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**Autor:** Duchen, Pablo / Höhna, Sebastian / Catalán, Ana  
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# Genomics of European fireflies : first results and next steps

Pablo DUCHEN<sup>1</sup>, Sebastian HÖHNA<sup>2,3</sup> & Ana CATALÁN<sup>4</sup>

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## Abstract

Climate change is affecting biodiversity significantly. Thus, finding the genes that allow organisms to adapt to such changes is a primary goal in evolutionary genetics. European fireflies constitute a novel system to study genetic adaptation to changing environments. They occur widespread South and North of the Alps, thus having adapted to various ecosystems. Unfortunately, genomic resources to study the genes responsible for this adaptation are not available for any European species. For this reason, we are currently sequencing the genomes of *Lampyrus noctiluca*, *Lamprohiza splendidula*, and *Luciola italica*, from which *L. noctiluca* and *L. italica* are represented by Swiss samples from the canton Vaud. In this report, we describe our collection and genomic DNA extraction methodologies, the planned sequencing and gene annotation strategies, and the planned data analyses.

**Keywords:** Adaptation, climate change, reference genome, genome annotation, latitudinal cline, Alps.

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## Résumé

Le changement climatique affecte considérablement la biodiversité. Ainsi, trouver les gènes qui permettent aux organismes de s'adapter à de tels changements est un des objectifs principaux de la génétique évolutive. Les lucioles européennes constituent un nouveau système pour étudier l'adaptation génétique aux environnements changeants. Elles sont distribuées au Sud et au Nord des Alpes, s'étant ainsi adaptées à divers écosystèmes. Malheureusement, des ressources génomiques pour étudier les gènes responsables de cette adaptation ne sont disponibles pour aucune des espèces européennes. Pour cette raison, nous séquençons actuellement les génomes de *Lampyrus noctiluca*, *Lamprohiza splendidula* et *Luciola italica*, dont *L. noctiluca* et *L. italica* sont représentés par des échantillons suisses du canton de Vaud. Dans ce rapport, nous décrivons nos méthodologies de collecte et d'extraction d'ADN génomique, les stratégies prévues de séquençage et d'annotation de gènes, ainsi que les analyses de données prévues.

**Mots-clés:** Adaptation, changement climatique, génome de référence, annotation des génomes, gradient latitudinal, Alpes.

<sup>1</sup> Department of Computational Biology, University of Lausanne, Switzerland.

<sup>2</sup> GeoBio-Center, Ludwig-Maximilians-Universität München, Richard-Wagner Str. 10, 80333 Munich, Germany.

<sup>3</sup> Department of Earth and Environmental Sciences, Paleontology & Geobiology, Ludwig-Maximilians-Universität München, Richard-Wagner Str. 10, 80333 Munich, Germany.

<sup>4</sup> Division of Evolutionary Biology, Ludwig-Maximilians-Universität München, Grosshaderner Straße 2, Planegg-Martinsried 82152, Germany.

Correspondence: pablo.duchenbocangel@unil.ch, ana.catalan@gmail.com.

## INTRODUCTION

Different ecosystem types can be found in Europe, including hot Mediterranean habitats, high altitude Alpine forests and cold temperate northern ecosystems. Some species are only able to inhabit a particular ecosystem, but others, have been able to colonize and thrive in different environments (IVITS *et al.* 2013). Widespread species, capable of inhabiting different ecosystems are ideal for the study of adaptation to different habitats. Additionally, environmental change has been accelerated as a result of human activity, further pushing populations to adapt to their ever-changing environments (FRANKS & WEIS 2008). Considerable theoretical and empirical work has been done on the genetics of local adaptation, but most of this work has largely focused on model organisms (NIELSEN 2005, CATALÁN *et al.* 2016, STEPHAN 2019). Therefore, studying multiple taxa outside of traditional model species is necessary to achieve a better and more general understanding of the complex process of adaptation.

We have identified a novel system for the study of adaptation: the firefly system. In Europe, two species show an ample geographical distribution, including regions south and north of the Alps: *Lampyrus noctiluca* (NOVAK 2017) and *Lamprohiza splendidula* (SCHWALB 1961; ÁLVAREZ & DE COCK 2011). Fireflies are conspicuous beetles that produce bioluminescence, which is used in larvae and adults as a warning signal, due to their toxicity and distastefulness, as well as for finding mates and prey (LEWIS *et al.* 2004, LEWIS & CRATSLEY 2008). *L. noctiluca* is the most widely distributed European firefly, being this species found from the Iberic and Balkan peninsula to Finland (NOVAK 2017). Similarly, *L. splendidula*'s distribution ranges from the Balkan peninsula to northern Germany (SCHWALB 1961; ÁLVAREZ & DE COCK 2011). In Switzerland, in addition to *L. noctiluca* and *L. splendidula*, we can find *Luciola italica* and *Luciola lusitanica* (GURCEL *et al.* 2020). In the case of *L. italica*, its habitat was, until recently, restricted from the Alps towards the south. However, after its introduction in the canton Vaud in the late 1930s (FAES 1941), *L. italica* started migrating and establishing populations north of the Alps (NEUMEYER 1991). Thus, *L. italica* offers us the opportunity to study a very recent colonization event and its impact at the genetic level. Even though, the ecology and behavior of European fireflies is well documented (SCHWALB 1961, DE COCK 2009), genomic resources are lacking for these species. By generating genomic resources (fully sequenced and annotated genomes), we will be able to study the genomic signatures left by colonization events, migration, and local adaptation.

Our goal is to generate the first population-level genomic data set for European fireflies, from several populations in Switzerland, Italy, and Germany, where they are widespread and form a natural south-north cline that crosses the Alps. We will then use these genomic resources to find the genes associated with adaptation to the different climatic conditions present in the sites where the fireflies were collected. By identifying genomic regions evolving under positive selection we will gain knowledge about the type of selective pressures fireflies are facing, including selective pressures encountered naturally in their habitats (e.g. parasitic load, predation) but also human-related selective pressures (e.g. insecticides, habitat loss, light pollution). With the firefly system, we will complement existing ecological information with genomic data for an integrative approach to understand adaptation in these species. In the present report, we summarize our first results concerning the collections and genomic DNA extractions, as well as describing our planned methods and analyses once the genomes become available.

## FIRST RESULTS

### Collection

Following a South/North latitudinal cline we collected in Arco, Cologno al Serio, and Turin in Italy (44.5-45.07° N), Lausanne and surroundings in Switzerland (46.52° N), and Munich in Germany (48.14° N) (figure 1). The Italian populations, which lie South of the Alps, provide a great opportunity to study adaptation to warmer climates and the migration capabilities across mountain ranges (figure 1). Additionally, populations from Switzerland constitute an ideal stepping-stone along this latitudinal cline (a complete description of the methods is given in the online Annexes).

To have a confident nucleotide polymorphism assessment, collections consisted of up to 20 individuals per population. Even though the time for collecting was restricted due to the Covid-19 pandemic, we were able to sample all five sites. In Lausanne, we collected 21 specimens of *L. noctiluca* and 24 of *Luciola italica*. The number of specimens collected for both species will allow us to generate a fully annotated genome and population level polymorphism data. In Cologno al Serio we found 28 specimens of *L. noctiluca* and 21 of *Luciola lusitanica*. In Arco (Italy) and Munich, we collected 25 individuals of *Lamprohiza splendidula* in each locality. From Turin, we have 20 individuals of *Luciola lusitanica*. For next-year collections we plan to start one month earlier (June), since population densities were already diminishing in July for *L. noctiluca* and *Luciola lusitanica*. In the case of *L. splendidula*, high population densities could still be found in July in the German populations.

### Genomic DNA extraction

Collected specimens were preserved in ethanol 96 % to ensure DNA stability for DNA extraction. As explained in the methods (see online Annexes), we tested four methods for DNA extraction, two for high molecular DNA (MagAttract HMW DNA and Genomic-tip 500/G, Qiagen) and two for regular DNA extraction (NEB Monarch No. T3010 and Qiagen DNAeasy No. 69504). With the MagAttract method we could extract the highest and longest DNA, with most of the DNA fragments being bigger than 50 kb long. The extraction of high molecular DNA will allow us to sequence using long range technology. For regular DNA the

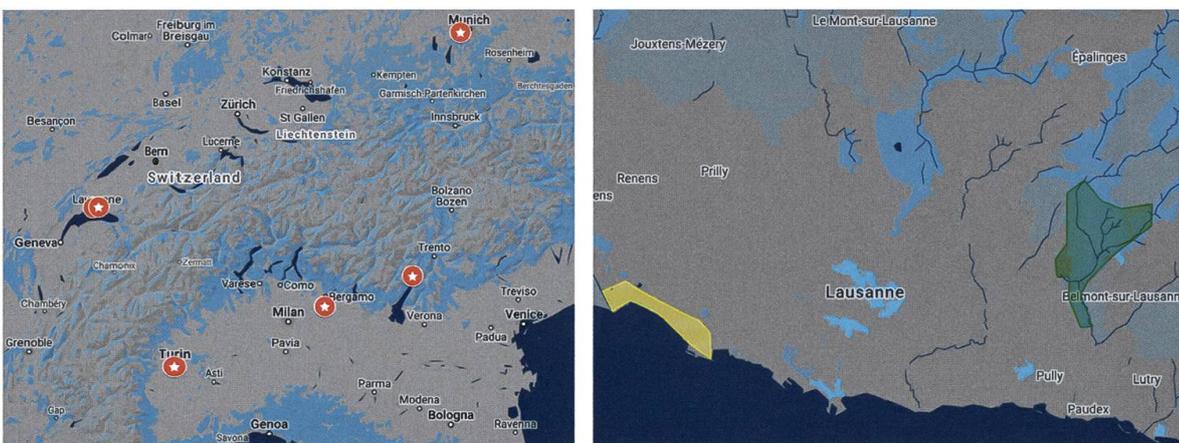


Figure 1. Collecting sites. Left panel: All sites sampled in 2020. Right panel: Collecting sites around Lausanne (yellow: *Luciola italica*; green: *Lamprohiza noctiluca*).

NEB Monarch method produced the best results. The average amount of total DNA that was extracted was  $\sim 3$   $\mu\text{g}$  for *L. splendidula*,  $\sim 8$ - $10$   $\mu\text{g}$  from *L. italica* and  $\sim 10$ - $15$   $\mu\text{g}$  from *L. noctiluca*.

## PLANNED ANALYSES

### Generating reference genomes

Since there are no genomic resources available for European fireflies, we are generating genome drafts for all of them, which is essential for the study of the evolutionary forces acting on these species. Fortunately, a high-quality genome has recently been published for the North American firefly *Photinus pyralis* (FALLON *et al.* 2018) which we will use as reference for gene annotation. Furthermore, having genome references for more than one species is useful to generate outgroups. The information gained from having an outgroup will be needed when applying tests for selection (PAVLIDIS *et al.* 2013, FU & LI 1993, McDONALD & KREITMAN 1991).

### Inferring the demographic history of *L. noctiluca*, *L. splendidula* and *Luciola italica*

After re-sequencing the genomes of *L. noctiluca*, *L. splendidula*, and *L. italica*, we will infer their population history, population structure, and migration events among the sampled populations. By quantifying the amount of genetic diversity obtained by demographic processes alone, we will generate a nucleotide diversity baseline which will serve to detect nucleotide diversity deviations putatively caused by natural selection. The demographic inference will be done under an ABC framework (DUCHEN *et al.* 2013, KOUSATHANAS *et al.* 2018), and for different possible demographic models. We applied this methodology in *P. pyralis* (RAD data) where we showed that model selection can be performed with good power (CATALÁN *et al.* 2019).

### Inference of loci under positive selection

To find genomic targets of positive selection, we will scan the genome for nucleotide diversity patterns that have the genetic footprint of positive selection (also known as selective sweeps). The identification of the genomic elements evolving under positive selection will allow us to further investigate their function and their role in adaptation (see CATALÁN *et al.* 2012, CATALÁN *et al.* 2016, and WILCHES *et al.* 2016 as a reference).

### Identifying orthologous loci under parallel evolution

After identifying loci affected by selection in European fireflies (particularly in *L. noctiluca*), we will compare the genomic locations of such loci to test for parallel evolution with the North American firefly *P. pyralis*. The ample spectrum of ecological niches and geographical latitudes that *L. noctiluca* and *P. pyralis* inhabit provides the opportunity to study the dynamics of parallel evolution to similar niches and of local adaptation to different environments. Thus, we will also be able to assess how repeatable responses to positive selection occur in wild insect populations. This study will help us understand how different species have adapted to their local environments in different parts of the world, despite having different demography, population dynamics, and life history traits. Having two completely diverged species of

fireflies both with a wide distribution range and inhabiting comparable habitats at northern and southern latitudes, offers an appropriate setup to study parallel evolution. The first step is to identify orthologous genomic regions between *L. noctiluca* and *P. pyralis*. The analysis on parallel evolution in two firefly species will give us a better insight on 1) how adaptation shapes nucleotide diversity when populations face comparable selective pressures, and 2) if evolution repeats itself and if so, how often does parallel evolution occur in truly wild non-hybridizing populations.

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