

The report of the summer school “**Livestock Conservation Genomics: Data, Tools and Trends**”, held at October 1-7, 2012, in Pag Island (Hotel "Luna"), Croatia.

### **Summary**

The summer school entitled “Livestock Conservation Genomics: Data, Tools and Trends”, jointly organised by the Faculty of Agriculture, University of Zagreb (Svetosimunska 25, 10 000 Zagreb, Croatia, Prof. Ino Curik) and University of Natural Resources and Life Sciences Vienna (Gregor Mendel Str. 33, A-1180 Vienna, Austria, Prof. Johann Sölkner) was held from 1<sup>st</sup> to 7<sup>th</sup> October in 2012, in Hotel "Luna" on the Pag Island, Croatia. The surrounding nature and quality of the hotel provided a pleasant working atmosphere and ambient. With a total number of 31 participants and 13 lecturers representing 12 countries (including Argentina and USA) the school was quite international event. While mainly represented by animal scientists there were scientists from other disciplines of applied biology (ecology, veterinary, molecular genetics, etc...). Although participants were mainly PhD students, there were several professors (7) and senior scientists (3), as well as several students (3), attending the school. In addition, majority of lecturers have fully attended the school during all 7 days while three lecturers have attended the school for a considerable time. This positive mixture of young and experience researchers in the audience assured a number of stimulating discussions at the high scientific level. A total number of 8 grants were provided to the PhD students coming from institutions from Austria, Croatia (2), France (2), Germany, Netherlands and Sweden. The content of the school was structured into theoretical lectures, software demonstrations and paper discussions and was targeting to update basic topics of conservation genetics with respect to newly available information (SNP chips and sequence data). The school started with required introduction to the school program schedule and timetable (Prof. Ino Čurik), followed by the updating overview of molecular techniques and methods providing DNA information (Prof. Paolo Ajmone Marsan). In the afternoon, we continued with explanation and tips required when starting to analyse and manipulate data coming from SNP chips (Dr. Gabor Meszaros and Ana María Pérez O’Brien). This part has been supplemented with software demonstrations and practical exercises. In the second day, theoretical backgrounds, concepts and new possibilities on linkage disequilibrium and population effective size (Prof. Henner Simianer) and inbreeding and relatedness (Prof. Ino Čurik). Theoretical aspects were followed by the software demonstration related to the calculation of all those parameters (Christian Reimer, Maja Ferencakovic). In the evening we had quite stimulating journal club discussion on the effective population size. On the third day, Prof. Johannes Lenstra and Prof. Johann Sölkner provided comprehensive empirical overview on the population structure and admixture followed by software demonstrations (Anamarija Frkonja). Assist. Prof Gregor Gorjanc presented the concept of imputation and phasing together with demonstration of its calculations. The Wednesday has finished with the journal club on population structure of sheep populations. On Friday, whole day was devoted to the sequence data analyses and detection of selection signatures (Dr. Carl-John Rubin). The Friday finished with the journal club presenting possibilities of sequence analyses in poultry. On Saturday, Assoc. Prof. Patrik Waldmann made introduction to the theory and calculation used in GWAS, while Prof. Johann Sölkner (theory and examples) and Maja Ferencakovic (calculation demonstration) presented possibilities of autozygosity mapping in “detecting” deleterious genes. On the last day, Prof. Michelle Tixier-Boichard nicely presented future trends and perspectives. At the end Prof. Johann Sölkner made closing speech and together with Prof. InoCurik provided certificates of the school attendance. On Thursday, we had working excursion visit to the sheep breeding (Pag Islan Sheep), cheese factory and old olive trees.

## **Description of the scientific content of and discussions at the event**

The quality of the school was assured by the large number of lecturers (13) engaged. In choosing lecturers, particular care was taken on the fact that lecturers have been previously shown their expertise in the lectured subject (scientific publications and experience in working with SNP chips or/and sequencing data was required). The school started with introduction to the schedule and timetable of the school programme (Prof. Ino Curik). During the introduction each participant and lecturer provided individual presentation of the working subject of interest and institution he/she is coming from. Acknowledgments were made to all countries financing the program GENOMIC-RESOURCES as well as to all lecturers accepting to participate as there was no single rejection. Prof Paolo Ajmone-Marsan continued with introduction to genetic data explaining: basic DNA and genetic concepts reminder, related terminology, types of data, sequencing and SNP genotyping techniques together with types of variation (copy number variations, insertions, deletions and polymorphic sites). It was pointed that we are witnessing severe price reduction per genotyped SNP, so in the near future we can expect radical changes in the methodology of obtaining molecular information as well as in analysing such data. Additionally, structural variations such as are copy number variations can be important source of phenotypic variation which was, up to recently, not considered at all. Ana María Pérez O'Brien explained in details what information we do receive from the SNP Chip analyses while Dr. Gabor Meszaros explained the manipulation of data and provided R scripts useful when working with large SNP chip related data files. The concept of linkage disequilibrium and population effective size was explained by Prof. Henner Simianer who also have shown some new opportunities in calculating effective population size and demographic history from the SNP chip data. Later his PhD student Christian Reimer demonstrated calculation of linkage disequilibrium and population effective size using Beagle program. Prof. Ino Curik explained in details the concept of inbreeding and relatedness on the individual and population level together with presentation of the calculation of inbreeding from Runs of Homozygosity, so called ROHs, (theoretical explanation and empirical results). The new concept of shifting individual inbreeding level was shown graphically ("SpagettiNo graph"). His PhD student Maja Ferenakovic explained how to calculate inbreeding from molecular information (ROHs) as well as how to calculate genomic relationship matrix by the method of VanRaden. In the journal club, Belen Jimenez Mena presented the paper: "Flury et al. (2010). Effective population size of an indigenous Swiss cattle breed estimated from linkage disequilibrium. *J. Anim. Breed. Genet.*, 127: 339–347.". Huge discussion has arisen on the fragility of effective population size concept used by FAO as criteria for endangered populations as well as on the confidence interval in the estimating effective population size from the linkage disequilibrium. Prof. Johannes Lenstra has made detail description, including the newest molecular information (SNP chip), on the current population structure and history status for cattle, sheep, goat and horse populations. Prof. Johann Sölkner introduced the concept of admixture on the research examples related to cattle. The concept was further supplemented by Anamarija Frkonja demonstrating calculations required. Availability of the large number of markers is a powerful tool in detecting admixed individuals but there is still not reliable method to provide precise estimation of time (remote generations) since admixture. On the other side admixture provides potentials in detecting adaptive selection signals. Assist. Prof. Gregor Gorjanc has presented methods used in determining haplotype phases as well as methods used to impute missing genotypes. He further demonstrated AlphaPhase as a program involved in haplotype phasing. At the end of the day, PhD student Tesfaye Getachew presented paper "Kijas et al. (2012). Genome-Wide Analysis of the World's Sheep Breeds Reveals High Levels of Historic Mixture and Strong Recent Selection. *PLoS Biol.*, 10: e1001258."

Discussion was related to the paper but was later extended to the domestication origin and population structure of other domestic species. After three intensive days, on Thursday, we were visiting cheese factory "Sirana Gligora" famous for the production of the worldwide multi-awarded cheeses. After passing through all processing units of cheese production we tasted 7 types of cheese produced there. Following, we have visited a classical sheep breeder in the Pag Island. Our excursion ended by visiting one of the oldest olive tree orchard (50 000 – 80 000 trees with estimated number of 300 subspecies based on DNA analyses) with trees being up to 1600 years old. In his first talk, Dr. Carl-Johan Rubin provided a nice overview over several methods used for detection of selection; a) based on the referent genome (differentiation on  $F_{ST}$  values; levels of heterozygosity; long haplotype tests, EHH; site frequency spectrum, Tajima's D test; and comparison with divergence, HKA and McDonalds-Kreitman test) and b) based on the absence of referent genome. The later approach was presented in details on the analyses performed on the sequences data derived from the pooled poultry populations samples. The second theoretical talk of Dr Carl-John Rubin was related to the structural variants, particularly to copy number variations influencing phenotypes. Both talks were extremely stimulating and introducing quite new approaches and methodologies, not regularly used by animal scientists. In the afternoon, there was demonstration of software's used in the analyses of sequences. All demonstrations were based on the poultry sequence data provided from the research published in the paper "Rubin et al. (2010). Whole-genome re-sequencing reveals loci under selection during chicken domestication. *Nature*, 464: 587-593.". The same paper was later presented in a journal club discussion by a PhD student Mirte Bosse. Very interesting discussion has been arisen, questioning what should be the main goal of an conservation program as well as discussion questioning usefulness of adaptive based approach versus neutral variation (mainly used in the last decade). Later discussion become more imaginative, for example, what if we would base long-term conservation strategy on the storing of sequence information of endangered breeds or what if we would target the individuals with the highest DNA content in the conservation, etc... On Saturday, Assoc. Prof. Patrik Waldmann continued with critical presentation of widely applied strategies known as GWAS in estimating SNPs affecting phenotypic variation. His theoretical presentation was supported by exercises using written R scripts and simulated data. Large number of extremely useful R scripts has been provided. The power of SNP chip information to detect detrimental single gene defects has been demonstrated by Prof. Johann Sölkner on the example where degenerative axonopathy in Tyrolain Grey cattle has been associated to the gene mutation. Furthermore, some strategies to perform mapping of inbreeding depression due to single gene were also shown, although methodology was still not fully developed. The practical issues and software demonstration in autozygosity mapping were demonstrated by Maja Ferencakovic. On the last day, Prof. Michelle Tixier-Boichard nicely presented future trends and perspectives related to the conservation genetics. Firstly, conceptual framework for conservation genomics in livestock has been explained. Secondly, the important research questions and major issues to be addressed using genomic approaches have been pointed. Thirdly, current technological trends and challenges has been addressed. Furthermore, societal challenges have been considered and at the end future conservation strategies were discussed from the point of gene-banking, biotechnology and up to definitions of phenotypes. The lecture was supported by the number of literature citations. At the end Prof. Johann Sölkner made closing speech and together with Prof. Ino Curik provided certificates of attendance. Through the usage of dropbox, as well as the usage of USB stick (given in "the school materials bag"), all participants were provided with all presentations (pdf or/and pptx), together with all software's applied and training datasets.

## **Assessment of the results and impact of the event on the future directions of the field**

The thematic of the school was very much on the cutting edge technology, so, we expect benefits from the educational, research and industrial sides. The intensive program lasted seven days and systematically covered a number of basic topics in conservation genetics (molecular genotyping and sequencing, linkage disequilibrium, effective population size, inbreeding, relatedness, population structure, admixture, detection of adaptive variation, detection of detrimental defects, estimation of complex trait variability) from the aspects of genomics and newly developed possibilities (SNP chips and sequences). In this way, the curriculum of one subject at the master and/or PhD level, covering the field of "Livestock Conservation Genomics", has been built. Thus, we expect that a number of lectured subjects will be incorporated in the future academic programs taught at EU Universities, particularly as considerable number of relevant academic staff has attended whole school program. Topics describing achievements in sequencing technology and development of molecular technologies were highly stimulating and surprising (in a positive way) for the majority of participants. At least in my institution we are re-evaluating our current development strategies. For example how much efforts and to what extent should we invest into building computing and calculation facilities and skills (developments/platforms/languages) versus how much should we invest into building of molecular genetics capacities? For a number of participants, practical's and software demonstrations were helpful, further, encouraging and facilitating future researches. The location and low price of the school enabled participation of a large number of East European participants (Croatia, Hungary, Slovenia, and Slovakia) to catch up with new scientific developments. We hope this is bringing to the creation of a more homogenized scientific research space within the Europe. The internationality of the participants and lecturers provided certain level of networking among researchers that have not been collaborated previously. Thus, we have already been informed about initialisations of several projects and research exchanges established during the school, particularly with respect to the incoming grant applications within the GENOMIC-RESOURCES program (it would be interesting to monitor next grant applications related to the participants attending the school). Discussions were simulative and quite opened, enabling young scientists as well as experienced scientists to critically confront ideas and arguments. Probably, the best example is discussion on the effective population size where there was general agreement that current numbers used ( $N_e=50$ ) are not sufficient criteria in determining the conservation status of the breed. The conclusion was that it is necessary to develop better approaches, measures and methods to evaluate endangeredness of a breed. Finally, we believe that the summer school was successful in providing Ph.D. students and researchers in animal genetics, conservation and breeding not yet conversant the analysis of SNP and sequence data with a toolbox of methods and softwares to address questions relevant in the conservation of livestock.

## **Annexes:**

### **a) Programme of the meeting with timetable**

#### **Monday 1st of October**

**9:30 - 10:30** Welcome & introduction

**Presenter:** Ino Curik

**10:30 - 11:00** Tea / Coffee Break

**11:00 - 12:30** Introduction to Genetic Data

**Presenter:** Paolo Ajmone-Marsan

**12:30 - 14:00** Lunch Break

**14:00 - 15:30** Basic Data Manipulation - Practical Workshop

**Presenter:** Gabor Meszaros

**15:30 - 16:00** Tea / Coffee Break

**16:00 - 18:00** Descriptive Statistics and Quality Control - Practical Workshop

**Presenter:** Ana María Pérez O'Brien

#### **Tuesday 2nd of October**

**9:00 - 10:30** Linkage Disequilibrium and Effective Population Size

**Presenter:** Henner Simianer

**10:30 - 11:00** Tea / Coffee Break

**11:00 - 12:30** Inbreeding and Relatedness

**Presenter:** Ino Curik

**12:30 - 14:00** Lunch Break

**14:00 - 15:30** Linkage Disequilibrium and Effective Population Size - Practical Workshop

**Presenter:** Henner Simianer / Christian Reimer

**15:30 - 16:00** Tea / Coffee Break

**16:00 - 18:00** Inbreeding and Relatedness - Practical Workshop

**Presenter:** Maja Ferencakovic

**18:00 - 20:00** Dinner Break

**20:00 - 21:30** Paper Discussion – Flury et al., (2010): Journal of Animal Breeding and Genetics

**Presenter:** Belen Jimenez

#### **Wednesday 3th of October**

**9:00 - 10:30** Population Structure and Breed History

**Presenter:** Johannes Lenstra

**10:30 - 11:00** Tea / Coffee Break

**11:00 - 12:30** Admixture

**Presenter:** Johann Sölkner

**12:30 - 14:00** Lunch Break

**14:00 - 15:30** Imputation and Phasing - Practical Workshop

**Presenter:** Gregor Gorjanc

**15:30 - 16:00** Tea / Coffee Break

**16:00 - 18:00** Admixture - Practical Workshop

**Presenter:** Anamarija Frkonja

**18:00 - 20:00** Dinner Break

**20:00 - 21:30** Paper Discussion – Kijas et al., (2012): PLOS Biology

**Presenter:** Tesfaye Getachew Mengistu

#### **Thursday 4th of October**

**9:00** - Group Excursion:

Visit to the hard cheese production

Visit to the Pag Island sheep breeding

**12:30 - 14:00** Lunch Break

**14:00 – 16:00** Visit to the old olive trees

**Organisers:** Vlatka Cubric Curik, Ivana Kovac & Ino Curik

**Friday 5th of October**

**9:00 - 10:30** Signatures of Selection

**Presenter:** Carl Rubin

**10:30 - 11:00** Tea / Coffee Break

**11:00 - 12:30** Structural Variations of DNA

**Presenter:** Carl Rubin

**12:30 - 14:00** Lunch Break

**14:00 - 15:30** Working with Sequence Data - Practical Workshop

**Presenter:** Carl Rubin

**Friday 5th of October**

**15:30 - 16:00** Tea / Coffee Break

**16:00 - 18:00** Applying Signatures of Selection approaches to sequence data - Practical Workshop

**Presenter:** Carl Rubin

**18:00 - 20:00** Dinner Break

**20:00 - 21:30** Paper Discussion – Rubin et al., (2010): Nature

**Presenter:** Mirte Bosse

**Saturday 6th of October**

**9:00 - 10:30** Genome-wide Association Studies (GWAS)

**Presenter:** Patrik Waldmann

**10:30 - 11:00** Tea / Coffee Break

**11:00 - 12:30** Autozygosity Mapping

**Presenter:** Johann Sölkner

**12:30 - 14:00** Lunch Break

**14:00 - 15:30** GWAS - Practical Workshop

**Presenter:** Patrik Waldmann

**15:30 - 16:00** Tea / Coffee Break

**16:00 - 18:00** Autozygosity Mapping - Practical Workshop

**Presenter:** Johann Sölkner / Maja Ferencakovic

**18:00 - 20:00** Dinner Break

**20:00 - 24:00** Closing Party

**Sunday 7th of October**

**9:00 - 10:30** Trends and Future perspectives

**Presenter:** Michelle Tixier-Boichard

**10:30 - 11:00** Tea / Coffee Break

**11:00 - 11:30** Final Discussion

**Presenter:** All

**11:30 - 12:00** Closing Remarks

**Presenter:** Johann Sölkner

## **b) full list of speakers and participants.**

Overall there were 44 participants, 13 lecturers/speakers and 31 participants out of which 8 have been nominated by ESF grant. Thirteen lecturers/speakers were involved in the teaching and software demonstration and they were representing scientific institutions from Austria (4), Croatia (2), France (1), Germany (1+1), Italy (1), the Netherlands (1), Sweden (2) and Slovenia (1). One participant (**Christian Reimer**), on the proposal of his supervisor prof. Henner Simianner, had lectured software demonstration for the calculation of linkage disequilibrium. There were 19 participants coming from six European countries financially supporting the program (GENOMIC-RESOURCES) while 7 participants were from other EU countries (Italy, Hungary, Slovakia and Slovenia). There were 5 participants coming from Argentina (1) and USA (4).

### **Speakers**

•Prof. **Paolo Ajmone-Marsan**: Internal Department of Zootechnology, Faculty of Agriculture of the Università Cattolica del S. Cuore, Piacenza, Italy. •Prof. **Henner Simianer**: Department of Animal Sciences, Georg-August-University Göttingen, Göttingen, Germany. •Prof. **Johann Sölkner**: Division of Livestock Sciences, Department of Sustainable Agricultural Systems, University of Natural Resources and Life Sciences Vienna, Vienna, Austria. •Prof. **Johannes Lenstra**: Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht, The Netherlands. • Prof. **Michelle Tixier-Boichard**: Institut National de la Recherche Agronomique INRA, France. •Prof. **Ino Curik**: Department of Animal Science, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia. •Assoc. Prof. **Patrik Waldmann**: Division of Statistics, Department of Computer and Information Science, Linköping University, Linköping, Sweden. •Ass. Prof. **Gregor Gorjanc**: Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Slovenia. •Dr. **Carl Rubin**: Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden. •Dr. **Gabor Meszaros**: Division of Livestock Sciences, Department of Sustainable Agricultural Systems, University of Natural Resources and Life Sciences Vienna, Vienna, Austria. •PhD student **Ana María Pérez O'Brien**: Division of Livestock Sciences, Department of Sustainable Agricultural Systems, University of Natural Resources and Life Sciences Vienna, Vienna, Austria. •PhD student **Maja Ferencakovic** (practical): Department of Animal Science, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia. •PhD student **Anamarija Frkonja** (practical): Division of Livestock Sciences, Department of Sustainable Agricultural Systems, University of Natural Resources and Life Sciences Vienna, Vienna, Austria. • PhD student **Christian Reimer** (practical-also participant) Department of Animal Sciences, Georg-August-University Göttingen, Göttingen, Germany.

### **Participants**

**Argentina (1)**: Joaquin Mueller; **Austria (4)**: Solomon Antwi Boison (native Ghana), Silvia Fluch, Tesfaye Getachew (native Ethiopia), Kahsa Tadel Gebre (native Ethiopia); **Croatia (10)**: Relja Beck, Kristina Budimir, Vlatka Čubrić Čurik, Ivana Kovač, Boris Lukić, Maja Maurić, Nikola Raguž, Dragica Šalamon, Marija Špehar, Anamarija Štambuk; **France (2)**: Badr Benjelloun (native Morocco), Belén Jimenez Mena; **Germany (1)**: Christian Reimer; Jimenez Mena (native Spain); **Italy (2)**: Lorenzo Bomba, Marco Milanese; **Hungary (2)**: Istvan Nagy, Attila Zsolnai; **The Netherlands (1)**: Mirte Bosse; **Slovakia (1)**: Radovan Kasarda; **Slovenia (2)**: Peter Dovč, Petra Filipović; **Sweden (1)**: Sangeet Lamichhaney (native Nepal); **USA (4)**: Janise Evans, Heather Huson, Olivia McCaskell, Brian Sayre.