## **Title:** Genomic correlates of disease recovery in natural populations of mountain yellow-legged frogs

**Running Title**: Genomics of recovery in mountain yellow-legged frogs

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#### **Abstract**

In our rapidly changing world, evolution is likely to play an important role in facilitating the resilience of wildlife populations. The mountain yellow-legged frog *(Rana muscosa/Rana sierrae*) provides a rare example of recovery following severe declines caused by the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). However, the role of evolution in facilitating this recovery remains circumstantial. In this study, we sought to gain insights into the potential role of evolution by comparing genomes of frogs from naïve and recovering populations located in relatively close proximity. Using multiple methods to scan frog genomes for signatures of selection, our study reveals several genomic variants associated with frog recovery. Specifically, we identify gene variants in interferon-related genes and genes associated with the complement system and major histocompatibility complex (MHC). Additionally, we identify a recovery-associated variant in RIN3, a gene that may play a critical role in disease defense and wound healing. Finally, we report no differences in genetic diversity between naïve and recovering populations. We provide a rare example from natural populations that suggests that evolution can produce individuals that harbor adaptive alleles and allow population recovery in a novel environment. These findings complement recent research on amphibian immune evolution and provide mechanistic hypotheses for how individuals from populations can recover from disease.

**Introduction**

Species across the tree of life are increasingly threatened with extinction. For example, an estimated 48% of animal species are currently declining (Finn et al., 2023), and models predict an accelerated rate of extinction in the next century as the effects of climate and other global change stressors intensify (Pereira et al., 2024). To persist, affected taxa will often need to adapt to their altered environment or face further decline and possible extinction. The process whereby rapid evolutionary change increases the frequency of adaptive alleles and restores positive population growth is known as “evolutionary rescue” (Carlson et al., 2014; Gomulkiewicz & Holt, 1995). During this process individuals with favorable traits within a declining population can persist and reproduce in the face of ongoing disturbance, passing on favorable characteristics to their offspring and therefore staving off extinction. In addition to preventing the extinction of declining populations, individuals from rescued populations could also disperse, or be reintroduced, to empty habitats and promote larger-scale recovery. Although this overall scenario is potentially of critical importance for the conservation of an ever-growing number of imperiled taxa, descriptions of its occurrence and effectiveness in the wild are lacking.

One particularly important threat to wildlife is the increasing incidence of emerging infectious diseases (Fisher et al., 2012; Jones et al., 2008), which have gravely impacted a variety of taxa (e.g., Hewson et al., 2014; Wake & Vredenburg, 2008). Amphibians in particular have suffered massive declines from disease, mostly due to outbreaks of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Bd) (Longcore et al., 1999; Luedtke et al., 2023). Bd is a generalist fungal pathogen, infecting a wide variety of host species across all continents where amphibians are found (Olson et al., 2013). Bd can persist in aquatic environments for extended periods of time (Walker et al., 2007) and can be spread between water bodies by tolerant hosts (Burns et al., 2021), making it a particularly challenging threat to manage. While the impacts of Bd have been of great conservation concern in amphibian systems, amphibian recoveries have provided critical insights into what role rapid evolutionary change in populations can play in disease resilience.

Laboratory studies have investigated the host mechanisms underlying Bd survival, revealing that multiple pathways may exist for amphibian species to persist despite Bd exposure. Bd is known to actively suppress amphibian immune systems (Fites et al., 2013; Rosenblum et al., 2012), and some amphibians that mount robust immune response are highly susceptible (e.g., *Atelopus zeteki* , Ellison et al., 2014). However, elevated immune responses have been linked to lower Bd susceptibility in cascades frog (*Rana cascadae*) (Gervasi et al., 2014). While the early activation of the immune system was linked to higher survival in a long-exposed population of alpine tree frogs (*Litoria verreauxii alpina*) (Grogan, Cashins, et al., 2018). Additionally, certain major histocompatibility complex (MHC) alleles have been associated with survival in frogs challenged with Bd (Bataille et al., 2015; a. E. Savage & Zamudio, 2011). These findings indicate that there may be a specific timing, type, or intensity of immune response that is most effective against Bd. In addition to immune mechanisms, there are many other factors may contribute to Bd survival including behavioral avoidance (McMahon et al., 2014), the genotype of Bd infecting the host (McDonald et al., 2023), and/or the presence of certain reservoir species (Wilber et al., 2020). Therefore, while there is mounting evidence that the timing, intensity, and type of immune response is critical to Bd-related disease outcomes, other factors make understanding the mechanisms of resilience in natural systems challenging.

A quintessential example of the impacts of Bd can be seen in the mountain yellow-legged (MYL) frog, composed of the sister species *Rana muscosa* and *Rana sierrae* (Vredenburg et al., 2007). This frog was once the most common amphibian in high alpine regions of the Sierra Nevada mountains (Grinnell & Storer, 1924), but has been extirpated from more than 90% of its historical range due in part to widespread Bd outbreaks in the region (Vredenburg et al., 2007). The arrival and spread of Bd in the mid-1900s (Vredenburg et al., 2019) caused large-scale extirpations (Rachowicz et al., 2006; Vredenburg et al., 2010), resulting in the listing of both *R. sierrae* and *R. muscosa* as federally endangered. Since Bd can persist in aquatic habitats without amphibian hosts (Walker et al., 2007), it represents a long-term threat that MYL frogs will need to overcome to persist in this system.

Most Bd-naive populations of MYL frogs are extirpated once Bd arrives, however some populations have persisted after outbreaks (Briggs et al., 2010) and are now recovering (Knapp et al., 2016). MYL frogs in recovering populations have lower susceptibility to Bd (characterized by low infection intensities) in both natural populations and when infected with Bd in the lab (Briggs et al., 2010; Joseph & Knapp, 2018; Knapp et al., 2011, 2016). These finding indicate that MYL frog resistance against Bd infection is a host-specific characteristic and is not strictly due to environmental conditions. The observed resistance of MYL frogs could be the result of several mechanisms, including natural selection (Grogan, Cashins, et al., 2018; Savage et al., 2016), acquired immunity (Grogan et al., 2018), and/or between-population differences pre-dating Bd exposure. The possible evolution of MYL frog resistance and population recovery is consistent with that expected under an “evolutionary rescue” scenario, opening the door to conservation interventions such as reintroducing resistant frogs to vacant habitats (Joseph & Knapp, 2018; Mendelson III et al., 2019).

Critically, a recent study of *Rana sierrae* reported that frogs collected from naturally recovering populations and translocated into vacant habitats can reestablish populations despite the ongoing presence of Bd (Knapp et al., 2024). Furthermore, survival of reintroduced frogs differed between source population, suggesting that some populations may have traits (e.g., disease resistance) that confer higher fitness Given the importance of resistance for frog survival, population establishment, and long-term viability (Knapp et al., 2024), in this study we aim to determine whether MYL frogs in recovering populations show evidence of selection and whether these genomic changes are associated with disease resistance.

Comparisons of whole exome sequences from Bd-naïve and Bd-exposed recovering populations of MYL frogs allowed us to characterize the genetic diversity of each population and search for gene variants associated with recovering populations. Furthermore, by comparing genes identified in this study to the growing body of research on evolutionary responses to disease, our study adds to a more comprehensive understanding of how rapid evolutionary change can shape amphibian population resilience in disease-impacted systems and inform management strategies.

#### **Materials and Methods**

#### Study design

To determine whether MYL frog populations show genomic patterns consistent with an evolutionary response to Bd, we compared frog exomes (i.e., coding region of a genome) between populations with contrasting histories of Bd exposure. Specifically, we compared frog exomes sampled in 4 populations that have not yet experienced a Bd-caused epizootic (“naive”) (Zhou et al., 2015) versus in 5 populations that experienced a Bd epizootic during the past several decades and have since recovered to varying degrees (“recovering”)(Knapp et al., 2016; Vredenburg et al., 2010). Bd-exposure histories of the 9 study populations are based on 10-20 years of visual encounter surveys and Bd surveillance using skin swabbing (e.g., (Knapp et al., 2016; Wilber et al., 2022; Zhou et al., 2015). Naive populations are characterized by large numbers of adults (i.e, typically 1000s), Bd prevalence that is generally 0% except during occasional Bd failed invasions (during which Bd loads remain very low, (Wilber et al., 2022)), and no history of Bd epizootics since we first surveyed these populations in the late 1990s and early 2000s (Zhou et al., 2015). In contrast, recovering populations exist in an enzootic state (Briggs et al., 2010), characterized by smaller numbers of adults (generally < 500), high Bd prevalence (often > 80%, (Knapp et al., 2011)), and, in adults, moderate Bd loads that are typically well below the level expected to cause mortality (Vredenburg et al., 2010).

Comparing populations with different infection histories allowed larger sample sizes and replication across the landscape. The alternative approach of comparing samples from the same populations before and after Bd exposure is not feasible in this system because Bd arrived in most MYL frog populations decades ago and population persistence/recovery is rare and unpredictable. As a result, samples from recovering populations collected before and after Bd exposure are not available and are unlikely to be available in the future.

*Sample Collection and Sequencing*

We collected DNA samples via buccal swabbing (Goldberg et al., 2003) from 53 *Rana muscosa*/*Rana sierrae* individuals: 24 from 4 naive populations, and 29 from 5 recovering populations. These populations are located in the southern Sierra Nevada, from northern Yosemite National Park to northern Sequoia National Park (Figure 1a). Samples were collected from 5-6 frogs per population. To minimize potential confounding effects caused by variation in frog genotypes across latitude (Byrne et al., 2023), we selected sampling sites such that both population types were represented across similar latitudinal ranges and were often in close proximity.

DNA was extracted following Qiagen DNEasy manufacturer’s protocols. We sequenced the samples using an exome capture assay as described in (Byrne et al., 2023). Briefly, genomic libraries were prepared and captured using a custom Nimblegen capture pool. Capture baits were designed based on the coding regions of the *R. muscosa* transcriptome (GenBank accession GKCT00000000). Captured libraries were then pooled and sequenced on a NovaSeq 6000 150PE Flow Cell S1 at the Vincent J. Coates Genomics Sequencing Lab at UC Berkeley.

##### *Data pre-processing and cleaning*

Raw reads were filtered for adapters and contaminants using fastp (Chen et al., 2018) and aligned to the *Rana muscosa* genome (NCBI SRA: SRS6533475 (Hon et al., 2020)), with repetitive elements masked using bwa (“mem” mode) (Li, 2013). Exact PCR duplicates were marked using Picard (Broad Institute, 2019). Variants were then called following GATK best practices (v.4.2.0.0 (McKenna et al., 2010)). Briefly, raw variants were called for each sample using HaplotypeCaller and combined using CombineGVCFs. Next, genotypes were jointly called using GenotypeGVCFs. Variants were then hard filtered using gatk VariantFiltration using the following parameters to remove low-quality sites: QD < 2.0, FS > 40.0, SOR > 3.0, MQ < 50.0, MQRankSum > 3.0, MQRankSum < -3.0, ReadPosRankSum > 3.0, ReadPosRankSum < -3.0. This initial filter resulted in 1,595,206 variant sites across 53 individuals.

We then further filtered our dataset at the individual and variant level. First, we trimmed our variants to only include those with minor allele frequency > 0.03, a maximum depth of 250 and minimum depth of 5, a minimum genotype quality of 20, and a maximum missing proportion of 0.5. This filter resulted in 427,038 sites, of which 353,172 were SNPs and 73,866 were INDELS. Finally, we trimmed samples with an average depth across filtered sites < 7x (n = 3). Our final dataset included 50 samples, 23 from naive and 27 from recovering populations, with an average depth of 16.7x (range = 7.4x – 26.1x).

*Population genetic analyses*

First, to visualize the population genetic relationships between sampling sites we conducted a PCA using the glPCA function in the adegenet R package (Jombart, 2008) using the set of 427,038 SNPs described above. Next, to characterize general patterns of genetic diversity between naive and recovering populations, we conducted three analyses. First, we calculated heterozygosity for each sampled frog using VCFtools (Danecek et al., 2011). Second, to characterize genome-wide patterns of diversity, we used VCFtools to calculate nucleotide diversity () in 100kb sliding windows along the genome for each population. Third, we calculated average per population within each of the 9 outlier windows identified in the splined window analysis (see below).

*Identifying gene variants associated with recovering populations*

To detect regions of the genome that differed between naive and recovering populations, i.e., putative regions under selection, we used two approaches: (1) a multivariate linear mixed model to evaluate individual variants (SNPs and INDELs), and (2) a splined window analysis to evaluate larger genomic regions (Beissinger et al., 2015). For the variant analysis, we first used a stringent data filter to include only variants with < 5% missing data (missing for no more than 2 individuals), and then calculated the likelihood ratio statistic for the resulting set of 148,307 high quality variants across 127 contigs using GEMMA (Zhou & Stephens, 2014). GEMMA calculates and incorporates a relatedness matrix for input samples, allowing us to account for relatedness and population structure when calculating likelihood ratio statistics. We identified variants showing different allele frequencies between naive versus recovering populations (“outliers”) using a Bonferroni-corrected significance level of 0.01. We visualized the results using a Manhattan plot and qqplot. We developed a more liberal set of outlier variants using a Bonferroni-corrected significance level of 0.05 and used this set solely for the gene ontology (GO) analysis (see below; Dataset S1, S2).

In the splined window analysis, we identified outlier regions using *FST* and differences in nucleotide diversity () between naive and recovering populations. First, we calculated per-site *FST* between the naive and recovering individuals for all bi-allelic SNPs in the 30 largest contigs (98% of all SNPs) using VCFtools (Danecek et al., 2011). Next, we calculated per-site nucleotide diversity separately for individuals from the naive and recovering populations using VCFtools, then calculated for each population (). We concatenated the values for *FST* and in order of size-sorted chromosome number and adjusted the SNP position based on the relative position in the genome (for more efficient data processing and to better contextualize the strength of the outlier signals in different regions of the genome). We then used the GenWin R package (Beissinger et al., 2015) to conduct a splined discrete window analysis for *FST* and . This method calculates where non-overlapping window boundaries should occur by identifying inflection points in the spline fitted to *FST* and values along the genome, therefore balancing false positive and false negative results that occur using other window-based methods (Beissinger et al., 2015). This method also calculates a W-statistic allowing for outlier identification. We identified outliers as those with a W-statistic greater than 4 standard deviations above the mean for *FST* or above/below the mean for . These standard deviations represent strict criteria to select only the top ~ 0.3% of windows. Shared outliers were then identified as those that were outliers in both analyses, meaning that they showed (i) high differentiation between naive and recovering populations, and (ii) differential patterns of nucleotide diversity in the same region. Finally, we extracted gene transcripts mapped within each region and retrieved annotation for that region using BLAST (Datasets S3, S4).

#### GO analysis and predicted effect of outlier variants

Using the liberal set of outlier variants (identified using a Bonferroni-corrected p-value of 0.05, which included 38 outliers: 35 SNPs and 3 INDELS from 30 distinct genes across 16 contigs), we determined if any GO biological functions, molecular functions, or cellular processes were overrepresented. To do this, we retrieved the BLAST hits and mapped GO terms for each gene in our targeted transcriptome. We then conducted a statistical overrepresentation test (Fisher’s exact test) using Blast2GO (Götz et al., 2008) to compare the 30 unique outlier genes to the complete set of genes in our target transcriptome. We repeated this process for the set of 35 genes located in the 9 shared regions of the *FST* and splined windows.

In the analysis of individual variants, for each outlier variant we determined whether the variant was synonymous (protein sequence the same for each variant) or non-synonymous (protein sequence differs between variants), and where in the gene it was located. To do this, we first extracted the reference genome sequence surrounding the variant using the bedtools “getfasta” function (Quinlan & Hall, 2010). Next, we re-annotated each sequence using BLAST to get the predicted gene location based on the closest annotated reference (Altschul et al., 1997). We then translated each variant to amino acids and aligned this translation to that of the gene annotation to ensure proper frame of reference using Geneious (Kearse et al., 2012). After ensuring proper translation, we characterized variants as within or outside the coding sequence of the gene and as either synonymous or non-synonymous.

**Results**

Individual frogs clustered into 3 separate groups in the first two principal components (Figure 1b), and clusters reflected the species split (i.e., *R. muscosa* versus *R. sierrae*) and the strong signature of isolation-by-distance that is characteristic of MYL frogs (Byrne et al., 2023; Poorten et al., 2017; Rothstein et al., 2020). Importantly, each cluster contained at least one population from both the naive and recovering groups, allowing us to distinguish allelic associations of individuals sampled in the 2 population types versus allelic associations resulting from population structure and genetic drift. Due to the scarcity of naive populations, we could not conduct replicated analyses among pairs of geographically proximate persistent and naive populations. Instead, to obtain adequate power, we conducted a pooled analysis where we looked for genes with a strong and consistent signature of selection across population types.

Results from the individual variant and splined window analyses show that recovering populations have signatures of selection on multiple regions of the genome. The analysis of individual variants identified 11 “outlier” SNPs (i.e., showing significantly different allele frequencies between naive versus recovering populations) from 7 distinct genes across 4 contigs (Figure 1C,D) (see Supplementary Data). One of the 7 identified genes (LOC108802036) does not have an associated annotation. For the outlier SNPs, frequency differences between the naive and recovering populations ranged from 0.41 to 0.86. Most of these SNPs showed frequency differences in only a subset of the sampled populations ([Figure](#fig-allelefrequencies) 2 A,B), but the SNP in the RIN3 gene showed consistent differences in frequencies across all populations ([Figure](#fig-allelefrequencies) 2C). This is suggestive of parallel selection at this locus across multiple populations. The other 6 outlier variants showed less consistent frequency differences across the study populations, but for these we still found a statistically significant signal of selection in 2 of the 3 genetic clusters (containing populations 5–9; [Figure](#fig-allelefrequencies) 2). Therefore, although some outlier variant associations have a more limited geographic extent than RIN3, they still describe results that suggest parallel evolutionary changes following Bd exposure.

The splined window analysis identified 33 outlier regions for and 58 outlier regions for *FST* ([Figure](#fig-spline-manhattan) 3A, B). Of these, 9 regions were outliers for both metrics (“shared regions”) and 2 of these shared regions also contained one or more of the outlier SNPs described above. A total of 35 annotated genes were found in the 9 shared regions. Given this large number of genes, here we focus on those with the strongest signal of selection and/or immune-related functions. The largest , indicative of directional selection, occurred in a 163kb region on Contig19, 12.9Mb upstream of the RIN3 outlier SNP ([Figure](#fig-spline-manhattan) 3C). This region contains approximately 500 SNPs and one annotated gene called “interferon-induced very large GTPase 1-like” (GVINP1). Additionally, a shared outlier region on Contig1 contained two complement factor genes (C6 and C7). Interestingly, this region had a large negative value, consistent with balancing selection. Finally, one shared outlier region on Contig8 contained one outlier SNP (TCF19) and was within 360kb of another outlier SNP (VARS) (Figure 3D). This region (854kb from the beginning of the outlier window to the VARS SNP) contained a total of 8 annotated genes. In *Xenopus*, five of these genes occur in the extended major histocompatibility complex (MHC) Class I region (FLOT1, TUBB, MDC1, CCHCR1, TCF19) and three occur in the extended MHC Class III region (HSP70, LSM2, VARS) (Ohta et al., 2006). Therefore, this region under selection is part of the extended MHC Class I and III complex and shows synteny with other amphibian genomes (Figure 4).

Although the joint processes of Bd-caused population declines and selection in response to Bd exposure could affect genetic diversity of recovering populations, we found no consistent differences in individual-level heterozygosity or population-level between naive and recovering populations (Figure 5). Thus, despite localized selection in particular regions of the genome, we did not find evidence for reduced genetic diversity across the genome in recovering populations. In addition, no GO biological functions, molecular functions, or cellular processes were over-represented in either the outlier variants or the 35 genes located in the overlapping *FST* and splined windows.

In summary, our genomic results indicate that frogs from naive and recovering populations show differences at several immune-function loci, consistent with selection following Bd exposure. The regions under selection contain several immunologically-relevant genes and gene families that are directly linked to disease resistance in other taxa (see below).

**Discussion**

Results from our genomic analyses suggest that natural selection for adaptive alleles is at least partially responsible for the increased resistance of frogs in recovering populations. We identified multiple alleles and genomic regions showing signatures of selection between adjacent naive and recovering MYL frog populations, consistent with selection following Bd exposure. These analyses are based on samples collected from virtually all of the MYL frog populations remaining in a naive state, as well as adjacent recovering populations. This study design produced genetic clusters that each contained at least one naive and one recovering population, allowing us to infer selection while attempting to minimize the confounding effects of population structure. In addition, we did not find a reduction in overall genetic variation in the recovering populations, suggesting that despite localized selection in the genome, some of these populations likely retain adequate genetic diversity for long-term persistence.

Importantly, some genomic regions that we identified as under selection are associated with cellular and immunological mechanisms known to contribute to disease resistance, including in amphibians (Zamudio et al., 2020). For example, the MHC plays an important role in immunity. In our study, we identified a region that shows evidence of selection in recovering populations and contains eight genes associated with either the MHC Class I or Class III regions. These results corroborate numerous previous studies linking MHC genes to amphibian resistance against Bd (e.g., (Bataille et al., 2015; a. E. Savage & Zamudio, 2011)). Similarly, the region with the strongest indication of directional selection (as measured by ) contains the interferon-related gene GVINP1. Several previous studies of amphibians have found this gene to be differentially expressed during Bd infection (e.g., (Ellison, Tunstall, et al., 2014; Grogan, Cashins, et al., 2018)) and in populations differing in Bd susceptibility (Grogan, Cashins, et al., 2018). This gene is also strongly linked to amoebic gill disease in salmon, explaining a notable 20% of the resistance phenotype (i.e., gill damage and amoebic load as heritable host resistance traits (Robledo et al., 2018, 2020)). Finally, the outlier variant with the lowest p-value was the uncharacterized gene LOC108802036. In the genome of another frog species (*Nanorana parkeri*), this gene is located adjacent to a type I interferon gene (Np-IFNi2) (Gan et al., 2018), and together with GVINP1 further suggests the importance of interferon-related genes in this system. Therefore, our study contributes to a growing body of evidence that MHC and interferon genes play a critical role in amphibian responses to Bd.

We also identified a region, characterized by high *FST* and low , that contained the complement genes C6 and C7. The complement system plays an important role in innate immunity (Riera Romo et al., 2016), and our results could indicate that balancing selection is acting in this region of the genome to favor a diverse set of alleles, as is a known pattern for C6 in humans (Soejima et al., 2005). Based on the analysis of individual outlier variants, the RIN3 gene showed a consistent pattern of allele frequency differences across all nine of the frog populations sampled in this study (Figure 2c), indicating consistent selection in populations distributed across a wide geographic area. This gene is associated with immune response and in *Xenopus* is expressed during appendage regeneration (Fukazawa et al., 2009). Therefore, RIN3 could play a critical role in skin repair in Bd-damaged tissue. Collectively, the genes associated with these genomic differences may confer at least some degree of resistance against Bd infection, an attribute that may be critically important to population resilience and recovery in the presence of Bd.

It is important to note that our genomics work cannot provide a direct assessment of the strength or timing of natural selection at particular loci due to sampling constraints. As with many studies of highly endangered species, we do not have time-series data or a paired sampling design. However, comparing exome-wide signatures of selection between population types allow us to identify specific regions of the genome that merit further study for their potential contribution to survivorship in recovering populations.

In light of a recent complementary study documenting strong associations between source populations and the probability of successful reestablishment following MYL frog translocations despite the presence of Bd (Knapp et al., 2024) our findings provide key insights into the mechanisms that may be at work in this conservation success story. In addition to addressing fundamental questions regarding evolutionary responses to disease, our study has real-world conservation applications. For example, the ability to identify genomic correlates of Bd resistance in MYL frogs could enhance our ability to screen populations for predicted reintroduction success. Therefore, our study provides an example of how we can use genomics in wildlife conservation to better understand eco-evolutionary dynamics that have real-world applications.

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**Data Accessibility and Benefit-Sharing**

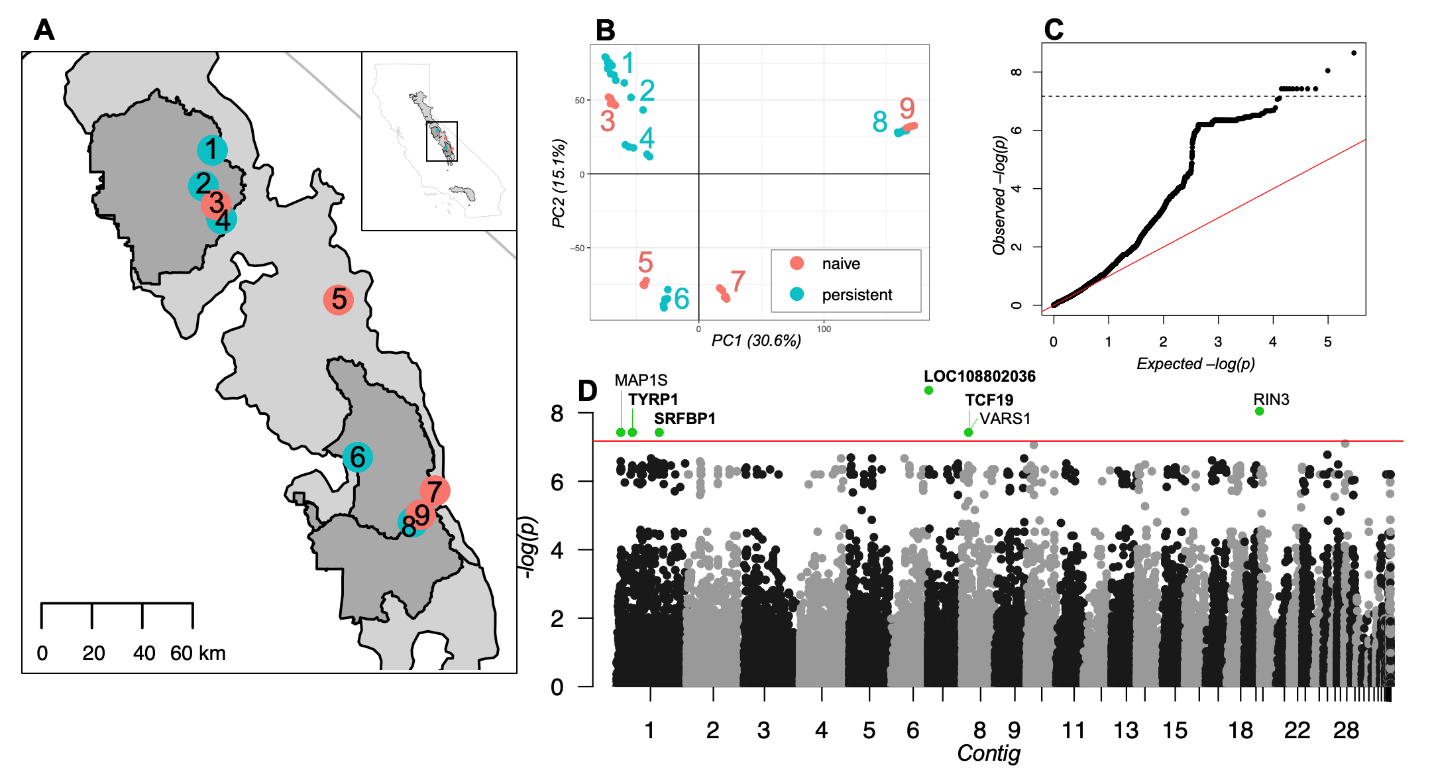
Raw sequencing reads are available from NCBI SRA (PRJNA870451). Code used for genomic analyses and to create figures is available from the following GitHub repository: <https://github.com/allie128/mylf-selection>.

Benefits Generated: Benefits from this research accrue from the sharing of our data and results on public databases as described above.

**Author Contributions**

AQB, EBR, APR, and RAK designed the study. APR produced the genetic data and AQB conducted the analyses. AQB, RAK, and EBR wrote the manuscript, and all authors provided helpful feedback.

**Figures**

Figure 1: (A) Map of sampling locations showing Bd-naïve populations in red and recovering populations in blue. Dark gray outlines represent Yosemite National Park in the north and Sequoia and Kings Canyon National Parks in the south. The light gray polygon shows the historical range of mountain yellow-legged frogs. (B) PCA calculated from binary SNPs showing the population genetic relationship of samples from each location in (A). Populations 1-7 are *R. sierrae* and populations 8 and 9 are *R. muscosa*. (C) qqplot showing observed and expected p-values for 148,307 SNPs and INDELS. Dashed line shows the p-value that identifies outliers. (D) Manhattan plot showing the p-value for each SNP. SNPs are sorted by genomic position and contigs are sorted by size. Red line shows the p-value that identifies outliers. Outlier SNPs above this threshold are highlighted and labeled. Bold labels indicate the presence of at least one non-synonymous SNP in that gene.

Chart, bubble chart

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| Figure 2: Evidence for selection on individual variants in recovering MYL frog populations at the landscape scale. For each of the 9 naive and recovering MYL frog populations (indicated by numbered points), adjacent pie charts show allele frequencies for the 11 outlier SNPs from 7 distinct genes in our GEMMA analysis: (A) LOC108802036, (B) TCF19, VARS, MAP1S, TYRP1, and SRFBP1, and (C) RIN3. Charts are superimposed on a map of the Sierra Nevada study area, with Yosemite, Kings Canyon, and Sequoia National Parks (from north to south) shown in dark gray, and the range boundary for MYL frogs shown in light gray. |

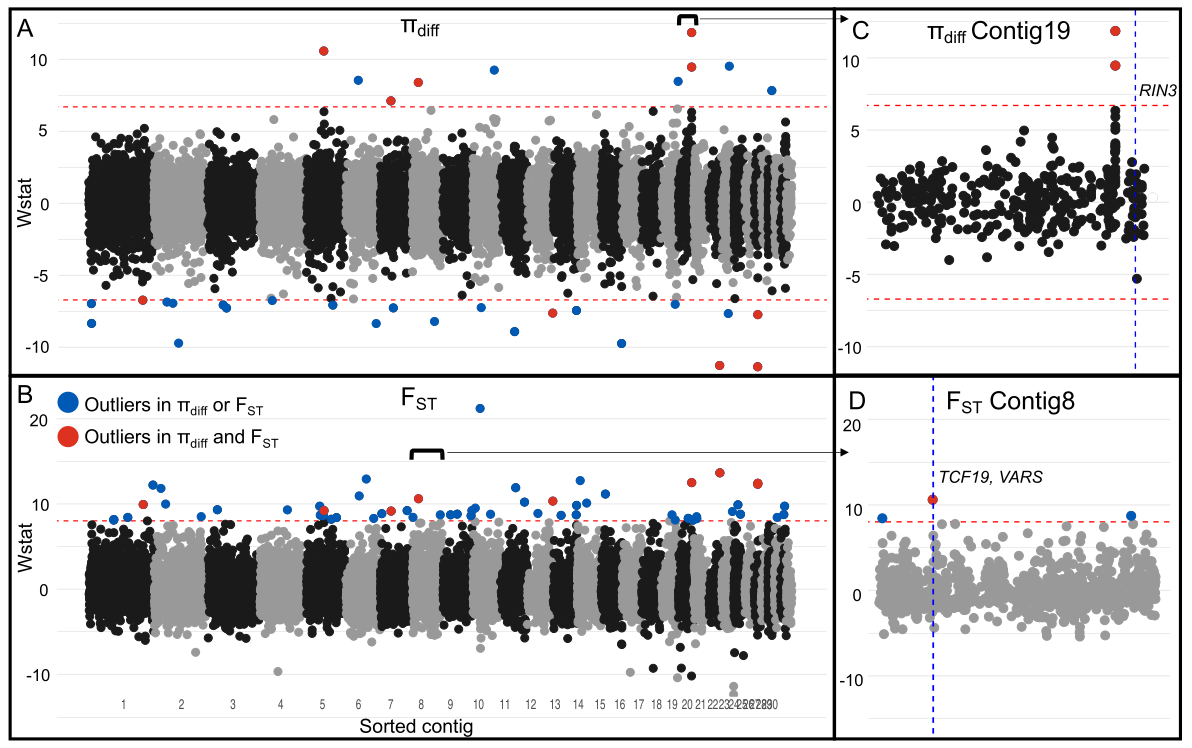


Figure 3: Evidence for selection on genomic regions in recovering MYL frog populations based on splined window analyses. Note that several key candidate genes (like RIN3 and TCF19) are outliers in both the GEMMA analyses (Figure 1D) and the splined window analysis (this figure). Manhattan plot of the results from the splined window analysis showing outlier regions for the difference in (A) nucleotide diversity and (B) FST. In (A), outlier regions are shown above the upper red dashed line and below the lower red dashed line. In (B), outlier regions are shown above the single dashed red line. Outlier regions for either or FST are shown in blue and outlier regions for both and FST are shown in red. (C) Magnified Contig19 from (A) showing two adjacent outlier regions for 12.9Mb upstream of the RIN3 outlier SNP (indicated with a dashed vertical blue line). (D) Magnified Contig8 from from (B) showing the FST outlier region that includes the outlier SNPs TCF19 and VARS. This region of the genome contains 8 annotated genes known to occur in the extended MHC Class I and III regions.

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| Figure 4: Synteny plot showing conserved gene order in Xenopus tropicalis and Rana muscosa for the outlier region containing MHC Class I Classical and MHC Class III gene regions. The plot was created with SimpleSynteny (Veltri et al., 2016) using Xenopus tropicalis Chromosome 8 (NC\_030684.2, genbank accession GCA\_000004195.4) and Rana muscosa Contig19. Asterisks indicate the location of SNP outliers in the TCF19 and VARS1 genes. Gap sizes for each contig representation are labeled. |

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| Figure 5: Violin plots showing individual heterozygosity for the Bd-naive and recovering populations. Individual data points are represented by their corresponding site number (from [Figure](#fig-allelefrequencies) 1A). |