog populations to have  $N_e$  less than 2000, we used only  $\hat{N}_{e(ABC,2000)}$  in our

**Table 3.** Results of tests for correlation between genetic parameters ( $\hat{N}_e$ ,  $H_e$ , and  $AR$ ) and geographic factors (latitude, longitude, elevation) for populations of the four frog species. In cases in which the data were normally distributed, standard Pearson correlation was performed and the test statistic is  $r$ . In all other cases, Spearman rank correlation was performed and the statistic is  $\rho$ . Significant outcomes are marked with asterisks. Significance was determined using a Bonferroni-adjusted cutoff  $\alpha$  of 0.0042 (0.05 divided by 12; 4 species  $\times$  3 geographic factors = 12).

Correlations within species									
	<i>Rana pretiosa</i>		<i>Rana luteiventris</i>		<i>Rana cascadae</i>		<i>Lithobates pipiens</i>		
	$\rho$ (or $r$ )	$P$ -value	$\rho$ (or $r$ )	$P$ -value	$\rho$ (or $r$ )	$P$ -value	$\rho$ (or $r$ )	$P$ -value	
$\hat{N}_{e(ABC)}$ vs. Lat	0.01	0.9721	0.55*	0.0042	0.23 ( $r$ )	0.3241	0.24	0.2606	
$\hat{N}_{e(ABC)}$ vs. Lon	0.42	0.0648	-0.61*	0.0012	0.22	0.3438	0.85* ( $r$ )	<0.0001	
$\hat{N}_{e(ABC)}$ vs. Elev	-0.05	0.8087	-0.57*	0.0025	0.17 ( $r$ )	0.4802	-0.75*	<0.0001	
Elev vs. Lat	-0.55	0.0132	-0.78*	<0.0001	-0.02 ( $r$ )	0.7028	-0.59*	0.0039	
Elev vs. Lon	-0.12	0.6169	0.49	0.0112	0.34	0.1360	-0.81*	<0.0001	



**Figure 4.** Plots of  $\hat{N}_e$  (on a logarithmic scale) from the LD, ABC, and SA methods, grouped by species. Gray lines between points connect estimates from two different methods for the same population. Populations for which  $\hat{N}_e$  from the LD method was negative (i.e., infinity) were converted to large positive numbers ( $\sim 20,000$ ). For the northwestern (first three) species, the ABC and SA estimates appear to have less variance within species, but do not have a consistent pattern of being greater than or less than the LD estimates. Estimates from all three methods are mostly larger and have greater variance in *L. pipiens* than in the other species.

statistical analyses. There was some variation of  $\hat{N}_e$  across independent, replicate runs of ONeSAMP (Supporting Information). This variation was most pronounced for the largest of the eight populations for which we had replicate estimates.

**TWO-SAMPLE ESTIMATES OF EFFECTIVE POPULATION SIZE—TM METHOD**

The two  $\hat{N}_{e(TM)}$  values for *R. pretiosa* populations RP1 and RP10 were both between 60 and 70, regardless of whether the census size of the population at the time of the first sample was assumed to be 500 or 2000. With  $N$  set at 500,  $N_e$  was 65 for RP1 (95% CI: 21–1281) and 61 for RP10 (95% CI: 13–infinity). With  $N$  set at 2000, the respective  $N_e$  estimates were 70 (95% CI: 21–infinity) and 66 (95% CI: 13–infinity). These point estimates from the temporal method are consistent with estimates from the single-sample methods.

Hoffman et al. (2004) previously estimated  $N_e$  for *L. pipiens* populations LP1–LP4 using the TM method (Waples 1989), the temporal method of Wang (2001), and the method of Wang and Whitlock (2003). Our single-sample estimates of  $N_e$  for these populations (from 2001) are mostly larger than the estimates of Hoffman et al. (2004; Table 5). Although there is a strong, positive correlation between  $\hat{N}_{e(ABC,2000)}$  and  $\hat{N}_{e(TM)}$  for these populations ( $r = 0.96, P = 0.008$ ), other correlations comparing the single-sample estimates and the temporal estimates for these populations were nonsignificant, as were the correlations between single-sample estimates from the two time periods.

**GENETIC DIVERSITY**

Expected heterozygosity ( $H_e$ ) and allelic richness ( $AR$ ) for each population are shown in Appendix 1 and are plotted against each other in Fig. 5. The most notable pattern here is the much greater

**Table 4.** Results of tests for correlation between the three single-sample  $N_e$  estimators. Spearman rank correlation was performed and the statistic is  $\rho$ . Significant outcomes are marked with asterisks. Significance was determined using a Bonferroni-adjusted cutoff  $\alpha$  of 0.0042 (0.05 divided by 12; 4 species  $\times$  3  $N_e$  estimators = 12).

Correlations between $N_e$ estimators								
	<i>Rana pretiosa</i>		<i>Rana luteiventris</i>		<i>Rana cascadae</i>		<i>Lithobates pipiens</i>	
	$\rho$	<i>P</i> -value	$\rho$	<i>P</i> -value	$\rho$	<i>P</i> -value	$\rho$	<i>P</i> -value
$\hat{N}_{e(LD)}$ vs. $\hat{N}_{e(ABC)}$	0.01	0.9811	0.73*	0.0001	0.39	0.1535	0.68*	0.0003
$\hat{N}_{e(LD)}$ vs. $\hat{N}_{e(SA)}$	0.18	0.4792	0.71*	0.0001	0.48	0.0718	0.71*	0.0002
$\hat{N}_{e(ABC)}$ vs. $\hat{N}_{e(SA)}$	0.36	0.0898	0.88*	<0.0001	0.59	0.0067	0.96*	<0.0001

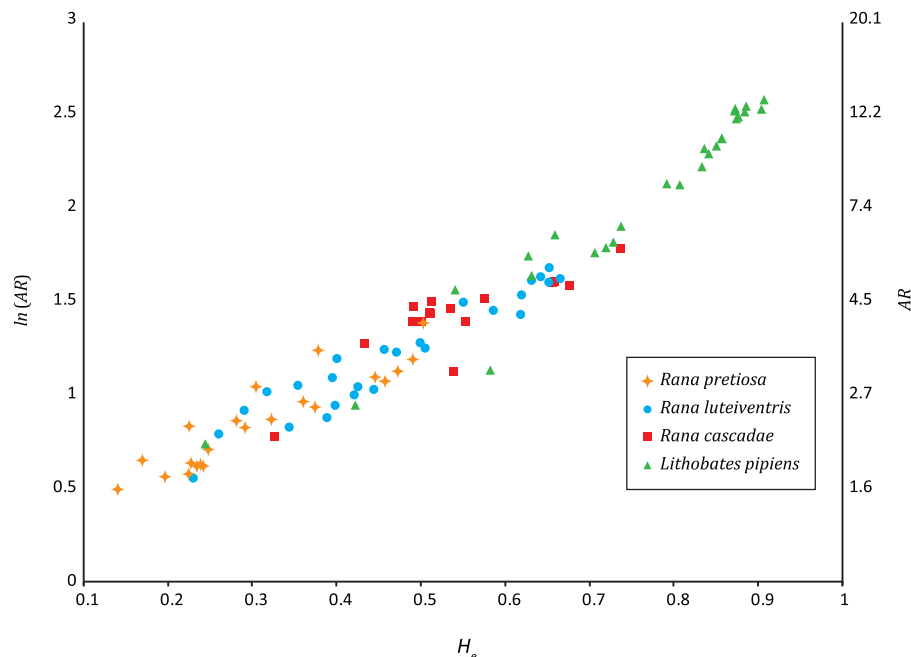
**Table 5.** Single-sample and temporal method estimates of  $N_e$  for five *L. pipiens* populations. The first column contains estimates from the single-sample ABC method, with an upper prior of 2000. ABC estimates from only the more recent sample for each population are shown. The last two columns are estimates reported in Hoffman et al (2004), from the temporal moments method of Waples (1989;  $\hat{N}_{e(TM)}$ ) and the method of Wang (2001;  $\hat{N}_{e(W)}$ ). The latter method assumes that  $N$  (census size) is large relative to  $N_e$ , such that  $N$  can be ignored and does not need to be estimated in the Type I sampling used for these frog populations. The single sample estimates,  $\hat{N}_{e(ABC,2000)}$ , apply to approximately one or a few generations preceding the time of sampling. The temporal method estimates apply to approximately all the generations between the first sample up to the generation before the second sample.

Population	$\hat{N}_{e(ABC,2000)}$	$\hat{N}_{e(TM)}$	$\hat{N}_{e(W)}$
LP1	1870 (641–9572)	588 (378–1355)	324 (230–488)
LP2	7737 (2154–64223)	1820 (660–∞)	469 (313–786)
LP3	97 (52–304)	410 (222–940)	102 (71–152)
LP4	2032 (797–13683)	1019 (490–∞)	243 (165–395)
LP5	764 (393–2262)	420 (245–837)	205 (150–295)

genetic diversity within many *L. pipiens* populations relative to the other three species. Results of the Kruskal–Wallis and Mann–Whitney U tests indicate that  $H_e$  and  $AR$  differ significantly between all species except between *R. luteiventris* and *R. cascadae* (Table 2). The *R. cascadae* populations generally have the highest levels of genetic diversity ( $H_e$ : 0.33–0.74;  $AR$ : 2.17–5.90) among the northwestern species. The populations of *R. pretiosa* tend to have very low levels of genetic diversity ( $H_e$ : 0.14–0.50;  $AR$ : 1.64–3.98). The diversities of the *R. luteiventris* populations ( $H_e$ : 0.23–0.66;  $AR$ : 1.74–5.33) span the range of values seen in

*R. cascadae* and *R. pretiosa*, although none are as low as the least genetically diverse *R. pretiosa* populations.

These differences in genetic diversity among species may reflect actual biological differences or they may be due to differences in the microsatellite loci used. There is some overlap in the loci used across species (Appendix 2), but most of the loci used for *L. pipiens* were not used for the other species. If the *L. pipiens* loci tend to be more polymorphic, this might explain why populations of this species appear to have higher  $H_e$  and allelic richness  $AR$ . Nevertheless, given the ranges of genetic diversities



**Figure 5.** Plot of allelic richness ( $AR$ ) versus expected heterozygosity ( $H_e$ ) for populations of the four frog species. A log transformation was applied to  $AR$  to improve the linear fit. *Lithobates pipiens* populations have the highest levels of genetic diversity. Among the other three species, *Rana cascadae* populations show the highest diversity. The populations with the lowest levels of diversity overall belong to *R. pretiosa*.

and effective population sizes estimated for the *L. pipiens* populations, we suspect that the loci used for only this species are not systematically given to higher polymorphism than the other loci.

## Discussion

The most salient finding of our study is that the three northwestern ranid frog species (and the western populations of *L. pipiens*) appear to have very small contemporary effective population sizes (<50) and correspondingly low genetic diversities. Based on our results, a good estimate for the effective size of a northwestern ranid frog population would be about 20 or 30 individuals (Fig. 2). However, it is worth considering violations of assumptions of the methods and caveats about precision that affect how much faith to put in these numbers (Luikart et al., 2010; see Waples and Do 2010 for in-depth discussion).

The ABC method is expected to suffer from less imprecision than the LD method, given that  $\hat{N}_{e(ABC)}$  is based on linkage disequilibrium plus seven other parameters that are related to  $N_e$  (Tallmon et al. 2008; Luikart et al. 2010). Wang (2009b) suggested that the SA method may also be more accurate than the LD method, at least under some scenarios. In our study,  $\hat{N}_{e(ABC)}$  and  $\hat{N}_{e(SA)}$  were less variable among populations within species than  $\hat{N}_{e(LD)}$  (Fig. 4).  $\hat{N}_{e(ABC)}$  and  $\hat{N}_{e(SA)}$  values were positively correlated in all four species (significantly so in two; Table 4), had mostly similar values, and had smaller nominal confidence intervals than  $\hat{N}_{e(LD)}$  (Appendix 3).

Some researchers have found that, in some datasets, estimates of  $\hat{N}_e$  from the ABC method are positively correlated with sample size (Haag et al. 2010; R. Waples, pers. comm.). We tested for correlations of each single-sample estimator (ABC, LD, and SA) with sample size within each species and found that only *L. pipiens* shows a positive correlation between sample size and  $\hat{N}_{e(ABC)}$  ( $r = 0.53$ ,  $P = 0.005$ ). Future evaluations of the ABC method will hopefully determine how such correlations arise.

The bias and precision of the ABC and SA methods under different biological situations have yet to be thoroughly evaluated, although our results suggest that these methods may be more reliable than the LD method. Nevertheless,  $\hat{N}_{e(LD)}$  was positively correlated with both  $\hat{N}_{e(ABC)}$  and  $\hat{N}_{e(SA)}$  in two species, so estimates from the LD method may be capturing much of the same information as the newer methods.

In using the SA method, we have assumed that our population samples do not include parent–offspring or other close, cross-generation pairs of relatives. The SA method derives an estimate of  $N_e$  from the inferred frequency of full- and half-sibling pairs in a sample (Wang 2009b). Any parent–offspring pairs in a sample would downwardly bias  $\hat{N}_{e(SA)}$ . We believe it is unlikely that our samples contain many cross-generation pairs of close relatives, but cannot rule out this possibility. The SA method had the

narrowest confidence intervals in our study. Wang (2009b) warns that when information content in a dataset is low, the SA method can give biased estimates with overly narrow confidence intervals. Although this may be an issue in our dataset, corroboration with the other, independent  $N_e$  estimation methods suggests that the SA method estimates are not grossly biased.

Samples from ranid frog populations are almost always in Hardy–Weinberg equilibrium (as they are in our study), so non-random mating or cryptic subdivision should not contribute to LD. Populations of the species in this study do violate the assumptions of discrete generations and (in some cases) closed populations. Given overlapping generations, the LD method actually estimates the effective number of breeding individuals ( $N_b$ ) that produced the sampled cohort(s), which may not be the true, per-generation  $N_e$ . How single-sample estimates of  $N_e$  and  $N_b$  are related in age-structured populations is still unclear (Waples 2010). A small rate of immigration can cause mixture disequilibrium (downward biased estimates of  $N_e$ ), although this effect is thought to be small at equilibrium (Waples and Do 2010). Even with gene flow as high as 10% the LD method should provide estimates of local  $N_e$  (Waples 2010). Extremely high gene flow (e.g., >10%) could cause the estimate from each local population to approximate the value for the metapopulation. However, this will not be the case for most amphibians, which have highly structured metapopulations.

Different estimators of  $N_e$  (LD, ABC, SA, and TM) gave fairly similar results for many of these frog populations, and our estimates are similar to those from other published studies of  $N_e$  in ranid frogs (Zeisset and Beebe 2003; Brede and Beebe 2006; Schmeller and Merila 2007; Ficetola et al. 2010; Phillipson et al. 2009). Thus, there is no evidence to suggest that our single-sample estimates are strongly biased upwards or downwards. The TM method can return  $\hat{N}_e$  that is biased upward when the assumption of nonoverlapping generations is violated and the sample consists of only adults (Waples and Yakota 2007), as it is with our data. However, the  $\hat{N}_{e(TM)}$  for the two *R. pretiosa* populations seem reasonable, given their similarity to values of the other estimators.

Our estimates should also be reasonably precise. Even for the sample sizes used in this study, the LD method is still considered reliable when true  $N_e$  is small (<50) (Waples and Do 2010). Furthermore, if our estimates were highly imprecise, it seems unlikely that our estimates would be as consistent among populations as they are (Fig 2). For these reasons, we believe it is reasonable to conclude that typical local effective sizes for populations of ranid frogs in western North America are in the range of a few tens of individuals.

We do not know the true  $N_e$  of our frog populations, and therefore cannot truly assess any biases in our estimates. Another consideration is that there is an interaction between bias and precision in the LD and TM methods (and possibly the other methods). As noted above, the CV of  $\hat{N}_e$  is an increasing function of true

$N_e$  (Waples and Do 2010). Thus, estimates that are downwardly biased will appear to be more precise, and vice versa.

A striking result is that *L. pipiens* populations show a much wider range of effective sizes than the three northwestern species (Fig. 2), with very large populations in the East and small ones in the West. Why might *L. pipiens* be so different? One possibility is that because the northwestern species diverged relatively recently from a common ancestor (Hillis and Wilcox 2005), they share some characteristics (e.g., breeding behaviors) that predispose them to small  $N_e$ . Another possibility is that something about the habitat in the Northwest causes low effective sizes. That western populations of *L. pipiens* have effective sizes similar to those of the three northwestern *Rana* species is consistent with a habitat effect. The montane wetland habitats occupied by the northwestern *Rana* species exist as small patches surrounded by a matrix of rugged landscape. Census population size (and thus  $N_e$ ) is likely to be restricted in small habitat patches, whereas inhospitable terrain between patches limits gene flow and can reduce  $N_e$  in isolated populations by preventing the introduction of new alleles through immigration. Evidence of gene flow limitation among populations of montane amphibians has been found in several studies (Monsen and Blouin 2004; Funk et al. 2005; Spear et al. 2005; Giordano et al. 2007; Kosciński et al. 2009). Landscape features that have been associated with gene flow restriction include high ridges (Funk et al. 2005) and elevational differences between populations (Funk et al. 2005; Spear et al. 2005; Giordano et al. 2007).

Restricted gene flow coupled with small effective population sizes in montane habitats is a key feature of the Valley-Mountain Model of amphibian population structure proposed by Funk et al. (2005). This model was originally a generalization of the pattern of genetic structure found for the *R. luteiventris* populations included in the present study. By estimating  $N_e$  explicitly for these populations, we confirm that high-elevation populations of *R. luteiventris* do indeed have smaller  $\hat{N}_e$ . Also, for *R. pretiosa*, we found that genetic diversity was highest in two of the lower elevation, valley populations, RP1 and RP4. The RP4 population also had the highest  $\hat{N}_e$  in *R. pretiosa*, and egg mass count survey data suggest that it has one of the largest census sizes (M. Hayes, pers. comm.). In contrast to the northwestern species, *L. pipiens* generally occupies lowland valley habitats. If *L. pipiens* populations in these habitats maintain larger census sizes and experience high levels of gene flow they may tend to maintain larger effective sizes.

#### **PATTERNS IN $N_e$ AMONG POPULATIONS WITHIN EACH SPECIES**

##### *Rana pretiosa*

The low genetic diversity and small  $\hat{N}_e$  found in *R. pretiosa* highlight its status as an imperiled species. Genetic connectivity among populations of this species is also very low (Blouin et al. 2010). It is possible that recent habitat fragmentation has

decreased genetic connectivity among *R. pretiosa* populations resulting in reduced  $N_e$  and genetic diversity. The low values of  $H_e$  we observed in *R. pretiosa* support a scenario of limited population connectivity. Alternatively, small  $N_e$  may be a natural characteristic of this species, in which case genetic diversity might have been lost from these populations over thousands of years.

##### *Rana luteiventris*

Populations of this species located at higher elevations (>1000 m) have smaller  $N_e$  than those found in valley habitats. In Funk et al. (2005), lower genetic diversities were reported for montane populations, but  $N_e$  was not estimated explicitly. Here, we show that  $N_e$  is smaller for montane populations, suggesting that low genetic diversity is due in part to small  $N_e$ , not simply restricted gene flow among populations. In another study of *R. luteiventris*, Davis and Verrell (2005) estimated  $N_e$  directly for four populations using demographic data and found values in the range of what we report here (3.2–37.8). Latitude and longitude are also correlated with the genetic parameters in *R. luteiventris* (Table 3), suggesting that genetic signatures of historical demographic processes are present in our data. However, latitude is also correlated with elevation for this species. Our regression analysis using residuals failed to identify which of the two variables is the most important associate of  $N_e$ . Nevertheless, the evidence suggests that elevation is the driving factor in this species. Within drainage basins at the same latitude, high elevation populations have much lower genetic diversity than low elevation populations (Funk et al. 2005).

##### *Rana cascadae*

Although most populations of this species have small  $N_e$ , they have generally higher genetic diversity than populations of the other two northwestern frogs. Given that *R. cascadae* and *R. pretiosa* are co-distributed in the Cascades Mountains and they have similarly small  $N_e$  estimates, the higher levels of genetic diversity seen in *R. cascadae* may reflect a greater level of connectivity among *R. cascadae* populations. *Rana cascadae* populations are more numerous across the Cascades Mountains than those of *R. pretiosa* and there is a strong pattern of isolation by distance among *R. cascadae* populations (Monsen and Blouin 2004), indicating stepping-stone gene flow among populations. Movements of up to 5.2 km between habitat patches have been documented for *R. cascadae* (Garwood and Welsh 2007) and the potential for gene flow among populations at a regional scale may be relatively high (Brown 1997).

##### *Lithobates pipiens*

There is a remarkable west-to-east pattern of increasing  $N_e$  and genetic diversity in *L. pipiens*. A similar pattern was found previously with independent data from mitochondrial DNA: western

populations have lower haplotype and nucleotide diversities than eastern populations (Hoffman and Blouin 2004a; Wilson et al. 2008). The largest  $\hat{N}_e$  for *L. pipiens* were found in populations of the Midwest and eastern regions identified by Hoffman and Blouin (2004a) as possible glacial refugia during the Pleistocene. Long-term environmental stability in these regions may have allowed large populations to persist for thousands of years. In contrast,  $N_e$  of the western populations has likely been influenced by extreme population bottlenecks and habitat fragmentation during the glacial cycles of the Pleistocene and during the drying/warming of western North America in the Holocene (Thompson et al. 1993). Moreover, the westernmost populations sampled in this study are located at the species' historical range margin. The small  $N_e$  and low diversities of these populations may also reflect their peripheral positions (Hoffman and Blouin 2004b). The extent to which historical signatures are present in these  $N_e$  estimates depends on which time scale the estimates apply to. If, as we have assumed, our estimates reflect "contemporary"  $N_e$ , then historical factors may not be driving the patterns we discovered.

Our single-sample estimates for the five *L. pipiens* populations with two temporal samples were only roughly similar in scale to the TM method estimates obtained by Hoffman et al. (2004). Although there is a correlation between  $\hat{N}_{e(ABC, 2000)}$  and  $\hat{N}_{e(TM)}$ , the latter are mostly smaller than the former. This could be because  $\hat{N}_{e(TM)}$  applies to a longer timeframe of 11–15 generations (Hoffman et al., 2004) whereas  $\hat{N}_{e(ABC, 2000)}$  may apply to only a few, recent generations (although this is uncertain; Wang, 2009b). The  $\hat{N}_{e(TM)}$  is an estimate of the harmonic mean  $N_e$  over the 11–15 generations. If  $N_e$  was small for even a single generation during that time, the estimate could be reduced significantly. This might explain the discrepancy between  $\hat{N}_{e(TM)}$  and  $\hat{N}_{e(ABC, 2000)}$ .

#### HOW DO OUR ESTIMATES OF $N_e$ COMPARE TO THOSE OF OTHER RANID FROG SPECIES?

In a summary of amphibian  $N_e$  estimates by Schmeller and Merila (2007), most of the estimates were less than 100, suggesting that amphibians tend to have small effective population sizes. Several recent, single-species studies had similar results (BeeBee 2009; Wang 2009a; Mullen et al. 2010). Estimates of  $N_e$  based on microsatellite markers have been obtained for several ranid frog species. Estimates for three European species—*R. ridibunda* (two populations; Zeisset and Beebee 2003), *R. temporaria* (two populations, Brede and Beebee 2006; two populations, Schmeller and Merila 2007) and *R. latastei* (eight populations, Ficetola et al. 2010)—were in the range of sizes we found for our northwestern species. The values of  $\hat{N}_e$  we found for the eastern populations of *L. pipiens* are the largest reported for a ranid frog and are much higher than most estimates for amphibians (but see Funk et al. 2009). Based on our results and those of previous studies,

it now seems reasonable to conclude that typical effective sizes for populations of most ranid frogs in western North America are in the tens, rather than in the hundreds or thousands. It will be interesting to explore what it is about the life history or habitat of *L. pipiens* in Eastern North America that make it an exception.

#### IMPLICATIONS FOR AMPHIBIAN EVOLUTION AND CONSERVATION

The small effective population sizes of amphibians, combined with their typically low levels of gene flow among populations, should have important evolutionary consequences. Populations with small  $N_e$  will tend to lose genetic diversity more rapidly than large populations. If few migrants are exchanged among populations with small  $N_e$ , the populations are likely to rapidly become genetically differentiated at neutral loci through genetic drift. If selective pressures differ among populations, phenotypic or behavioral divergence could also occur. The historical interactions of local selection pressures, strong genetic drift, and restricted gene flow may have been important factors leading to the present-day genetic and taxonomic diversity of amphibians.

These same factors should be key considerations in the conservation and management of amphibians. Conservation practices should aim to preserve the natural evolutionary forces that distribute and maintain genetic diversity in amphibians. When possible, species (or populations) that have naturally small effective population sizes and low levels of among-population gene flow should be distinguished from those that have experienced recent, anthropogenic reductions in  $N_e$  and/or gene flow. For the former species, maintaining natural population sizes, habitat quality, and habitat connectivity would be most important. For species that have naturally larger  $N_e$  and higher gene flow, but have suffered from human activities, the focus should be on identifying and restoring the environmental (or other) features that allow for these genetic traits.

#### SUMMARY

Most empirical studies of effective population size based on molecular genetic data have reported estimates of  $N_e$  (or  $N_b$ ) from only a small number of populations per species, and few comparisons have been made among species (e.g., Jehle et al. 2001; Brede and Beebee 2006; Fraser et al. 2007). In this study, we estimated  $N_e$  for 90 populations across four frog species. The three northwestern species appear to have very small  $N_e$ , fitting the general pattern found in most previous studies on amphibians. Some populations of the fourth species, *L. pipiens*, have considerably larger  $N_e$ . There are significant differences in  $\hat{N}_e$  and genetic diversity among species, and geographic trends in  $\hat{N}_e$  among populations within species. In particular, there is a strong east-to-west trend of decreasing  $N_e$  in *L. pipiens*. We discovered these patterns by using a comparative approach, analyzing data from a large number

of populations within and among species. More studies like this might be useful for revealing the most important intrinsic (e.g., shared biological characteristics, shared evolutionary histories of populations in the same geographic region) and extrinsic (e.g., landscape influences such as elevation and topography) factors that control  $N_e$  in different taxa.

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## Appendix 1

Collection locality information for populations of the four frog species used in this study. Sample size after exclusion of monomorphic loci and individuals with missing data is given by  $n$ . Spatial data are given by latitude (Lat), longitude (Lon), and elevation (Elev). Measures of genetic diversity are given as expected heterozygosity ( $H_e$ ) and allelic richness ( $AR$ ; rarefied to common sample size of 15). Separate, temporally spaced samples taken from a single population have names ending with “-A” (first sample) or “-B” (second sample). The collection year is shown as a superscript for each of these samples.  $H_e$  and  $AR$  are not reported for populations that had too much missing data or small sample size.

Species	Population	Original name*	<i>n</i>	Lat	Lon	Elev (m)	$H_e$	AR
<i>Rana pretiosa</i>								
	RP1-A <sup>1999</sup>	DC	30	46.9667	-123.0000	42	0.472	3.07
	RP1-B <sup>2006</sup>	DC	34	46.9667	-123.0000	42	0.446	2.98
	RP2	BC	26	46.8833	-122.9167	77	0.491	3.27
	RP3	TL	36	46.0167	-121.5333	596	0.170	1.91
	RP4	CB	39	45.9500	-121.3167	555	0.503	3.98
	RP5	CP	29	45.1373	-121.5690	962	0.140	1.64
	RP6	HL	31	43.9701	-121.7730	1518	0.239	1.87
	RP7	LC	34	43.8030	-121.8738	1451	0.248	2.02
	RP8	LL	37	43.9108	-121.7572	1445	0.225	1.78
	RP9	WI	32	43.7003	-121.7708	1325	0.242	1.85
	RP10-A <sup>1999</sup>	SR	38	43.8684	-121.4536	1269	0.292	2.28
	RP10-B <sup>2006</sup>	SR	32	43.8684	-121.4536	1269	0.227	1.88
	RP11**	-	208	43.8512	-121.4474	1269	0.234	1.85
	RP12	LP	31	43.6827	-121.5161	1282	0.281	2.36
	RP13	DL	29	43.6356	-121.8571	1346	0.323	2.38
	RP14	BM	31	43.3916	-121.9539	1443	0.225	2.29
	RP15	GL	30	43.6326	-122.0432	1466	0.197	1.75
	RP16	JC	34	43.1514	-121.5367	1497	0.374	2.54
	RP17	KE	27	42.9625	-121.5856	1381	0.457	2.92
	RP18	KW	31	42.9464	-121.7485	1379	-	-
	RP19	AR	40	42.9333	-121.4833	1387	0.361	2.61
	RP20	WR	44	42.6233	-121.9714	1263	0.378	3.44
	RP21	BL	31	42.2518	-122.2043	1506	0.305	2.83
<i>Rana luteiventris</i>								
	RL1	KSM	28	48.3178	-115.9796	1581	0.499	3.58
	RL2	KFG	29	48.3347	-115.9737	884	0.586	4.25
	RL3	KLB	19	48.3309	-115.9215	785	0.631	4.99
	RL4	KSF	29	48.3267	-115.9187	824	0.550	4.44
	RL5	KUSF	19	48.3241	-115.9232	812	0.618	4.16
	RL6	SSCL	27	48.2347	-115.9177	1485	0.401	3.29
	RL7	MUB	25	47.8899	-115.8350	833	0.652	5.34
	RL8	MMB	55	47.8910	-115.8300	819	0.665	5.04
	RL9	MUBDG	26	47.8903	-115.7964	769	0.619	4.62
	RL10	MHB	25	47.8876	-115.7938	839	0.651	4.95
	RL11	MBAM	30	47.8745	-115.7551	769	0.642	5.08
	RL12	OSOH	30	46.6620	-114.2366	2251	0.444	2.78
	RL13	SWDPE	30	46.6415	-114.2526	2244	0.471	3.40
	RL14	SWDPW	30	46.6406	-114.2534	2241	0.456	3.45
	RL15	SWPLW	30	46.6326	-114.2359	1982	0.425	2.82
	RL16	SWRR	30	46.5746	-114.0867	999	0.421	2.70
	RL17	SWSFS	30	46.6131	-114.2700	2238	0.398	2.56
	RL18	RURC	30	46.0202	-114.4159	2133	0.389	2.40
	RL19	RRCP	25	46.0746	-114.2105	1250	0.505	3.48
	RL20	LFP	21	46.0127	-114.3797	2256	0.318	2.75
	RL21	LBLP	30	46.0069	-114.3668	2139	0.291	2.49
	RL22	LLRCL	24	46.0229	-114.3420	1995	0.344	2.28
	RL23	SKFP	28	45.1121	-114.5989	2484	0.354	2.85
	RL24	SKB	30	45.1202	-114.5826	2652	0.395	2.97
	RL25	TT	31	45.0896	-114.6116	2548	0.261	2.20
	RL26	TA	30	45.0756	-114.6161	2560	0.231	1.74

Continued.

Species	Population	Original name*	<i>n</i>	Lat	Lon	Elev (m)	<i>H<sub>c</sub></i>	AR
<i>Rana cascadae</i>								
	RC1	Many Lakes	25	43.8155	-121.9068	1563	0.538	3.07
	RC2	Berkeley Park	22	46.9131	-121.6872	1971	0.490	4.01
	RC3	Gold Lake	31	43.6332	-122.0464	1467	0.736	5.90
	RC4	Todd Lake	25	44.0250	-121.6821	1895	0.553	4.02
	RC5	Benson Lake	25	44.2322	-121.9157	1684	0.513	4.46
	RC6	Reflection Lakes	25	46.7680	-121.7264	1520	0.511	4.20
	RC7	Paradise River	22	46.7786	-121.7368	1498	0.501	4.02
	RC8	North Waldo	23	43.7623	-122.0131	1600	0.656	4.93
	RC9	Melakwa Lake	15	44.1973	-121.9089	1497	-	-
	RC10	Seven Mile	14	42.7161	-122.1278	1448	-	-
	RC11	Elysian Fields	30	46.9435	-121.7554	1792	0.433	3.56
	RC12	McKenzie Pass	30	44.2448	-121.8414	1585	0.535	4.30
	RC13	Waldo Lake	30	43.7623	-122.0131	1664	0.676	4.85
	RC14	Illabot Creek	22	48.4402	-121.3876	1331	0.326	2.17
	RC15	Mt. Ranier	30	46.9160	-121.6531	1922	0.492	4.33
	RC16	Olympic	29	47.9163	-123.7814	1083	0.512	4.19
	RC17	Big Frank	18	42.4422	-122.2416	1780	-	-
	RC18	Crystal Springs	22	43.3123	-122.1404	1275	-	-
	RC19	Breitenbush	27	44.7716	-121.9495	732	0.658	4.97
	RC20	Colby Creek	32	40.1113	-121.4846	1496	0.576	4.52
<i>Lithobates pipiens</i>								
	LP1-A <sup>1971</sup>	NONQ	44	44.037	-78.979	317	0.885	12.66
	LP1-B <sup>2001</sup>	NONQ	40	44.037	-78.979	317	0.872	12.49
	LP2-A <sup>1971</sup>	MONTZ	42	42.98925	-76.77146	117	0.903	12.46
	LP2-B <sup>2001</sup>	MONTZ	46	42.98925	-76.77146	117	0.907	13.10
	LP3-A <sup>1971</sup>	HAPVY	54	43.4679	-76.01	182	0.884	12.28
	LP3-B <sup>2001</sup>	HAPVY	22	43.4679	-76.01	182	0.856	10.67
	LP4-A <sup>1979</sup>	FAIRM	39	45.06865	-75.65299	86	0.874	11.84
	LP4-B <sup>2001</sup>	FAIRM	38	45.06865	-75.65299	86	0.876	11.96
	LP5	CAMPB	40	43.518	-79.997	306	0.872	12.34
	LP6	17	36	46.9208	-92.1555	416	0.728	6.12
	LP7	16	36	44.2153	-93.5931	315	0.856	10.64
	LP8	15	32	45.1917	-93.6917	289	0.836	10.09
	LP9	14	37	45.1264	-94.0306	297	0.850	10.24
	LP10	13	32	45.7097	-94.8931	381	0.841	9.82
	LP11	12	26	42.0006	-97.0064	426	0.833	9.16
	LP12	11	34	42.1734	-98.1558	541	0.807	8.32
	LP13	10	23	42.2371	-100.0883	820	0.737	6.66
	LP14	9	25	41.9467	-102.4334	1183	0.582	3.09
	LP15	8	22	41.9652	-102.3390	1181	0.706	5.79
	LP16	7	31	41.9393	-102.3872	1180	0.719	5.95
	LP17	6	25	42.4005	-100.7893	919	0.791	8.37
	LP18	-	22	42.1385	-111.2623	1877	0.659	6.37
	LP19	5	23	42.2568	-112.0131	1451	0.627	5.69
	LP20	4	31	42.6198	-113.2837	1280	0.631	5.12
	LP21	3	23	42.6120	-113.2473	1279	0.540	4.75
	LP22	2	27	49.0500	-116.5017	626	0.422	2.56
	LP23	1	29	47.07613	-119.35362	318	0.244	2.08

\*Original population names from Blouin et al. (2010; *R. pretiosa*), Funk et al. (2005; *R. luteiventris*), Monsen and Blouin (2003; *R. cascadae*), Hoffman et al. (2004; *L. pipiens* populations LP1–5), and Hoffman et al. (2006; *L. pipiens* populations LP6–LP22).

\*\* Data for this population were collected in the study of Phillipsen et al. (2010).

## Appendix 2

Microsatellite loci used for each frog species in this study.

Locus	<i>R. pretiosa</i>	<i>R. luteiventris</i>	<i>R. cascadae</i>	<i>L. pipiens</i>	References*
RC287			•		c
RP3	•	•			a,b
RP15		•			a,b
RP17	•	•	•		a,b,c
RP22	•				a
RP23	•	•			a,b
RP26	•				a
RP193	•		•	•	c
RP415	•			•	a
RP461	•				a
SFC104	•				a
SFC134	•	•	•		a,b,c
SFC120	•		•		a,c
SFC128			•		c
SFC139		•			b
Rpi100				•	d
Rpi101				•	d
Rpi103				•	d
Rpi104				•	d
Rpi106				•	d
Rpi107				•	d
Rpi108				•	d
<i>n</i>	11	6	6	9	

\*Locus references: a = Blouin et al. (2010), b = Funk et al. (2005), c = Monsen and Blouin (2003), d = Hoffman et al. (2003).

## Appendix 3

Estimates of effective population size for populations of the four frog species. Estimates were obtained using the single-sample linkage disequilibrium (LD) method and approximate Bayesian computation (ABC) method. For estimates from the latter, the upper Bayesian prior for  $\hat{N}_e$  is given in the subscript. Confidence limits are shown in parentheses. See Table 1 for notation used.

Species	Population	$\hat{N}_{e(LD)}$	$\hat{N}_{e(ABC,2000)}$	$\hat{N}_{e(ABC,10000)}$	$\hat{N}_{e(SA)}$
<i>Rana pretiosa</i>					
	RP1-A	44.3 (19.4–772.7)	30 (21–79)	50 (55–394)	41 (23–86)
	RP1-B	30.1 (15.7–82.5)	28 (22–56)	38 (24–121)	34 (19–65)
	RP2	44.5 (19.3–1325.1)	28 (19–71)	48 (25–143)	50 (26–127)
	RP3	42.8 (6.2-Inf)	21 (12–61)	37 (16–185)	32 (18–60)
	RP4	1319.6 (67.1-Inf)	45 (29–163)	194 (97–911)	62 (35–153)
	RP5	124 (8.2-Inf)	5 (4–8)	1 (1–2)	23 (11–52)
	RP6	37.3 (6.3-Inf)	30 (18–102)	140 (55–582)	20 (11–39)
	RP7	18.1 (8.4–48.7)	9 (7–29)	26 (15–93)	26 (14–54)
	RP8	29.8 (4-Inf)	35 (19–73)	16 (9–37)	23 (13–44)
	RP9	20.6 (5.9–234.9)	30 (20–93)	38 (20–147)	28 (16–54)
	RP10-A	–57.2 (63.8-Inf)	42 (24–136)	57 (30–196)	35 (21–64)
	RP10-B	–82.1 (14.8-Inf)	27 (15–66)	62 (29–196)	22 (8–32)
	RP11	32.6 (14.9–69.2)	34 (19–66)	28 (15–56)	54 (36–81)

Continued.

Species	Population	$\hat{N}_{e(LD)}$	$\hat{N}_{e(ABC,2000)}$	$\hat{N}_{e(ABC,10000)}$	$\hat{N}_{e(SA)}$
	RP12	-3216.9 (32.5-Inf)	48 (27-153)	36 (20-112)	41 (23-83)
	RP13	25.5 (6.9-Inf)	23 (15-57)	41 (21-118)	32 (18-65)
	RP14	-66.1 (18.2-Inf)	33 (19-128)	50 (27-363)	30 (16-63)
	RP15	-318.6 (10.9-Inf)	23 (15-68)	17 (9-56)	21 (11-43)
	RP16	15.9 (8.2-37.9)	33 (23-93)	60 (32-244)	24 (13-49)
	RP17	86.3 (22.2-Inf)	32 (22-104)	9 (5-20)	28 (16-53)
	RP18	-12.9 (-46.3-Inf)	19 (13-45)	28 (15-90)	32 (17-77)
	RP19	63.9 (21.2-Inf)	32 (22-97)	87 (43-390)	31 (17-66)
	RP20	83 (21.8-Inf)	33 (22-95)	54 (31-195)	29 (17-56)
	RP21	173.8 (39.8-Inf)	17 (10-40)	22 (11-61)	25 (14-46)
<i>Rana luteiventris</i>					
	RL1	21.6 (4.7-Inf)	20 (12-54)	19 (10-57)	26 (14-52)
	RL2	36.5 (8.1-Inf)	27 (18-64)	30 (17-102)	30 (15-74)
	RL3	104.9 (16.7-Inf)	32 (21-76)	18 (10-52)	34 (16-121)
	RL4	86.5 (17.2-Inf)	35 (19-98)	42 (20-122)	39 (21-99)
	RL5	-673.4 (9.8-Inf)	32 (19-108)	21 (11-66)	29 (13-81)
	RL6	65.9 (15.5-Inf)	32 (19-102)	17 (8-43)	27 (14-59)
	RL7	10.5 (2.7-66.4)	24 (14-53)	7 (4-13)	27 (14-60)
	RL8	31.4 (13.7-126.3)	65 (35-186)	51 (26-124)	39 (25-65)
	RL9	32.7 (17.1-106.4)	45 (25-153)	36 (17-104)	32 (18-66)
	RL10	92 (10.7-Inf)	38 (21-107)	34 (17-105)	38 (20-84)
	RL11	249.8 (15.9-Inf)	30 (19-78)	32 (18-100)	27 (14-53)
	RL12	3.8 (1.6-17.2)	12 (8-27)	12 (7-35)	15 (8-32)
	RL13	35.6 (8.9-Inf)	23 (13-69)	31 (15-144)	22 (12-46)
	RL14	-61.9 (71.5-Inf)	19 (13-39)	36 (20-136)	24 (13-47)
	RL15	21.2 (0.8-Inf)	12 (9-29)	10 (5-34)	19 (10-42)
	RL16	2.3 (1.1-8.4)	9 (6-18)	7 (4-19)	11 (5-27)
	RL17	24.7 (3.1-Inf)	14 (8-29)	73 (32-324)	21 (11-44)
	RL18	11.8 (1.5-Inf)	13 (9-31)	11 (7-39)	15 (8-32)
	RL19	21.8 (3.1-Inf)	23 (14-56)	25 (12-63)	26 (14-59)
	RL20	80.1 (6.9-Inf)	30 (15-82)	63 (25-193)	28 (15-74)
	RL21	5.3 (1.2-79.8)	13 (7-31)	69 (30-381)	17 (9-38)
	RL22	-41.5 (2.2-Inf)	28 (16-60)	24 (14-59)	23 (12-54)
	RL23	27.1 (1.3-Inf)	26 (16-69)	38 (19-199)	13 (7-31)
	RL24	8.4 (2.6-27.3)	29 (14-74)	62 (108-2816)	23 (12-49)
	RL25	72.1 (0.7-Inf)	14 (9-42)	11 (7-41)	18 (10-38)
	RL26	2 (0.1-Inf)	9 (6-27)	16 (9-59)	12 (6-30)
<i>Rana cascadae</i>					
	RC1	67.3 (2.1-Inf)	23 (13-59)	10 (6-26)	32 (16-92)
	RC2	28.7 (7.1-Inf)	23 (15-55)	6 (4-13)	27 (14-67)
	RC3	15.4 (10.4-23.5)	54 (35-159)	20 (13-43)	42 (25-91)
	RC4	17.5 (7.4-75.1)	23 (14-64)	23 (11-63)	22 (11-53)
	RC5	-69 (34.5-Inf)	37 (23-108)	38 (20-128)	40 (21-113)
	RC6	116 (15.9-Inf)	51 (28-177)	28 (14-97)	38 (20-90)
	RC7	39.6 (4.3-Inf)	30 (19-91)	35 (17-106)	33 (17-89)
	RC8	45.1 (8.7-Inf)	40 (27-137)	40 (21-139)	28 (14-63)
	RC9	-38.6 (11.6-Inf)	24 (14-71)	19 (11-61)	42 (15-Inf)
	RC10	2.2 (1.1-8.1)	18 (12-42)	5 (3-10)	18 (8-54)
	RC11	584.8 (39.3-Inf)	43 (22-151)	22 (11-58)	31 (17-62)
	RC12	18.3 (11.6-32.3)	36 (21-98)	36 (17-106)	40 (22-83)
	RC13	-76.4 (436.3-Inf)	38 (25-86)	43 (26-144)	41 (23-83)
	RC14	-14.9 (-124.1-Inf)	27 (15-81)	45 (23-178)	18 (10-41)
	RC15	45.9 (14.1-Inf)	47 (28-174)	37 (19-117)	36 (20-80)

Continued.

Species	Population	$\hat{N}_{e(LD)}$	$\hat{N}_{e(ABC,2000)}$	$\hat{N}_{e(ABC,10000)}$	$\hat{N}_{e(SA)}$
	RC16	1187.2 (32.5-Inf)	27 (18–64)	25 (15–73)	41 (24–88)
	RC17	33.4 (4.1-Inf)	19 (13–44)	10 (7–26)	22 (10–65)
	RC18	10.6 (3.3–34.3)	17 (12–44)	29 (17–102)	24 (12–55)
	RC19	–43.8 (-104.1-Inf)	29 (16–68)	10 (6–18)	33 (18–70)
	RC20	8.3 (4–15.3)	37 (21–128)	33 (18–111)	28 (16–51)
<i>Lithobates pipiens</i>					
	LP1-A	324.5 (150.9-Inf)	12681 (3728–142875)	9105 (1654–288893)	631 (240-Inf)
	LP1-B	1292.5 (318-Inf)	1870 (641–9572)	22934 (3011–1075876)	390 (165-Inf)
	LP2-A	647.9 (203.3-Inf)	209 (111–614)	103 (49–419)	191 (109–664)
	LP2-B	–2053.9 (275-Inf)	7737 (2154–64223)	4861 (1326–106474)	345 (168-Inf)
	LP3-A	1086.3 (240.5-Inf)	751 (252–4424)	4719 (1153–73993)	409 (212–4881)
	LP3-B	–786 (736.3-Inf)	97 (52–304)	132 (65–530)	116 (52-Inf)
	LP4-A	372.6 (140.3-Inf)	732 (309–2582)	3412 (945–60889)	185 (100–1525)
	LP4-B	9336.1 (341.9-Inf)	2032 (797–13683)	4436 (1039–69271)	201 (112–1187)
	LP5	93.6 (42.9-Inf)	764 (393–2262)	6941 (1484–92461)	246 (130–1463)
	LP6	–102.1 (-1446.5-Inf)	64 (41–169)	85 (44–315)	60 (34–118)
	LP7	209.7 (113.2–991.6)	764 (393–2262)	1962 (616–20480)	126 (72–392)
	LP8	151.2 (73.6–3233.1)	572 (263–2401)	477 (200–2761)	198 (92-Inf)
	LP9	254.1 (119-Inf)	350 (178–922)	1137 (353–8848)	148 (82–563)
	LP10	144.3 (79.9–547.1)	183 (100–422)	478 (201–2193)	124 (70–481)
	LP11	54.2 (33.6–117.4)	115 (68–347)	130 (64–484)	65 (35–201)
	LP12	50.2 (35.1–80.6)	116 (70–324)	216 (91–743)	102 (54–293)
	LP13	10.9 (8.1–14.9)	33 (22–70)	42 (22–136)	13 (7–30)
	LP14	220 (2.8-Inf)	16 (12–30)	20 (13–58)	9 (4–24)
	LP15	167.8 (32.9-Inf)	48 (30–120)	39 (21–133)	58 (30–224)
	LP16	38.3 (23.5–79.8)	48 (32–121)	69 (35–270)	52 (29–111)
	LP17	68.8 (40.8–176.8)	80 (50–189)	96 (49–300)	60 (33–158)
	LP18	70.3 (26.6-Inf)	58 (34–169)	99 (50–473)	92 (44–1868)
	LP19	224.5 (38.4-Inf)	41 (25–99)	49 (25–160)	51 (27–142)
	LP20	27.7 (17.4–49.4)	41 (27–119)	35 (17–121)	46 (25–96)
	LP21	5 (2.1–14.5)	21 (15–46)	38 (19–126)	30 (15–73)
	LP22	7.9 (2.6–23.4)	23 (16–56)	38 (21–133)	23 (11–53)
	LP23	–4.3 (15.4-Inf)	25 (15–79)	29 (15–102)	13 (7–30)

## Supporting Information

The following supporting information is available for this article:

**Table S1:** Replicates of  $\hat{N}_{e(ABC,10000)}$  for eight populations (two populations per species, four replicates per population).

Supporting Information may be found in the online version of this article.

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