Introduction - study species/system and relevant background

The swallow bug (*Cimex vicarious*) is a hematophagous, wingless ectoparasite distributed throughout North America (Brown et al. 2017; Hamlili et al., 2023). Swallow bugs parasitize the cliff swallow (*Petrochelidon pyrrhonota*) which is a migratory and highly colonial passerine that nests in gourd-shaped mud nests attached to cliff faces and rocky outcrops, underneath bridges, and inside culverts (Moore et al. 2007, Brown et al. 2013). Swallow bugs actively disperse within a colony which indicates potential panmixia at the colony level (Rannala 1995; Reinhardt and Siva-Jothy, 2007). However, less is known on their dispersal across cliff swallow colonies. Within the study area in SW Nebraska, colonies are typically separated by 1-10km (Brown and Brown 2005). The adult swallow bugs disperse by attaching to the legs and toes of cliff swallows while instars attach to the brood patch (Rannala 1995; Brown and Brown 2004b), but it is unknown how frequently or successful bugs disperse.

Research question(s)

My study aims at understanding the processes structuring the population of an ectoparasite by analyzing the genetic structure of *Cimex vicarius* in a spatial context. Specifically, for this class project I will evaluate the genetic differentiation with respect to distance from ancestral cliffs and neighboring colonies <u>to determine if gene flow between colonies is reduced by geographic distance.</u>

Methods

The data for the project was generated using a double-digest restriction associated sequencing (ddRAD-seq) approach following that of Peterson *et al*. with modifications (2012). The libraries were pooled in equimolar amounts for multiplexed, single-end sequencing performed on an Illumina HiSeq4000 at the University of Oregon Genomics and Cell Characterization Core Facility (GC3F).

Post-sequencing, raw illumina sequences were inspected for quality using FastQC (Andrews, 2014). The raw sequences were then demultiplexed and filtered using the *process_radtags* pipeline in *STACKS 2.65* (Catchen et al., 2011). I used an integrated alignment method for assembling reads with the reference genome of *Cimex lectularius*. The populations module in *STACKS* was used to filter the data and export loci for downstream analyses. Each SNP had to be present in 70% of individuals within a subpopulation and in a minimum of two populations. A minimum allele count of 3 and maximum heterozygosity of 0.8 were required to process a nucleotide site. Finally, a single SNP was exported to avoid linkage disequilibrium (--write-random-snp).

For this project, I will be using the R package *adegenet v2.1.1* (Jombart, Devillard, & Balloux, 2010) to evaluate the presence and magnitude of putative isolation by distance of swallow bugs across cliff swallow colonies. The *mantel.randtest* function will be used to compute the correlation between the estimated genetic differences and the respective geographic distance (Euclidean).

Predictions/expected outcomes

I expect there to be a degree of isolation by distance - especially along the West-East gradient as the ancestral colony sites are located in the west of the study area.