PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XXXV cycle, 2019-21)

Project draft

1.Field of interest

BIO/03 Botanica Ambientale e Applicata, AGR/11 Entomologia Generale e Applicata

2.Project title

Impact of climate oscillations on the population genomics of cold-adapted endemic plants and their pollinators

3.Tutor

Dr. Matteo Montagna (AGR/11); **Dr. Simon Pierce** (BIO/03)

Relatore esterno: **Dr. Cristiano Vernesi** (Fondazione Edmund Mach; www.fmach.it; Dichiarazione d'intento in allegato)

4.Relevance of the topic and state of the art:

The risk of species extinction is closely linked to endemism and geographic range restriction. For cold-adapted species, ongoing restriction of geographic ranges is particularly severe as increasing average global temperature diminishes the extent of suitable bioclimatic envelopes[1]. Indeed, the main response of species to rapid, large-magnitude climatic changes are geographical distribution changes, with 8% of plants, 6% of insects and 4% of vertebrates projected to lose over half of their climatically-determined geographic range following global warming of 1.5°C[1].

Anthropogenic climate forcing forms part of an ongoing history of oscillations in both climate and species distributions. Species responses to past warming can be informative with regard to the velocity and extent of predicted future changes. For example, cold-adapted species with Alpine/Apennine or Boreo-Alpine distributions are known to have suffered highly fragmented distributions during warmer interglacial periods, surviving as isolated populations in montane refugia. As reduced local population size can limit genetic variability and population viability, knowledge of changes in population genomics in response to climate oscillations, evident from genomic, distribution and ecological information, promises a detailed view of population responses and thresholds for local extinction, or extinction risk.

Additionally, plants exhibit minimum viable population sizes affecting the capacity to attract sufficient pollinators[2]. Bumblebees are key pollinators for many endemic or rare plant species, and fragmentation of plant populations is known to alter bumblebee flight behaviour and pollination patterns[3]. Climate change is predicted to induce species-specific shifts in bumblebee distributions and changes in the structure of bumblebee communities[4], and the extent to which these changes will mirror those of plants is unknown.

5.Layout of the project (draft)

5.1. Materials & Methods:

The proposed doctoral project will infer Pleistocene demography and distribution of a model an Italian endemic cold-adapted plant species, *Campanula raineri* (Campanulaceae), and its bumblebee pollinators, to forecast their population structures and distributions under global warming. Detailed results for the model plant and animal species will then be used to create predictions for similar endemic, cold-adapted rocky outcrop plant species and their pollinators. The project will: 1) update existing records of species distribution, 2). employ a 2b-restriction site-associated DNA (2b-RAD) sequencing approach to infer demographic characteristics of the target species during the Quaternary oscillations, 3). integrate these data with paleoclimate data, current and likely future climatic conditions to reconstruct past distributions and predict extinction risks for local subpopulations, 4). extend these results to similar endemic, cold-adapted plant and animal species.

Specifically, species distribution records will be achieved by the collation of published georeferenced distribution records (e.g., using CKmap, Osservatorio della Biodiversità-Regione Lombardia). This activity will be combined with field excursions throughout the historic range of the species to simultaneously confirm/update historical records and initiate the sampling effort.

Past demographic reconstruction will be achieved by selecting appropriate restriction enzymes and initial *in silico* simulation to predict the number of loci obtained after a genome-wide enzymatic double digestion using *RestrictionDigest* perl module (or similar pipelines) and the most widely used digestion enzymes. MSs genome sizes and GC contents will be inferred from data for close relatives available in the Animal Genome Size Database (www.genomesize.com) and the Plant DNA C-values Database (http://data.kew.org/cvalues/). High-molecular-weight genomic DNA will be extracted by plant and animal tissues using protocols previously adopted and *ad hoc* optimized in our laboratories, namely C-TAB for plant tissues and E.Z.N.A.R Insect DNA Kit in the case of insects. After surface sterilization of sampled material, DNA will be extracted from the legs in the case of *Bombus* species.

For 2b-RAD wet lab and sequencing, DNAs will be digested using enzymes selected above and libraries prepared following the 2b-RAD genotyping protocol available at the Meyer Lab website (http://people.oregonstate.edu/~meyere/tools.html). The obtained libraries will be sequenced by Illumina NextSeq500 using TG NextSeqR500 High Output Kit v2 (75cycles).

Sequence data analyses: in order to obtain the SNP matrices, raw sequences will be processed using 2bRAD utilities v3.0 (https://github.com/Eli-Meyer/2bRAD_utilities) with the implementation of *ad-hoc* scripts. Population structure and the existence of distinct populations will be inferred by fastSTRUCTURE[5]. Past demography of each population will be estimated using the coalescent-based multilocus analysis implemented in BEAST2[6], setting the EBSP as the tree prior model, and adopting maximumlikelihood framework using δaδi[7,8]. To test the hypothesized evolutionary scenarios, different methods will be adopted such as those implemented in DIYABC[9] and in Migrate[10]. Some of the analyses will be performed using *adegenet* and *ape* R packages.

Past, present, future 5MSs distribution modelling will employ datasets with the species' presence localities will be generated at GPS-precision points, from species' distributions as determined above. Bioclimatic variables will be downloaded from WorldClim.org at 30" spatial resolution and cut to the extent of the European Alps. After this process, variables will be tested for possible multicollinearity and the modelling process will be performed through the *biomod2* R package. Past projections will be performed once that model is calibrated using target species' occurrence localities and current climatic conditions.

5.2. Schedule and major steps (3 years):

First year: planning of the research activities, bibliographic research and delimitation of known historical distributions of target species, also to guide fieldwork. Field collecting activities organized on the Southern ridge of the Central and Eastern Alps where the target species are present. *Bombus* species will be collected by malaise traps or directly by net on flowers. All plant tissues and insects will be stored at -20 °C to preserve the DNA.

Second year: Selection of the most appropriate restriction enzyme pairs for the target species. DNA extraction.2b-RAD wet lab and sequencing. Sequence data analysis. Past, present, future distribution modeling for target species.

Third year: writing manuscripts, manuscript submission and presentation of the achieved results in national and international congresses.

6. Available funds (source and amount)

Pierce: € 2500
Montagna: € 1000
Vernesi (Fondazione Edmund Mach): "I declare that I'm willing to provide support in terms of reagents for the wet lab activities, along with logistic support for field trips" (vedi "*Letter of commitment*" in allegato).

7. Literature:

[1]IPCC (Intergovernmental Panel on Climate Change). 2018. *Global warming of 1.5°C*. Switzerland: IPCC.

[2]Pierce, S., Spada, A., Caporali, E., Puglisi, F., Panzeri, A., Luzzaro, A., Cislaghi, S., Mantegazza, L., Cardarelli, E., Labra, M., Galimberti, A. & Ceriani, R.M. 2018. Identifying population thresholds for flowering plant reproductive success: the marsh gentian (*Gentiana pneumonanthe*) as a flagship species of humid meadows and heathland. *Biodiversity and Conservation* **27:** 891–905. (https://vimeo.com/241145178/0aa594360d)

[3]Goverde, M., Schweizer, K., Baur, B. & Erhardt, A. 2002. Small-scale habitat fragmentation effects on pollinator behaviour, experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. *Biological Conservation* **104:** 293–299.

[4]Prandervand, J.-N., Pellissier, L., Randin, C.F. & Guisan, A. 2014. Functional homogenization of bumblebee communities in alpine landscapes under projected climate change. *Climate Change Responses* **1:** 1.

[5]Raj, A., Stephens, M. & Pritchard, J. K. 2014. Variational inference of population structure in large SNP datasets. *Genetics* **197:** 573-589.

[6]Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., Suchard, M. A., Rambaut, A. & Drummond, A.J. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS computational biology* **10(4):** e1003537.

[7]Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H. & Bustamante, C. D. 2009. Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS genetics* **5(10):** e1000695.

[8]Jouganous, J., Long, W., Ragsdale, A. P. & Gravel, S. 2017. Inferring the joint demographic history of multiple populations: beyond the diffusion approximation. *Genetics* **206(3):** 1549-1567.

[9]Cornuet, J. M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., Martin, J. M. & Estoup, A. 2014. DIYABC v2. 0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *Bioinformatics* **30(8):** 1187-1189.

[10]Beerli, P. & Felsenstein, J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences* **98(8):** 4563-4568.