



Gene Pool Boundaries for the Yosemite Toad (*Anaxyrus canorus*) Reveal Asymmetrical Migration Within Meadow Neighborhoods

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The Yosemite toad (*Anaxyrus [Bufo] canorus*) is a federally threatened species of meadow-specializing amphibian endemic to the high-elevation Sierra Nevada Mountains of California. The species is one of the first amphibians to undergo a large demographic collapse that was well-documented, and is reputed to remain in low abundance throughout its range. Recent phylogeographic work has demonstrated that Pleistocene toad lineages diverged and then admixed to differing extents across an elevational gradient. Although lineage divisions may have significant effects on evolutionary trajectories over large spatial and temporal scales, present-day population dynamics must be delineated in order to manage and conserve the species effectively. In this study, we used a double-digest RADseq dataset to address three primary questions: (1) Are single meadows or neighborhoods of nearby meadows most correlated with population boundaries? (2) Does asymmetrical migration occur among neighborhoods of nearby meadows? (3) What topographic or hydrological variables predict such asymmetrical migration in these meadow neighborhoods? Hierarchical STRUCTURE and AMOVA analyses suggested that populations are typically circumscribed by a single meadow, although 84% of meadows exist in neighborhoods of at least two meadows connected by low levels of migration, and over half (53%) of neighborhoods examined display strong asymmetrical migration. Meadow neighborhoods often contain one or more large and flat “hub” meadows that experience net immigration, surrounded by smaller and topographically rugged “satellite” meadows with net emigration. Hubs tend to contain more genetic diversity and could be prioritized for conservation and habitat management and as potential sources for reestablishment efforts.

Keywords: population genetics, conservation, asymmetrical migration, source-sink dynamics, isolation-by-distance, amphibian conservation, meadows, RADseq

INTRODUCTION

Identifying the unit at which a population is ecologically and evolutionarily independent is important because this forms the basis for many conservation and management actions, and for monitoring species status. Often, two types of conservation units are delineated: Management Units (MUs; Dizon et al., 1992; Moritz, 1994; Palsbøll et al., 2007) and Evolutionarily Significant Units (ESUs; Ryder, 1986; Avise, 1994; Moritz, 1994). Present-day independence is captured by MUs, defined as demographically independent populations with sufficiently high growth rates and low connectivity to neighboring units (Palsbøll et al., 2007). Conceptually, MUs should be panmictic units that can persist in spite of low immigration rates (e.g., $m < 10\%$) or genetic migration rates (e.g., $N_e m < 1$; Waples and Gaggiotti, 2006). The category of MUs is used to reflect present-day population boundaries, and contrasts with ESUs, which are lineages reflecting long-term or ancient isolation sufficient to create fixed genetic or morphological differences, and perhaps even reproductive isolation. In practice, MUs are delineated based on genetic discontinuity or local adaptation to unique environmental conditions (Dizon et al., 1992). Moritz (1994) defined MUs as having significant nuclear or mitochondrial allele frequency divergence, representing present-day genetic or demographic cohesion. Advances in genomics have allowed an unprecedented level of precision to delimit population boundaries, and manage adaptive evolution (Funk et al., 2012; Shafer et al., 2015; Peters et al., 2016; Hendricks et al., 2018; Waples and Lindley, 2018; Waters et al., 2018).

There are still many technological challenges involved in accurately delineating MU boundaries. For example, it has been acknowledged that different processes underlie demographic and genetic connectivity (Lowe and Allendorf, 2010). The number of migrants ($N_e m$) is solely responsible for genetic connectivity, while demographic connectivity is influenced by m relative to birth and death rates. Additionally, genetic connectivity may be disproportionately impacted by rare and unobservable dispersal events, or historical colonization followed by disequilibrium of drift and gene flow (i.e., Slatkin's Paradox; Slatkin, 1985, 1987; Mallet, 2001; Yu et al., 2010). Extrapolating the number of migrants from estimates of genetic structure using $F_{ST} \approx 1/(1 + 4N_e m)$ is therefore non-trivial, particularly under the strict assumptions of the island model (McCauley and Whitlock, 1999). Despite these caveats, dispersal ability is still highly correlated with genetic structure estimates such as F_{ST} (Bohonak et al., 1998; Bohonak, 1999), and population genomic datasets have proven very effective compared with small or single-locus datasets for delineating population structure. For example, population genomic approaches have been successfully used in delineating reproductive isolation amongst salmonids (Waples et al., 2020), tracing complex admixture in gray wolf populations (VonHoldt et al., 2016), and identifying invasive introgression in California tiger salamanders (Fitzpatrick et al., 2010).

The Yosemite toad (*Anaxyrus [Bufo] canorus*) is a federally threatened species of meadow-specializing amphibian that is in need of MU delineation (U.S. Fish Wildlife Service, 2014; Brown et al., 2015). The species is an endemic to the Sierra Nevada Mountains of California, where it underwent

a 10-fold demographic decline at multiple localities during the 1970s–1990s, and remains in low abundance today (Sherman and Morton, 1993; Green and Sherman, 2001). Recent phylogeographic work has demonstrated that Pleistocene toad lineages diverged and then admixed to differing extents across an elevational gradient (Maier et al., 2019); however, present-day population boundaries have only been assessed using several markers (Shaffer et al., 2000), and with limited spatial sampling (Wang, 2012). In this study, we aimed to delineate population boundaries for the Yosemite toad (*Anaxyrus canorus*) that can be used for management within two national parks (Yosemite and Kings Canyon). We used thousands of targeted nuclear markers generated using double-digest restriction-site associated DNA sequencing (ddRADseq), and a sampling scheme that densely covers all known occupied locations, based on a recent 6-year survey (Lee et al.¹).

Given the previously identified pattern of isolation-by-distance (IBD), and the statistical issues caused by stepping-stone migration likely taken by toads between meadows (Meirmans, 2012; Perez et al., 2018), we used a hypothesis-driven approach to identify which biologically plausible unit of population best explains gene pool boundaries. Evidence exists for at least three possible levels of population structure: (1) single meadows (mean radius in Yosemite National Park: 266 m), since on average toads travel short yearly distances (275–279 m), have small home ranges (radius: 92–166 m), and display high rates of philopatry (Sherman, 1980; Martin, 2008; Liang, 2010); (2) neighborhoods of nearby meadows, since previous survey and census efforts have found toad meadows to be spatially aggregated (Brown et al., 2012; Berlow et al., 2013; Matchett et al., 2015); and (3) phylogenetically distinct lineages (Maier, 2018; Maier et al., 2019; **Figures 1A,B**). We used several tests to determine which hierarchical level best matches the population definition of Waples and Gaggiotti (2006), i.e., panmictic units that are demographically or genetically independent from other populations.

Next, we examined potential asymmetrical migration among meadows. Occupancy and breeding frequency of Yosemite toads is known to partly depend on the quality of other meadows in a local neighborhood (Berlow et al., 2013), and a previous microsatellite study attributed 42.6% of genetic structure between meadows to topography and moist climate (Wang, 2012). We therefore hypothesized that neighborhoods of nearby meadows consist of “hubs” with high quality breeding area, minimal topographic complexity, and high site fidelity, resulting in a pattern of net genetic immigration from poor quality “satellite” meadows. We avoid the terminology of “source” and “sink” habitats, because populations may be demographically viable and independent, yet have a net influx of migrants that makes them sinks relative to nearby populations (i.e., “pseudo-sinks;” Watkinson and Sutherland, 1995; Thomas et al., 1996; Kawecki and Holt, 2002). Asymmetrical migration has been detected in many organisms, including fish (Pinhal et al., 2020), wind pollinated trees (Kling and Ackerly, 2021), sea stars (Iguchi

¹ Lee, S. L., Ostojka, S.M., Maier, P. A., Matchett, J. R., McKenny, H. C., Brooks, M. L., et al. (in preparation). Distribution and spatio-temporal variation of Yosemite toad populations in Sierra Nevada national parks.

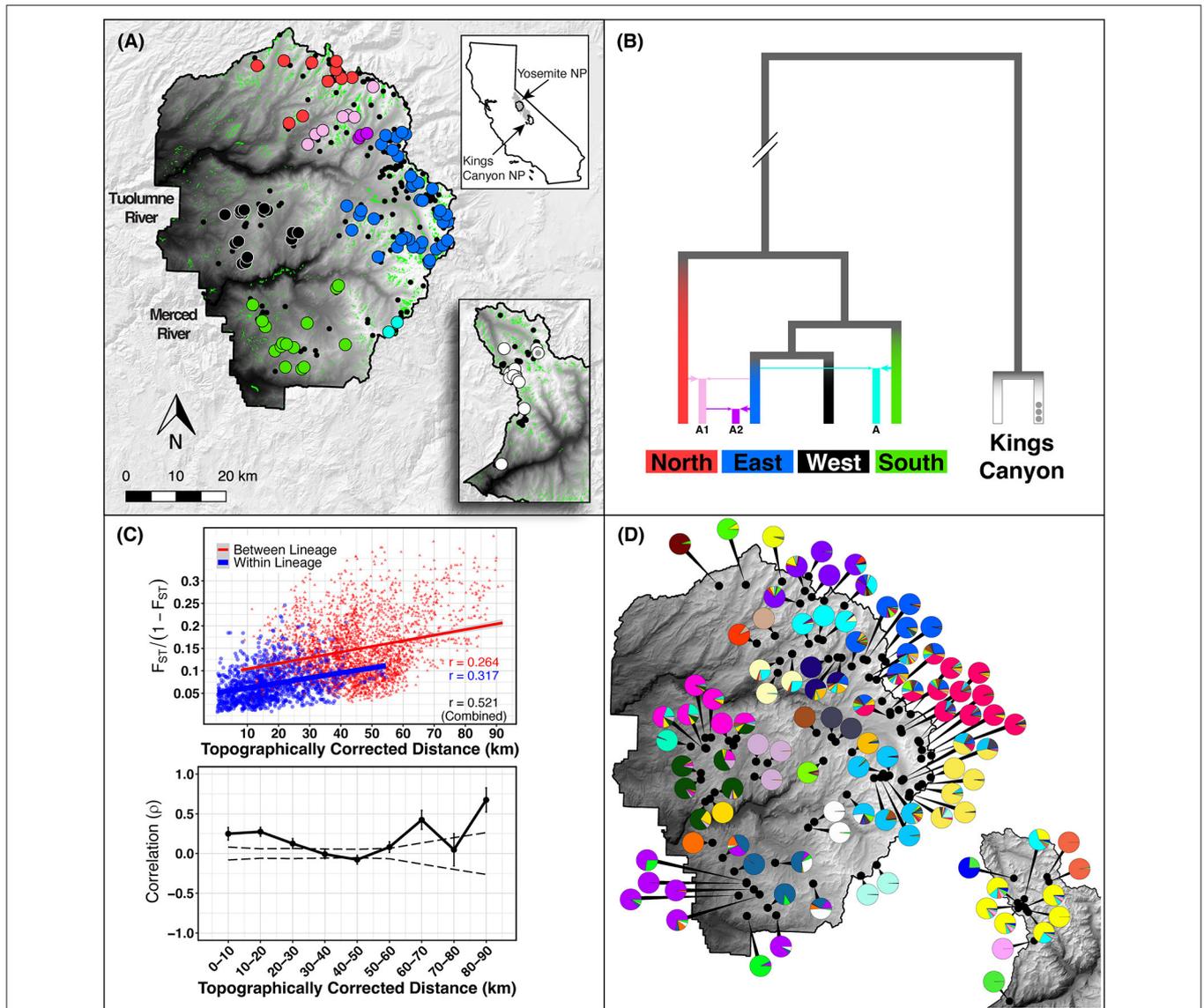


FIGURE 1 | Study area. **(A)** Study area in Yosemite NP (YOSE) and Kings Canyon NP (KICA), California. Top right inset shows the range of Yosemite toads in gray, and the boundaries of YOSE and KICA in black. Green polygons are all meadows within the parks. Small black circles indicate all known Yosemite toad meadows identified between 1915 and the present. Random noise is added to locations in order to protect the locations of this threatened species. Large circles indicate the meadows with data available in the present study, in YOSE ($n = 90$) and KICA ($n = 12$). Colors correspond to phylogenetic lineages, shown in **(B)**, reproduced from Maier et al. (2019). **(C)** Isolation by distance result based on $F_{ST}/(1 - F_{ST})$ and topographically corrected distances between meadows. Mantel correlogram shows Mantel correlation coefficients for successive 10 km distance categories. Solid lines are actual correlations, error bars represent bootstrapped confidence intervals, and stippled lines represent permuted confidence intervals (null distribution). **(D)** Meadow neighborhoods defined by $K = 28$ (YOSE) and $K = 6$ (KICA) gene pools from ADMIXTURE cross-validation. Each pie chart represents individual ancestry (i.e., q -values) from each meadow neighborhood, aggregated and displayed at the meadow scale.

et al., 2021), and amphibians (Cortázar-Chinarro et al., 2017; Hantschmann et al., 2021). Such migrational dynamics may play an important role in population persistence, as it has been shown that demographic stability is influenced by the long-term persistence of hubs, and continued genetic input from satellite populations (Dias, 1996; Gaggiotti and Smouse, 1996).

Our results provide information that can be used to plan and develop effective mitigation for habitat and climate related disturbance. Identifying hub-satellite dynamics may

pinpoint hub meadows that will be more robust to ongoing declines, and help inform specific conservation actions, such as relocations.

MATERIALS AND METHODS

Spatial Extent of Sample Collection

Sites (=meadows) within Yosemite National Park (YOSE) and Kings Canyon National Park (KICA) were chosen to maximize

representation across all known breeding locations from a recent 6-year survey effort (Lee et al., see text footnote 1, respectively), and overlap with previous studies (Shaffer et al., 2000; Wang, 2012; Berlow et al., 2013; Maier, 2018; Maier et al., 2019). YOSE includes ~33% of known Yosemite toad meadows, whereas KICA includes the southern-most 4% of toad meadows. Tadpoles were sampled across all available egg clutches, ponds, meadows, and two separate years to maximize inclusion of available genetic diversity and reduce potential bias of oversampling close relatives relative to the total local population. A minimum of five samples was used per meadow if additional meadows were included within 1 km, otherwise 10 samples per meadow were used, unless insufficient samples were available. This scheme maximized intra- and inter-meadow sampling representation across the study area.

We collected tissue samples during the summers of 2011–2013 in YOSE and KICA, and delimited sampling meadows using a park-wide vegetation map with 0.5 ha resolution (Keeler-Wolf et al., 2012; Berlow et al., 2013). Tail tissue was collected from larval toads using a sterilized razor blade.

Molecular Methods, ddRAD Sequencing, and Bioinformatics

We used a ddRADseq haplotype dataset (previously described in Maier, 2018; Maier et al., 2019) for all analyses of genetic differentiation and structure. Details about the library preparation, sequencing, and bioinformatic parameters used to identify variable loci are described therein, and in the **Supplementary Materials**. Briefly, genomic DNA was extracted using a combination of 96-well glass fiber plate (Ivanova et al., 2006) and DNeasy blood and tissue spin column (Qiagen) protocols. A total of 535 individual Yosemite toad (*Anaxyrus canorus*) tadpoles sampled from 90 meadows across YOSE, and 109 individual tadpoles sampled from 12 meadows in KICA, were prepared using a double-digest RADseq protocol (Peterson et al., 2012; S1 Protocol), then sequenced using 2×100 bp sequencing on an Illumina HiSeq 2500.

Stringent quality thresholds were used in the Stacks v1.19 pipeline (Catchen et al., 2011, 2013) to avoid recovering markers from paralogous sequences. Filtering thresholds were: minimum $10\times$ locus coverage, 75% complete loci, and minor allele frequency of 0.05. SNPs were called using the multinomial likelihood algorithm of Stacks, which calculates the likelihood of the observed genotypes given the per-base sequencing error rate. Two datasets were generated from a total of 3,261 loci (2,318 YOSE-polymorphic loci, and 1,914 KICA-polymorphic loci): (1) SNPs (one SNP kept per locus), and (2) full RAD-locus haplotypes (concatenated paired-end reads were used where possible, otherwise Read 1 was used). Some analyses required SNP data, whereas others could accommodate the full diversity present among multiple SNPs at a locus due to recombination. Therefore, we used a custom python script previously described in Maier et al. (2019) to output RAD haplotypes in addition to SNPs (fasta2genotype.py; <https://github.com/paulmaier/fasta2genotype>).

Addressing Potential Sampling Bias

Removing close relatives from population genetic datasets is often a standard practice (Peterman et al., 2016), although it may introduce biases if the original sampling was random. Small and/or inbred populations naturally contain many close relatives, and artificially distorting this composition can bias results toward a pattern of higher genetic diversity (Waples and Anderson, 2017). Removing data inevitably reduces precision and statistical power. Also, kinship estimators such as COLONY (Wang, 2004; Jones and Wang, 2010) and KING (Manichaikul et al., 2010) cannot accurately distinguish between close relatives and unrelated individuals from inbred populations, especially if the markers are low-density, unmapped SNPs (Chen, Personal Communication). To assess the impact of data removal, we used COLONY to estimate full-sib relationships, and calculated pairwise F_{ST} with PLINK (Purcell et al., 2007) pre- and post-removal of putative siblings. We used the SNP dataset with YOSE individuals only.

Delineating Population Boundaries

We initially checked all loci for deviations from Hardy-Weinberg expectations at the meadow scale, using the adegenet v2.1.5 package (Jombart, 2008) in R v4.1.2 (R Core Team, 2021). We then used the ade4 v1.7.18 package (Dray and Dufour, 2007) to test for isolation-by-distance (IBD) between meadows with a Mantel test. We used the F_{ST} dataset derived from YOSE individual as described above. F_{ST} values were linearized into $F_{ST}/(1 - F_{ST})$ as recommended by Rousset (1997). Since a pattern of IBD or hierarchical structure can confound population delineation algorithms that attempt to assign individuals into a set of clearly differentiated Hardy-Weinberg populations (Meirmans, 2012; Perez et al., 2018), we delineated current population boundaries in three distinct ways.

First, we asked which level of population structure is generally the smallest detectable level: meadows or neighborhoods of nearby meadows. We used a hierarchical STRUCTURE analysis (Vähä et al., 2007), which accounts for IBD by re-running each clustering analysis recursively until no further sub-structure is found. We ran STRUCTURE v2.3.4 (Pritchard et al., 2000) three times for 4.0×10^5 steps and 1.0×10^5 burn-in with K (number of ancestral populations) ranging from 1 to 10. The optimal K was chosen using the ΔK method of Evanno et al. (2005), and individual runs were combined using CLUMPP (Jakobsson and Rosenberg, 2007). This method can only test optimal K values greater than one, so the hypothesis that $K = 1$ was tested using a paired Wilcoxon test on log-likelihood values (Rosenberg et al., 2001; Emanuelli et al., 2013), to address inherent bias of ΔK toward finding $K = 2$ when no further substructure exists in reality (Janes et al., 2017). We used the haplotype dataset with YOSE individuals only for this analysis, so as to focus on the general pattern rather than finding every unit.

Second, we estimated which number of ancestral populations most reliably partitions the data. We used ADMIXTURE v1.3.0, which can efficiently apply the STRUCTURE algorithm for multi-locus SNP genotypes at large values of K (Alexander et al., 2009). The cross-validation procedure employed in ADMIXTURE determines which K has the most predictive accuracy, while

withholding data points (Alexander and Lange, 2011). We ran ADMIXTURE with 10-fold cross-validation on K ranging from one to the total number of meadows 1–90 (YOSE) or 1–12 (KICA). Whereas, the hierarchical STRUCTURE analysis was used to recursively find the smallest possible unit of population (to account for IBD), this analysis was used to find population units that best describe the entire dataset, and thus, likely an intermediate unit.

Finally, we took a hypothesis-driven approach to population delineation. Given the hierarchical nature of population structure previously described (Shaffer et al., 2000; Wang, 2012), there may be no single “correct” level. Consequently, we hypothesized that one level has a disproportionately large effect on partitioning toads into genetically/demographically independent gene pools. We used a hierarchical analysis of molecular variance (AMOVA) on haplotype data to quantify the relative contribution of meadows, meadow neighborhoods, and lineages to population differentiation. Four distinct phylogenetic lineages with median coalescent ages spanning 296–724 kya, as well as three “fused” lineages formed by inter-lineage hybridization 254–473 kya, were spawned by glacial pulses during the Pleistocene (Figure 1B; Maier et al., 2019). “Meadow neighborhoods” were defined as a group of nearby meadows with >50% assignment to the genetic clusters derived by ADMIXTURE. We used the poppr v2.6.1 function “poppr.amova” with 100 permutations to test for significance. In order to correctly partition the variance components of interest, we included all known levels: individuals, meadows, meadow neighborhoods, lineages, and parks. We compared the ϕ -statistics to values expected under Wright’s island model (Wright, 1931), i.e., $F_{ST} \approx 1/(1 + 4N_e m)$, using values of N_e calculated by the single-sample linkage disequilibrium method of NeEstimator v2.01 (Do et al., 2014). We assessed population independence with the 10% migration rate (m) demographic guideline (Hastings, 1993) and one migrant per generation (OMPG; i.e., $N_e m = 1$) genetic guideline (Wright, 1951; Mills and Allendorf, 1996).

Environmental Drivers of Asymmetrical Migration

We hypothesized that meadow neighborhoods (see “Delineating Population Boundaries”) included “hub” meadows that receive and retain the majority of migrants, and lower quality “satellite” meadows with net emigration. We tested this hypothesis by estimating directional migration rates between meadows using the method of Sundqvist et al. (2016). This method calculates geometric means of allele frequencies for a theoretical pool of migrants between each pair of meadows using alleles present in both meadows. Then asymmetrical G_{ST} values (Nei and Chesser, 1983) are calculated by comparing each meadow to the migrant pool. Finally, migration rates are estimated from G_{ST} values: $N_e m \approx ((\frac{1}{G_{ST}}) - 1)/4$ (Wright, 1931). We extracted pairwise differences between emigration and immigration to yield a unitless measure of net relative migration δM . Relative values are less sensitive to assumptions of the island model, such as drift-migration equilibrium, because they only infer differential migration without explicitly modeling any parameter. For any

comparison between two meadows, the meadow with $\delta M < 0$ has net immigration, and meadows with $\delta M > 0$ have net emigration. For each meadow neighborhood we examined, δM estimates were mapped onto igraph (Csárdi and Nepusz, 2006) objects to display the extent of asymmetrical migration in each neighborhood of meadows.

From previous research, Yosemite toads are most likely to occupy meadows based on a network of environmental attributes at nearby meadows (Berlow et al., 2013). Topographic relief also is known to have a large impact on meadow-to-meadow migration (Wang, 2012). Based on those two likely environmental drivers, we evaluated 10 meadow-scale environmental attributes as potential correlates of hubs and satellites (Table 1). We used ArcGIS 10 to extract four topographic variables derived from the Shuttle Radar Topography Mission (SRTM; Rabus et al., 2003), and five meadow network attributes derived from a LiDAR-based vegetation map from YOSE (Keeler-Wolf et al., 2012). The last variable was a network-boosted probability of toad breeding from previous work (Berlow et al., 2013). Any variables that did not approximate normality were transformed using either logarithmic, exponential, or root transformations. We identified environmental correlates of net migration (δM) individually, using Mantel correlations as implemented by the ncf v1.2.9 package (Bjørnstad, 2019), with 1,000 permutations for significance. We then identified correlations collectively for all variables using a multiple regression on distance matrices (MRDM; Legendre et al., 1994; Lichstein, 2007), using the method of Wang (2013), and 1,000 permutations.

We also gave “hubs” and “satellites” formal definitions based on extreme values of net migration. Hubs were defined as meadows with average δM that is negative, and at least one standard deviation (SD) less than zero. Satellites were defined as meadows with average δM that is positive, and at least one SD greater than zero. We performed a principal components analysis (PCA) and a linear discriminant analysis (LDA) using the MASS v7.3.54 package (Venables and Ripley, 2002) to identify environmental predictors of the two types. In order to determine the most important discriminatory variables, we performed stepwise variable selection to optimize the accuracy of predictors using the stepclass function of the klaR v0.6.15 package (Weihs et al., 2005). The process uses 10-fold cross-validated accuracy of the LDA to add or remove variables until improvement is <1%.

RESULTS

Addressing Potential Sampling Bias

We found high levels of inbreeding in the final dataset. Using 2,318 un-linked SNP markers variable across YOSE, we estimated that in any given meadow an average of 551 are polymorphic, and an individual is heterozygous for an average of 233 due to meadow-level inbreeding, with average $F_{IS} = 0.221$. COLONY estimated 173 first-degree relative pairs. Matrices of pairwise F_{ST} calculated with and without 173 putative siblings were highly correlated ($\rho = 0.948$), and therefore, should have little impact on landscape-level results. Based on this result and the fact that close relatives can be overestimated in systems with high inbreeding,

TABLE 1 | Hub-satellite predictive variables.

Category	Abbreviation	Source	Definition	GIS summary
Meadow network	BP	(Berlow et al., 2013)	Network-boosted breeding probability	Value
Meadow network	A	YNP LiDAR	Area of meadow	Value
Meadow network	BWA	YNP LiDAR	Breeding wet area	Value in 1 km radius
Meadow network	MA	YNP LiDAR	Area of meadows	Value in 1 km radius
Meadow network	P	YNP LiDAR	Length of meadow perimeter	Value
Meadow network	WAC	YNP LiDAR	Wet area of closest meadow	Value
Topography	ESD	SRTM	Elevation	SD in 500 m radius
Topography	R	SRTM	Roughness index	Mean in 500 m radius
Topography	S	SRTM	Slope	Mean in 500 m radius
Topography	SPI	SRTM	Slope position index	Mean in 500 m radius

we elected to keep all individuals in the dataset to retain a robust sample size for subsequent analyses.

Delineating Population Boundaries

Our suite of analyses supported meadows as the smallest distinguishable genetic and demographic population unit, and that populations were further structured by IBD among meadows. We found significant IBD up to 30 km distances (**Figure 1C**), suggesting that population structure might be heavily influenced by stepping-stone migration at multiple spatial scales. The hierarchical structure analysis revealed that 63 out of 90 (70.0%) individual meadows in YOSE are the smallest detectable population unit (**Figure 2**). The remaining units consisted of two meadows ($n = 7$), three meadows ($n = 1$), or 10 meadows ($n = 1$; Tioga). Seven phylogenetic lineages—four pure, three of admixed origin (Maier et al., 2019)—were distinct at high levels in the hierarchy (underlined in **Figure 2**). The ADMIXTURE analysis delineated units of genetic cohesion intermediate between meadow and lineage (**Figure 1D**). The lowest ADMIXTURE cross-validation error for YOSE was found at $K=28$, and for KICA was $K=6$, although subsequent K values elicited only gradual increases in cross-validation error (**Supplementary Figures S1, S2**). Most meadows (84.3%) were contained in neighborhoods of two or more meadows, with a mean size of 3.0 meadows, and a maximum of 12 meadows (Tioga). Out of 34 total meadow neighborhoods across both parks, 16 (47.1%) contained only one meadow. In most cases, these singleton “neighborhoods” were in terrain separated by canyons (e.g., Rancheria, Thompson, Wells, Tilden, and Twin Lakes in the Y-North lineage). Names and summary statistics for each neighborhood are in **Supplementary Table S1**.

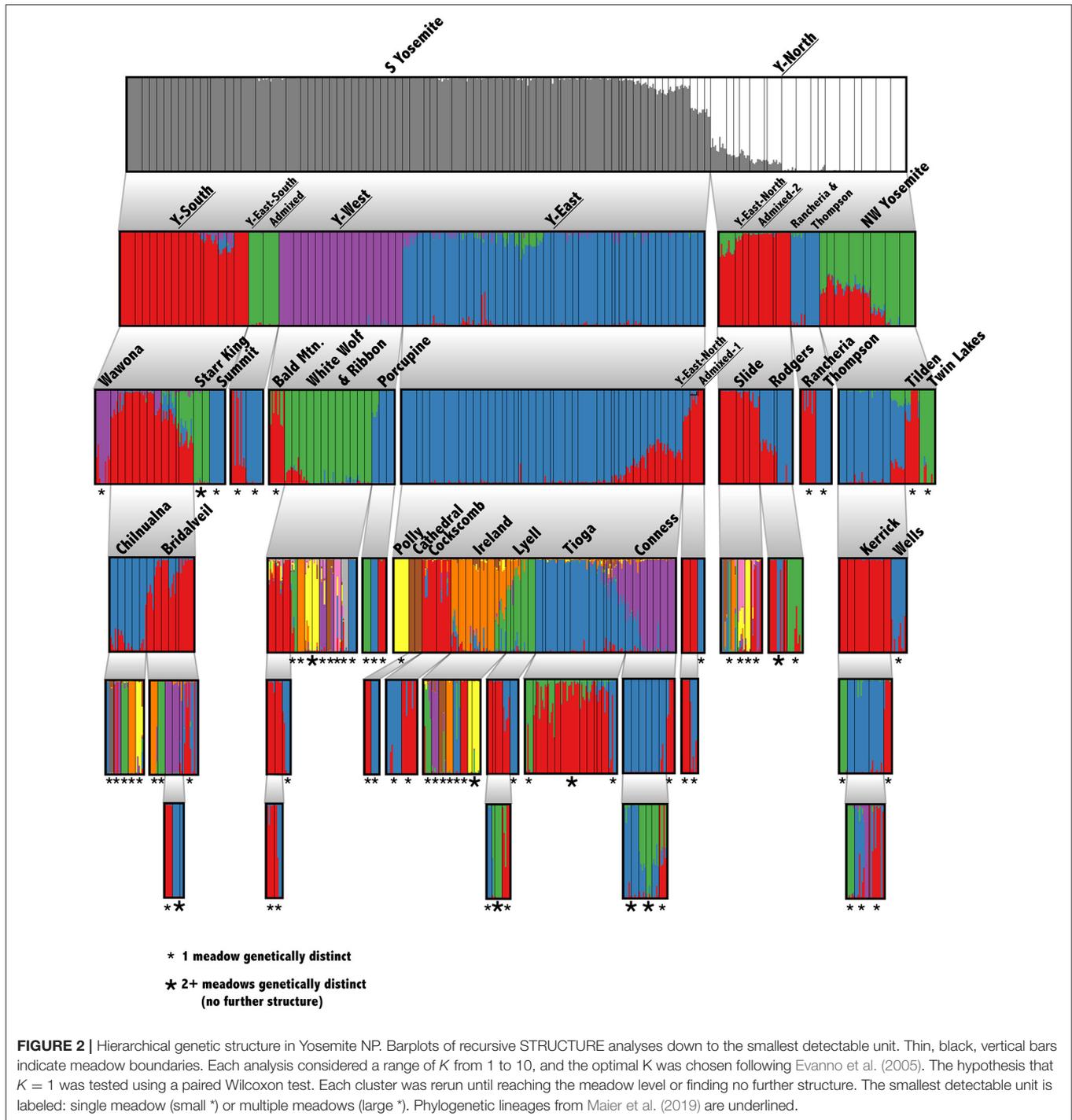
The hierarchical AMOVA found a large proportion of total variance between parks (30.46%; $\phi_{PT} = 0.305$) as expected (**Table 2**). Meadows within neighborhoods showed the next highest variance (23.54%) and had the highest ϕ -statistic ($\phi_{MN} = 0.406$), supporting the idea that much more of the genetic variation is partitioned among meadows than meadow neighborhoods (3.71%; $\phi_{NL} = 0.06$). Mean pairwise ϕ_{MN} for meadows within each neighborhood had comparable levels (mean: 0.409; sd: 0.08; **Supplementary Table S1**). Further, we estimated meadows to be demographically independent

($m < 10\%$) based on average observed meadow N_e of 29.13 (**Supplementary Table S1**), and $F_{ST} \geq 0.079$ ($\phi_{MN} = 0.406$). In contrast, we estimated neighborhoods to have average N_e of 23.33 and require $F_{ST} \geq 0.097$ for demographic independence, a value greater than the observed statistic $\phi_{NL} = 0.06$. The estimated threshold for genetic independence among either unit regardless of population size ($N_e m \leq 1$) is $F_{ST} \geq 0.2$, which is consistent with meadows but not meadow neighborhoods being genetically independent. Patterns of conformity to Hardy Weinberg expectations at the meadow and meadow neighborhood scales further supported meadow distinctiveness, with meadows deviating at 2.48% of genotypes, compared to 4.86% for meadow neighborhoods, a nearly two-fold difference (**Supplementary Figure S3**).

Environmental Drivers of Asymmetrical Migration

Out of 34 total meadow neighborhoods across both parks, nine contained a moderate sample size ($n \geq 5$) of meadows. We found that asymmetrical migration was strong in six (67%) based on the presence of at least one hub or satellite meadow, i.e., with migration greater than one standard deviation from symmetrical (**Supplementary Table S2; Figure 3**). Hub meadows showed high relative H_E (**Supplementary Table S1**) and much stronger genetic immigration than emigration. Out of these nine neighborhoods initially examined, three had a single hub meadow, and another three had two to three hubs. Distinct satellite meadows were only identified in three (33%) of these neighborhoods. A detailed example is depicted in **Supplementary Figure S4**.

We found environmental patterns characteristic of all 18 hubs and satellites more broadly (**Figure 4**), regardless of meadow count (i.e., $n \geq 2$). Generally, we found that hub meadows are significantly larger, and less topographically complex, than satellite meadows (**Figures 4A–D**). The first two principal components of the PCA accounted for 76.3% of the variance, and this variance was orthogonally split into the two categories of variables: meadow network attributes, and topography (**Figure 4E**). However, topographic variables had a much smaller boost to discriminatory power compared with meadow network variables. The LDA function based on all 10



variables had a 100.0% predictive accuracy rate, and an 83.5% cross-validated accuracy rate (Figure 4F). The stepwise variable selection for LDA identified four variables that significantly improved discrimination between hubs and satellites: meadow area, breeding wet area, wet area of the closest meadow, and SD of elevation (within 500 m of the sampled location).

Individual t -tests confirmed that hubs are significantly larger, and less topographically complex, than satellite meadows.

Compared with satellites, hub meadows (defined as having δM values ≤ 1 SD below zero) have significantly larger mean areas ($t = 3.10$, $df = 10.1$, $p = 0.006$), perimeters ($t = 3.56$, $df = 11.3$, $p = 0.002$), and breeding wet area within 1 km ($t = 2.35$, $df = 13.1$, $p = 0.017$). Satellites have (within 500 m of the sampled location): higher slope ($t = -2.51$, $df = 8.06$, $p = 0.081$), elevation SD ($t = -2.1$, $df = 7.65$, $p = 0.035$), and roughness index ($t = -3.03$, $df = 10.7$, $p = 0.006$). Hubs also had lower mean slope within

TABLE 2 | Analysis of Molecular Variance (AMOVA) results based on five hierarchical levels: park, lineage, meadow neighborhoods (defined by ADMIXTURE cross-validation), meadows, and individuals. Significance is based on 100 random permutations with one-tailed test.

Variance component	^a df	^b SS	^c MS	^d σ	^e % Total	^f ϕ -Statistic	^g P-value
Between parks (P)	1	50745.20	50745.20	116.94	30.46	$\phi_{PT} = 0.305$	**
Between lineages (L) within parks (P)	7	41768.02	5966.86	30.16	7.86	$\phi_{LP} = 0.113$	**
Between neighborhoods (N) within lineages (L)	25	53314.90	2132.60	14.25	3.71	$\phi_{NL} = 0.060$	**
Between meadows (M) within neighborhoods (N)	68	78941.58	1160.91	90.35	23.54	$\phi_{MN} = 0.406$	**
Between individuals (I) within meadows (M)	542	95376.63	175.97	43.78	11.41	$\phi_{IM} = 0.331$	**
Within individuals (I)	644	56931.50	88.40	88.40	23.03	$\phi_{IT} = 0.770$	**
Total	1287	377077.82	292.99	383.89	100.00		

^adf = degrees of freedom.

^bSS = sums of squares.

^cMS = mean squares.

^d σ = variance.

^e% = proportion of total of variance.

^f ϕ -statistic = ratio of variances.

^gp-value = permuted probability of the observed σ by chance; ** $p < 0.01$.

500 m of the sampled location ($t = -2.15$, $df = 9.11$, $p = 0.03$). Interestingly, although hubs had larger areas and larger nearby wet areas, their nearest neighboring meadow had significantly less wet area ($t = -2.37$, $df = 6.01$, $p = 0.028$). All tests remained significant after correcting for multiple comparisons with the FDR method (Benjamini and Hochberg, 1995) with a maximum p -value of 0.035.

We also found significant relationships between pairwise differences in meadow environment (δ environment) and net asymmetrical migration (δM) using an MRDM (Supplementary Table S3; Supplementary Figure S5). For pairwise regression analyses, we considered all meadows in a neighborhood, regardless of whether they were extreme hubs or satellites. The general result can be summarized as follows: for any two meadows in a neighborhood, the meadow with larger area and less topographic ruggedness tends to have net immigration (influx of gene copies). The five significant variables in the model were: meadow area, breeding wet area, wet area of the closest meadow, total area of meadows within 1 km, and percent slope (within 500 m of the sampled location). The MRDM results supported the above pattern of meadow area strongly influencing hub-satellite dynamics, and meadow ruggedness having a small to moderate influence (Figures 4A–D). We found that meadow area explained the largest percentage of the variance (30.0%), followed by wet area of the closest meadow (10.3%), while the remaining variables collectively only accounted for 5.1% of the variance [$R_{adj}^2 = 0.43$, $F_{(10,399)} = 31.79$, $p < 0.001$].

We performed a *post-hoc* test of Mantel correlations (with 1,000 permutations) for each variable and found each to be significant after correcting for multiple comparisons with the FDR method. Interestingly, this included a small but significant positive correlation between δM and δ (slope position index) meaning that meadows closer to hilltops have net genetic influx ($\rho = 0.11$, $p = 0.013$). We also found a small but significant positive correlation between δM and δ (breeding probability),

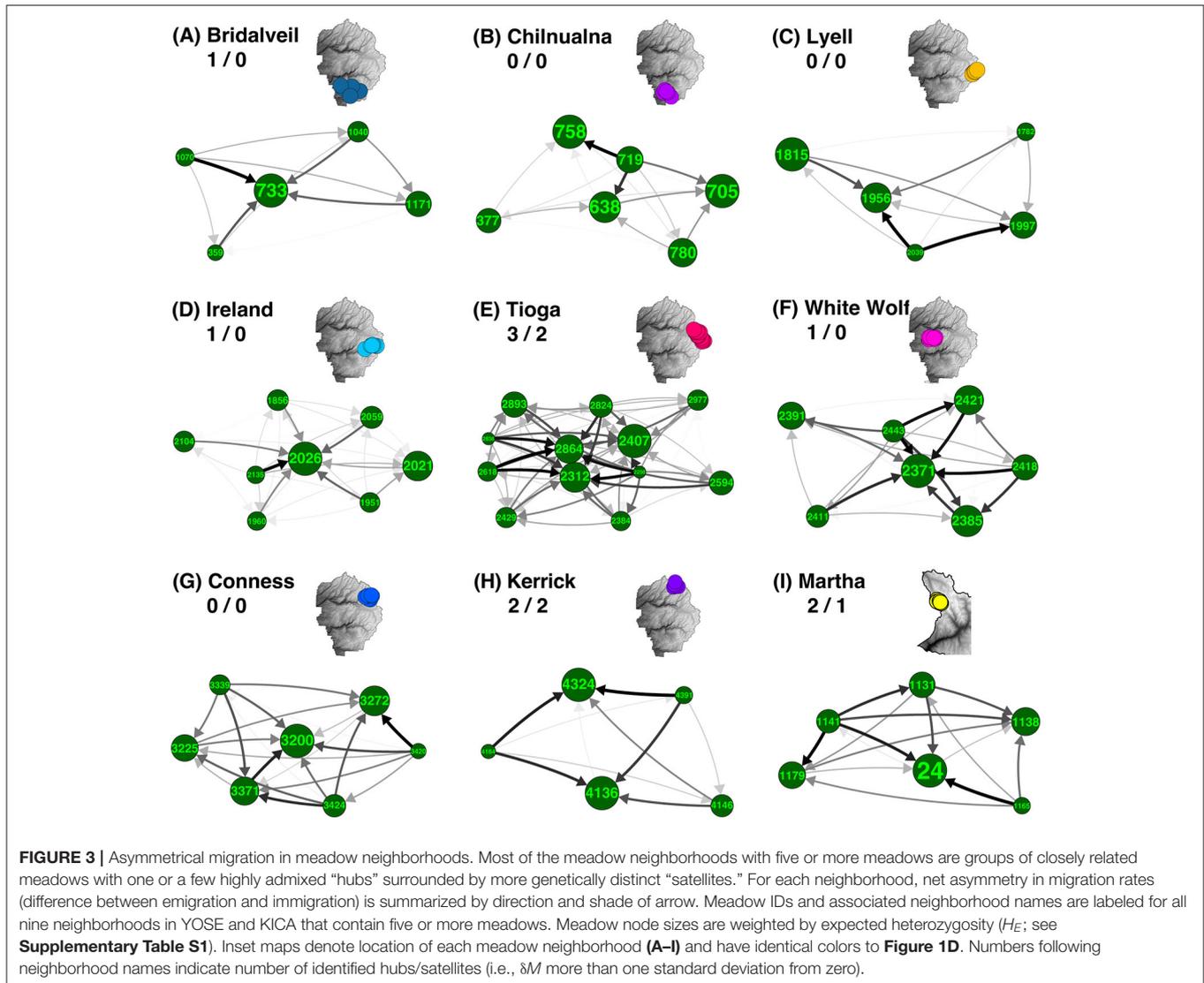
indicating that meadows with higher probability of breeding have net genetic influx ($\rho = 0.14$, $p = 0.007$).

DISCUSSION

Are Meadows a Useful Unit for Population Management?

We found that individual toad meadows are genetically distinguishable in 70% of cases, and that almost half (47.1%; $n = 16$) of meadow neighborhoods contain one meadow (Figures 1D, 2). Moreover, we found that meadows are the unit contributing most to population subdivision ($\phi_{MN} = 0.406$) compared with meadow neighborhoods ($\phi_{NL} = 0.06$) and lineages ($\phi_{LP} = 0.113$) (Table 2). Our results are broadly consistent with previous findings of significant among-“pond” (=meadow) variation (Shaffer et al., 2000); however, they are informed by substantially expanded sample size ($n = 102$; $n = 28$), marker set ($n = 3,261$; $n = 1$), and number of hierarchical levels ($n = 5$; $n = 3$). Our estimates of average meadow migration rate ($m \ll 10\%$) and number of migrants ($N_e m \ll 1$) are based on island model assumptions; however, our result (meadows are the most distinct unit) is robust to those assumptions.

Meadow distinctness makes sense given that toad-occupied meadows in YOSE have a mean radius of 266 m (Keeler-Wolf et al., 2012), and are typically surrounded by more xeric vegetation. Yosemite toads are observed to have small average home range size (radius: 92–166 m) and high rates of philopatry in short-term mark-recapture studies (Sherman, 1980; Martin, 2008; Liang, 2010). A sample of five other Nearctic toads (formerly “*Bufo*”) have comparable average home ranges, with radius between 15 and 390 m (Denton and Beebe, 1993; Kusano et al., 1995; Muths, 2003; Forester et al., 2006). Yosemite toads may occasionally travel long distances (maximum 1,261 m; Martin, 2008; Liang, 2010), but this may be infrequent enough for most meadows to remain independent population units.

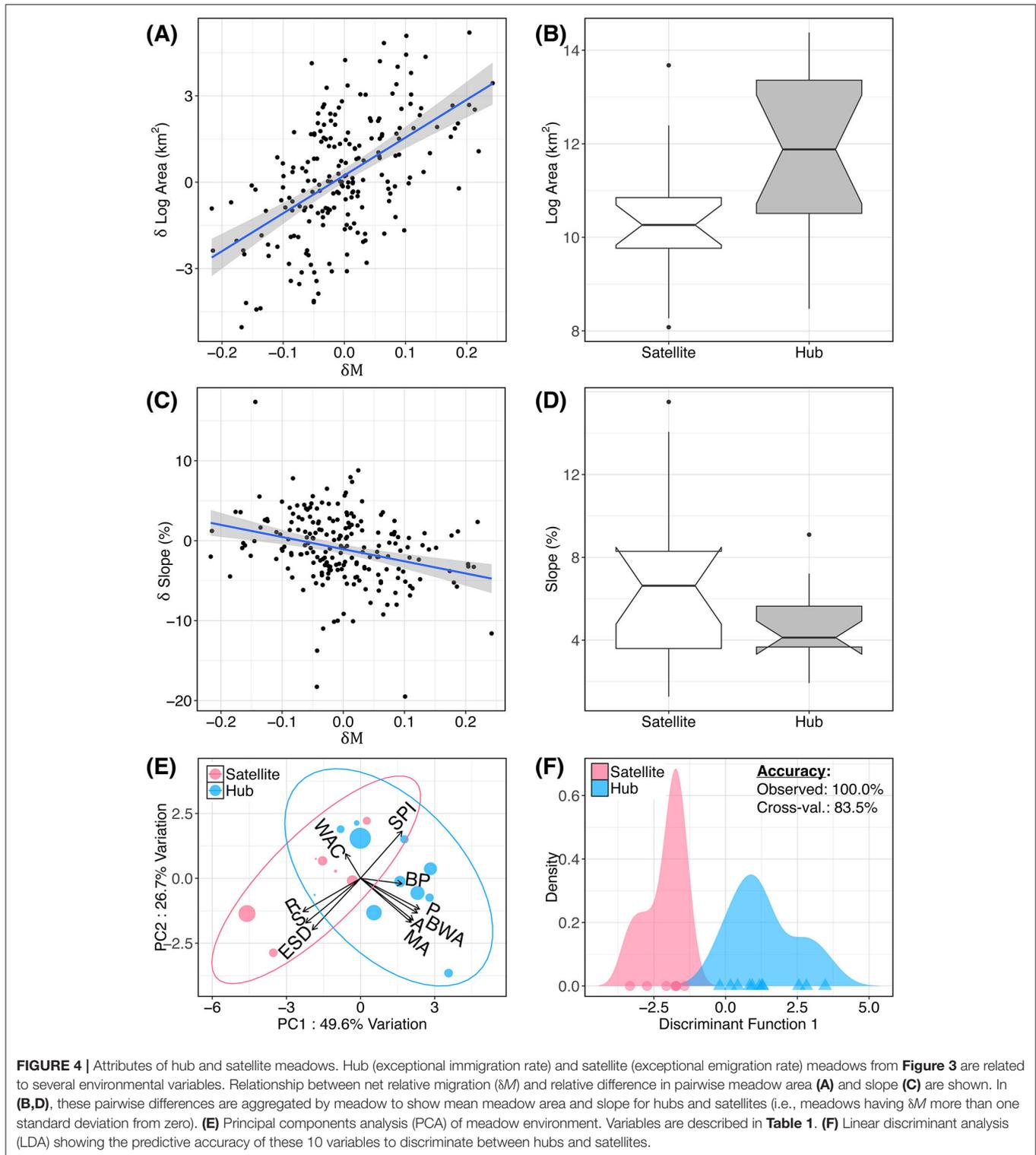


Although most meadows are genetically distinct, 84.3% of meadows are members of multi-meadow neighborhoods, and inter-meadow genetic rescue may be important over longer time scales. Also, several exceptions to this pattern exist: e.g., Tioga, Conness, Ireland, and White Wolf meadow neighborhoods appear undifferentiated, due to high gene flow or recent colonization (**Figure 2**). Two out of four of these undifferentiated neighborhoods (Tioga and White Wolf) are bisected by a major highway (CA-120), which has been identified as a barrier to connectivity (Maier, 2018). Future work should try to precisely estimate migration relative to demographic stability of these meadows, for example by using parentage analysis, genetic assignment, and simulations (Peery et al., 2008, 2010).

Meadow Attributes Impact Network Connectivity

Despite the distinctness of most meadows, we found low-level migration amongst meadows in neighborhoods. Strong

asymmetrical migration was found in over half (53%) of the neighborhoods containing two or more meadows. Our asymmetrical migration analysis found that larger and topographically flatter meadows predictably (with 83.5% cross-validated accuracy) act as regional genetic hubs (**Figures 3, 4**), and often these meadows are centrally positioned in the spatial arrangement of these meadow neighborhoods (e.g., **Supplementary Figure S4**). This means that one or several meadows within each neighborhood tend to receive a disproportionately large amount of genetic input and send a disproportionately small amount of genetic output. Hubs tended to be not only bigger and flatter, but have more wet area nearby, and be positioned closer to hilltops than satellites. Aside from large meadow area, the second-best discriminator of hubs was that they neighbored a meadow with minimal wet area. This may underscore the importance of juxtaposing breeding habitat with more xeric upland meadow habitat, which plays a vital role in Yosemite toad foraging and hibernation (Sherman, 1980;



Morton, 1981; Martin, 2008). It is important to note that due to collinearity, it is also possible that big, wet meadows necessarily exist adjacent to relatively dry neighbor meadows, but without a larger dataset these two variables are difficult to disentangle.

Contrasting environmental correlates have previously been found for persistent vs. intermittent meadows; proximity to fire perimeters and timber harvest were predictors of intermittently occupied meadows in one study (Liang and Stohlgren, 2011).

However, the present study offered the first insight into genetic hub-satellite structure, with 10 m resolution of meadow network attributes that predict hubs and satellites. High hub genetic diversity appears to reflect genetic admixture; multiple satellite meadows surround them and promote the mixing of genetically diverse toads in a central area. The ultimate cause for this is unclear: perhaps temporally fluctuating habitat quality or low carrying capacity in satellite meadows periodically sends migrants seeking refuge in hub meadows (Holt et al., 2004). Our result is consistent with the discovery that toad breeding detection in YOSE has increased at higher quality meadows, between two survey efforts spaced one decade apart (Lee et al., see text footnote 1, respectively). If climate change or other stressors are degrading marginal habitat in satellites, then toads may be consolidating at hubs in response.

By the same token, large hub meadows may act as genetic (pseudo-)sinks because breeding toads show higher rates of philopatry within superior breeding conditions. Populations below carrying capacity may be demographically independent if they do not require immigration to persist (Watkinson and Sutherland, 1995). For example, the pseudo-sink populations of the butterfly *Euphydryas editha* survived a severe frost in nearby Sequoia National Forest, after which they thrived and became sources (Thomas et al., 1996; Boughton, 1999). Thus, net immigration in hubs could reflect recent environmental disturbance. We also cannot rule out the possibility that hubs are actually source populations, but with excess private alleles. The premise of the private allele method used in this study is that populations with more unique alleles are more isolated. However, hubs may simply be larger and more stable populations that have accumulated more diversity, and the homogeneity of satellite populations may be due to recent founder effects (Sundqvist et al., 2016). This alternative is not easily disentangled using a single genetic dataset and is better tested by tracking studies. Regardless, hub admixture is a common pattern in our study. Since admixture is pervasive on a larger scale (i.e., between lineages; Maier, 2018; Maier et al., 2019; **Figures 1A,B**), it is unsurprising to find admixture plays an important role at a smaller scale. This result comes with the caveat that many neighborhoods have few meadows ($\bar{n} = 3.0$). Therefore, there are also many examples of isolated meadows and meadow pairs existing without a large, admixed meadow nearby.

Conclusions and Conservation Implications

Meadows are the most genetically distinct unit of population for Yosemite toads. However, our fine-scale sampling and environmental analysis showed that this is not the entire story. Most (84.3%) of toad meadows exhibit low levels of gene flow with neighborhood meadows, in many cases displaying hub-satellite structure. Genetic distinctness is consistent with the MU definition; however, recent demographic declines (Sherman and Morton, 1993; Green and Sherman, 2001; N_e estimates in **Supplementary Table S1**) suggest that present and future population viability of Yosemite toads may depend on connectivity between meadows.

Hub meadows may be robust to climate change and fluctuations, given their pattern of higher immigration and

genetic diversity. Therefore, hub meadows may be important refugia for nearby meadows with environmental instability, although this assumes hub meadows are not also in danger of demographic decline. Climate change (Brown et al., 2015; Maier, 2018), disease (Lindauer, 2018; Lindauer and Voyles, 2019), or synergistic effects between the two (Lindauer et al., 2020) may continue to reduce carrying capacity and growth rate, while reducing gene flow from satellites as well as foreign meadow neighborhoods, which could eventually force all meadows toward an extinction vortex.

Mitigation efforts in the short term could take advantage of the higher genetic diversity of hubs for translocation efforts or captive breeding programs. Although hubs are a potentially useful resource for medium to large meadow neighborhoods, many meadows exist in isolation. Out of 102 meadows in our study, 16 were singleton “neighborhoods” and a further eight were neighborhoods of only two. Isolated meadows could also be prioritized for protection efforts due to their potentially unique genotypes, which may play a role in future adaptation to climate change and/or disease.

Future research could investigate the population dynamics of hub and satellite meadows over multiple generations, to determine parameters such as growth rate and net migration rate. We found that hubs are identifiable 100% of the time in our dataset, and with 83.5% cross-validated accuracy, based primarily on meadow size, proximity to upland habitat, and gentle topography, to a lesser extent. Those variables may be useful proxies for genetic hubs in Yosemite toad meadow complexes across the species distribution.

DATA AVAILABILITY STATEMENT

No original genetic data were produced in this study. Demultiplexed fastq files of double-digest RADseq data are available at NCBI GenBank SRA under BioProject PRJNA558546 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA558546>). Scripts and genotype data used to perform analyses in this study are deposited at Dryad (<https://doi.org/10.5061/dryad.xsj3tx99h>). The U.S. Geological Survey metadata record associated with this study can be found at the USGS Science Data Catalog (<https://doi.org/10.5066/P9KABYPU>).

ETHICS STATEMENT

This animal study was reviewed and approved by San Diego State University Animal Care and use protocol #13-03-001B.

AUTHOR CONTRIBUTIONS

PM, SO, AA, AV, and AB designed the study. PM and SO collected tissue samples. PM performed the laboratory work and conducted the analyses, with guidance from AB, AV, and AA. PM wrote the manuscript, with input from AV, AB, SO, and AA. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcosc.2022.851676/full#supplementary-material>

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