# Exercises in Marine Ecological Genetics

## 06. Population genomics and genetic diversity

- Summarize and filter VCF files
- Reduce linkage disequilibrium in SNP data
- Assess genetic diversity in SNP data

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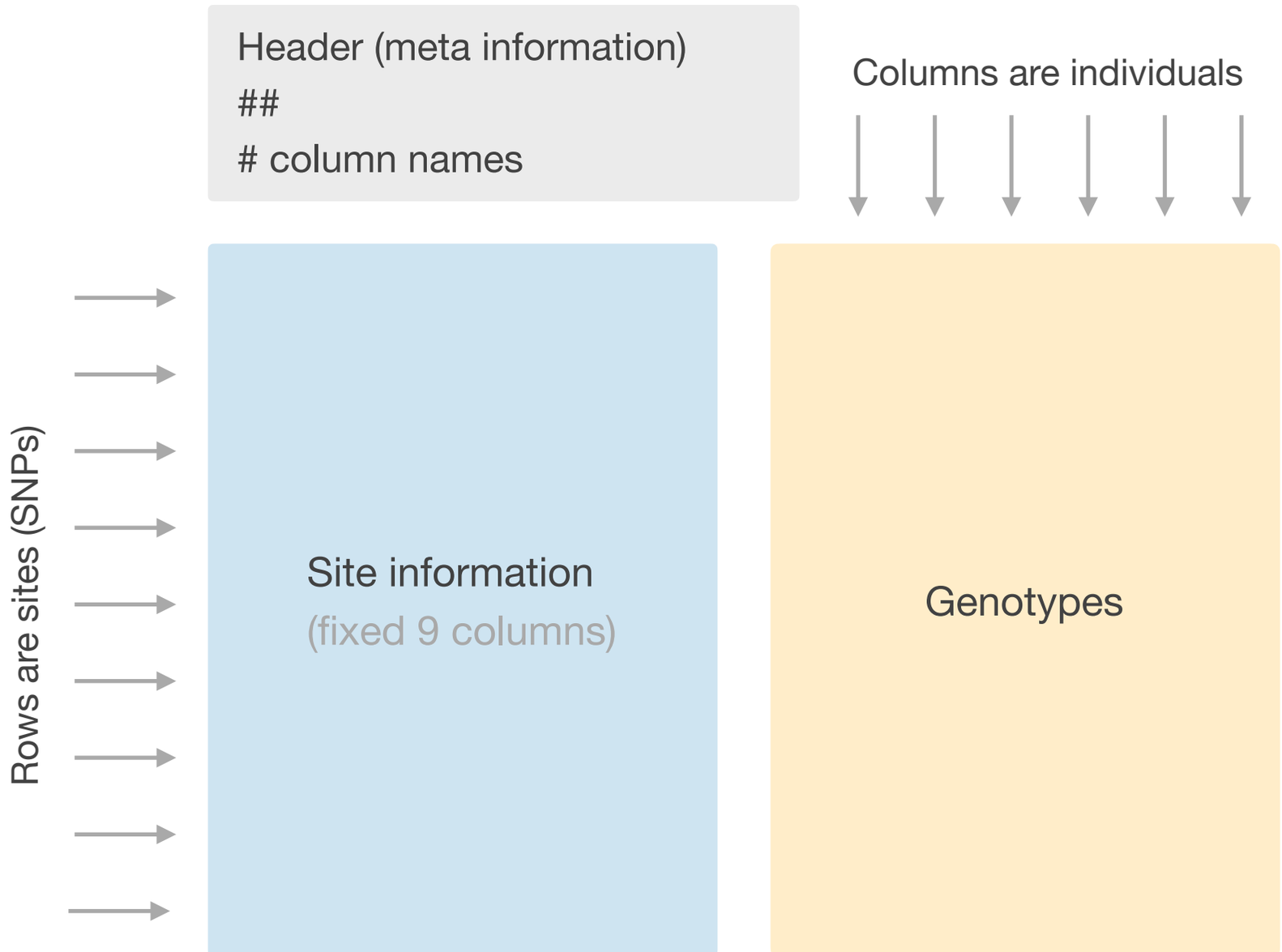
https://github.com/mhelmkampf/meg25

# Variant call format (VCF)

Recap




Efficiently stores

genetic variation data

Optional data e.g:

- allele frequencies
- no. samples with data
- read depth
- genotype likelihood

# Variant call format (VCF)

Recap

```
zcat < local/snps_hamlets_filtered.vcf.gz | head
```

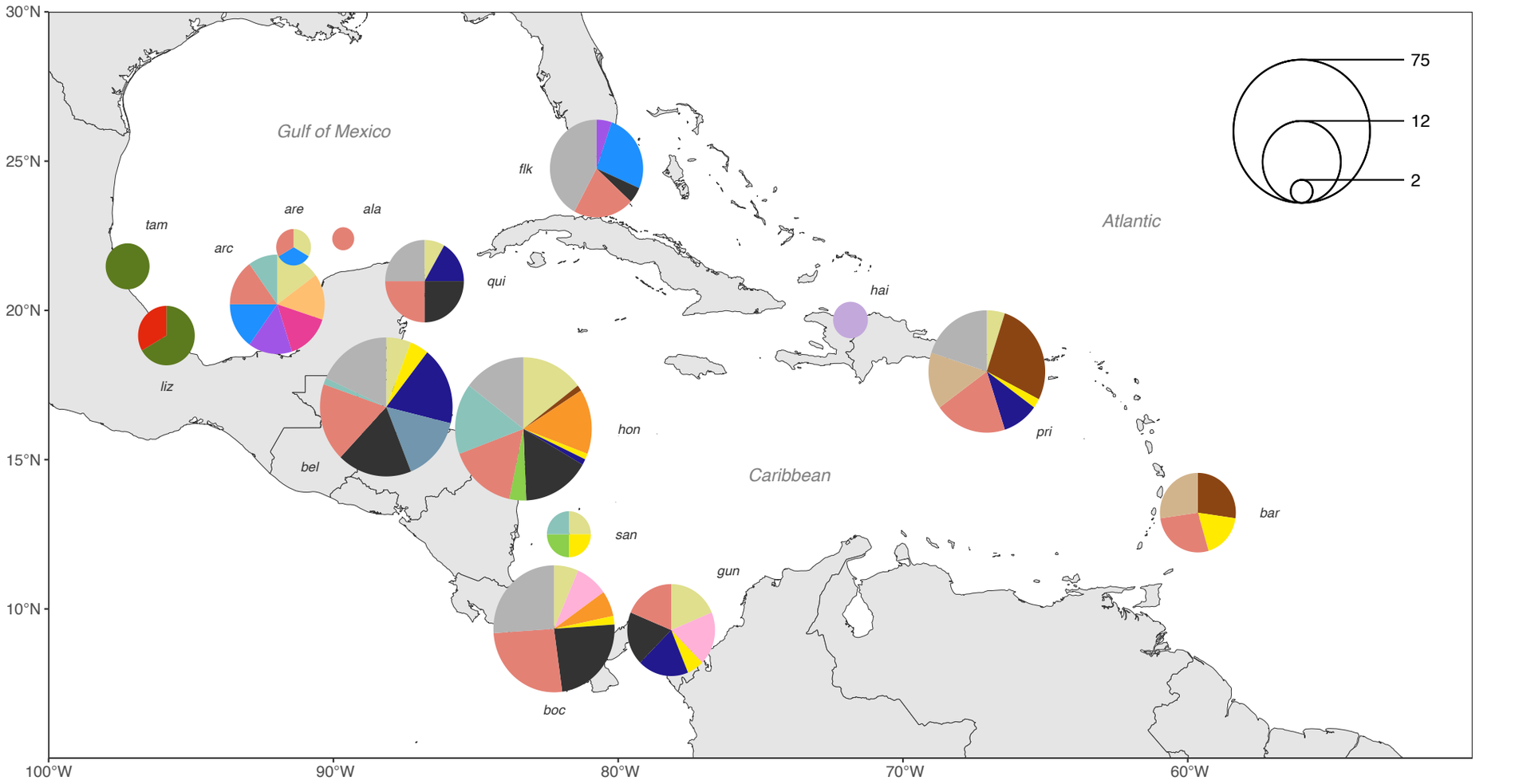
```
##fileformat=VCFv4.1
##fileDate=02012019_20h38m04s
##source=SHAPEIT2.v837
##log_file=shapeit_02012019_20h38m04s_959049fa-700a-4d37-a4ff-3b5db0353190.log
##FORMAT=<ID=GT,Number=1,Type=String,Description="Phased Genotype">
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT 18158nigbel 18159nigbel 18162nigbel …
LG12 4152 . T G . PASS . GT 0|0 0|0 0|0 …
LG12 4228 . C A . PASS . GT 0|1 0|0 0|1 …
LG12 4262 . A G . PASS . GT 1|0 0|1 1|0 …
LG12 4263 . C T . PASS . GT 0|1 1|0 0|1 …
```

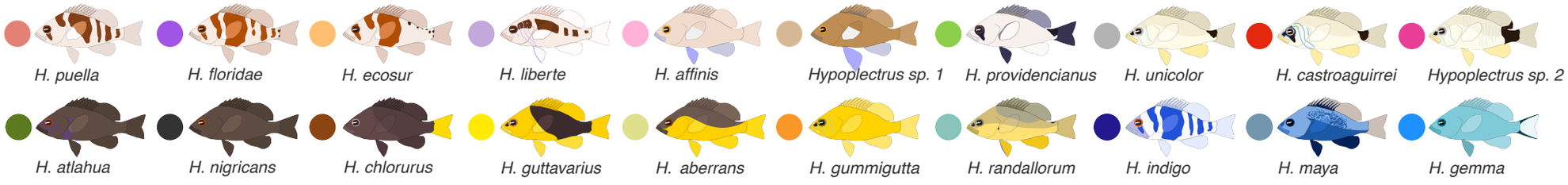
`0|0` Homozygous for reference (1st) allele

`1|1` Homozygous for alternate (2nd) allele

`0|1` and `1|0` Heterozygous

# Example dataset



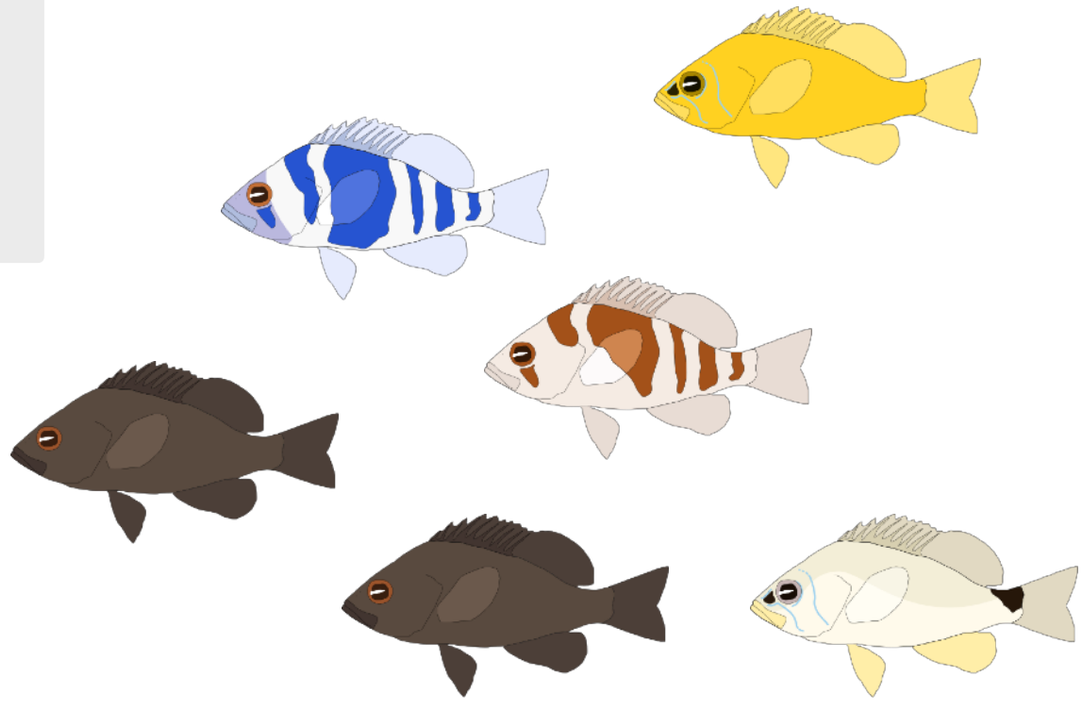



# Example dataset

- 19 species of hamlet (genus *Hypoplectrus*)
- 15 sites in Caribbean and Gulf of Mexico
- 327 hamlet samples total
- Illumina short-read resequencing (mean depth 17×)
- Genotyping with GATK
- High-quality reference genome of *H. puella*

**hamlets_LG12_snp.vcf.gz**

- Chromosome 12 only
- Subset to 36 samples from 6 populations



Illustrations by Kosmas Hench

# LD statistics

Exercise 2

$D_{AB} = p_{AB} - p_A p_B$

Coefficient of linkage disequilibrium between two alleles

0 to ±1, but constrained by allele frequencies

Product of allele frequencies

Haplotype frequency

$D' = D / D_{max}$

*D* normalized with respect to allele frequencies

0 to ±1, full range (0: no association, ±1: perfect LD)

Max value given allele frequencies

$$r^2 = \frac{D^2}{p_A (1 - p_A) p_B (1 - p_B)}$$

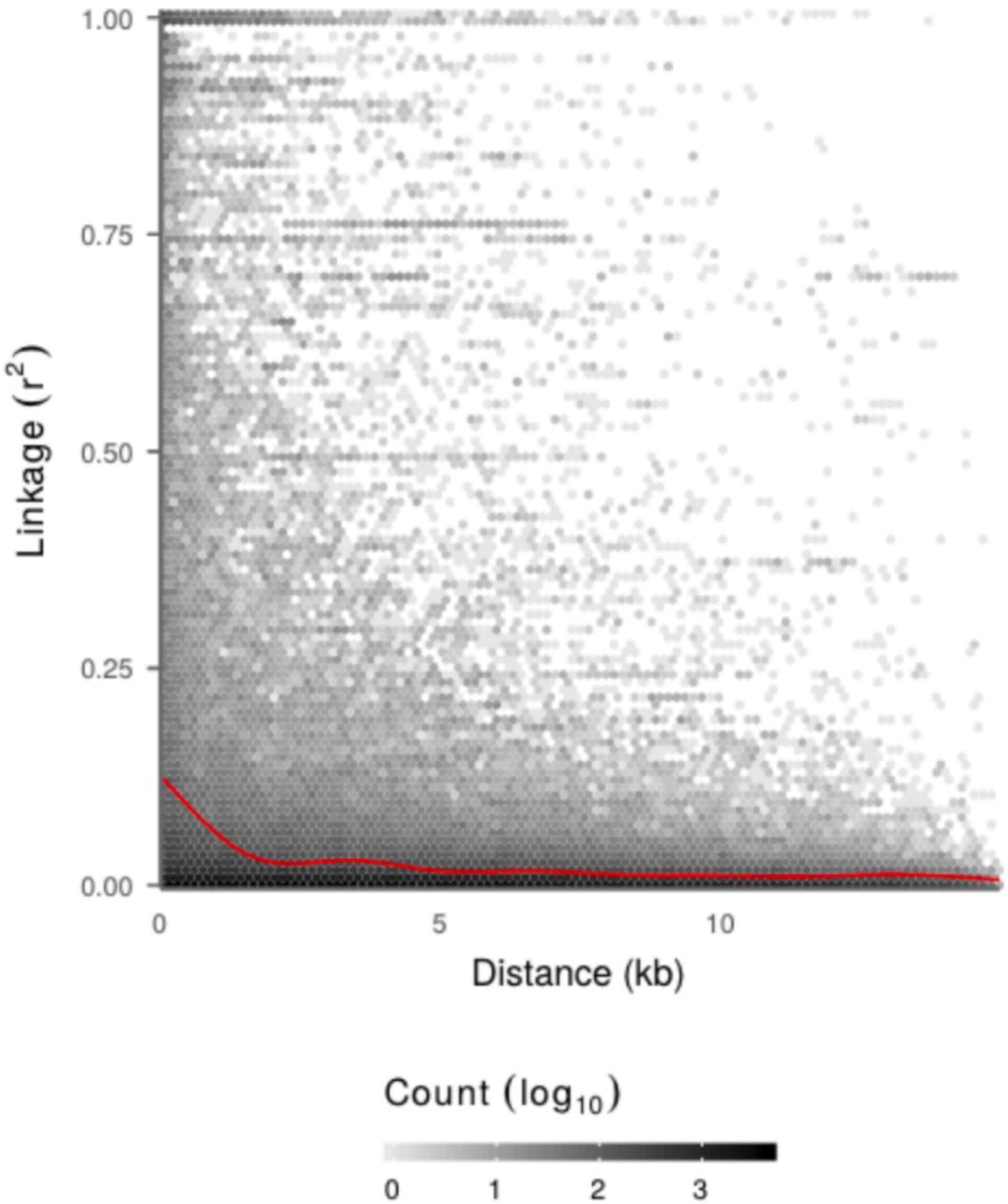
Correlation coefficient of linkage disequilibrium

0 to 1, but constrained by allele frequencies

a.k.a. *ρ* (*rho*)

# Decay of linkage with physical distance

Exercise 2




Hench et al. 2019 (Nat Ecol Evol)

# Nucleotide diversity (pi)

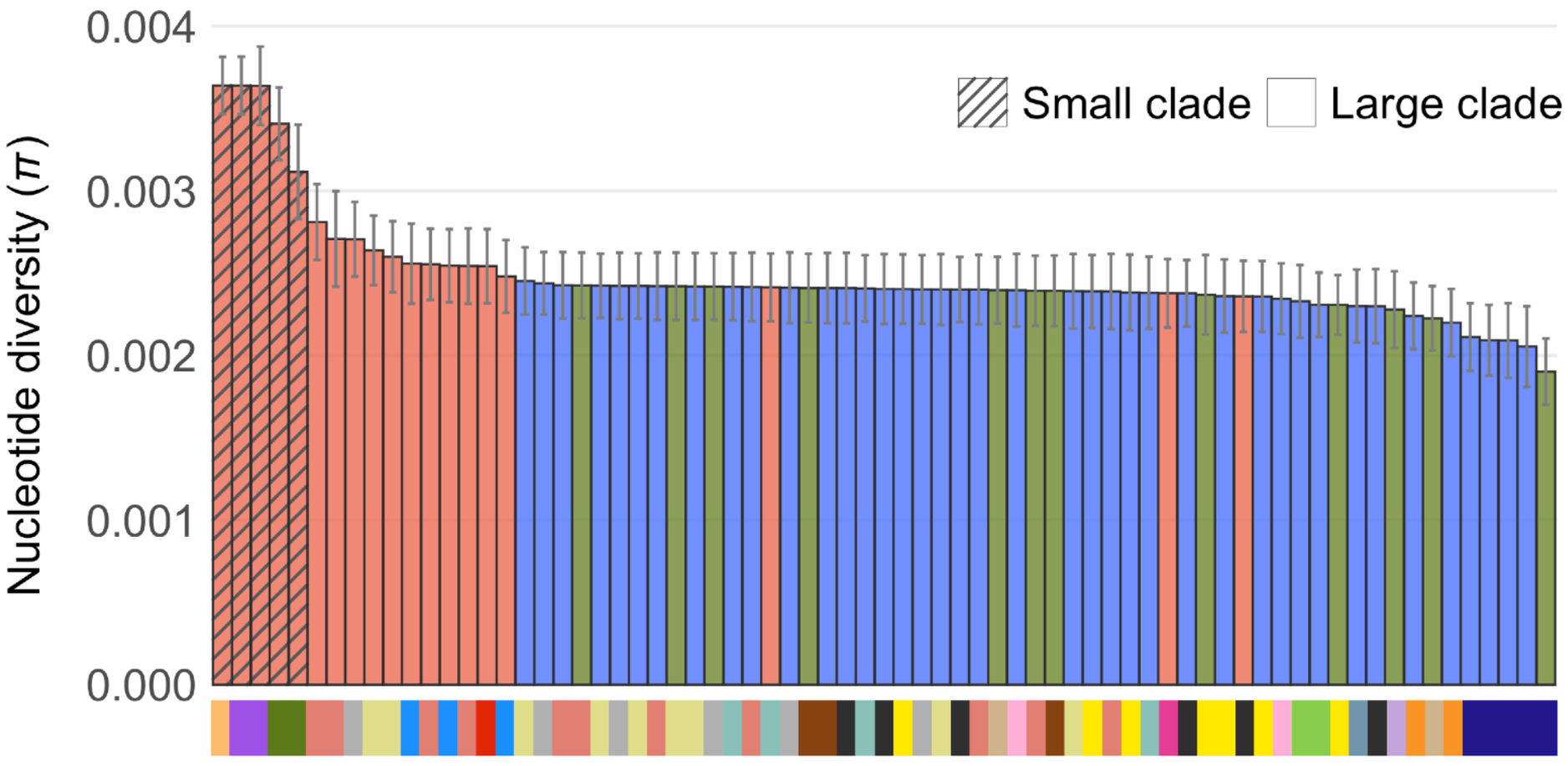
Exercise 3

Average number of nucleotide differences per site between all possible pairs of sequences

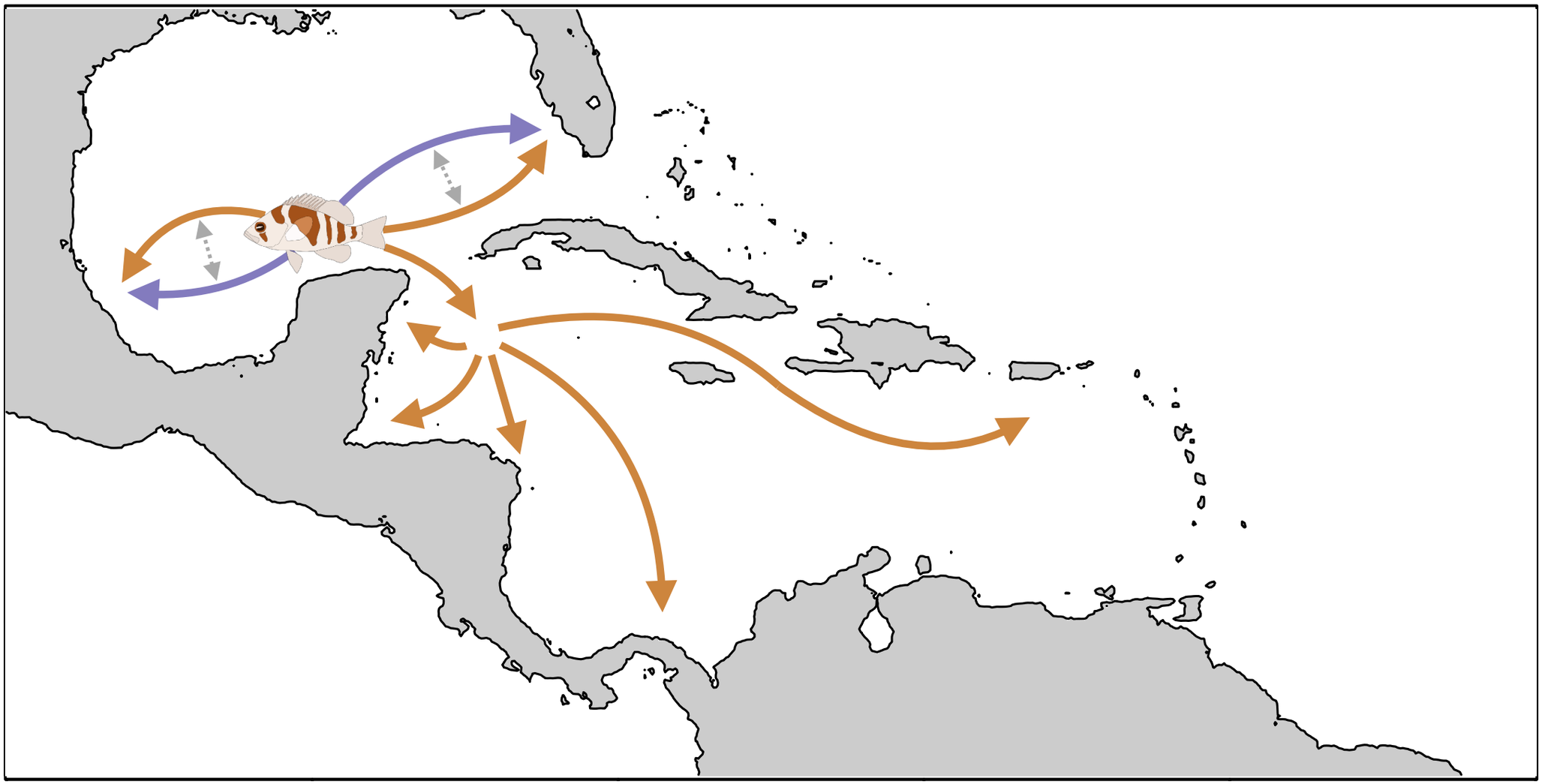
$$\hat{\pi} = \frac{n}{n-1} \sum_{ij} x_i x_j \pi_{ij} \;:$$

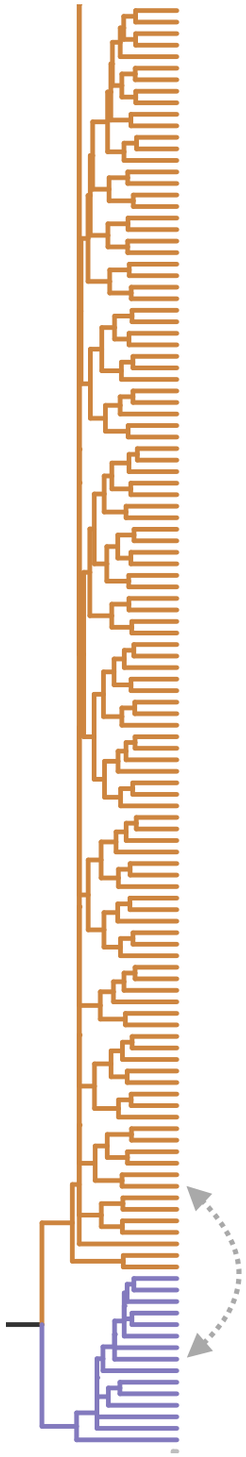
# Nucleotide diversity (pi)





# An emerging scenario





Map data provided by NOAA

# Population genomics and genetic diversity

Summary

```
vcftools --gzvcf … --mac2 --thin 2000 --rcode …    # Filter by MAC and distance

vcftools --gzvcf … --het --stdout > …                # Calculate heterozygosity

vcftools --gzvcf … --keep <pop.txt> --site-pi --out …            # Calculate pi
```

- SNPs must be filtered carefully, e.g. with respect to minor allele count or missing data, to ensure high-quality results
- Removing SNPs in linkage disequilibrium is important for analyses that assume independence between loci
- Genome-wide statistics like heterozygosity or nucleotide diversity (pi) provide valuable information about genetic variation within populations

# Nucleotide diversity (pi)

Exercise 3

Average number of nucleotide differences per site between all possible pairs of sequences

$$\hat{\pi} = \frac{n}{n-1} \sum_{ij} x_i x_j \pi_{ij}$$ :

| Site: | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Sample A: | A | G | C | T | T |
| | A | G | C | T | T |
| Sample B: | A | G | T | T | T |
| | A | G | T | T | T |
| Sample C: | G | G | C | C | T |
| | G | G | C | T | T |
| No. diff: | 8 | 0 | 6 | 5 | 0 |
| Pi: | 0.4 | 0 | 0.4 | 0.3 | 0 |