

The guppy (*Poecilia reticulata*) is a long-standing model of ecological genetics, yet little is known about population subdivision at the whole-genome level. To gain insights into divergence among populations of the guppy (*Poecilia reticulata*), we genotyped 1005 nuclear markers in 239 individuals from 37 sites in Trinidad and Venezuela. We sampled fish at three hierarchical levels: major geographic regions and river systems, rivers within each region, and sampling sites or habitats within a river.

We used three different clustering methods, Neighbor-net, principal components analysis (PCA) and STRUCTURE, to detect population sub-structure. We performed a phylogeographic analysis using the method Neighbor-net implemented in SplitsTree4 by concatenating all SNPs in an artificial nucleotide sequence. After performing PCA on the dataset, we estimated the maximal number of subpopulations that can be found within a dataset by determining the number (p) of statistically significant principal components (PCs) and applied the k -means clustering algorithm onto the first $k-1$ significant PCs as input given k clusters in order to reveal the structure that is described by these PCs. For direct assessment of admixture we used STRUCTURE (version 2.2). STRUCTURE implements a Bayesian model that infers sub-clustering based on assumptions about linkage disequilibrium and Hardy-Weinberg equilibrium.

We found that the hierarchical population substructure agrees with geographic vicariance and previously hypothesized patterns of historical colonization. We detected clear genomic signatures of previous introduction experiments and inferred potential admixture during occasional floods. The present study is an important first step to identifying genes under natural selection in wild vertebrate populations.