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Species boundaries, phylogeography and conservation genetics of the red-legged frog (*Rana aurora/draytonii*) complex

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Abstract

The red-legged frog, *Rana aurora*, has been recognized as both a single, polytypic species and as two distinct species since its original description 150 years ago. It is currently recognized as one species with two geographically contiguous subspecies, *aurora* and *draytonii*; the latter is protected under the US Endangered Species Act. We present the results of a survey of 50 populations of red-legged frogs from across their range plus four outgroup species for variation in a phylogenetically informative, ~400 base pairs (bp) fragment of the mitochondrial cytochrome *b* gene. Our mtDNA analysis points to several major results. (1) In accord with several other lines of independent evidence, *aurora* and *draytonii* are each diagnosably distinct, evolutionary lineages; the mtDNA data indicate that they do not constitute a monophyletic group, but rather that *aurora* and *R. cascadae* from the Pacific northwest are sister taxa; (2) the range of the *draytonii* mtDNA clade extends about 100 km further north in coastal California than was previously suspected, and corresponds closely with the range limits or phylogeographical breaks of several codistributed taxa; (3) a narrow zone of overlap exists in southern Mendocino County between *aurora* and *draytonii* haplotypes, rather than a broad intergradation zone; and (4) the critically endangered population of *draytonii* in Riverside County, CA forms a distinct clade with frogs from Baja California, Mexico. The currently available evidence favours recognition of *aurora* and *draytonii* as separate species with a narrow zone of overlap in northern California.

Keywords: conservation genetics, cytochrome *b*, declining amphibian, Pacific Northwest phylogeography, *Rana aurora*, *Rana draytonii*

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Introduction

Red-legged frogs (*Rana aurora*) comprise a wide-ranging species complex of western North American anurans that have been a long-standing source of confusion with respect to species boundaries and composition. The group is restricted to the Pacific coast of North America from southern British Columbia, Canada to northern Baja California, Mexico (Linsdale 1932; Stebbins 1985). Within this range, the frogs have variously been considered two distinct species or two conspecific subspecies, with or without a broad zone of intergradation between them. Described originally as two distinct species, the northern red-legged frog *R. aurora* and the California red-legged frog *R. draytonii* (Baird & Girard 1852), *R. aurora* was reclassified subsequently as a single polytypic species with two subspecies (Camp 1917). Two decades later, *R. cascadae* was recognized as a species with close phylogenetic affinities to *R. aurora* (Slater 1939) that some authors have considered to be a subspecies of *aurora* (Stejneger & Barbour 1943; Stebbins 1962). Currently, *R. a. aurora* and *R. a. draytonii* are

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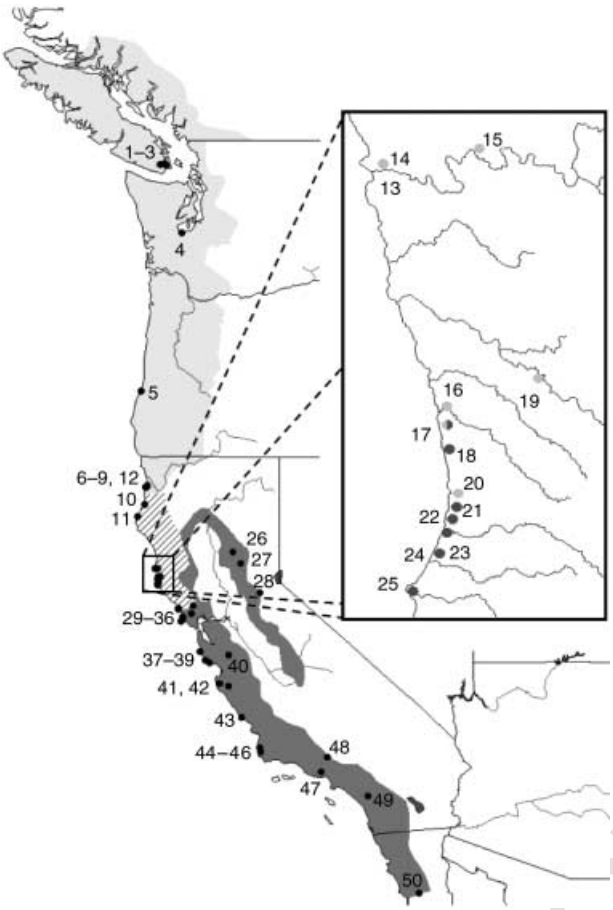

Fig. 1 Map showing the currently accepted ranges of *Rana aurora aurora, R. a. draytonii* and intergrade populations. Light shading is *aurora*, dark shading is *draytonii* and hatched shading is the area that has been considered to be an intergrade zone. Our sampling sites for mtDNA are mapped, with our dense sampling around the mitochondrial contact zone shown in the insert. Locality numbers are the same as in Appendix I.

recognized as conspecific subspecies (following Camp 1917), and *R. cascadae* as a distinct species (Frost 1985; Stebbins 1985), with considerable geographical substructure (Monsen & Blouin 2003). Consistent with this interpretation, *aurora* and *draytonii* have been considered generally to have a broad zone of intergradation spanning several hundred kilometres from Marin to Humboldt Counties, CA (Fig. 1), although the precise boundaries vary among authors (Stebbins 1985; Jennings & Hayes 1994). Recent studies of variation in allozymes, morphology, advertisement calls and oviposition behaviour (Hayes & Miyamoto 1984; Green 1986a; Hayes & Kremples 1986) have led some authors to suggest that *aurora* and *draytonii* may be distinct species (Hayes & Miyamoto 1984).

In addition to the basic problem of species boundaries, the red-legged frogs are of interest from both biogeographical and conservation perspectives. The contact between *aurora* and *draytonii* occurs in northern California, a region that has been identified as a significant biogeographical suture zone for a number of vertebrate and plant taxa (Good 1989; Soltis *et al*. 1997; Redenbach & Taylor 2002), and may represent a phylogeographical contact zone of general importance. From a conservation perspective, the red-legged frog is one of the best-known examples of a declining amphibian (Davidson *et al*. 2001; Davidson *et al*. 2002). *R. aurora* was once widespread and abundant in the Sierra Nevada and the southern San Joaquin Valley (Jennings & Hayes 1985), and is probably the species described by Mark Twain in 'The Celebrated Jumping Frog of Calaveras County'. However, the species is now extirpated from the San Joaquin Valley and has declined to near extinction in the Sierra Nevada, with only six recently discovered populations known to still be extant; of these, only one is known to have more than 10 breeding adults. The species has also declined dramatically south of Los Angeles, CA, with a single population known from the Santa Rosa Plateau in Riverside County, although it still persists in northern Baja California, Mexico. In 1996, the US Fish and Wildlife Service (USFWS) listed *R. a. draytonii* as threatened under the US Endangered Species Act (United States Fish & Wildlfe Service 1996). This listing has potentially enormous economic and ecological consequences, because the frog coexists with at least 20 additional threatened species in coastal California (United States Fish & Wildlfe Service 2002). Based in part on the current subspecies concept and on a presumed broad area of intergradation, frogs along the coast in the Walker Creek drainage (Marin County, CA) and south are assumed to be pure *draytonii* and are therefore protected under the US Endangered Species Act, whereas all coastal frogs to the north are considered to be intergrades or pure *aurora* and are not so protected (Fig. 1).

In this study, we present the results of a range-wide survey of the *R. aurora* complex for variation in ~400 base pairs of the mitochondrial cytochrome *b* gene. We also include sequence data for representative material from a broad geographical sampling of three of the other four members of the *R. boylii* species group (Zweifel 1955; Case 1978; Macey *et al*. 2001) as outgroups for our *R. aurora* samples, and a single eastern bullfrog (*R. catesbeiana*) as a distant outgroup. Although the DNA fragment that we used was small, it was phylogenetically informative and allowed us to examine the contact zone dynamics, conservation genetics and phylogeography of this important group of frogs.

Materials and methods

Specimens

We analysed 108 frogs from six taxa. Specimens included whole tadpoles (generally < 1 cm total length), tadpole fin clips and adult toe clips. Tissue samples were either frozen

in liquid nitrogen or preserved in the field in 95% ethanol and transferred later to a –20 °C freezer. We sampled 50 sites that span the range of *aurora* and *draytonii*, with one to four individuals per site (Fig. 1, Appendix I). This sampling effort covered the entire geographical range of the species from British Columbia to Baja California, and included material from three of the six known populations from the Sierra Nevada (sites 26–28) and from the three remaining populations known from southern California (sites 47–49), including the Santa Rosa Plateau (site 49). Our sampling was particularly dense in the contact region between *aurora* and *draytonii* in southern Mendocino County, CA (Fig. 1, Appendix I).

We included geographically widespread samples of three presumptive outgroup taxa, including all but two members of Case's (1978) *Rana boylii* species group. For *R. cascadae*, Monsen & Blouin (2003) identified three major clades based on mtDNA sequence data, and we included at least one individual from each of their clades in our analyses (sites 59–63). For *R. boylii*, we included individuals from eight sites encompassing most of the geographical range of the species (sites 51–58). For *R. muscosa*, we included individuals from eight sites covering the range of the species (sites 64–71) except for the distinct, endangered clade from the southern Sierra Nevada (Macey *et al*. 2001). Finally, we included one *R. catesbeiana* (from site 15; Appendix I) as an additional, distant outgroup to root the *R. boylii* species group. We used this species for consistency with other studies, and based on recent evidence suggesting that it is approximately as divergent as several other candidate outgroup ranid species (Macey *et al*. 2001).

Molecular methods

DNA was extracted with standard chloroform–phenol methods (Hillis *et al*. 1996a). Based on existing *Rana* sequences downloaded from GenBank and our preliminary sequence analyses, we developed four primers that amplify an approximately 400 base pairs (bp) fragment of the cytochrome *b* mitochondrial DNA (mtDNA) gene from all taxa. The fragment is located at the 5′ end of the gene, begins at about position 96 in the *R. nigromaculata* cytochrome *b* sequence, and corresponds to approximately positions 16 662–17 158 in the full *R. nigromaculata* mtDNA genome (Sumida *et al*. 2001). Three of these are species-specific primers at the 5′ end of our fragment *b*: cyt*b*1-ra (aactttgggtctctcctagg) was used for *R. aurora*/*draytonii* and *R. cascadae*, cyt*b*1-rm (aacttcgggtcactcctagg) for *R. muscosa* and cyt*b*1-rb (aactttggctcacttctggg) for *R. boylii*. At the 3′ end of the fragment, we used the primer cyt*b*2-ra (5′–3′: tgaggacaaatatcattctgaggg) for all taxa. We sequenced all individuals in both directions, and used fully confirmed sequences (most cases) with occasionally unconfirmed, unambiguous ends of sequences (determined by visual inspection). Sequences ranged in length from 297 to 397 bp, with most sequences about 350 bp long. We conducted all sequencing on ABI 377 or ABI 3100 automated sequencers at the UC Davis Division of Biological Sciences Sequencing Facility. Sequences were examined for signal quality and confirmed for complementarity using SEQUENCHER version 3.0, and aligned with CLUSTAL X (Thompson *et al*. 1997). All unique sequences will be deposited in GenBank, and the alignment is available from H. B. S.

Analysis

We conducted parsimony and likelihood analyses using PAUP* version 4.0b8 (Swofford 1998). Because our sequence lengths varied, we ran all analyses on both a short 312 bp fragment (that represents full sequence for all but a few base pairs of a few individuals) and on the full 397 bp fragment with missing data at the ends of many sequences. In all cases the two sets of analyses yielded virtually identical results; therefore, we report only the analyses based on the longer sequences. There are potential disadvantages to including taxa with incomplete data; however, these problems are usually outweighed by the advantages of their inclusion (Wiens 1998). For likelihood analyses, we used MODELTEST (Posada & Crandall 1998) for a 287 bp fragment that was common to all sequences to determine the optimal model for our data and to parameterize that model, but ran the analysis in PAUP* on the full-length data set. We used this strategy because the actual model is optimized across all sequences and we did not want a few long sequences to unduly affect model fitting. We assessed the strength of parsimony (775 pseudoreplicates) and likelihood (100 pseudoreplicates) trees using bootstrap proportions (BP), and we tested a priori hypotheses of relationships using parametric bootstrapping (Hillis *et al*. 1996a; Huelsenbeck *et al*. 1996a; Huelsenbeck *et al*. 1996b).

Results

Sequence variation

Of the 107 sequences, 47 were unique including 26 *aurora*/*draytonii* sequences and 21 outgroup sequences. Base composition of the 287 bp of sequence common to virtually all individuals shows the low frequencies of guanine typical of vertebrate mitochondrial sequences [f(A) = 0.25, f(C) = 0.28, f(G) = 0.15, f(T) = 0.32], a transition:transversion ratio of 4.99, and a gamma distribution shape parameter of 0.30, leading to an optimal model selection of HKY + G (Posada & Crandall 1998). Within taxa, HKY + G corrected sequence divergence ranged from 0 to 0.027 substitutions/site in *R. a. draytonii* to approximately 0.05 substitutions/site in *R. cascadae* (Table 1). Among-taxon, corrected sequence divergences for members of the *R. boylii* group were much larger, ranging from 0.076 substitutions/site (the lowest

Table 1 Sequence divergence (substitutions/site), corrected for multiple substitutions (HKY + G), among five species of western ranid frogs and the bullfrog, R. catesbeiana

Sequence divergence between	*aurora*	*draytonii*	*cascadae*	*muscosa*	*boylii*
aurora	0–0.035				
draytonii	0.16–0.24	0–0.03			
cascadae	0.08–0.15	0.16–0.24	0–0.05		
muscosa	0.12–0.19	0.11–0.17	0.13–0.22	0–0.03	
boylii	0.27–0.46	0.22–0.36	0.28–0.38	0.26–0.41	0–0.04
catesbeiana	0.22–0.27	0.25–0.29	0.26–0.33	0.20–0.23	0.35–0.47

divergence between two *R. a. aurora* and *R. cascadae* sequences) to 0.463 substitutions/site (the greatest divergence between *R. a. aurora* and *R. boylii*). Corrected sequence divergence to the more distant outgroup *R. catesbeiana* were also large, ranging from 0.205 to 0.466 substitutions/site.

Phylogeny

Maximum parsimony and bootstrap analyses of the full data set (107 sequences, a maximum of 397 characters, all sites weighted equally, tree-bisection–reconnection (TBR) branch swapping, full heuristic searches, 775 bootstrap replicates with 10 random addition replicates per bootstrap replicate) revealed several well-supported clades (Fig. 2), including: (1) *R. a. aurora*, including all populations from southern Mendocino County, Ca, USA north to British Columbia, Canada; (2) *R. cascadae*; (3) *R. a. draytonii*, including all populations from southern Mendocino County, CA, USA south to Baja California, Mexico; (4) *R. muscosa*; and (5) *R. boylii*. These results also demonstrate a sister-group relationship between *R. a. aurora* and *R. cascadae* to the exclusion of *R. a. draytonii* (BP = 93), and therefore that (*aurora* + *draytonii*) is not a monophyletic group. Finally, although most branches within these clades are weakly supported, the clade including the southern-most populations from Baja California, Mexico (population 50) and the Santa Rosa Plateau, CA, USA (population 49) is strongly supported (BP = 98, Fig. 2).

To examine relationships further among these five major clades, and particularly the apparent nonmonophyly of (*aurora* + *draytonii*), we conducted bootstrap likelihood searches on a subset of the 47 unique sequences in the analysis. We chose 15 sequences, including three representatives (two for *R. cascadae*) that span the sequence divergence in each of the five major groups (Fig. 3) to conduct bootstrap likelihood searches (100 pseudoreplicates). This analysis also strongly supported the monophyly of each individual taxon and the monophyly of the (*R. a. aurora* + *R. cascadae*) clade (BP = 96), although all other relationships were weakly supported among the five primary taxa.

Finally, we performed parametric bootstrap analysis to test the hypothesis that *Rana a. aurora* and *R. a. draytonii* are sister taxa (Hillis *et al*. 1996b; Huelsenbeck *et al*. 1996a; Huelsenbeck *et al*. 1996b). We used the full data set of the 47 unique sequences to search for a model tree, in which *aurora* + *draytonii* formed an exclusive clade. We used this tree and parameters estimated from it and the data set to simulate 1000 matrices in MESQUITE (Maddison & Maddison 2003), with the model of evolution corresponding to HKY + G. We conducted two parsimony tree-searches on each of these matrices: with and without the constraint of an exclusively monophyletic (*aurora* + *draytonii*), and compared the differences in constrained and unconstrained treelengths between the simulated and original data. The treelength of a monophyletic *aurora* + *draytonii* was significantly longer ($P << 0.01$) than expected if these two taxa did form an exclusive clade, leading us to reject the hypothesis that *R. a. aurora* and *R. a. draytonii* are sister taxa.

The aurora/draytonii *contact zone*

As sequence analysis progressed during the course of this study, we supplemented our sampling effort to determine the approximate width of the contact zone, and identify any biogeographical barriers that may be separating the two taxa. We found that *aurora* and *draytonii* overlap over a several-km region south of Elk Creek in southern Mendocino County, CA, USA (Fig. 1). We found only pure *aurora* from Big River (localities 13/14 and 15) north, only pure *draytonii* from Mills Creek (locality 21, 9.1 km SE Elk) south, and *draytonii* and *aurora* interspersed in between. In this area of overlap (about 5 km in extent), we found both types (of two individuals sequenced) in one pond at locality 17 (5.3 km SSE of Elk), *aurora* at localities 16 (3.2 km SSE of Elk), 19 (8.5 km ESE of Elk) and 20 (8.3 km SSE of Elk) and *draytonii* at locality 18 (7.1 km SSE of Elk). Because we sequenced only one or a few individuals per site, we cannot say whether the zone of overlap is restricted to this 5-km area, or describe the breeding dynamics within the overlap zone. However, our survey does indicate that the mtDNA contact zone between *aurora* and *draytonii* is narrow and does not correspond with any obvious habitat barriers to gene flow.

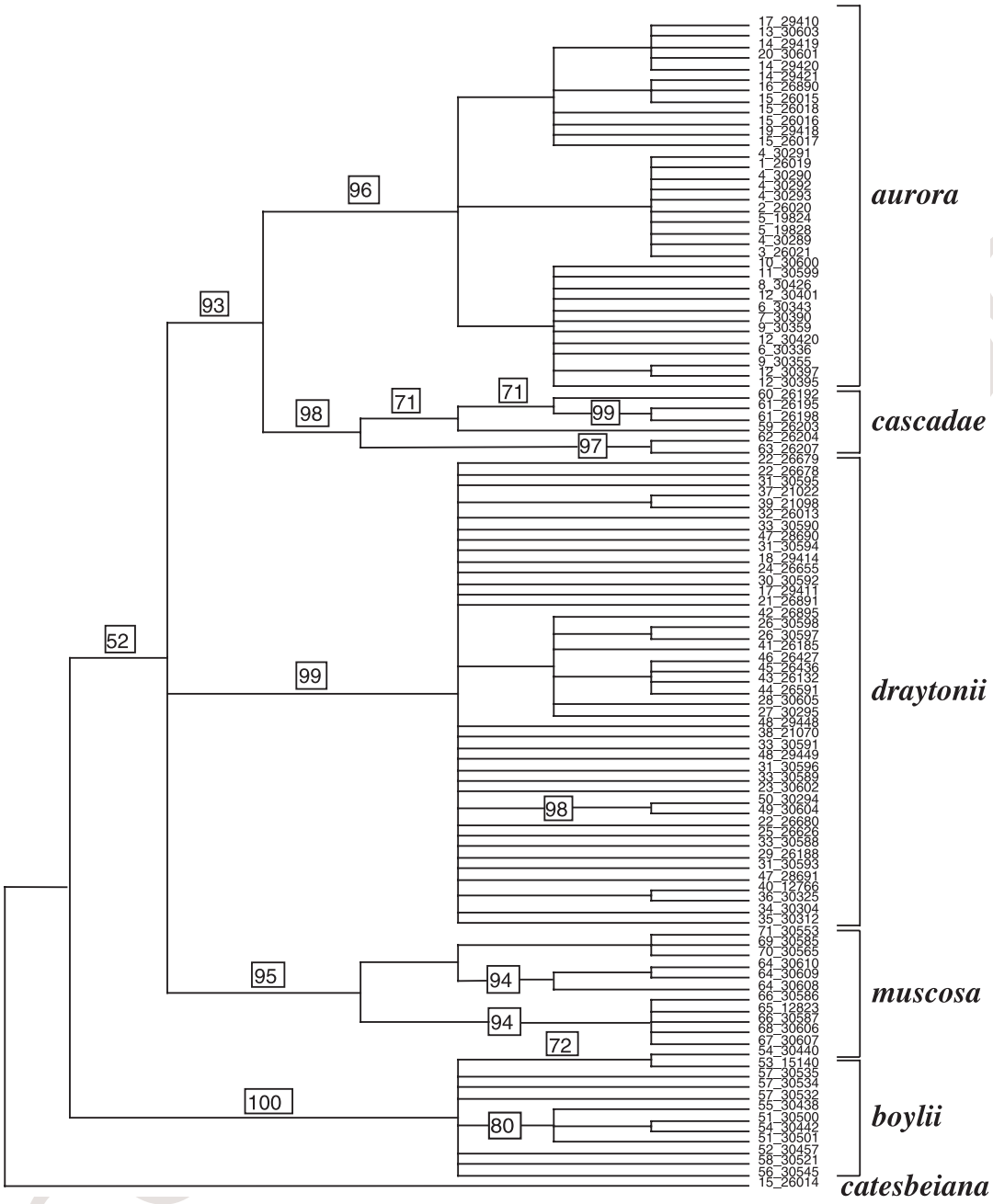

Fig. 2 Maximum parsimony bootstrap phylogeny for *R. aurora* plus outgroups. The tree is based on 775 pseudoreplicates for 107 full-length sequences. Numbers in boxes are bootstrap proportions. Specimen identification numbers at the tips of the tree are locality number_HBS number; for example, 17_29140 is from population 17, HBS 29140.

Discussion

Species boundaries in the R. aurora *complex*

Several papers published in the last two decades have provided evidence relevant to defining species boundaries in the *R. aurora* complex. These data include allozymes (Hayes & Miyamoto 1984; Green 1986b), karyotypes (Green 1985, 1986a), vocal sac structure (Hayes & Kremples 1986) and body size, calling and oviposition behaviour (Hayes & Miyamoto 1984); our mtDNA data add a new, detailed dimension to this information. Published data indicate that most populations of *aurora* and *draytonii* are diagnosably distinct taxa, but previous workers have disagreed on the extent of intergradation and whether the data indicate that the two should be considered separate species (Hayes &

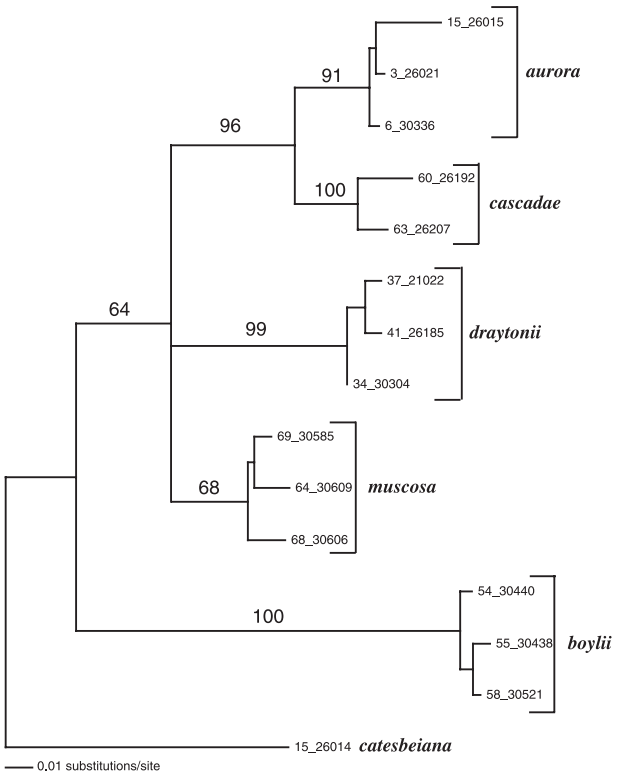

Fig. 3 Maximum likelihood bootstrap tree for *R. aurora* plus outgroups. The tree is based on 100 pseudoreplicates for 15 representative ingroup and outgroup taxa using full-length sequences. Numbers along branches are bootstrap proportions. Specimen identification numbers as in Fig. 2.

Miyamoto 1984; Hayes & Kremples 1986) or are best considered subspecies exhibiting clinal variation (Green 1985). Our mtDNA data support separate species recognition, based on (1) the relatively deep differentiation [characteristic of vertebrate species, see (Avise & Walker 1999)] and reciprocal monophyly of *aurora* and *draytonii*, and (2) the apparent sister-group relationship of *aurora–cascadae* to the exclusion of *draytonii*.

Separate species status for *aurora* and *draytonii* was proposed originally by Baird & Girard (1852), and accumulating evidence favours that interpretation. All evidence indicates that over most of their respective ranges, *aurora* and *draytonii* are biologically quite different frogs. Average allozyme differentiation is reasonably large between the two taxa, and this has been replicated in two relatively small studies with Nei's D_n = 0.123 (Green 1986b) and 0.151 (Hayes & Miyamoto 1984). These values are at the characteristic intra-/interspecific boundary identified by Highton (D_n = 0.15) for species recognition (Highton 1990; Sites & Marshall 2003). Green's study is the larger of the two (four native and one introduced population), and he found 'a general north–south pattern of divergence, with a break between samples representative of the two subspecies' (Green 1986b: 293). However, Macey *et al*. (2001) reanalysed Green's allozyme data and found that the data yielded low bootstrap support for the monophyly of the two populations of *draytonii* (from two nearby localities in Contra Costa County, CA) and no support for the monophyly of the two populations of *aurora* (from Mendocino County, CA and Vancouver, BC). An extensive analysis of vocal sac variation (a morphological character associated sometimes with mate recognition in anurans) in 280 adult frogs indicated that frogs from San Francisco, CA south to Baja California, Mexico have paired vocal sacs, frogs from Del Norte County, CA north to BC, Canada lack vocal sacs, and frogs from the intervening 480 km often have an 'intermediate' condition of asymmetric or rudimentary vocal sacs (Hayes & Kremples 1986). Because of the presumed biological importance of the vocal sac and its covariation with calling behaviour and body size (*draytonii* have paired vocal sacs, typically call in the air, and are large, whereas *aurora* lack vocal sacs, call mainly under water and are smaller), Hayes and Kremples suggested that *aurora* and *draytonii* are biologically divergent taxa that may represent separate species. Their hypothesis that the broad zone of vocal sac heterogeneity reflects past natural and human-mediated hybridization (Hayes & Kremples 1986) appears to be at odds with the narrow zone suggested by our mtDNA results, as such hybridization would lead presumably to a widespread mixture of haplotypes in far northern California. In addition, vocal sacs and slits can be labile evolutionarily; among the eight species in the *R. palmipes* group, some are fixed for the presence of vocal sacs and slits, others lack vocal sacs and slits and both *R. vaillanti* and *R. palmipes* (which are sister species) are variable for the presence of both features (Hillis & de Sá 1988). Thus, the biological role of vocal sacs and slits and their importance as diagnostic features may vary, even among closely related frog species, casting some doubt on the relevance of these features for species and hybrid identification in the *R. aurora* complex.

Assuming that our mtDNA phylogeny reflects the correct order of speciation events, the first split in the red-legged frog complex was between northern (*aurora, cascadae*) and southern (*draytonii*) frogs, with a more recent split between the northern frogs from the coast range (*aurora*) and the interior cascade mountains (*cascadae*). Experimental crosses utilizing hand-fertilizations between allopatric Oregon populations of *aurora* and *cascadae* demonstrate that complete postzygotic reproductive isolation has evolved, with a species-specific asymmetry in the mode of reproductive isolating mechanism (Porter 1961). In the single experimental cross that has been published between *cascadae* eggs and *draytonii* sperm, only one of 177 fertilized eggs survived to metamorphosis (Zweifel 1955). Unfortunately, no crossing data are available between *draytonii* and *aurora*, although the evolution of essentially complete

postzygotic isolation between *aurora* and *cascadae*, and *draytonii* and *cascadae* may imply that *aurora* and *draytonii* are similarly isolated reproductively.

Biogeography

One of the most compelling results from comparative phylogeographical analyses is the occasional geographical concordance of clade boundaries across diverse taxa (Remington 1968; Redenbach & Taylor 2002). In western North America, phylogeographical and biogeographical analyses have focused recently at very broad geographical scales (Soltis *et al*. 1997; Zink *et al*. 2001), as well as more fine-scale analyses within California (Calsbeek *et al*. 2003). Although most of these studies are based on relatively sparse population sampling, two general patterns appear to be emerging regarding primary phylogeographical splits along the Pacific coast. One, identified recently as the most general pattern in California (Calsbeek *et al*. 2003) is a north–south break centred on the Transverse ranges separating southern from central California. Second, a number of studies (Barrowclough *et al*. 1999; Rodriguez-Robles *et al*. 1999; Conroy & Cook 2000; Bronikowski & Arnold 2001; Maldonado *et al*. 2001; Rodriguez-Robles *et al*. 2001) have identified a deep phylogeographical break in northern California, often at or north of San Francisco Bay. Several amphibian species demonstrate either species-level distributional breaks in southern Mendocino or northern Sonoma counties (Good 1989), or the southern-most range limit of a northerly distributed species in this immediate area (*Rhacotriton variegatus*, *Ambystoma gracile*, *Ascaphus truei* and *Aneides vagrans*; http://elib.cs.berkeley.edu/aw/). Assuming that the gene trees presented in these studies represent the history of species, the contact zone area between *aurora* and *draytonii* coincides generally with the northern California contact zone, and precisely with the southern distributional limit for many (but by no means all) species of amphibians in the Pacific Northwest. As comparative phylogeographical and range limit studies continue to accumulate, we may be able to postulate mechanistic hypotheses to account for this strong pattern of concordant range boundaries. However, the concordance among species is compelling evidence that the *aurora*/*draytonii* break seen in our mtDNA data reflects the history of the species and not just the mitochondrion (Irwin 2002).

Conservation

The previous taxonomic interpretation of *R. aurora*/*draytonii* implied that there were large geographical areas that contain pure populations of each taxon, and a broad intergradation zone where they meet. Because of this, the USFWS has recognized three classes of red-legged frog populations: pure *draytonii* (which are protected under the US Endangered Species Act), pure *aurora* (which are not protected) and intergrade populations (which are also not protected) (United States Fish & Wildlfe Service 2002). Based on mtDNA, our data indicate that most populations that were considered to be intergrades are not — some are pure *draytonii* and some pure *aurora*. It is always possible that our results reflect mtDNA dynamics rather than evolutionary history and genetic interactions of the entire genomes of these frogs (Hudson & Coyne 2002; Shaw 2002), and we are testing nuclear markers to determine the placement of a nuclear contact zone (Shaffer, Fujita and Picco, unpublished). However, assuming that the mtDNA reflects the history of the organisms, it appears that the potential zone of intergradation/hybridization is much more narrowly circumscribed and that *draytonii* extends about 100 km further north than thought previously. If this result is confirmed with nuclear markers, it suggests that the species and conservation status of frog populations in northern California may require adjustment.

A final conservation issue centres on the *draytonii* populations from the extreme southern portion of the range. In California south of Los Angeles, a single *draytonii* population still persists on the Santa Rosa Plateau in Riverside County (population 49; Appendix I). Populations of *draytonii* in southern California have declined precipitously (Davidson *et al*. 2001; Davidson *et al*. 2002), and since 2000 the Santa Rosa Plateau population has been reduced to a total of three adult males (R. Smith, personal communication). Managers have considered the possibility of captive breeding and repatriation (R. Smith, personal communication), but there are questions about what populations might best serve as a source. Although based on relatively sparse sampling, and only on mtDNA, the Santa Rosa Plateau frogs appear to be related most closely to the geographically distant populations from the Sierra San Pedro Martir of northern Baja California (population 50, 322 km south-southwest of population 49), rather than the geographically closest ones from Los Angeles and Ventura counties (populations 47 and 48, 150 and 161 km northwest, respectively). This southern clade consisting of populations (49, 50) is strongly supported (98% BP, Fig. 2), reflecting the relatively large (0.016–0.026 substitutions/site) sequence divergence between populations 49 and 50 and all other *draytonii*. We acquired material recently from three new populations from northern Baja California (11.7, 41.7 and 66.8 km east of population 50), and they were identical to populations 49/50, confirming that the Baja/Santa Rosa Plateau relationship extends across the region. Assuming that phylogenetic diversity reflects ecological differentiation (which has not been demonstrated in these animals), our results suggest that the geographically distant populations from Baja California may be appropriate candidates for reintroductions to the Santa Rosa Plateau.

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This work was carried out as part of a multispecies project on the genetics of declining amphibian species in California. Brad Shaffer's laboratory group has research interests in amphibian genetics, conservation and systematics; the molecular work for this project was conducted while Voss, Oliver and Pauly were part of that laboratory group. Randal Voss's primary research interests are in amphibian genomics and population genetics. Jeff Oliver and Greg Pauly are pursuing their PhD research on insects and amphibians, respectively. Gary Fellers and his research team conducted some of the field work for this project; the remainder was conducted by members of the Shaffer laboratory.

Appendix I

Locality data for all samples of *R. aurora* and three outgroup taxa. The *aurora* samples are arranged in a roughly north–south numerical sequence

Rana aurora	
1. HBS 26019	Prospect Lake Road., Vancouver Island, British Columbia, Canada. 48 30 5 N 123 26 34 W
2. HBS 26020	Sooke Reservoir, Vancouver Island, British Columbia, Canada. 48 33 4 N 123 42 14 W
3. HBS 26021	Fork Lake, Vancouver Island, British Columbia, Canada. 48 31 9 N 123 29 8 W
4. HBS 30289, 30290, 30291, 30292, 30293	Grass Lake, Olympia, Thurston Co., WA. 47 3 17 N 122 57 23 W
5. HBS 19824, 19828	Saunders Lake off Hwy 101, Coos Co., OR. 43 32 9 N 124 13 0 W
6. HBS 30336, 30343	Redwood Creek oxbow at Redwood National Park Visitor Center, 2.4 km (air) W of Orick, Humboldt Co., CA. 41 17 10 N 124 5 17 W
7. HBS 30390	Strawberry Creek Pond, 0.4 km (air) S of Orick, Redwood National Park, Humboldt Co., CA. 41 16 54 N 124 3 35 W
8. HBS 30426	Prairie Creek, 3.4 km (air) NNE of Orick, Redwood National Park, Humboldt Co., CA. 41 18 49 N 124 2 39 W
9. HBS 30355, 30359	1.25 km (air) S of Orick, Redwood National Park, Humboldt Co., CA. 41 16 32 N 124 4 4 W
10. HBS 30600	Centerville Road., 4.5 km (air) ENE of Ferndale, Humboldt Co., CA. 40 34 55 N 124 19 7 W
11. HBS 30599	Arcata Marsh at the foot of I St., Arcata, Humboldt Co., CA. 40 51 42 N 124 5 42 W
12. HBS 30395, 30397, 30401, 30420	South side of Redwood Creek, 1.6 km (air) NE of Orick, Redwood National Park, Humboldt Co., CA. 41 17 49 N 124 2 42 W
13. HBS 30603 (collection 1)	Unnamed pond 50 m SE of junction of Hwy 1 and North Big River Road., Mendocino, Mendocino Co., CA. 39 18 15 N 123 47 27 W
14. HBS 29419, 29420, 29421 (collection 2)	Unnamed pond 50 m SE of junction of Hwy 1 and North Big River Road., Mendocino, Mendocino Co., CA. 39 18 15 N 123 47 27 W
15. HBS 26015, 26016, 26017, 26018	Beaver pond adjacent to Little North Fork, Mendocino Woodlands Outdoor Center, 0.3 km (air) NE of confluence with Big River, 8.4 km (air) ENE of Mendocino, Mendocino Co., CA. 39 18 59 N 123 42 10 W
16. HBS 26890	Hwy 1 at Elk Creek, 3.2 km (air) SSE of Elk, Mendocino Co., CA. 39 6 7 N 123 42 5 W
17. HBS 29410, 29411	Unnamed stock pond, 5.3 km (air) SSE of Elk, Mendocino Co., CA. 39 5 2 N 123 42 8 W
18. HBS 29414	Unnamed stock pond, 7.1 km (air) SSE of Elk, Mendocino Co., CA. 39 4 8 N 123 41 31 W
19. HBS 29418	Morrison House Pond, 8.5 km (air) ESE of Elk, Mendocino Co., CA. 39 7 26 N 123 37 3 W
20. HBS 30601	Hwy 1 at mile marker 27.75, 8.3 km (air) SSE of Elk, Mendocino Co., CA. 39 3 32 N 123 41 13 W
21. HBS 26891	Hwy 1 at Mills Creek, 9.1 km (air) SSE of Elk, Mendocino Co., CA. 39 3 4 N 123 41 1 W
22. HBS 26678, 26679, 26680	Pomo Lake, 0.55 km W of Hwy 1 on Pomo Lake Dr, 5.9 km (air) N of Manchester, Mendocino Co., CA. 39 1 24 N 123 40 54 W
23. HBS 30602	Hwy 1, 0.3 km S of Alder Creek, 2.9 km (air) N of Manchester, Mendocino Co., CA. 38 59 47 N 123 41 21 W
24. HBS 26655	Lagoon at Manchester Beach State Park, 2.3 km (air) NW of Manchester, Mendocino Co., CA. 38 59 17 N 123 42 2 W
25. HBS 26626	Unnamed creek draining into Hathaway Creek, 50 m W of Hwy 1, 4.3 km (air) SW of Manchester, Mendocino Co., CA. 38 56 5 N 123 42 30 W
26. HBS 30597, 30598	Hughes Place Pond, 1.9 km (air) NE of North Fork Feather River, 9.0 km (air) N of Madrone Lake, Plumas NF, Butte Co., CA. 39 43 42 N 121 24 3 W
27. HBS 30295	Pond adjacent to Little Oregon Creek, 7.6 km (air) SE of Challenge, Yuba Co., CA. 39 25 47 N 121 10 27 W
28. HBS 30605	Spivey Pond, 2.1 km (air) SW of Pollock Pines, El Dorado Co., CA. 38 44 44 N 120 35 53 W
29. HBS 26188	Western Dr, 0.5 km E of Chileno Valley Road., 2.5 km (air) W of Petaluma, Sonoma Co., CA. 38 13 32 N 122 39 58 W
30. HBS 30592	Unnamed pond, 0.2 km (air) SE of junction of Hwy 1 and Salmon Creek, 2.2 km (air) NW of the town of Bodega Bay, Sonoma Co., CA. 38 21 1 N 123 3 30 W
31. HBS 30593, 30594, 30595, 30596	Unnamed pond, 0.3 km (air) SE of junction of Hwy 1 and Salmon Creek, 2.2 km (air) NW of the town of Bodega Bay, Sonoma Co., CA. 38 21 6 N 123 3 34 W
32. HBS 26013	University of California Bodega Marine Laboratory, 2.6 km (air) SW of the town of Bodega Bay, Sonoma Co., CA. 38 19 2 N 123 4 10 W
33. HBS 30588, 30589, 30590, 30591	Ludson Marsh, Annadel State Park, 2.2 km (air) SE of Bennett Mtn., Santa Rosa, Sonoma Co., CA. 38 24 31 N 122 35 59 W
34. HBS 30304	Pond S of Abbotts Lagoon Trail, 7.7 km (air) NW of Inverness, Point Reyes National Seashore, Marin Co., CA. 38 7 22 N 122 56 17 W
35. HBS 30312	Unnamed pond 0.3 km N of Sir Frances Drake Hwy, 6.6 km (air) ESE of Inverness, Point Reyes National Seashore, Marin Co., CA. 38 5 41 N 122 55 44 W
36. HBS 30325	Unnamed pond on C Ranch, 1.7 km SW of junction of Sir Frances Drake Hwy and Drakes Beach Road., Point Reyes National Seashore, Marin Co., CA. 38 1 59 N 122 58 40 W
37. HBS 21022	Pescadero Road., 0.4 km (air) E of Hwy 1, 2.7 km (air) ENW of Pescadero, San Mateo Co., CA. 37 15 29 N 122 24 33 W
38. HBS 21070	Hwy 1, 0.075 km N of mile marker SCR24.04, 9.8 km (air) SW of junction of Hwy 1 and Hwy 17 in Santa Cruz, Santa Cruz Co., CA. 36 58 22 N 122 7 41 W

Appendix I *Continued*

39. HBS 21098	Hwy 1, 70 m S of Scott Creek, 19 km (air) WNW of junction of Hwy 1 and Hwy 17 in Santa Cruz, Santa Cruz Co., CA. N. 37 2 24 N 122 13 43 W
40. HBS 12766	Pond at Henry Coe State Park, 7.8 km (air) ENE of Anderson Lake dam, Santa Clara Co., CA. 37 11 11 N 121 32 44 W
41. HBS 26185	Colander Pond, 15 km (air) SE of Carmel on Rancho San Carlos Road., Monterey Co., CA. 36 27 29 N 121 48 3 W
42. HBS 26895	Blomquist Pond, 18 km SE (air) town of Carmel Valley, adjacent to Hastings Natural History Reservation, Monterey Co., CA. 36 23 8 N 121 33 24 W
43. HBS 26132	San Simeon Beach State Park, 5.2 km (air) NW of Cambria, San Luis Obispo Co., CA. 35 35 43 N 121 7 32 W
44. HBS 26591	Vandenberg Air Force Base, pond 91, Santa Barbara Co., CA. 34 48 34 N 120 34 51 W
45. HBS 26436	Vandenberg Air Force Base, pond 48, Santa Barbara Co., CA. 34 41 23 N 120 33 49 W
46. HBS 26427	Vandenberg Air Force Base, pond 47, Santa Barbara Co., CA. 34 48 6 N 120 34 2 W
47. HBS 28690, 28691	East Las Virgenes Creek, Ahmanson Ranch, 2.9 km (air) N of Brents Junction, 13 km (air) E of Thousand Oaks, Ventura Co., CA. 34 10 27 N 118 41 56 W
48. HBS 29448, 29449	San Francisquito Creek, in San Francisquito Canyon, 8.8 km (air) NE of Castaic Lake dam, Los Angeles Co., CA. 34 32 45 N 118 30 59 W
49. HBS 30604	Owl Pool, Cole Creek, Santa Rosa Plateau Ecological Reserve, 5.6 km (air) SE of Murrieta, Riverside Co., CA. 33 31 51 N 117 16 8 W
50. HBS 30294	Upper Rio San Telmo drainage, NW part of the Sierra San Pedro Martir Mountains, Estado Baja California, Mexico. 30 58 18 N 115 41 50 W
Rana boylii	
51. HBS 30500, 30501	Redwood Creek at junction with Bond Creek, 6.6 km (air) SE of Orick, Redwood National Park, Humboldt Co., CA. 41 14 1 N 124 1 16 W
52. HBS 30457	Redwood Creek at junction with Tom McDonald Creek, 9.7 km (air) SE of Orick, Redwood National Park, Humboldt Co., CA. 41 12 23 N 124 0 40 W
53. HBS 15140	Wheatfield Fork, Gualala River, at intersection of Skaggs Springs and Annapolis Rds., 7.6 km (air) ENE of Stuarts Point, Sonoma Co., CA. 38 39 56 N 123 18 49 W
54. HBS 30440, 30442	Halleck Creek, 3.1 km (air) NE of Nicasio, Marin Co., CA. 38 4 35 N 122 40 8 W
55. HBS 30438	Nicasio Creek, 1.0 km (air) SE of town of Nicasio, Marin Co., CA. 38 3 15 N 122 41 36 W
56. HBS 30545	Coyote Creek, upstream from Natural Bridge, 5.6 km (air) SE of Angels Camp, Calaveras Co., CA. 38 3 18 N 120 28 37 W
57. HBS 30532, 30534, 30535	Orestimba Creek, 17.3 km (air) WSW of Newman, Stanislaus Co., CA. 37 17 34 N 121 12 50 W
58. HBS 30521	Arroyo Leona Creek, 27 km (air) NW of junction of Hwy 5 and route 145, Fresno Co., CA. 36 23 40 N 120 32 15 W
Rana cascadae	
59. HBS 26203	Clear Lake, c. 8 km SE of Sol Duc Hot Springs, Clallam Co., WA. 47 55 13 N 123 46 45 W
60. HBS 26192	Pond at fork of trails to Hidden Lake and Upper Palisades Lake, c. 1.6 km north of Clover Lake, NE of Sunrise, Pierce Co., WA. 46 56 25 N 121 35 40 W
61. HBS 26195, 26198	South end of Waldo Lake, ENE of Heather, Lane Co., OR. 43 40 54 N 122 3 41 W
62. HBS 26204	Jake Spring, 5.8 km (air) W of Lake Britton dam on Pit River, Shasta Co., CA. 41 1 21 N 121 44 45 W
63. HBS 26207	Colby Creek, 1.9 km (air) NW of Jonesville, Tehama Co., CA. 40 7 7 N 121 29 13 W
Rana muscosa	
64. HBS 30608, 30609, 30610	Unnamed pond 0.8 km (air) W of Roosevelt Lake, Yosemite National Park, Tuolumne Co., CA. 37 58 14 N 119 20 42 W
65. HBS 12823	Hwy 120 at Dry Creek, 1.9 km W of Sagehen Summit, 22 km (air) SW of Lee Vining, Mono Co., CA. 37 52 54 N 118 53 6 W
66. HBS 30586, 30587	Mono Meadow, 6.2 km (air) SSW of Glacier Point, Yosemite National Park, Mariposa Co., CA. 37 40 30 N 119 34 57 W
67. HBS 30607	Unnamed lake, 2.3 km (air) SW of Merced Peak, Yosemite National Park, Tulare Co., CA. 37 37 23 N 119 24 57 W
68. HBS 30606	Stream draining out of unnamed lake 2.3 km (air) SW of Merced Pass, Yosemite National Park, Tulare Co., CA. 37 37 27 N 119 25 6 W
69. HBS 30585	Pools N of Golden Bear Lake, Center Basin, Sequoia National Park, Tulare Co., CA. 36 43 48 N 118 21 36 W
70. HBS 30565, 30572	Unnamed ponds 2.2 km (air) ESE of Mt. Jordan, Upper Kern River drainage, Sequoia National Park, Tulare Co., CA. 36 40 47 N 118 25 31 W
71. HBS 30553	Unnamed pond 4.8 km (air) ENE of Table Mountain, Upper Kern River drainage, Sequoia National Park, Tulare Co., CA. 36 39 46 N 118 25 14 W
Rana catesbeiana	
15. HBS 26014	Beaver pond adjacent to Little North Fork, Mendocino Woodlands Outdoor Center, 0.3 km (air) NE of confluence with Big River, 8.4 km (air) ENE of Mendocino, Mendocino Co., CA. 39 18 59 N 123 42 10 W

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