A next generation sequencing approach for targeted Varroa destructor (Acari: Varroidae) mitochondrial DNA analysis based on honey derived environmental DNA

Honey contains DNA from many different organisms that are part of hive micro-environmental niches and honey bee pathospheres. In this study, we recovered and sequenced mite mitochondrial DNA (mtDNA) from honey from different locations around the world (Europe, Asia, Africa, North and South America). DNA extracted from 17 honey samples was amplified with eight primer pairs targeting three mite mtDNA genes, obtaining 88 amplicons that were sequenced with an Ion Torrent sequencing platform. A bioinformatic pipeline compared produced reads with Varroa spp. mtDNA sequence entries available in GenBank and assigned them to different mitotypes. In all honey samples, the highest percentage of reads was attributed to the K1 lineage, including a few variants derived from it, in addition to J1 reads observed in the two South American samples and C1-1 reads obtained from the Chinese honey. This study opens new possibilities to analyse mite lineages and variants and monitor their geographical and temporal distribution, simplifying surveillance against this damaging honey bee parasite.

General information
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Organisations: National Institute of Aquatic Resources, Technical University of Denmark, Section for Marine Living Resources, University of Bologna
Contributors: Utzeri, V. J., Schiavo, G., Ribani, A., Bertolini, F., Bovo, S., Fontanesi, L.
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.47 SJR 0.929 SNIP 1.122
Detection of Co-expressed Pathway Modules Associated With Mineral Concentration and Meat Quality in Nelore Cattle

Meat quality is a complex trait that is influenced by genetic and environmental factors, which includes mineral concentration. However, the association between mineral concentration and meat quality, and the specific molecular pathways underlying this association, are not well explored. We therefore analyzed gene expression as measured with RNA-seq in Longissimus thoracis muscle of 194 Nelore steers for association with three meat quality traits (intramuscular fat, meat pH, and tenderness) and the concentration of 13 minerals (Ca, Cr, Co, Cu, Fe, K, Mg, Mn, Na, P, S, Se, and Zn). We identified seven sets of co-expressed genes (modules) associated with at least two traits, which indicates that common pathways influence these traits. From pathway analysis of module hub genes, we further found an over-representation for energy and protein metabolism (AMPK and mTOR signaling pathways) in addition to muscle growth, and protein turnover pathways. Among the identified hub genes FASN, ELOV5, and PDE3B are involved with lipid metabolism and were affected by previously identified eQTLs associated to fat deposition. The reported hub genes and over-represented pathways provide evidence of interplay among gene expression, mineral concentration, and meat quality...
traits. Future studies investigating the effect of different levels of mineral supplementation in the gene expression and meat quality traits could help us to elucidate the regulatory mechanism by which the genes/pathways are affected.

**General information**

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Organisations: Department of Health Technology, Statistics and Data Analysis, Department of Applied Mathematics and Computer Science, Section for Marine Living Resources, National Institute of Aquatic Resources, Federal University of São Carlos, University of São Paulo, City University of New York, Empresa Brasileira de Pesquisa Agropecuária


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- Web of Science (2018): Indexed yes
- BFI (2017): BFI-level 1
- Scopus rating (2017): CiteScore 3.78 SJR 2.274 SNIP 1.032
- Web of Science (2017): Impact factor 4.151
- Web of Science (2017): Indexed yes
- Scopus rating (2016): CiteScore 3.44 SJR 2.067 SNIP 0.884
- Web of Science (2016): Impact factor 3.789
- Scopus rating (2015): CiteScore 3.38 SJR 2.021 SNIP 0.84
- Web of Science (2015): Indexed yes
- Scopus rating (2014): CiteScore 3.1 SJR 1.798 SNIP 0.758
- Web of Science (2014): Indexed yes
- Scopus rating (2013): CiteScore 2.57 SJR 1.342 SNIP 0.596
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**Genome-wide patterns of homozygosity provide clues about the population history and adaptation of goats**

Background: Patterns of homozygosity can be influenced by several factors, such as demography, recombination, and selection. Using the goat SNP50 BeadChip, we genotyped 3171 goats belonging to 117 populations with a worldwide distribution. Our objectives were to characterize the number and length of runs of homozygosity (ROH) and to detect ROH hotspots in order to gain new insights into the consequences of neutral and selection processes on the genome-wide homozygosity patterns of goats. Results: The proportion of the goat genome covered by ROH is, in general, less than 15% with an inverse relationship between ROH length and frequency i.e. short ROH (< 3 Mb) are the most frequent ones. Our data also indicate that ~ 60% of the breeds display low F<sub>ROH</sub> coefficients (< 0.10), while ~ 30 and ~ 10% of the goat populations show moderate (0.10 < F<sub>ROH</sub> < 0.20) or high (> 0.20) F<sub>ROH</sub> values. For populations from Asia, the average number of ROH is smaller and their coverage is lower in goats from the Near East than in goats from Central Asia, which is consistent with the role of the Fertile Crescent as the primary centre of goat domestication. We also observed that local breeds with small population sizes tend to have a larger fraction of the genome covered by ROH compared to breeds with tens of thousands of individuals. Five regions on three goat chromosomes i.e. 11, 12 and 18, contain ROH hotspots that overlap with signatures of selection. Conclusions: Patterns of homozygosity (average number of ROH of 77 and genome coverage of 248 Mb; F<sub>ROH</sub> < 0.15) are similar in goats from different geographic areas. The increased homozygosity in local breeds is the consequence of their small population size and geographic isolation as well as of founder effects and recent inbreeding. The existence of three ROH hotspots that co-localize with signatures of selection
demonstrates that selection has also played an important role in increasing the homozygosity of specific regions in the goat genome. Finally, most of the goat breeds analysed in this work display low levels of homozygosity, which is favourable for their genetic management and viability.

**Geneve information**

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**Organisations:** National Institute of Aquatic Resources, Section for Marine Living Resources, Iowa State University, Autonomous University of Barcelona, Fondazione Parco Tecnologico Padano

**Contributors:** Bertolini, F., Cardoso, T. F., Marras, G., Nicolazzi, E. L., Rothschild, M. F., Amills, M.

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**Genome-wide SNP profiling of worldwide goat populations reveals strong partitioning of diversity and highlights post-domestication migration routes**

**Background:** Goat populations that are characterized within the AdaptMap project cover a large part of the worldwide distribution of this species and provide the opportunity to assess their diversity at a global scale. We analysed genome-wide 50 K single nucleotide polymorphism (SNP) data from 144 populations to describe the global patterns of molecular variation, compare them to those observed in other livestock species, and identify the drivers that led to the current distribution of goats. Results: A high degree of genetic variability exists among the goat populations studied. Our results highlight a strong partitioning of molecular diversity between and within continents. Three major gene pools correspond to goats from Europe, Africa and West Asia. Dissection of sub-structures disclosed regional gene pools, which reflect the main post-domestication migration routes. We also identified several exchanges, mainly in African populations, and which often involve admixed and cosmopolitan breeds. Extensive gene flow has taken place within specific areas (e.g., south Europe, Morocco and Mali-Burkina Faso-Nigeria), whereas elsewhere isolation due to geographical barriers (e.g., seas or mountains) or human management has decreased local gene flows. Conclusions: After domestication in the Fertile Crescent in the early Neolithic era (ca. 12,000 YBP), domestic goats that already carried differentiated gene pools spread to Europe, Africa and Asia. The spread of these populations determined the major genomic background of the continental populations, which currently have a more marked subdivision than that observed in other ruminant livestock species. Subsequently, further diversification occurred at the regional level due to geographical and reproductive isolation, which was accompanied by additional migrations and/or importations, the traces of which are still detectable today. The effects of breed formation were clearly detected, particularly in Central and North Europe. Overall, our results highlight a remarkable diversity that occurs at the global scale and is locally partitioned and often affected by introgression from cosmopolitan breeds. These findings support the importance of long-term preservation of goat diversity, and provide a useful framework for investigating adaptive introgression, directing genetic improvement and choosing breeding targets.
Signatures of selection and environmental adaptation across the goat genome post-domestication

Background: Since goat was domesticated 10,000 years ago, many factors have contributed to the differentiation of goat breeds and these are classified mainly into two types: (i) adaptation to different breeding systems and/or purposes and (ii) adaptation to different environments. As a result, approximately 600 goat breeds have developed worldwide; they differ considerably from one another in terms of phenotypic characteristics and are adapted to a wide range of climatic conditions. In this work, we analyzed the AdaptMap goat dataset, which is composed of data from more than 3000 animals collected worldwide and genotyped with the CaprineSNP50 BeadChip. These animals were partitioned into groups based on geographical area, production uses, available records on solid coat color and environmental variables including the sampling geographical coordinates, to investigate the role of natural and/or artificial selection in shaping the genome of goat breeds. Results: Several signatures of selection on different chromosomal regions were detected across the different breeds, sub-geographical clusters, phenotypic and climatic groups. These regions contain genes that are involved in important biological processes, such as milk-, meat- or fiber-related production, coat color, glucose pathway, oxidative stress response, size, and circadian clock differences. Our results confirm previous findings in other species on adaptation to extreme environments and human purposes and provide new genes that could explain some of the differences between goat breeds according to their geographical distribution and adaptation to different environments. Conclusions: These analyses of signatures of selection provide a comprehensive first picture of the global domestication process and adaptation of goat breeds and highlight possible genes that may have contributed to the differentiation of this species worldwide.
Entomological signatures in honey: an environmental DNA metabarcoding approach can disclose information on plant-sucking insects in agricultural and forest landscapes

Honeydew produced from the excretion of plant-sucking insects (order Hemiptera) is a carbohydrate-rich material that is foraged by honey bees to integrate their diets. In this study, we used DNA extracted from honey as a source of environmental DNA to disclose its entomological signature determined by honeydew producing Hemiptera that was recovered not only from honeydew honey but also from blossom honey. We designed PCR primers that amplified a fragment of mitochondrial cytochrome c oxidase subunit 1 (COI) gene of Hemiptera species using DNA isolated from unifloral, polyfloral and honeydew honeys. Ion Torrent next generation sequencing metabarcoding data analysis assigned Hemiptera species using a customized bioinformatic pipeline. The forest honeydew honeys reported the presence of high abundance of Cinara pectinatae DNA, confirming their silver fir forest origin. In all other honeys, most of the sequenced reads were from the planthopper Metcalfa pruinosa for which it was possible to evaluate the frequency of different mitotypes. Aphids of other species were identified from honeys of different geographical and botanical origins. This unique entomological signature derived by environmental DNA contained in honey opens new applications for honey authentication and to disclose and monitor the ecology of plant-sucking insects in agricultural and forest landscapes.
Exploiting phenotype diversity in a local animal genetic resource: identification of a single nucleotide polymorphism associated with the tail shape phenotype in the autochthonous Casertana pig breed

Casertana is a local pig breed mainly raised in Central-South regions of Italy. Pigs of this breed are considered the descendants of the ancient Neapolitan population that largely influenced the constitution of the modern commercial pigs. The pigs of this breed are usually curly-tailed, like several other domestic pig populations. However, Casertana population shows some variability for this trait, including animals having straight tail as observed in wild boars. In this study, we run, for the first time, a genome wide association study (GWAS) comparing the curly tailed (no.=53) and straight tailed (no.=19) Casertana pigs to identify genomic regions associated with the tail shape phenotype in Sus scrofa. All animals were genotyped with the Illumina PorcineSNP60 BeadChip v.2. GEMMA software was used in the GWAS for which we were able to correct for stratification in the analysed cohort. A single nucleotide polymorphism (rs81439488), located on porcine chromosome 12, was significantly associated with the investigated trait. This marker is close to the SRY-box 9 (SOX9) gene that encodes for a transcription factor that is required during sequential steps of the chondrocyte differentiation pathway, notochord maintenance and skeletogenesis. As the shape of the tail could be important in relation to the problem of tail biting in pigs, the obtained results might open new perspectives for defining selection programs answering indirectly animal welfare issues. This work demonstrated that autochthonous animal genetic resources might be used to disclose genetic factors affecting peculiar traits by exploiting segregating phenotypes and genetic variability.

General information
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Organisations: Department of Bio and Health Informatics, University of Bologna, Food and Agriculture Organization of the United Nations, Associazione Nazionale Allevatori Suini
Contributors: Bertolini, F., Schiavo, G., Tinarelli, S., Santoro, L., Utzeri, V. J., Dall'Olio, S., Costa, L. N., Gallo, M., Fontanesi, L.
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BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.58 SJR 0.817 SNIP 1.095
Web of Science (2016): Impact factor 1.377
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.35 SJR 0.838 SNIP 0.994
Web of Science (2015): Impact factor 1.293
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.56 SJR 0.827 SNIP 1.211
Web of Science (2014): Impact factor 1.171
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 1.4 SJR 0.733 SNIP 1.049
Web of Science (2013): Impact factor 1.1
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 1.46 SJR 0.847 SNIP 1.172
Web of Science (2012): Impact factor 1.249
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Precision Medicine in Targeted Therapies for Severe Asthma: Is There Any Place for "omics" Technology?

According to the current guidelines, severe asthma still represents a controversial topic in terms of definition and management. The introduction of novel biological therapies as a treatment option for severe asthmatic patients paved the way to a personalized approach, which aims at matching the appropriate therapy with the different asthma phenotypes. Traditional asthma phenotypes have been decomposing by an increasing number of asthma subclasses based on functional and physiopathological mechanisms. This is possible thanks to the development and application of different omics technologies. The new asthma classification patterns, particularly concerning severe asthma, include an increasing number of endotypes that have been identified using new omics technologies. The identification of endotypes provides new opportunities for the management of asthma symptoms, but this implies that biological therapies which target inflammatory mediators in the frame of specific patterns of inflammation should be developed. However, the pathway leading to a precision approach in asthma treatment is still at its beginning. The aim of this review is providing a synthetic overview of the current asthma management, with a particular focus on severe asthma, in the light of phenotype and endotype approach, and summarizing the current knowledge about "omics" science and their therapeutic relevance in the field of bronchial asthma.

General information
State: Published
Organisations: Department of Bio and Health Informatics, Azienda Ospedaliera Santa Maria Nuova di Reggio Emilia, Ospedale Policlinico
Contributors: Galeone, C., Scelfo, C., Bertolini, F., Caminati, M., Ruggiero, P., Facciolongo, N., Menzella, F.
Number of pages: 15
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Severe refractory asthma: current treatment options and ongoing research

Patients with severe asthma have a greater risk of asthma-related symptoms, morbidities, and exacerbations. Moreover, healthcare costs of patients with severe refractory asthma are at least 80% higher than those with stable asthma, mainly because of a higher use of healthcare resources and chronic side effects of oral corticosteroids (OCS). The advent of new promising biologicals provides a unique therapeutic option that could achieve asthma control without OCS. However, the increasing number of available molecules poses a new challenge: the identification and selection of the most appropriate treatment. Thanks to a better understanding of the basic mechanisms of the disease and the use of predictive biomarkers, especially regarding the Th2-high endotype, it is now easier than before to tailor therapy and guide clinicians toward the most suitable therapeutic choice, thus reducing the number of uncontrolled patients and therapeutic failures. In this review, we will discuss the different biological options available for the treatment of severe refractory asthma, their mechanism of action, and the overlapping aspects of their usage in clinical practice. The availability of new molecules, specific for different molecular targets, is a key topic, especially when considering that the same targets are sometimes part of the same phenotype. The aim of this review is to help clarify these doubts, which may facilitate the clinical decision-making process and the achievement of the best possible outcomes.

General information
State: Published
Organisations: Department of Bio and Health Informatics, University of Modena and Reggio Emilia, Verona University Hospital, L. Spallanzani National Institute for Infectious Diseases
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Shotgun metagenomics of honey DNA: Evaluation of a methodological approach to describe a multi-kingdom honey bee derived environmental DNA signature
Honey bees are considered large-scale monitoring tools due to their environmental exploration and foraging activities. Traces of these activities can be recovered in the honey that also may reflect the hive ecological micro-conditions in which it has been produced. This study applied a next generation sequencing platform (Ion Torrent) for shotgun metagenomic analysis of honey environmental DNA (eDNA). The study tested a methodological framework to interpret DNA sequence information useful to describe the complex ecosystems of the honey bee colony superorganism, its pathosphere and the heterogeneity of the agroecological environments and environmental sources that left DNA marks in the honey. Analysis of two honeys reported sequence reads from five main organism groups (kingdoms or phyla): arthropods (that mainly included reads from Apis mellifera, several other members of the Hymenoptera, in addition to members of the Diptera, Coleoptera and Lepidoptera, as well as aphids and mites), plants (that clearly confirmed the botanical origin of the two honeys, i.e. orange tree blossom and eucalyptus tree blossom honeys), fungi and bacteria (including common hive and
honey bee gut microorganisms, honey bee pathogens and plant pathogens), and viruses (which accounted for the largest number of reads in both honeys, mainly assigned to Apis mellifera filamentous virus). The shotgun metagenomic approach that was used in this study can be applied in large scale experiments that might have multiple objectives according to the multi-kingdom derived eDNA that is contained in the honey.

**General information**

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Organisations: Department of Bio and Health Informatics, University of Bologna
Contributors: Bovo, S., Ribani, A., Utzeri, V. J., Schiavo, G., Bertolini, F., Fontanesi, L.
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Scopus rating (2017): CiteScore 3.01 SJR 1.164 SNIP 1.111
Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 3.11 SJR 1.236 SNIP 1.101
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Scopus rating (2015): CiteScore 3.32 SJR 1.427 SNIP 1.136
Web of Science (2015): Indexed yes
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Scopus rating (2013): CiteScore 3.94 SJR 1.772 SNIP 1.153
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 4.15 SJR 1.982 SNIP 1.156
Web of Science (2012): Impact factor 3.73
ISI indexed (2012): ISI indexed yes
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BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.58 SJR 2.425 SNIP 1.233
Web of Science (2011): Impact factor 4.092
ISI indexed (2011): ISI indexed no
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.705 SNIP 1.178
Web of Science (2010): Impact factor 4.411
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.614 SNIP 1.046
Web of Science (2009): Indexed yes
Taking advantage from phenotype variability in a local animal genetic resource: identification of genomic regions associated with the hairless phenotype in Casertana pigs

Casertana is an endangered autochthonous pig breed (raised in south-central Italy) that is considered to be the descendant of the influential Neapolitan pig population that was used to improve British breeds in the 19th century. Casertana pigs are characterized by a typical, almost complete, hairless phenotype, even though a few Casertana pigs are normal haired. In this work, using Illumina PorcineSNP60 BeadChip data, we carried out a genome-wide association study and an FST analysis with this breed by comparing animals showing the classical hairless phenotype (n = 81) versus pigs classified as haired (n = 15). Combining the results obtained with the two approaches, we identified two significant regions: one on porcine chromosome (SSC) 7 and one on SSC15. The SSC7 region contains the forkhead box N3 (FOXN3) gene, the most plausible candidate gene of this region, considering that mutations in another gene of the same family (forkhead box N1; Foxn1 or FOXN1) are responsible for the nude locus in rodents and alopecia in humans. Another potential candidate gene, rho guanine nucleotide exchange factor 10 (ARHGEF10), is located in the SSC15 region. FOXN3 and ARHGEF10 have been detected as differentially expressed in androgenetic and senescent alopecia respectively. This study on an autochthonous pig breed contributes to shed some light on novel genes potentially involved in hair development and growth and demonstrates that local animal breeds can be valuable genetic resources for disclosing genetic factors affecting unique traits, taking advantage of phenotype variability segregating in small populations.

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Web of Science (2017): Impact factor 1.841
Web of Science (2017): Indexed yes
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Towards precision medicine: The application of omics technologies in asthma management

Asthma is a chronic obstructive respiratory disease characterised by bronchial inflammation. Its biological and clinical features have been widely explored and a number of pharmacological treatments are currently available. Currently several aspects of asthma pathophysiological background remain unclear, and this is represent a limitation for the traditional asthma phenotype approach. In this scenario, the identification of new molecular and clinical biomarkers may be helpful in order to better understand the disease, define specific diagnostic tools and highlight relevant novel targets for pharmacological treatments. Omics technologies offer innovative research tools for addressing the above mentioned goals. However, there is still a lot to do both in the fields of basic research and in the clinical application. Recently, genome-wide association studies, microRNAs and proteomics are contributing to enrich the available data for the identification of new asthma biomarkers. A precise approach to the patient with asthma, particularly with severe uncontrolled asthma, requires new and specific therapeutic targets, but also proper tools able to drive the clinician in
tailoring the treatment. On the other hand, there is a need of predictors to treatment's response, particularly in the field of biological drugs, whose sustainability implies a correct and precise selection of the patients. Translating acquired omics knowledge in clinical practice may address the unmet needs described above, but large-scale studies are required in order to confirm their relevance and effectiveness in daily practice. Thus in our opinion the application of omics is still lagging in the real-life setting.

**General information**

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Scopus rating (2015): CiteScore 0.87 SJR 0.62 SNIP 0.341
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**Projects:**

**Reproductive Physiology of Female European Eel**
Jørgensen, M. G. P., PhD Student, National Institute of Aquatic Resources
Tomkiewicz, J., Main Supervisor
Kjørsvik, E., Supervisor
Eg Nielsen, E., Supervisor
Bertolini, F., Supervisor
Technical University of Denmark
15/12/2016 → 14/12/2019
Award relations: Reproductive Physiology of Female European Eel
Project: PhD