

Introduction

- Atlantic Wood Industries is a superfund site on the Elizabeth River (VA), that was once used for wood processing and waste disposal and is highly contaminated with heavy metals and polycyclic aromatic hydrocarbons (PAH).
- One species that has been able to adapt to this highly polluted environment is the Atlantic killifish (*Fundulus heteroclitus*), a small brackish water fish.
- This study interrogated the genomes of *F. heteroclitus* populations collected from the Atlantic Wood Industries site and nearby sites with different levels of PAH pollution to identify potentially adaptively important genes.

Purpose and Methods

- Fish were collected, and PAH concentrations were recorded from various sites in the Elizabeth River (Fig 1) by Dr. DiGiulio's lab (Duke University)¹.
- The fish were sequenced with RAD-seq (Fig. 2), and the sequences were deposited in the National Center for Biotechnology Information².
- Computational approaches were used to call Single Nucleotide Polymorphisms (SNPs), determine pairwise F_{ST} values, and find genes of interest.
- Significant SNPs were those that had high F_{ST} values ($P < 0.01$), in the two most highly polluted sites (Republic and Atlantic Wood) and the least polluted site (Mains).

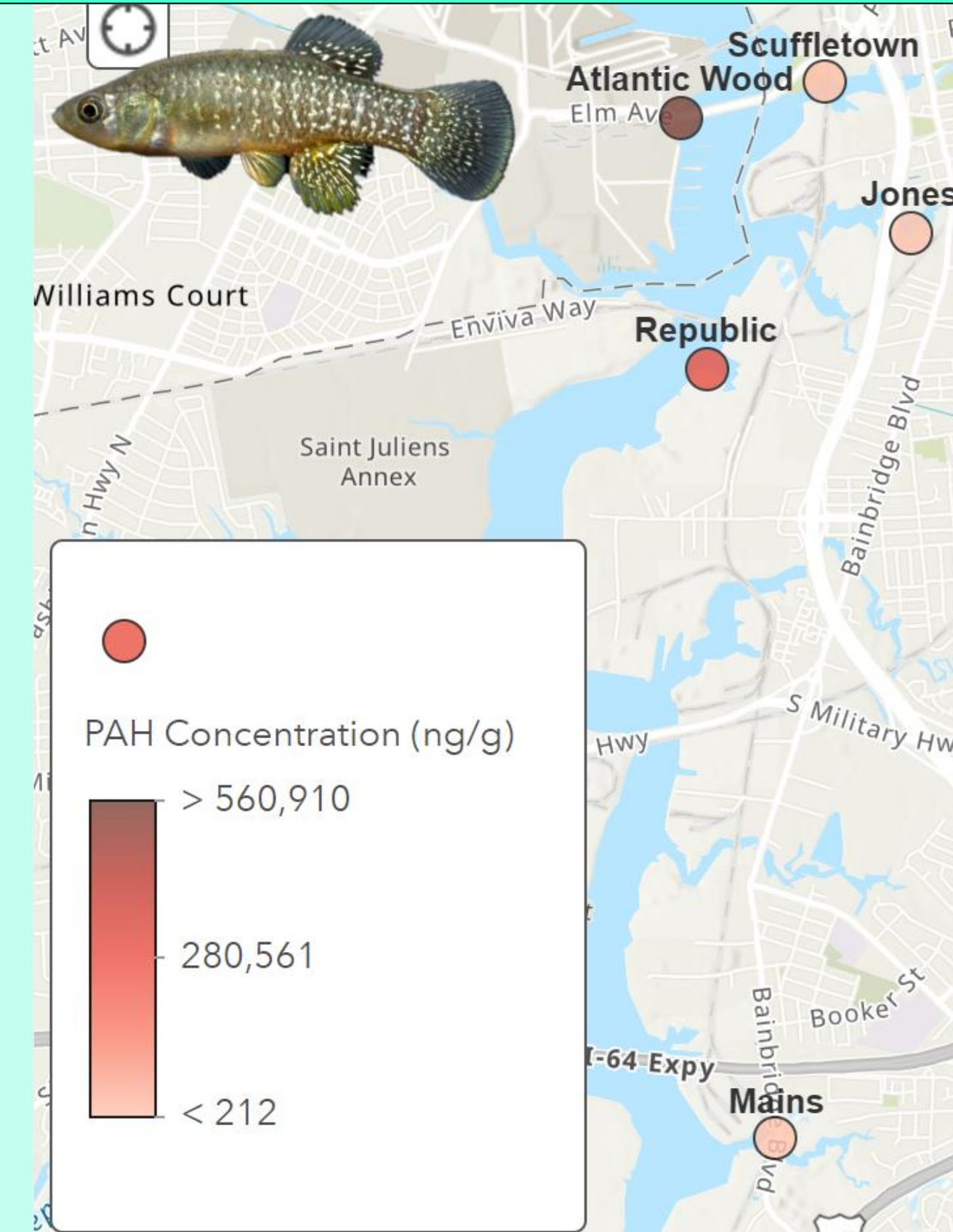


Fig 1. PAH concentration at sites where *F. heteroclitus* were collected.

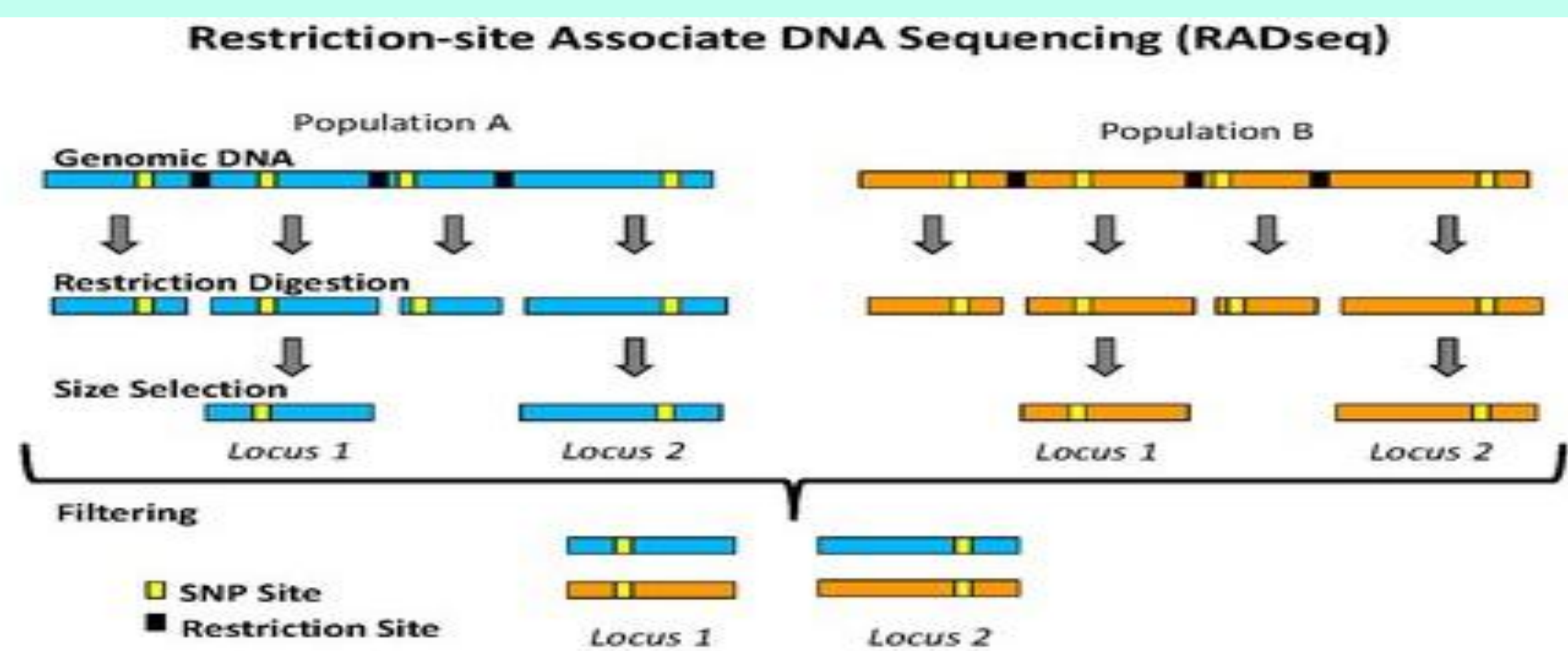


Fig 2. The RAD-seq method, in this process the DNA is cut at restriction sites and put into a database of genomes.

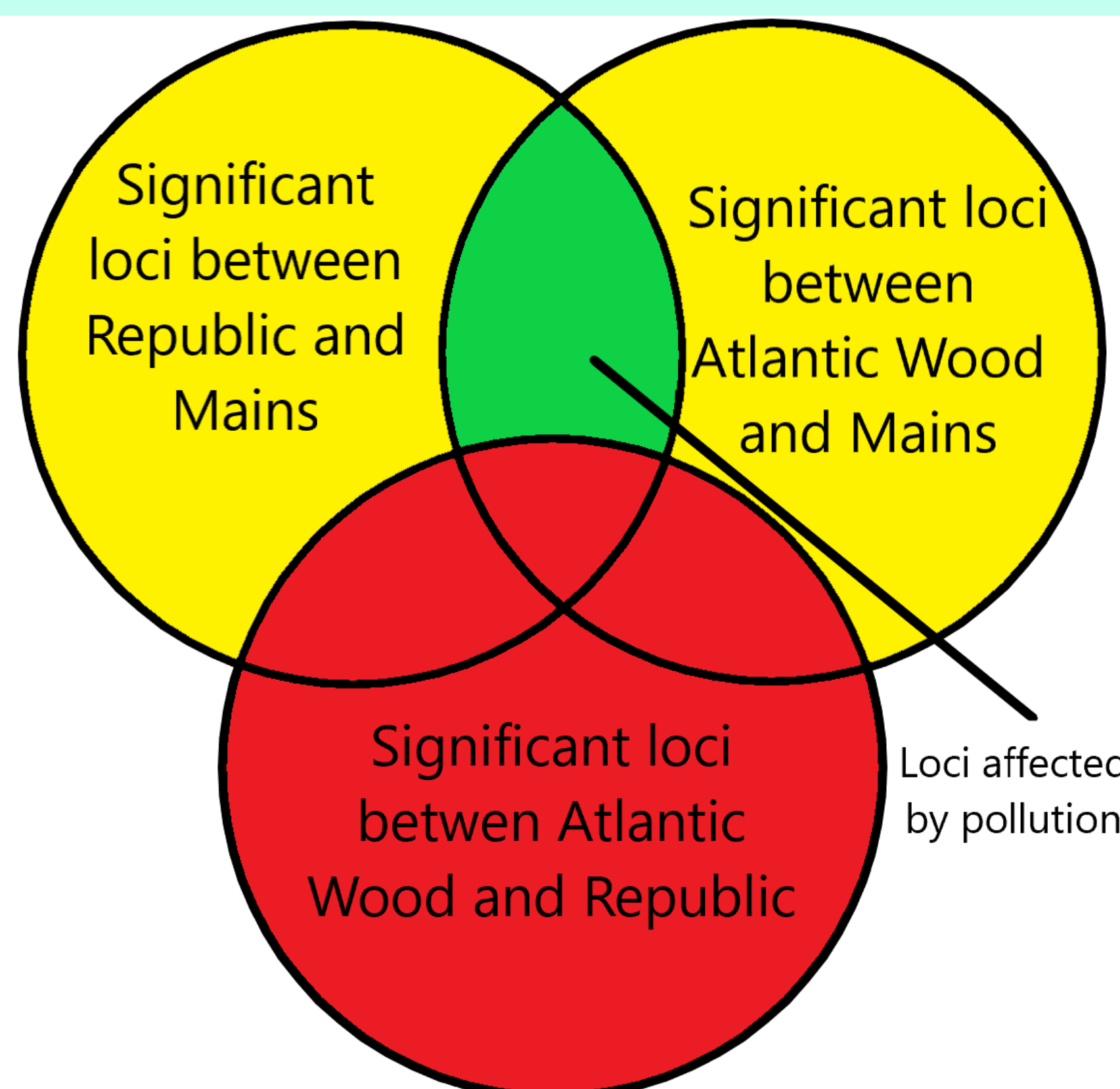


Fig 3. Visualization of which SNPs were used to find genes under selection. The loci in the green part of the Venn Diagram are expected have high F_{ST} because of pollution.

Results

- RAD-seq identified ~100,000 SNPs among 160 individuals in 5 populations.
- After filtering, 10,242 SNPs in 148 individuals were analyzed.
- 66 loci showed significant F_{ST} values ($P < 0.01$) in the two most highly polluted sites (Republic and Atlantic Wood) compared to the least polluted site (Mains, Fig. 4).
- 39 SNPs remained significant after a multiple test correction.

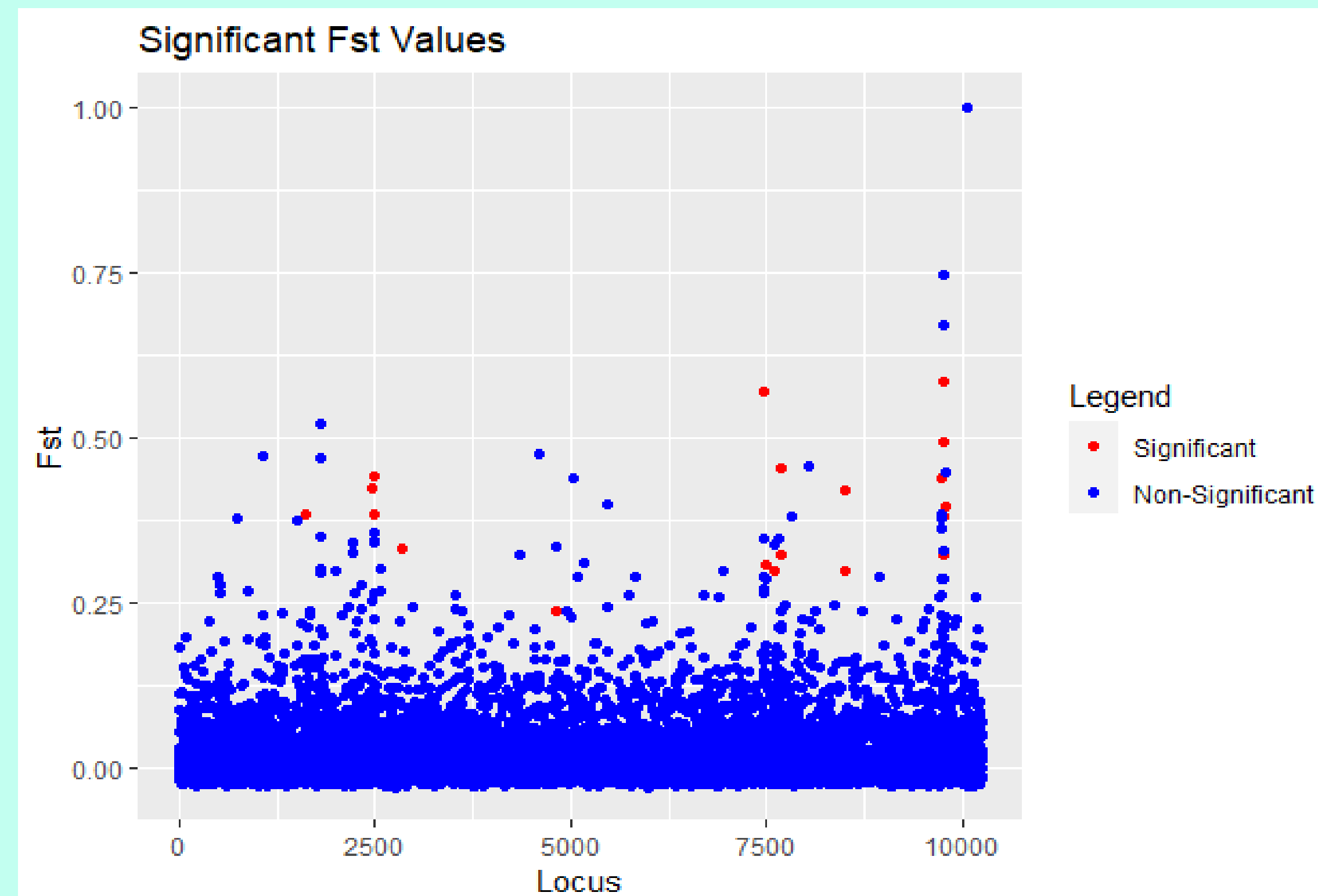


Fig 4. F_{ST} per Locus between Atlantic Wood and Mains. Loci are considered significant if they have an FDR corrected P-value less than 0.01. The red spots are loci with large F_{ST} values that wouldn't be due to random drift between populations.

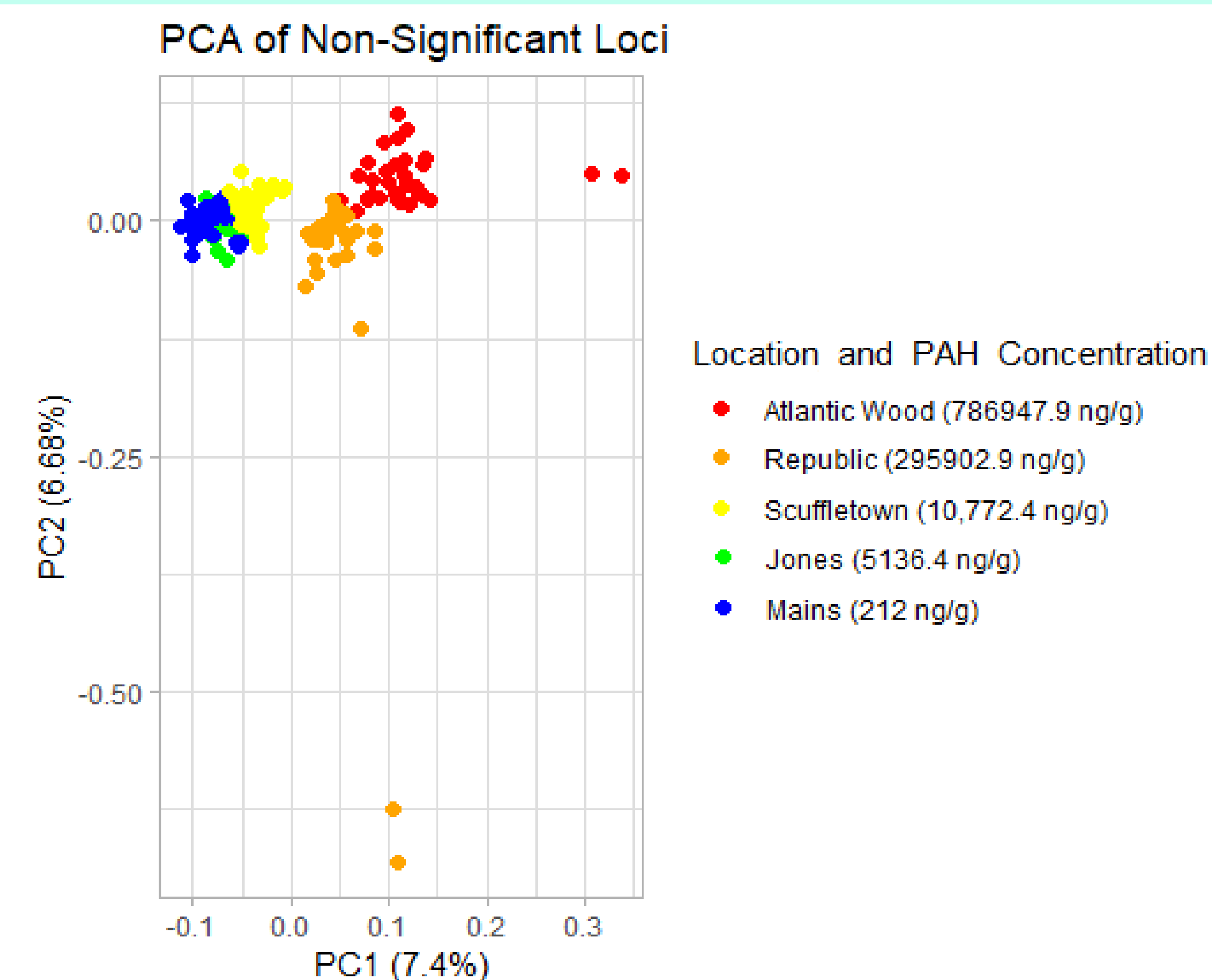


Fig 5. Principal Component Analysis of Non-significant Loci. This principal component analysis used the 10,203 loci that were not under selection and found that populations tend to cluster based on location.

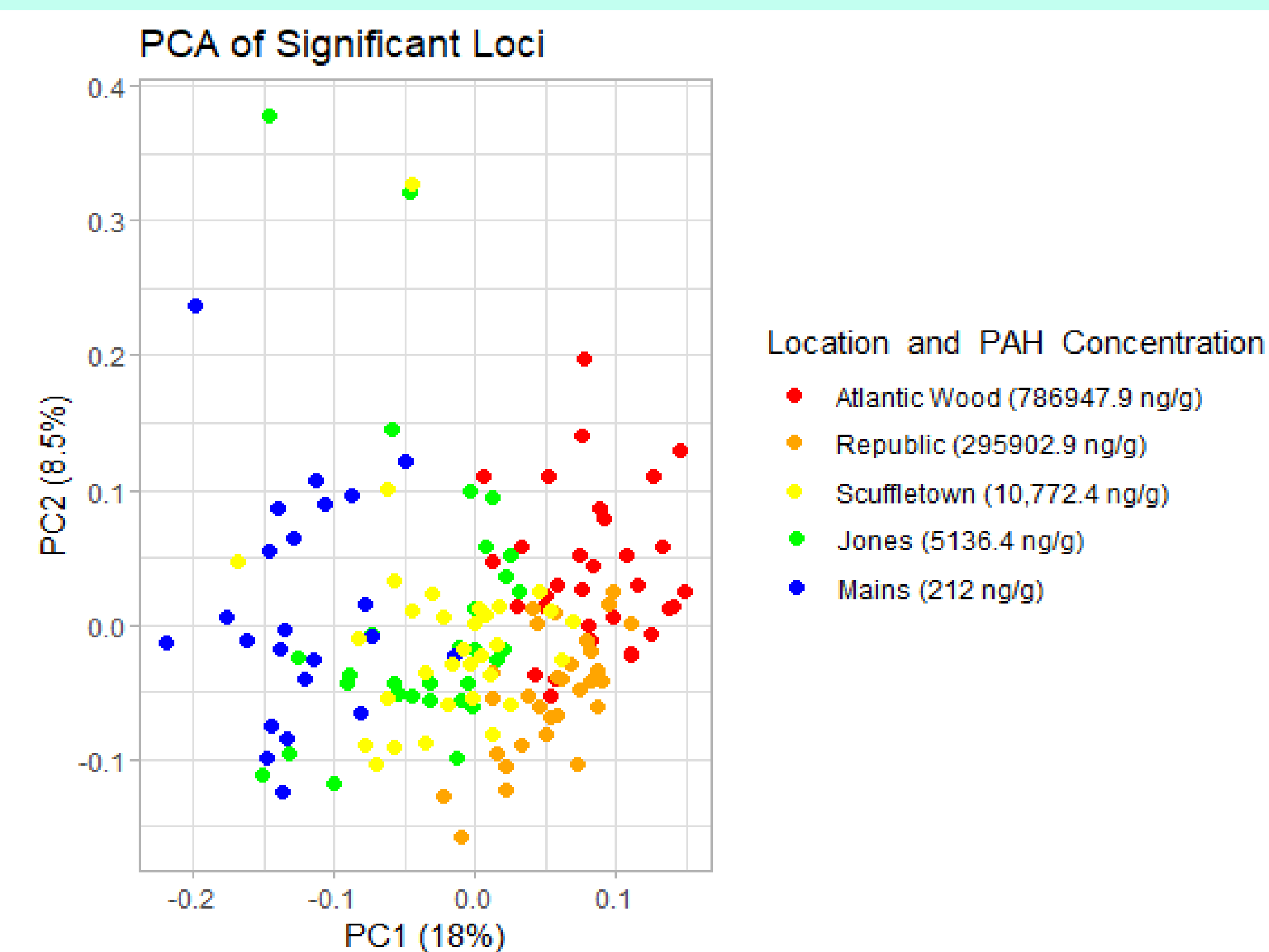


Fig 6. Principal Component Analysis of Significant Loci. This principal component analysis used the 39 loci that were putatively adaptively important showing a clustering based on pollution level.

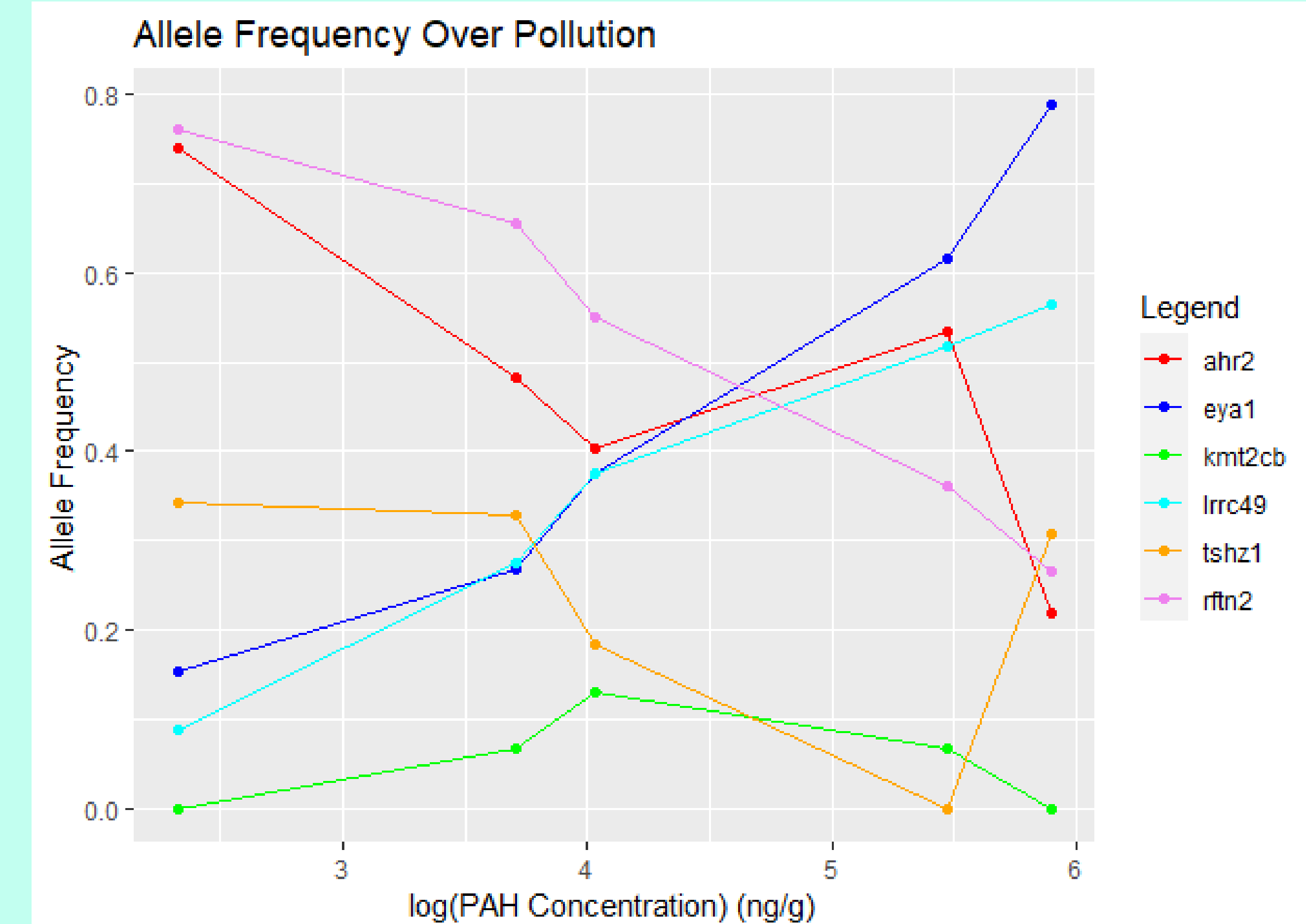


Fig 7. Minor allele frequency over \log_{10} (PAH Concentration) for the six genes that were found to be different between populations. In these genes we can see a correlation between allele frequency and pollution level.

Discussion and Conclusions

- From the 39 loci, 21 genes are associated with those sites.
- Of the genes found to be under selection because of pollution, two of them (*ahr2*³, and *lrcc49*⁴) are involved in detoxification of pollutants.
- The genes *kmt2cb*⁵ and *eya1*⁶ have higher allele frequencies in response to pollution.
- F. heteroclitus* spend their embryonic stage on the beds of rivers and estuaries. Because the pollutants found at Atlantic Wood Industries are found in the sediments, embryonic fish are the most exposed to them, and two developmentally important genes, *tshz1*⁷ and *rftn2*⁸ were identified as potentially adaptively important.
- In polluted sites, some genes may be fixed which can reduce genetic diversity in these populations.

Future Research

- Further investigation into genes associated with pollution at different superfund sites would help understand the genetic components of adaptation to environmental degradation.
- Future studies analyzing the phenotypic response to selection associated with these genes would create a better understanding of the importance of these genes.

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