ESF Research Networking Programme ConGenOmics

-Exchange Grant Scientific Report -

Application Reference nr: 4372

Applicant: Valentina Rovelli, PhD Student, Roma Tre University, Department of Science, Rome, Italy

Host institution: Dr. David Vieites, Museo Nacional de Ciencias Naturales (MNCN), Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain

Population and landscape genomics analysis for the conservation of the Italian stream frog, *Rana italica*

1. PURPOSE OF THE VISIT

One of the main interests of conservation biologists has always been to better understand the relationships between species and individuals with the environment where they occur (Schoville *et al*. 2012), and the consequences on his genetic structure and connectivity between populations. The genetic approach towards this topic has been focused on trying to infer the role of landscape and environment in driving and shaping the genetic structure and gene flow of species in a spatial context. Until recently, there was a limited number of available genetic resources for most species, and especially for non-model organisms those were even more scarce. As a consequence studies applying population and landscape genetics on those non-model organisms were limited to few speces and using a limited number of genetic markers. Nowadays, Next Generation Sequencing allows for the screening of thousands of genome-wide genetic markers, e.g. single nucleotide polymorphisms (SNPs) or whole genome sequences, in a very short time and with a relatively limited economic effort, which provide markers that allow performing population and landscape genomic analyses.

The whole genomes of some endangered species have been recently completed; however, these data will not automatically provide useful information for species conservation (Frankham 2010), especially because of the limited information on population variation that is possible to infer from single individual sequencing. Nonetheless, these new approaches provide a significant aid in identifying genetic markers that can be applied to the study of entire populations (Frankham 2010). This is particularly important in endangered species where data on their population genetics at the landscape level is needed to inform on connectivity, genetic diversity, population genetics, corridors, populations sizes, etc., of high conservation value. Therefore, these novel methods facilitate to deepen existing disciplines (e.g. Molecular Ecology and Genome-Wide Association Studies - GWAS), or even to open the way to the development of new branches, such as Conservation Genomics (Ouborg *et al*. 2010). This emerging discipline can be simply defined as the application "of new genetic techniques to solve problems in conservation biology" (Allendorf *et al*. 2010), such as genetic drift, hybridization, inbreeding or outbreeding depression, natural selection, loss of adaptive variation and fitness.

Genomic information will turn out to be useful especially to study species with small and isolated populations, such as the target species of the present project, *Rana italica*. The Italian stream frog, *Rana italica*, is an endemic amphibian to Italy. It is distributed in the western side of the Italian peninsula, ranging from 100 m to over 1500 m a.s.l. This species is strictly bounded to rivers, creeks and streams with perennial water, usually located in woodlands. Very few studies have been carried out on this species and the only genetic research is focused on its evolutionary history (Canestrelli *et al*. 2008). In the Latium region this species is facing a slow decline since the beginning of the'70s, mostly due to water pollution and habitat fragmentation (Bologna *et al*. 2000). Because of its intrinsic value as a declining endemic species and the

gap of knowledge about its ecology and conservation status, a deeper analysis is required, in order to get essential information for management actions.
In a conservation genetic context, most species of interest do not have sequence resources available (Ouborg *et al*. 2010); however, this issue can be overcomed by using NGS techniques, such as the novel genotyping by sequencing (GBS) technique (Elshire *et al.* 2011). The GBS procedure allows working also on species with large genome and high diversity, by using a bioinformatics pipeline for SNP discovery, based on a species wide network approach called the Universal Network-Enabled Analysis Kit (UNEAK) (Lu *et al*. 2013). The GBS protocol is a multiplexed, high-throughput, and low-cost method to explore the genetic diversity in populations (Elshire *et al.* 2011). It employs a reduced representation library (RRL) strategy (Altshuler *et al*. 2000) to target a fraction of the genome for sequencing, thereby decreasing cost and increasing the SNP-calling accuracy. GBS is the simplest of the RRL approaches developed thus far (Davey *et al*. 2011), and has already seen extensive application in a wide diversity of taxa, i.e., in barley and wheat (Poland *et al*. 2012), maize (Elshire *et al.* 2011; Hansey *et al*. 2012), rice, grape and cacao (many publications in progress; Mitchell pers. com.).
Tissue samples from adults individuals of *Rana italica* were collected from 7 different populations in Latium region (Italy), for a fine scale landscape genomic analysis. A 96-plate was sent to the Institute of Genomic Diversity (Cornell University) for genotyping by sequencing. I received those data in November 2013 and the purpose of the visit to the Vieites lab at the MNCN was to start analyzing them, improving my analytical skills by learning how to handle those NGS data and apply them to conservation.
The available genomic resources for amphibians are limited, with a single genome available (Hellsten *et al*., 2010), few transcriptomes, and virtually no population genomic datasets for this group. Hence, this project will built on the genomic resources available for this group. Moreover, this work will integrate into a wider research project, which the host institution is about to start, concerning the GBS analysis of other *Rana* species. This research will allow to gather a more comprehensive NGS dataset of several *Rana* species from southern Europe and to carry out analyses in an interspecific comparative framework.

The present project, and the relative exchange period, is strictly bounded to the scope of the ConGenOmics Programme, as it aims to "develop and transfer genomics knowledge and approaches in a conservation genetic context" and to "apply and develop data handling and processing strategies in conservation genomics".

In particular the **main aims** were:
1. Developping an effective panel of SNPs for *Rana italica.*
2. Learning how to analyze NGS data, in particular GBS data, in a non-model species.
3. Get essential information for the management of wild populations.
4. Share these knowledge with other conservation genetics research teams, to create a collaboration network about European amphibians.

2. DESCRIPTION OF THE WORK CARRIED OUT DURING THE VISIT

According to Canestrelli *et al*. (2008), allozyme and mitochondrial data for *Rana italica* populations from Latium region reveal a very low genetic diversity, with the different populations sharing the same genetic cluster and haplotype. However, we believed that this result was due to the limited resolution power of the markers used in this previous study. Therefore we choose as study area right the Latium region, in order to test the if SNPs ,obtained by Genotyping by Sequencing technique, would have been able to reveal the real genetic structure of the species in this area, not identified by the previous analysis. We have the hypothesis that the area of Rome, altered by humans for few thousands of years, would have an impact on limiting gene flow and shaping the genetic structure of surrounding populations of this stream breeding amphibian.

For the 7 populations used for this study, samples are so distributed: Veio = 14 individuals, Insugherata = 13 individuals, Cannuccete = 13 individuals, Tolfa = 14 individuals, Licenza = 14 individuals, Monterano = 13 individuals, Cineto = 14 individuals (see Fig. 1 for the distribution of the populations).

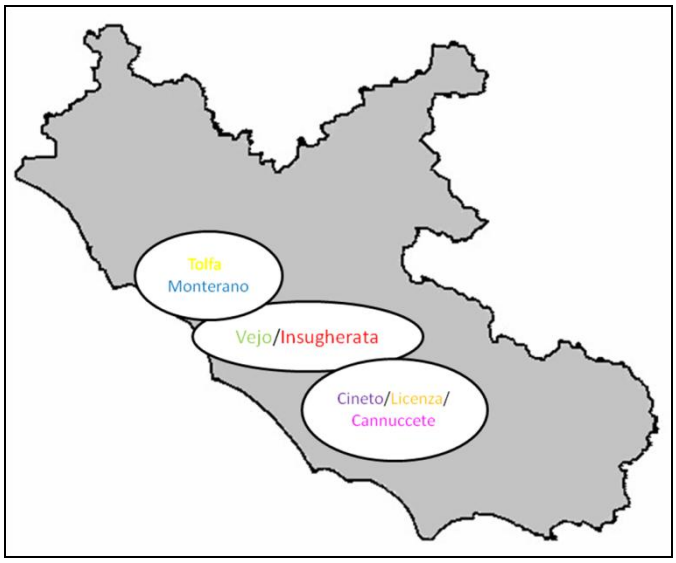

Fig. 1 – Distribution of the sampled populations in Latium region

Samples were shipped to the laboratory of Cornell University before my stay at the MNCN, and GBS results arrived two weeks before the beginning of the visiting period at the host institution. Ninety-five uniquely named samples and 1 blank were digested with enzyme EcoT22I, and data resulting from the UNEAK pipeline were provided in three folders, respectively containing .hapmap, .vcf and .xml files. HapMap is the standard file format generated by the TASSEL GBS pipeline. Cornell provided two sets of HapMap files: 1) a set without post SNP-calling filtering (located in the folder "hapmap/unfiltered") and 2) a set with additional filtering on missingness and allele frequency (located in the folder "hapmap/filtered). VCF is an alternative format for holding SNP information that retains information on depth of coverage for each allele, and can be output from the GBS pipeline. XML configuration files can be used (with the software versions indicated) to reproduce the results obtained from the UNEAK pipeline.

We first assessed the quality of the data by analyzing missing data, potential genotyping errors per individual and locus. An important characteristic of natural populations is their within population genetic diversity. The first objective of our study was to calculate individual heterozygosity, a very important index for conservation genetics as it is usually used to reveal inbreeding in natural populations. Inbreeding can indeed significantly contribute to the extinction risk of small populations, due to decrease juvenile survival, adult longevity and egg-hatching rate (Saccheri *et al*., 1998). Moreover, we aimed to calculate also population heterozygosity, in order to identifying possible levels of genetic variability between populations. The first step has been to identify the appropriate tool to perform this analysis, since actually there's no one software specifically developed for the heterozygosity's estimate from SNPs data.

We used the R package Rhh (Alho *et al.*, 2010) modifying the format of our panel of SNPs in which every individual has one column and genotypes are represented by using the IUPAC code (see Fig. 2). Since Rhh package, instead, requires as genotype's argument an array with each row representing an individual and every two columns representing genotype data for two alleles in a loci, we had to add a new column for every individual, and replace the IUPAC code with the real alleles. An example of the modified database is shown in Fig. 3.

As there is no previous work using large scale SNP's for (ranid) frogs, we wanted to evaluate several analytical approaches as well as the potential of those data to infer heterozygosity levels. To calculate the minimum number of loci needed to get a reliable estimate of heterozygosity, we performed different analyses with different subset of loci (5, 10, 20, 50, 100, 200, 300, 400, 500, 650, 800, 950, 1100, 1304, being 1304 the maximum number of loci available to us). These results have been achieved by creating an R script that

can selected a random number of loci within the whole dataset, and then run Rhh. The package Rhh has a function to calculate three estimates of individual Multilocus Heterozygosity: homozygosity by loci (Aparicio *et al*., 2006), internal relatedness (Amos *et al*., 2001) and standardized heterozygosity (Coltman *et al*., 1999) and to estimate heterozygosity-heterozygosity correlation based on these measures (Alho *et al*., 2010).

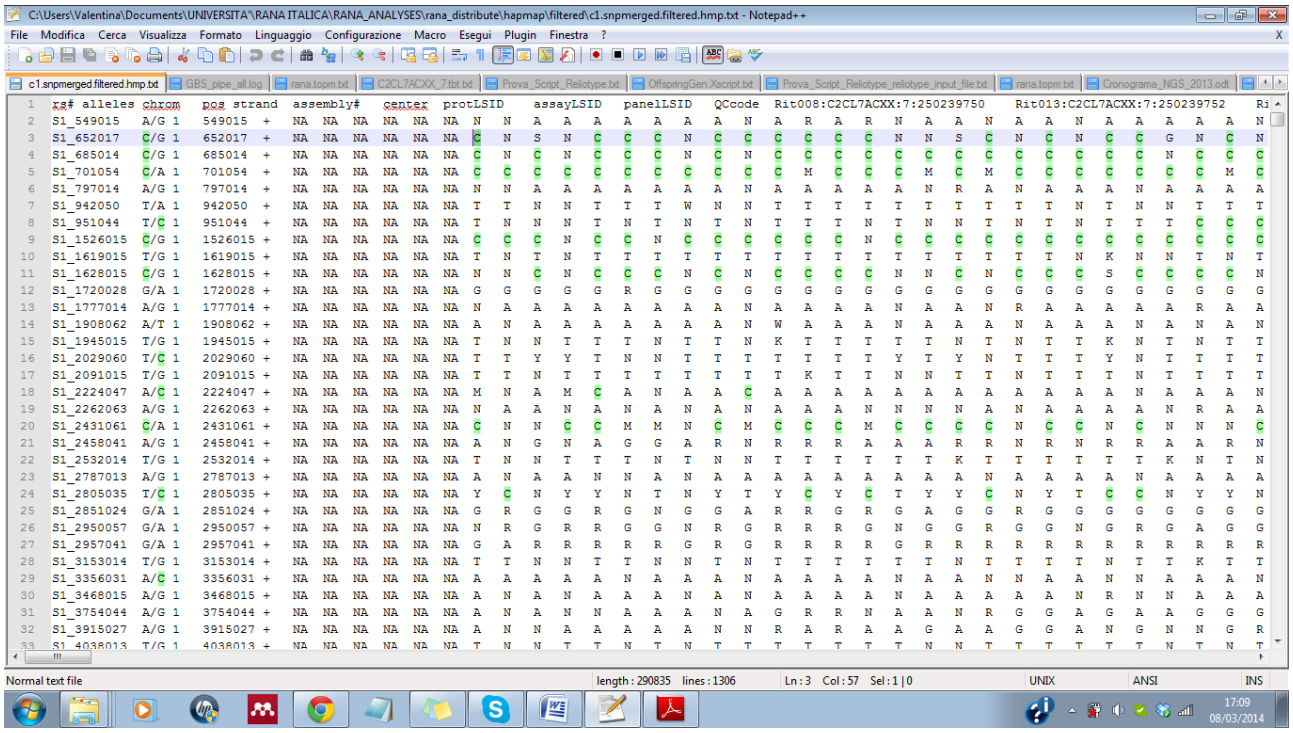

Fig. 2 – SNPs calls output as Hapmap filtered

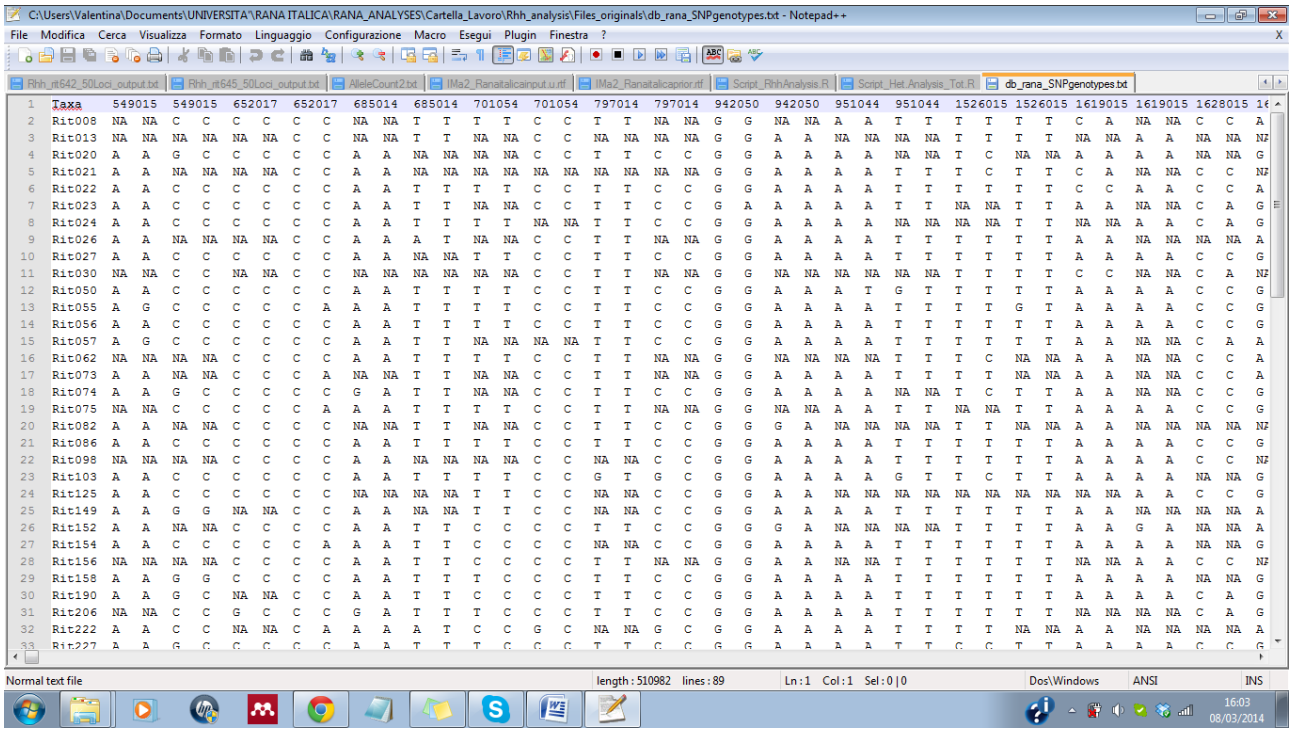

Fig. 3 – Genotypes modified database

Below is reported the R script used for the analysis.

```
# --- Script starts below --- #

object.name=read.table("inputfile_genotypes.txt",header=T,sep="\t",colClasses="character")  # rana genotypes is the file containing all individuals and SNPs, in two columns

listn <- sample(1:1304, x)*2   # in the second argument write the number of SNPs to extract

listr <- sort(c(listn, listn+1))

individual.name_xLoci <- rana[,listr]   # to save the data frame from which we selected the required number of SNPs (better write the name of the individual and the selected number of loci)

write.table(individual.name_xLoci,file="individual.name_xLoci.txt",row.names=rana$Taxa,col.names=F,quote=F,sep="\t")

require("Rhh")

h <- mlh("individual.name_xLoci.txt", "Rhh_ IndividualName _xLoci_output.txt", "NA", 4) # estimates the Multilocus Heterozygosity; for every output file specify the individual name and the number of loci

r <- h_cor("individual.name_xLoci.txt", "NA", 250, "hl") # estimates the Homozygosity by Loci

i <- h_cor("individual.name_xLoci.txt", "NA", 250, "ir") #  estimates the Internal Relatedness

s <- h_cor("individual.name_xLoci.txt", "NA", 250, "sh") # estimates the Standardized Heterozigosity

summary_ris <- list(r,i,s) # creates a list of the three heterozygosity estimates

write.table(summary_ris,file=" IndividualName _HL_IR_SH.txt",col.names=TRUE) # saves in a text file the three heterozygosity estimates
```

Due to the high number of randomizations used (250), the computation process required several weeks.
The second aim of the project was to estimate gene flow and migration rate between our populations, by using the computer program IMa2 (Hey and Nielsen 2007), which apply the Isolation with Migration model to genetic data drawn from a pair of closely related populations.
Since phylogeographic assumptions can be made only if the program is run with the whole tags sequences, instead of only SNPs, we had to ask for an additional kind of file, the tags on physical map (.topm) file, which lists tag sequences. We spent a lot of time of my research period at the MNCN trying to create a script to merge the two different files, and so obtain a file with sequences in place of SNPs, but we still haven't achieve our objective.
Moreover, since the program does not allow the presence of missing data, we had to remove some individuals and some loci from the analysis. After checking carefully our dataset, we were able to retain 42 individuals, out of 87, and 471 SNPs, out of 1304 with full datasets. However, even if with a reduced dataset, we will still have at least one individual for each locality, and in particular: Veio = 1 individual, Insugherata = 4 individuals, Cannuccete = 3 individuals, Tolfa = 13 individuals, Licenza = 10 individuals, Monterano = 6 individuals, Cineto = 5 individuals. In this way, we should be able to reveal gene flow between the three

main groups of populations: Veio-Insugherata (n=5), Tolfa-Monterano (n=19), Cannuccete-Licenza-Cineto (n=18).

3. DESCRIPTION OF THE MAIN RESULTS OBTAINED

From the application of the UNEAK pipeline for Genotyping by Sequencing on our samples we obtained the following results:

- HapMap SNPs (unfiltered) : 17188
- HapMap SNPs (filtered) : 1304
- VCF SNPs: 20399

Seven individuals (7,29%), out of 95, were excluded from the analysis because they have less than 10% of the mean reads per sample coming from the lane on which they were sequenced.
With the software TASSEL v. 3.0, we obtained a neighbor-joining tree using only simple parsimony substitution models (Fig. 4). As we expected, the 7 populations present a clear genetic structure, with three main groups: group 1, composed of Veio and Insugherata, group 2 composed of Tolfa and Monterano, group 3 composed of Cannuccete, Licenza and Cineto.

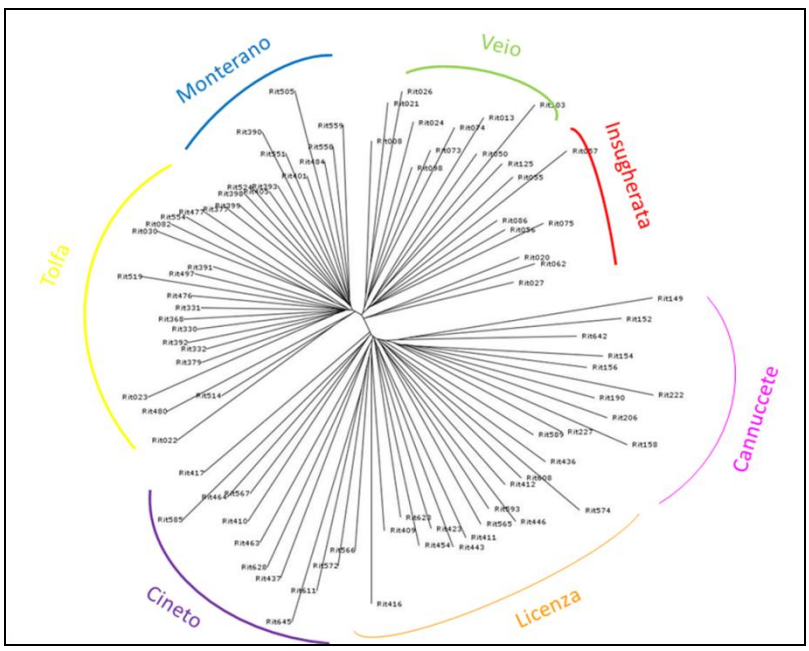

Fig. 4 – Genetic structure of the 7 populations from Latium region

For what concern the heterozygosity analysis, the first results show that estimates obtained by using Homozigosity by Loci and Internal Relatedness are affected by the number of loci. In Fig. 5 and 6 we can see a significant difference between estimates made with the totality of loci (1304) and the reduced datasets. This can be explained by the presence of very few loci that increase the heterozygosity levels. When the estimate is done with a reduced dataset, if these loci are selected by random sampling, they are very likely represented by outliers. On the contrary, when the estimate is done with the whole dataset these loci increase the mean value. In Fig. 7 are shown the results of the Standardized Heterozygosity estimates, that follow a different trend; this is probably due to the fact that Stardardized heterozygosity seeks to ensure that the

multilocus heterozygosity of all individuals is measured on an identical scale in cases where individuals have been typed for different loci.

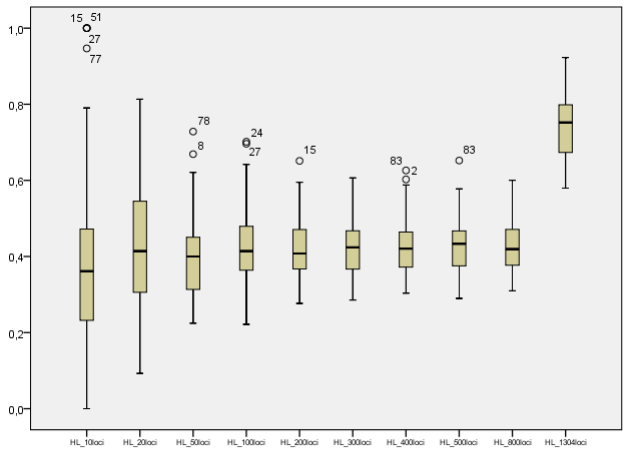

Fig. 5 – Homozygosity by the number of loci used

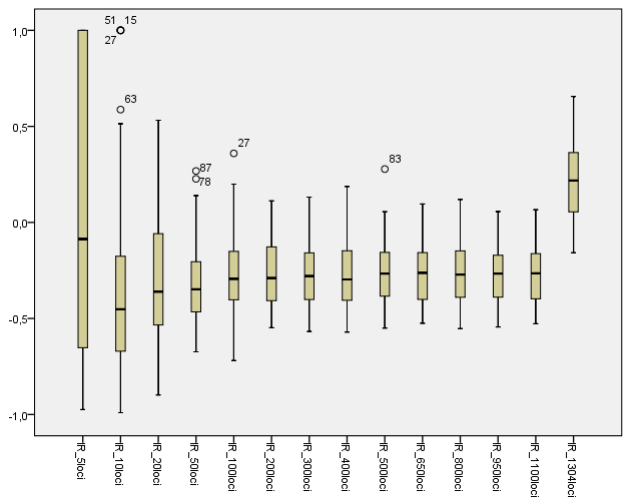

Fig. 6 – Internal Relatedness estimates by the number of loci used

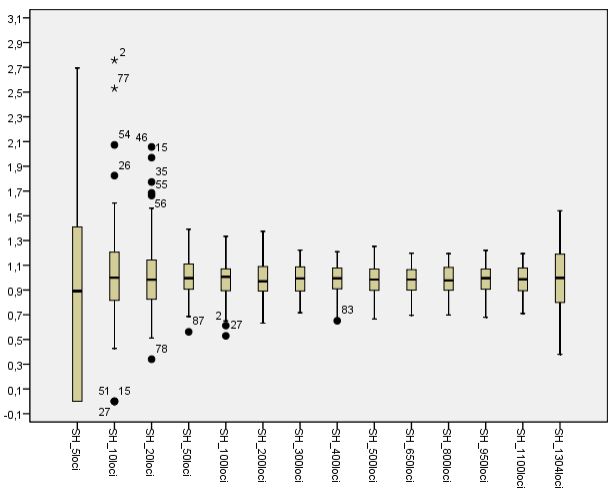

Fig. 7 – Standardized Heterozygosity estimates by the number of loci used

Heterozygosity analysis have been performed also on a dataset divided according to the three population groups: Veio-Insugherata, Tolfa-Monterano, Cannuccete-Licenza-Cineto. Our results show that Homozygosity by Loci (Fig. 8), Internal Relatedness (Fig. 9) and Standardized Heterozygosity (Fig. 10) estimates follow the same trend in the three population groups, and general values are quite similar. Interestingly, the values of all analyses are more or less stable if 100 loci or more are used, but when using the total number of loci the values are significantly higher. We are now looking carefully at those results to understand why this is happening.

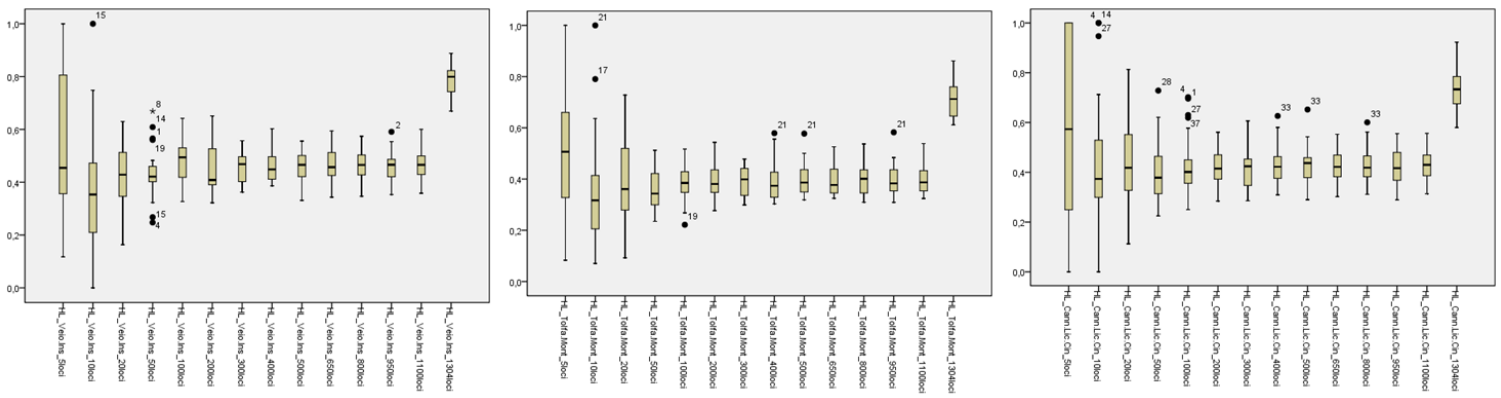

Fig. 8 – Heterozygosity by Loci estimates for the three population groups; from left to right Veio-Insugherata, Tolfa-Monterano, Cannuccete-Licenza-Cineto

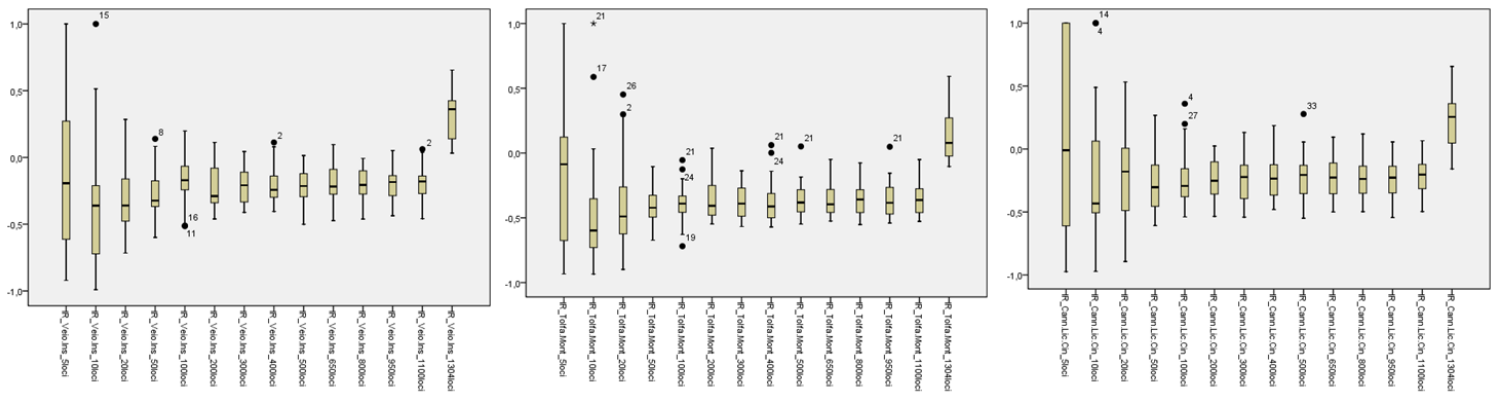

Fig. 9 – Internal Relatedness estimates for the three population groups; from left to right Veio-Insugherata, Tolfa-Monterano, Cannuccete-Licenza-Cineto

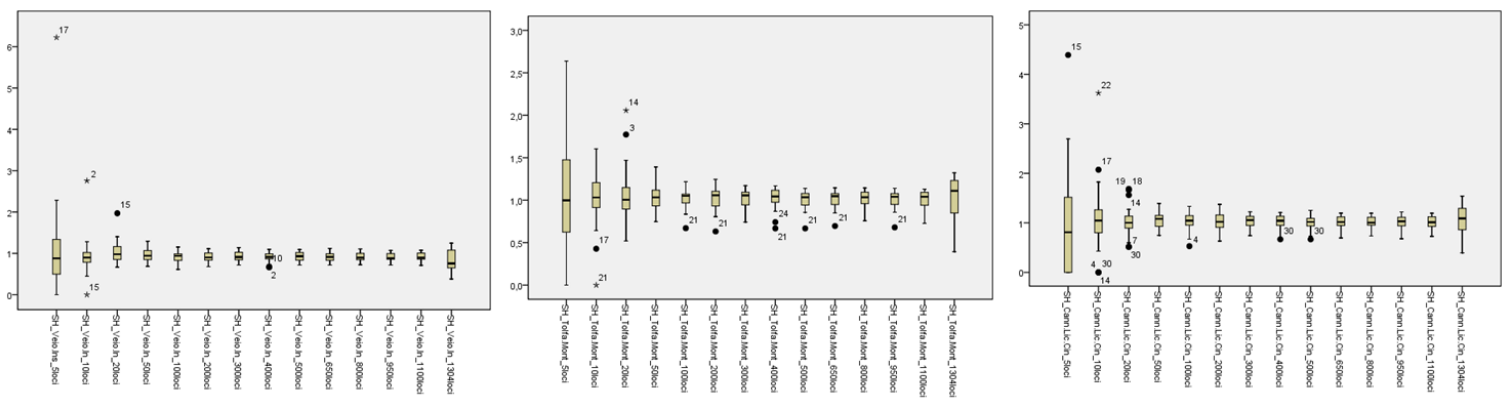

Fig. 10 – Standardized Heterozygosity estimates for the three population groups; from left to right Veio-Insugherata, Tolfa-Monterano, Cannuccete-Licenza-Cineto

During my stay at the MNCN, we worked on preparing the files for running IMa2 for the three main genetic groups detected to infer migration and gene flow. Those analyses have to run for a long time and will be completed during in the next months. Our results are in agreement with the hypothesis that there is a genetic signature that differentiates populations surrounding Rome (Fig. 4), and IMa2 analyses will tell us how much gene flow existed between them, allowing translating those results to the real impact of Rome area for the genetic conservation of those declining populations.

4. FUTURE COLLABORATION WITH HOST INSTITUTION (IF APPLICABLE)

The ESF visit grant has allowed the establishment of a very productive collaboration with the host institution. Apart from carrying on the beforehand analysis, we are also exploring the possibility of extending the sampling to all the distribution area of the species, increasing the number of individuals to analyze, in order to get a more comprehensive knowledge about the genetic variability of the species across its whole range. I will have access to the computer clusters at the Vieites lab to perform my analyses of genomic data.

5. PROJECTED PUBLICATIONS/ARTICLES RESULTING OR TO RESULTS FROM THE GRANT (ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant)

We have outlined three potential papers out of my work at the MNCN, which may be finally merged in two depending on results. Considering that our dataset is likely the first large available SNP dataset for anurans using GBS, we will focus one of the papers on the potential of such technique using the study species as a case study. The tentative papers would be:

1st Individual heterozygosity and demographic estimates by GBS in a non-model amphibian species: *Rana italica*.

2nd Impact of human development in migration rates and gene flow between populations of *Rana italica* from the Latium region

3rd Landscape genomics of a stream-breeding anuran in a highly developed landscape area.

6. OTHER COMMENTS (IF ANY)

7. REFERENCES

Alho J.S., Välimäki K. and J. Merila, 2010. Rhh_an R extension for estimating multilocus heterozygosity and heterozygosity-heterozygosity correlation. Molecular Ecology Resources 10: 720–722

Allendorf, F.W., Hohenlohe P., and G. Luikart. 2010. Genomics and the future of conservation genetics. Nature reviews. Genetics 11:697-709.

Altshuler D., Pollara V.J., Cowles C.R., Van Etten W.J., Baldwin J., Linton L., and E.S. Lander. 2000. An SNP map of the human genome generated by reduced representation shotgun sequencing. Nature 407:513–516.

Amos W., J. Worthington Wilmer, K. Fullard, T. M. Burg, J. P. Croxall, D. Bloch and T. Coulson, 2001. The influence of parental relatedness on reproductive success. Proceedings of the Royal Society 268: 2021-2027

Aparicio M., Ortego J.and P.J. Cordero, 2006. What should we weigh to estimate heterozygosity, alleles or loci? Molecular Ecology 15: 4659–4665.

Bologna, M.A., Capula, M., and G.M. Carpaneto. 2000. Atlante degli Anfibi e Rettili del Lazio. Roma: Fratelli Palombi Editori.

Canestrelli, D., Cimmaruta, R. and G. Nascetti. 2008. Population genetic structure and diversity of the Apennine endemic stream frog, *Rana italica* – insights on the Pleistocene evolutionary history of the Italian peninsular biota. Molecular Ecology 17:3856–3872.

Coltman D.W., Pilkington J.G., Smith J.A. and J.M. Pemberton, 1999. Parasite mediate selection against inbred soay sheep in a free living island population. Evolution 53: 1259-1267

Davey J.W., Hohenlohe P.A., Etter P.D., Boone J.Q., Catchen J.M., and M. L. Blaxter. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Review Genetics 12:499–510.

Elshire R.J., Glaubitz J.C., Sun Q., Poland J.A., Kawamoto K., Buckler E.S., and Mitchell S.E. 2011. A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. PLoS ONE 6(5): e19379.

Frankham, R. 2010. Challenges and opportunities of genetic approaches to biological conservation. Biological conservation 143:1919-1927.

Hansey C.N., Vaillancourt B., Sekhon R.S., de Leon N., Kaeppler S.M., and C.R. Buell. 2012. Maize (Zea mays L.) Genome Diversity as Revealed by RNA-Sequencing. PLoS ONE 7: e33071

Hansey CN, Vaillancourt B, Sekhon RS, de Leon N, Kaeppler SM, et al. (2012) Maize (Zea mays L.) Genome Diversity as Revealed by RNA-Sequencing. PLoS ONE 7: e33071.

Hellsten, U., Harland, R.M., Gilchrist, M.J., Hendrix, D., Jurka, J., Kapitonov, V., Ovcharenko, I., Putnam, N.H., Shu, S., Taher, L., Blitz, I.L., Blumberg, B., Dichmann, D.S., Dubchak, I., Amaya, E., Detter, J.C., Fletcher, R., Gerhard, D.S., Goodstein, D., Graves, T., Grigoriev, I.V., Grimwood, J., Kawashima, T., Lindquist, E., Lucas, S.M., Mead, P.E., Mitros, T., Ogino, H., Ohta, Y., Poliakov, A.V., Pollet, N., Robert, J., Salamov, A., Sater, A.K., Schmutz, J., Terry, A., Vize, P.D., Warren, W.C., Wells, D., Wills, A., Wilson, R.K., Zimmerman, L.B., Zorn, A.M., Grainger, R., Grammer, T., Khokha, M.K., Richardson, P.M. and D.S. Rokhsar. 2010. The genome of the Western clawed frog Xenopus tropicalis. Science 328:633–636.

Hey J. and R. Nielsen, 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. PNAS 104: 2785-2790.

Lu, F., Lipka, A.E., Glaubitz, J., Elshire, R., Cherney, J.H., Casler,M.D., Buckler, E.S.,and D.E. Costich. 2013. Switchgrass genomic diversity, ploidy, and evolution: novel insights from a Network-Based SNP Discovery Protocol. PLoS Genetics 9(1): e1003215.

Ouborg, N. J., Pertoldi C., Loeschcke V., Bijlsma R.K., and P. W. Hedrick. 2010. Conservation genetics in transition to conservation genomics. Trends in genetics 26:177-87.

Poland J.A., Brown P.J., Sorrells M.E., and J.L. Jannink. 2012. Development of High- Density Genetic Maps for Barley and Wheat Using a Novel Two-Enzyme Genotyping-by-Sequencing Approach. PLoS ONE 7: e32253.

Saccheri I., Keessaari M., Kankare M., Vikman P., Fortelius W., and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491-494

Schoville S.D., Bonin A., François O., Lobreaux S., Melodelima C., and Manel. 2012. Adaptive Genetic Variation on the Landscape: Methods and Cases. Annual Review of Ecology, Evolution, and Systematics 43: 23-43.

MINISTERIO
DE ECONOMÍA
Y COMPETITIVIDAD

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

mncn

MUSEO NACIONAL
DE
CIENCIAS NATURALES
SALIDA Nº 1196
Día 6 Mes 11 Año 13

VALENTINA ROVELLI

En respuesta a su solicitud de permiso de estancia temporal en el Museo Nacional de Ciencias Naturales, con el objetivo de mejorar su formación científica y ampliar su *Curriculum Vitae,* le comunico que esta Dirección ha tenido a bien autorizar dicha estancia, desde el **4 de noviembre de 2013 al hasta el 5 de marzo de 2014**, de acuerdo al caso nº 4 de la norma reguladora de la permanencia temporal en Institutos y Centros propios del Consejo Superior de Investigaciones Científicas, de personal ajeno al Organismo.

Tal estancia, es en su propio beneficio y su actividad dentro del Museo no estará sujeta a horario y dependerá de las Vicedirecciones de Investigación y Colecciones.

La validez de esta autorización quedaría sin efecto inmediato en el caso de incorrecta acreditación de las razones que la aconsejan o por informe expreso del tutor asignado o de la Vicedirección correspondiente.

Sin otro particular y deseando que la ocasión que se le brinda le resulte provechosa, le saluda atentamente.

Madrid, 4 de noviembre de 2013

MUSEO NACIONAL DE CIENCIAS NATURALES - MADRID
C.S.I.C.

Santiago Merino Rodríguez
Director

C/ Serrano, 115 Dpdo.
28006 MADRID ESPAÑA
TEL.: 91 745 25 00 – Ext. 981160
FAX: 91 564 08 00
Correo electrónico rvillar@mncn.csic.es

MINISTERIO
DE CIENCIA
E INNOVACIÓN

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

mncn
museonacionaldecienciasnaturales

ANEXO I

SOLICITUD DE PERMISO DE ESTANCIA EN CENTROS DEL CSIC

Don/Doña: Valentina Rovelli
con DNI nº: AU 9730391 Fecha de nacimiento: 14/02/1984
Teléfono de contacto: 914111328
Titulación: Licenciada en Biología Universidad: Roma Fecha:22/9/2010

Solicita del CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS permiso de Estancia en el **Museo Nacional de Ciencias Naturales (MNCN-CSIC)** siendo su caso:

- ☐ 1. Personal de las Administraciones Públicas y personal empleado en alguna Empresa, en servicio activo, que pretendan realizar trabajos de investigación o aprendizaje por tiempo limitado, manteniendo su retribución en la correspondiente Administración o Empresa.
- ☐ 2. Perceptores de becas o ayudas distintas de las contempladas en el apartado 3.2. e) de la Norma. (Duración máxima: tiempo de vigencia de la beca o ayuda)
- ☐ 3. Personas que vayan a realizar trabajos preliminares de Tesis Doctoral cuando por razones de plazos y fechas no hubieran podido solicitar beca predoctoral. (Duración máxima: 12 meses)
- ☒ 4. Estudiantes, Licenciados o Ingenieros de Escuelas Superiores o de Grado Medio que deseen realizar Prácticas, Tesina o Proyecto Fin de Carrera. (Duración máxima: 12 meses)
- ☐ 5. Licenciados, Arquitectos, Ingenieros o equivalentes u homologados a ellos que deseen realizar cursos, seminarios y trabajos de investigación tutelados del programa de doctorado correspondiente (R.D. 778/1998, de 30 de abril, BOE de 1 de mayo) (Duración máxima: tiempo estipulado para su realización)
- ☐ 6. Personas que soliciten realizar el aprendizaje de técnicas (Duración máxima improrrogable: 12 meses)
- ☐ 7. Personas que habiendo terminado el disfrute de una beca predoctoral deseen o necesiten permanecer en el CSIC. (Duración máxima: 12 meses)
- ☐ 8. Personas que durante estancias temporales de corta duración en el Museo Nacional de Ciencias Naturales o en la Estación Biológica de Doñana, se inicien en el conocimiento de la investigación mediante actividades de colaboración voluntaria y desinteresada. (Duración máxima: 6 meses)

Trabajo a realizar o Técnica a aprender:
Aprendizaje de procesado de imágenes 3D del CT-Scan recientemente adquirido por el Museo.

Nombre o Categoría del Investigador del CSIC con el que se realizará la estancia:
David Vieites, Científico titular

Periodo de Estancia solicitado: Desde 4-11-2013

Hasta 5-3-2014

MINISTERIO
DE CIENCIA
E INNOVACIÓN

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

mncn
museonacionaldecienciasnaturales

En caso de ser personal de la Administración Pública:	**En caso de ser personal de Empresas:**
Categoría	Categoría
Departamento	Empresa
Organismo	Dirección Postal

En el caso de beca recogida en el apartado 2 indicar entidad financiadora

El solicitante DECLARA que en caso de autorizarse su Estancia CONOCE y ACEPTA las siguientes condiciones:

a) Que su permanencia en dependencias del CSIC no tiene significado de puesto de trabajo en el Consejo Superior de Investigaciones Científicas, ni establece relación laboral alguna con dicho Organismo.

b) Que se compromete a suscribir una Póliza Individual de Seguro de Accidentes (en caso de carecer de esta cobertura), cuyo pago correrá a su cargo.

c) En el caso de Personal de la Administración Pública o Empleados de Empresas se acompaña a esta solicitud una carta oficial de la dependencia de la Administración o de la Gerencia de la Empresa donde el interesado presta sus servicios, justificando esta situación.

d) En el caso de perceptores de ayudas o becas financiadas por Organismos públicos o privados, tanto nacionales como extranjeros, en cuya solicitud conste la autorización o visto bueno del Centro o Instituto correspondiente, siempre que la actividad pre o postdoctoral se lleve a cabo en el Organismo, se acompaña a esta solicitud documento oficial de concesión de la ayuda donde se exprese claramente el periodo de disfrute de la misma y la Entidad financiadora.

En Madrid a 4 de November de 2013

Firma del Solicitante

Valentina Rastelli

Vº Bº del Responsable

U (Mensaje 1 de 98)

Asunto: RE: Alta en seguro Valentina Rovelli
Fecha: Hoy, 13:05:28 CET
De: Alicia Larrubia <alicia.larrubia@gmail.com>
Para: '"RAIMUNDO.VILLAR.MARTINEZ.14599"' <rvillar@mncn.csic.es>

En relación con la póliza n° 14830301 colectiva de accidentes, les comunico que queda asegurado/a:

VALENTINA ROVELLI - FECHA DE NACIMIENTO: 14/02/1984
PASAPORTE: AU 9730391
PERMISO DE ESTANCIA (4/11/2013 AL 5/03/2014)

Un saludo,

Menú

1. Eliminar
2. Responder
3. Reenviar
4. Redirigir
5. Modificar como nuevo
6. Mensaje siguiente
7. Para Entrada
8. Redactar
9. Carpetas
10. Salir

AirEuropa.com

Codice: **Z8RUJW**

Voli					
Origine	Destinazione	Num. Volo	Classe	Data	Ora
Roma	Madrid	UX1040	R	02/11/2013	10:30
Madrid	Roma	UX1043	Q	02/03/2014	07:05

Passeggeri		
Passeggeri	Num. Identità	Num. Biglietto
VALENTINA ROVELLI	AU9730391	9960001180152

BAGAGLIO CONTRATTE:		
	QUANTITÀ	PREZZO
VALENTINA ROVELLI	1 Bagaglio per la Andata e Ritorno	100.00EUR

Importo				
Base	Tasse	Gestione	Bagaglio	Totale
98.00 EUR	128.42 EUR	12.00 EUR	100.00 EUR	338.42EUR

Prenotazione		
Tipo di biglietto	Codice autorizzazione	Riferimento acquisto
E-Ticket	H7A243	3002664

Condizioni

Tipo di ritiro:

Dovuto all'incremento delle procedure di controllo sicurezza aeroportuale e per rispettare la puntualità del volo, il limite di accettazione al check-in, salvo che ne venga specificato un altro maggiore, sarà di 45 minuti prima della partenza prevista del volo per tutti i voli nazionali ed europei e di 60 minuti per i voli intercontinentali.