Genetic Variation in Captive Populations of Barrens Topminnow

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INTRODUCTION

The Barrens topminnow (*Fundulus julisia*), a freshwater killifish native to the Barrens Plateau in Tennessee, has suffered continual population declines since the 1980's. These declines can be attributed to drought, habitat loss, and harassment by the invasive Western Mosquitofish (*Gambusia affinis*), which have been widely introduced across the Southeastern United States. As of 2019, this species was federally listed under the Endangered Species Act.

In order to mitigate further population declines, a number of captive ark populations were established, beginning in 1983, to serve as a source for future introductions. There is concern about the adaptive potential and genetic health of re-introduced populations as loss of genetic diversity can accumulate rapidly in captivity. Genetic monitoring of ark populations can inform management strategies to better retain genetic variation.

Here we used genotype data from 14 microsatellite loci to investigate genetic variation in ark populations of the *F. julisia* and compare measures of genetic variation to estimates from native source populations. Results from these genetic surveys can be used to inform captive breeding strategies that will best preserve the long-term persistence and adaptive potential of this species.

MATERIALS AND METHODS

1.) Fin clips were collected from ~20 individuals from two captive population maintained at CFI. Ark populations were founded from brood stocks obtained from Pond Spring and Benedict Spring (Type locale).We also resampled an introduced site (Merkle Spring) to estimate allele frequency shifts in natural populations over time and compare these to shifts experienced in captivity.

2.) PCR primers for 14 microsatellite loci were designed from whole-genome sequence data from *F. julisia*.

3.) Fluorescently labeled primers were used for PCR amplification. Alleles sizes were determined using the software Peak Scanner 2.0.

4.) Estimates of genetic variation including observed and expected heterozygosity (H_O) and (H_e) were compared between native and stock populations. Bayesian Assignment tests were used to assess structure of genetic variation across sampled populations.

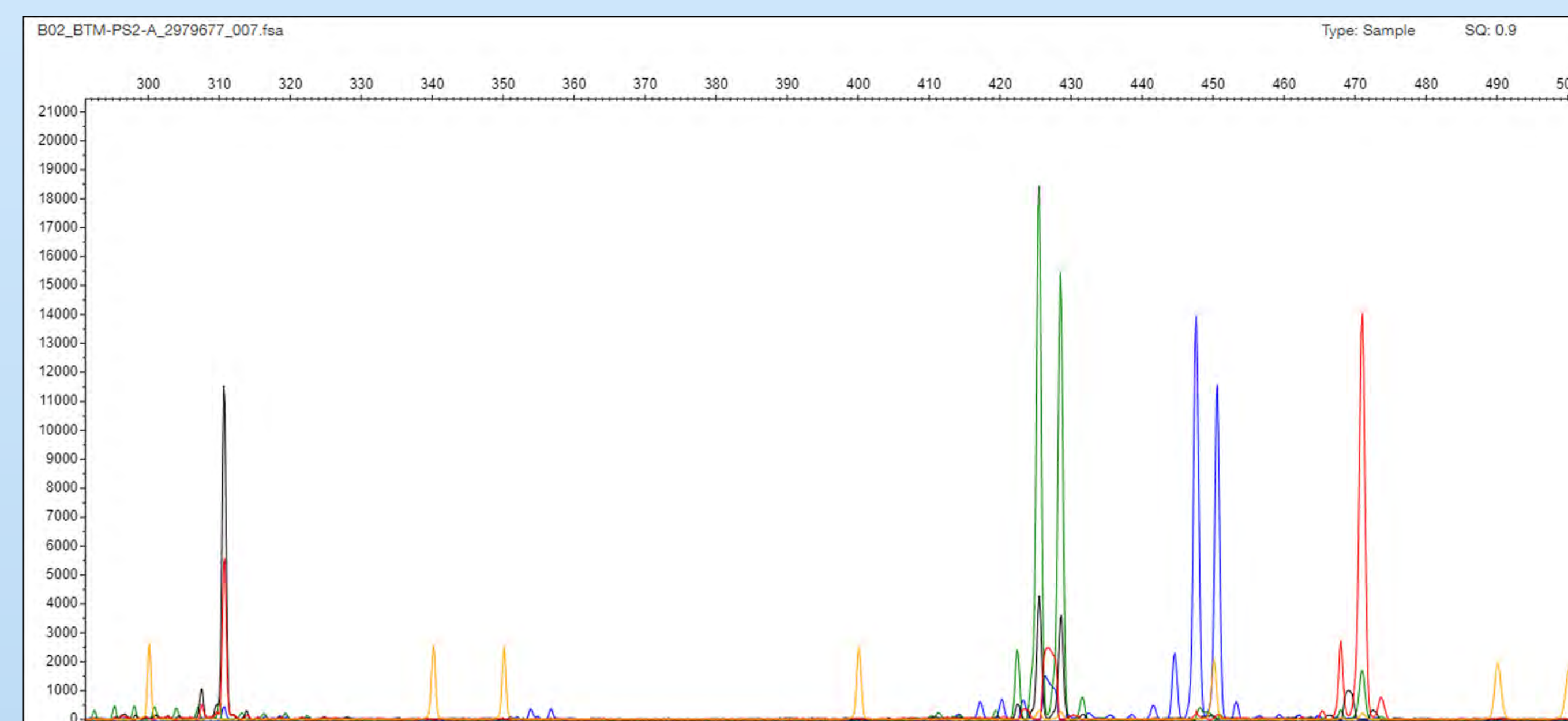**Figure 1:** Photograph of male Barrens Topminnow in nuptial coloration.

Figure 2. Microsatellite Chromatogram for Ark Pond Spring individual 2. Four loci are depicted in different colors. The x-axis indicates allele size as determined by the ladder shown in yellow. Fuji001 labeled with FAM Dye (blue) is genotype 448/451, Fuji002 labeled with HEX Dye is genotype 426/429, Fuji004 labeled with ROX (red) is genotype 472/472. Fuji013 is 311/311.
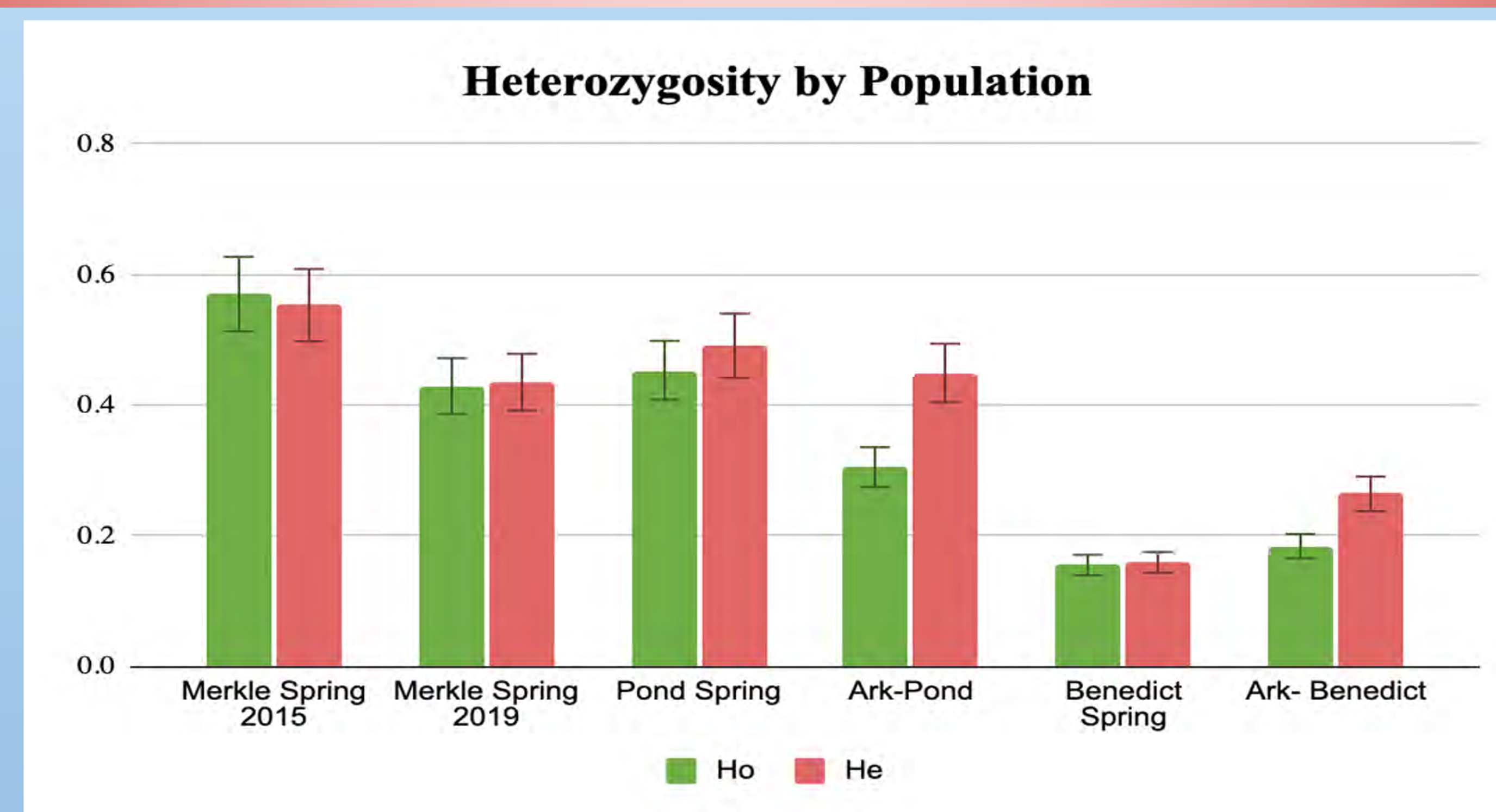
RESULTS AND DISCUSSION

Figure 3. Observed and expected heterozygosity (H_o and H_e) for temporal samples taken from introduced Merkle Spring, and for native Pond Spring and Benedict Spring (type locality) and their respective captive population. Bars indicate standard error.

Genetic Diversity. Merkle Spring demonstrated a significant loss of heterozygosity in temporal samples taken from 2015 and again in 2019, suggesting that this population had suffered a loss of genetic variation due to drift over this four-year period. The Pond Spring Ark population demonstrated reduced genetic variation compared to estimates from samples obtain from the native site. Unfortunately, the native population at Pond Spring is now extirpated due to presence of invasive Western Mosquitofish at this site. Interestingly, native Benedict Spring had lower estimates of genetic variation compared to its Ark population. A reduction of variation at this site is likely due to several bottleneck events that resulted from a series seven droughts that occurred beginning in 1980.
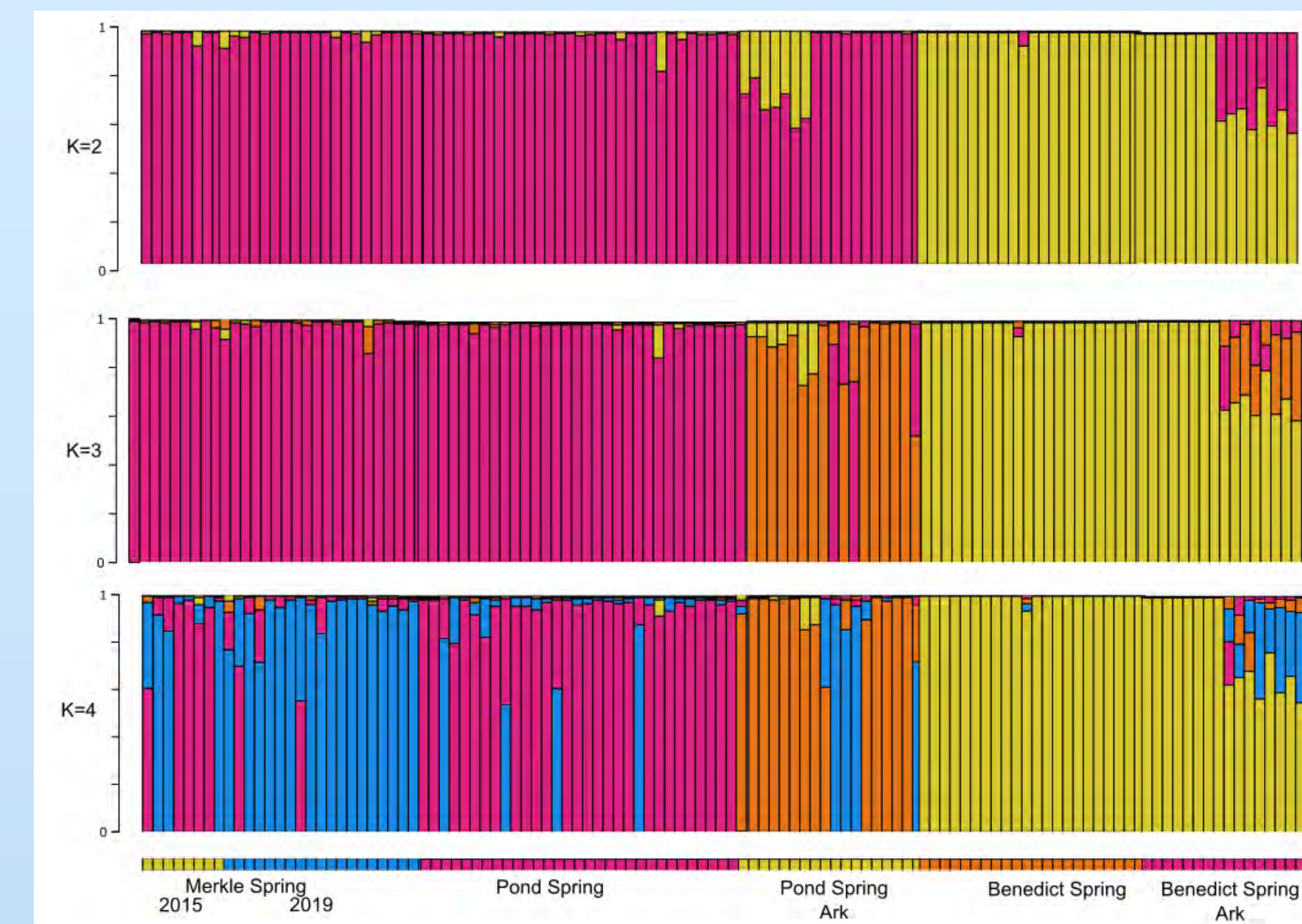

Figure 5. Assignment probabilities based on allele frequency variation at 14 microsatellite loci as performed in STRUCTURE. K indicates the number of assumed populations for each graph. The optimal number of populations as determined by the Evanno K method was three.

Assignment Tests. Results from Bayesian assignment tests showed that native sites Benedict Spring and Pond Spring are genetically distinct, consistent with earlier surveys of these populations. Both ark populations (established beginning in the 1980s) were largely assigned to the same group as their original founding population, although a handful of individuals in both the Benedict Ark population and the Pond Spring Ark population demonstrate admixture.

In the K=3 plot, both Merkle populations were found to be similar to Pond Spring. This result is expected as Merkle was originally founded from Pond Spring fish. However, when K is increased to four, samples from Merkle Spring split from samples obtained in 2014/2015, providing further evidence of drift in this population.

CONCLUSIONS AND FUTURE DIRECTIONS

Our results demonstrate the utility of genetic monitoring for the management of *F. julisia*. We found evidence of reduced genetic variation, both in captivity and in natural habitats. We also demonstrated that the ark population that originated from Benedict spring brood stock effectively maintained variation that had been lost in the wild.

Future monitoring efforts are being planned that will incorporate genome-wide SNP data that will allow more robust conclusions to be made regarding genomic changes in response to recovery efforts. These genomic techniques, combined with regular sampling efforts, can be used to inform breeding strategies aimed at maintaining population fitness and adaptive potential.

Acknowledgements