Genomic adaptation of Mediterranean and Alpine local cattle breeds to the climate variables

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ABSTRACT. – Adaptive ability of livestock to climate variations is a factor of first importance in the context of global climate change. Domestic species such as cattle represent attractive biological models to characterize the genetic basis of short domestication history. Local cattle breeds are valuable genetic resources to decipher the molecular mechanisms of cattle adaptation to their specific environments. Using newly generated dense SNP genotyping data, we assessed the structuring of genetic diversity of 21 Mediterranean and 24 Alpine cattle breeds and performed genome-wide association analyses with covariables discriminating the different Mediterranean and Alpine climate subtypes. This provided insights into both the demographic and adaptive histories. A detailed functional annotation of genes surrounding variants associated with climate variations highlighted several biological functions involved in Mediterranean and Alpine climate adaptation such as thermotolerance, UV protection, pathogen resistance or metabolism with strong candidate genes identified. Accordingly, our results suggest that main selective pressures affecting cattle in Mediterranean and Alpine area may have been related to variation in heat and UV exposure, in food resources availability and in exposure to pathogens. Taken together, our results highlight the genetic uniqueness of local Mediterranean and Alpine cattle breeds and strongly support conservation of these populations.

INTRODUCTION. – In recent decades, changes in climate have caused impacts on natural and human systems on all continents. The Mediterranean region is a global "hot-spot" of climate change both in the South (southern Mediterranean regions) where climate conditions are already stressful, and the North (Europe) where droughts and heat waves are recurrent. During this century, the climate will profoundly

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change, and with it the context of livestock production. The forecasted global climate change will deeply impact ecosystems and will not spare agriculture and livestock productions. In the border lands of the Mediterranean sea, the so-called "Mediterranean climate", characterized by the wet winter/dry summer seasonality of rainfall will be affected in these following ways: i) a significant warming, ii) an increase of pre-cipitations in winter and a decrease in summer, iii) a decline of annual precipitations over much of the region, exacerbating drought conditions, and iv) an increased frequency of extreme events (droughts or heat-waves). As a consequence, the regional livestock production systems will have to face various stresses, such as: i) feeding and water resources scarcity, ii) heat stress, which reduces male and female reproduction and production abilities, increases mortality risk and increases water require-ments and iii) disease stress, due to the spread of pathogens or the spatial and seasonal changes in disease distributions that may expose livestock populations to new diseases. According to these forecasts, adaptive traits, such as body reserve management, disease and heat resistance, feed and water scarcity tolerance related to a poor-quality environ-ment will gain a dramatic importance. In the different Mediterranean environments, for centuries farmers have developed production systems adapted to the local resources availability along the seasons and to their own objectives (milk, meat, manure...), taking advantage of the adaptive traits of local breeds. However, in order to adapt to the effects of climate change, management practices have to be modified.

A production system embeds a breeding system into the whole food chain process at the local level. The production objectives determine a set of decisions to make at each step of the livestock activity. In a breed-ing program adapted to a harsh environment, breeders have: i) to choose the most appropriate breeds to use in a given production system and ii) to follow suitable selection goals that match the production system constraints (Mirkena *et al.* 2010). The use of animal genetic diversity in relation to system management diversity in contrasted agroecological situations should allow the identification of the most adapted genotypes and management practices capable of coping with the environmental challenges posed by production systems. Adaptability is the ability to survive, grow and reproduce itself within a defined environment, or the degree to which an animal can remain adapted to a wide range of environments (Mirkena *et al.* 2010). Adaptation abilities include adap-tation to heat stress (thermotolerance), nutritional stresses (low meta-

bolic requirements, ability to reduce metabolism, fat deposition as feed reserve) and presence of parasites and diseases. All these adaptation abilities mainly rely on functional traits such as reproductive, metabolic, neurological, immunological traits and hair coat characteristics and depend on complex interactions among anatomical and physiological factors. From a breeder's point of view, these traits are usually characterized by: measurement problems, low heritability, important genotype/environment interactions and underlying antagonistic biological relationships with production traits (Mirkena *et al.* 2010). As, the causal physiological or genetic mechanisms of adaptation traits are largely unknown, the direct study of adaptive traits is difficult to perform. This is particularly the case in southern Mediterranean countries where performance and pedigree recordings are far from being set up.

Understanding how species and ecosystems respond to climate change has become a crucial focus in ecology, biodiversity conservation and management. The recent availability of genome-wide SNP panels (high density SNP chips) allows providing background information concerning genome structure in domestic animals, opening new perspectives to livestock genetics. Several approaches have been performed in landscape genomics to detect adaptation to different climate pressure by correlations between genomic data and climate variables. The International Project GALIMED "Genetic Adaptation of Bovine Livestock and production systems in MEDiterranean region", has developed an integrated approach that combines the analysis of SNP markers and bioclimatic variables to identify genotypes able to respond to climate change and provided a genomic map of climate adaptation in Mediterranean cattle breeds.

A GENOMIC MAP OF CLIMATE ADAPTATION IN MEDITERRANEAN CATTLE BREEDS (FLORI, L., MOAZAMI-GOUDARZI, L., ALARY, V. *ET AL.* CONTRIBUTOR: ROBERTA CIAMPOLINI. MOLECULAR ECOLOGY 2019; 28: 1009-1029. © 2019 JOHN WILEY & SONS LTD. *HTTPS://DOI.ORG/10.1111/MEC.15004*). – Domestic species represent attractive biological models to characterize the genetic basis of short-term evolutionary response to abiotic selective pressure such as climate. Indeed, during their human mediated migration from restricted domestication centres, they colonized a wide range of new environments and differentiated into a variety of populations or breeds shaped by the combined action of natural and artificial selection, and genetic drift. For instance, cattle (*Bos tau-*

rus) have been domesticated ca. 10,000 years before present (YBP) in two independent domestication centers, located in the Fertile Crescent for taurine (*Bos t. taurus*) and in the Indus Valley for the zebu (*Bos t. indicus*) (Loftus *et al.* 1994). These cattle populations then migrated with farmers on the different continents leading to more than 800 different cattle breeds now recognized worldwide (Felius *et al.* 2014). Standing at a crossroad of several early cattle migration routes, the Mediterranean basin appeared as a central area for European and African population exchanges. Indeed, the Near East Neolithic farmer populations followed two main routes through Europe migrating from 9,000 YBP to Central Europe via the so-called Danubian route (*i.e.*, through Anatolia, Thrace and the Balkans), and to Southern Europe via the so-called Mediterranean route (*i.e.*, using a maritime route by the Mediterranean sea) reaching Italy, France and Spain, 6,000 to 6,500 YBP (Payne & Hodges 1997). Conversely, in Africa, taurine cattle were introduced through Egypt along the Mediterranean littoral, 6,500 YBP, although migrations from Southern Europe via Mediterranean islands also possibly occurred (Payne & Hodges 1997). Finally, if zebu cattle were first introduced in Africa via the Suez route, 3,500 YBP, their two major introductions occurred later via maritime routes to the Horn of Africa with Muslim expansion during the 7th century AD and following the rinderpest epidemics of 19th century (Payne & Hodges 1997). Zebu then spread gradually to the entire African continent, including Mediterranean regions and probably in Southern Europe, as suggested by the indicine ancestry detected in some Italian and Greek breeds (D'Angelo *et al.* 2006; Gautier *et al.* 2010). During this complex colonization history, and over the course of less than 1,500 generations, cattle became adapted to a variety of local climatic conditions across the Mediterranean basin (Gautier *et al.* 2007). Indeed, although the Mediterranean climate is mainly characterized by dry summers and mild and moist winters, a wide range of climate subtypes still persists from "dry-summer temperate" in Southern Europe to "dry-summer subtropical" in North Africa (Peel *et al.* 2007). Capitalizing on the genomic resources available for cattle, and in particular in SNP genotyping assays, it is now possible to scan the genome for regions involved in adaptation to local environment. To that end, Genome-Environment Association (GEA) analyses have facilitated identification of genetic variants associated with population-specific environmental covariables (Gautier 2015). When combined with a functional annotation of candi-

date genes or variants, such a population genomics approach has proved to be efficient at highlighting candidate genes or physiological pathways affected by climate variation in both animal (Gao *et al.* 2017; Lv *et al.* 2014) and plant (Frachon *et al.* 2018) species. Here, using a newly developed genotyping data set, we aimed at providing insights into both the demographic and adaptive histories of Mediterranean cattle, with a focus on climate adaptation. More precisely, was analyzed the structure of the genetic diversity across 21 local cattle breeds inhabiting eight different countries on both sides of the Mediterranean sea (Spain, France, Italy, Greece, Cyprus, Egypt, Algeria and Morocco) and infer their relationships with other breeds. The bovine genome was then scanned to identify footprints of adaptation to the Mediterranean climate using the methods implemented in the Baypass software (Gautier 2015) and climatic variables associated with the cattle sampling locations. A detailed functional annotation of the identified candidate genes using system biology tools (Flori *et al.* 2014) uncovered the main physiological pathways mobilized during cattle adaptation to the Mediterranean climate. The newly developed medium density SNP genotyping data set demonstrates a clear structuring of the genetic diversity of local breeds across the Mediterranean basin resulting from a complex recent demographic history with admixture events involving the three major cattle groups. Yet, most of the local breeds we considered can be viewed as stabilized in the sense that they displayed a high degree of within population genetic homogeneity. This led us to consider the population or breed as the appropriate level of organization to draw a bovine genomic map of short-term adaptive response to local Mediterranean climate variation that was mainly captured by climatic covariables related to temperature, precipitation, radiation and humidity (Fig. 1). Strikingly, the indepth functional analysis we carried out based on the 55 candidate genes found associated with climatic covariables highlighted several functions or physiological pathways (*e.g.,* cancer, pigmentation, metabolism or infection and immunity) already reported in other similar GEA performed in human and sheep to be important in climate adaptation (Lv *et al.* 2014). More specifically, our results suggested a central role of genes involved in cancer, cellular growth and proliferation while highlighting the impact of heat stress and UV exposure on physiology. Some plausible candidate genes were identified, such as METTL3, which regulates the UV-induced DNA damage response (Xiang *et al.* 2017) and LEF1, which could be related to thermotolerance and UV protection, as sug-

gested by its central role in hair pigmentation (Guenther *et al.* 2014) and its location in a QTL for UV protective eye area pigmentation in cattle (Pausch *et al.* 2012). In addition, LEF1 was also found under adaptive differentiation across Mediterranean breeds in our study and across Chinese local cattle breeds (Gao *et al.* 2017). The functional annotation of the candidate genes also identifies genes involved in the development and function of the nervous system such as CTNNA2, NRG1 and RFX4 (Dominguez *et al.* 2018), the latter being also reported as associated with climate covariable in Chinese cattle (Gao *et al.* 2017). These genes might be important to adapt to varying Mediterranean climatic conditions via their action on thermal regulation, behavioural and sensory system development. Other candidate genes play also a role in development such as NDUFB3 and FBN1 that are involved in morphology and stature (Alston *et al.* 2016). This might be viewed as support for the hypothesis that climate strongly influences body size with positive correlations between heat and aridity, and smaller size (Franks & Hoffmann 2012). Finally, several candidate genes were found to be involved in amino acid metabolism (*e.g.,* GADL1, GLDC, NRG1, SLC46A1) and in cardiovascular system or in development of urinary tract (*e.g.*, ALDH1A2, AMER1, CDH4, EYA1, FBN1, LAMC1, MST1, MYC, NOG, PTGS2, PTPRF, SHH, SLC19A1, SMO, SST, TP53, VEGFA, VHL) which could be speculatively interpreted as resulting from physiological adaptations required to cope with drought in some Mediterranean areas. Interestingly, some of the genes found associated with climate were actually involved in infectious disease resistance (*e.g*., ANTXR2, MAP3K8, MLST8 and SMYD3), perhaps implicating pathogens with distributions that are influenced by climate. The most striking example is represented by ANTXR2 that was found both under selection and associated with climate covariables, suggesting that anthrax could have exerted a strong selective pressure on Mediterranean cattle breeds. Indeed, this gene encodes the major receptor mediating in vivo lethality of the toxin produced by Bacillus anthracis (Arévalo *et al.* 2014) responsible for anthrax, the oldest known zoonosis with a worldwide distribution, severely affecting human and ruminants. This disease, is thought to have originated in Egypt and Mesopotamia and to be depicted in ancient writings since 1,491 BC (Schwartz 2009). In Europe, it was described in a 10th century collection of veterinary writings, was responsible for enormous domestic livestock losses in Europe from the 17th to the 19th century (Schwartz 2009) and also occurred more recently, at the end of

the last century, in the Mediterranean countries of Southern Europe, especially in Spain, Italy, Turkey and Greece. A link between climatic factors (*i.e*., temperature, precipitation pattern and solar radiation) and the onset of anthrax outbreaks have been established, as the spores of Bacillus anthracis are especially resistant in contaminated soils, where they can survive for years. In particular, areas in Europe with a pronounced dry season, such as Mediterranean countries, have a higher prevalence of animal anthrax. This disease could also have exerted strong selective pressure in cattle from other areas, ANTXR2 being found under selection in West-African cattle, and in other susceptible species since this gene was also found associated with climate variables in humans and sheep (Lv *et al.* 2014) and correlated with pathogen diversity in humans. In addition to ANTXR2 and LEF1, were identified other genes, VDAC1, TCF7 and SKP1, that were all associated with climatic covariables and located in a genomic region displaying strong evidence of adaptive differentiation on BTA7. Interestingly, the strongest differentiation signal in this region is obtained close to TCF7, which, as LEF1, is a main downstream effector of a signalling pathway (*i.e.,* Wnt/β catenin) and both genes have recently been found to be involved in a regulatory feedback loop controlling taste cell renewal in the circumvallate papilla epithelium and loss of gustatory nerve fibres in mice (Gaillard *et al.* 2017). Similarly, a SNP proximal to LEF1 is also associated with feeding behaviour and eating efficiency in Duroc pigs (Ding *et al.* 2017). Although difficult to characterize, it is nevertheless important to note that other human-mediated or natural selective pressures may also have driven the adaptive genetic diversity of the Mediterranean local cattle breeds. Overall, genome scan for association with climate covariable on breeds representative of various local Mediterranean conditions provided a global picture of the main targeted candidate physiological adaptations. This illustrates in turn the originality and genetic potential of Mediterranean cattle breeds in particular in the context of global warming (Segnalini *et al.* 2013). In addition to the identification of the genetic variants underlying adaptive response of Mediterranean cattle breeds to local climatic variation, characterizing their origin may thus be critical to promote the conservation of genetic resources and the associated traditional herding systems that are threatened by the increasing use of a small number of commercial breeds (Bruford *et al.* 2015). The structuring of genetic diversity of Mediterranean local cattle breeds and their recent post-domestication history makes it unlikely that locally

favourable genetic variants arose by new mutations. Instead, adaptation to local climate conditions may have rather relied on standing genetic variation that existed in both domesticated and wild European cattle during the period when domestication took place and as a consequence of early farmer migration, or may be the result of more recent adaptive introgression of indicine origin (both hypotheses being of course mutually nonexclusive). Zebu that diverged from taurine between 84,000 and 275,000 YBP (Ho *et al.* 2008), have been subjected for a longer time to tropical and arid conditions and are now well adapted to these specific conditions while African taurine, which diverged from European taurine several thousand years ago (Stock *et al.* 2013), are also well adapted to tropical humid climate. Although the current lack of genetic data on local aurochs individuals prevents us from assessing their contribution, our characterization at the genome-wide scale demonstrates an admixture with (a) zebu ancestry in Southern Europe with an East to West decreasing gradient for Cyprus, Greek, Maremmana and Romagnola Italian and Corsican breeds, which confirms and refines previous studies (Gautier *et al.* 2010), (b) African taurine ancestry in Maghreb (Tidili, Oulmes Zaër, Brune de l'Atlas and Chelifienne breeds) and (c) both zebu and African taurine ancestries in Maghreb (Cheurfa and Guelmoise breeds), Egypt and Cyprus. Taken together, this observed pattern of cattle genetic ancestry for the Mediterranean breeds remains concordant with the known migration history of Neolithic farmers from the taurine domestication centre in the Fertile Crescent toward the West through the Mediterranean coasts and major islands (*e.g.,* cattle arriving in Corsica around 5,000 YBP), following the Mediterranean route; the migration of taurine from North-Africa to Spain after their introduction in Africa through Egypt 6,500 bc; and the crossing in Egypt of the various migration routes taken by settled communities towards Europe and Africa presumably leading to interbreeding of the cattle populations. Cattle with indicine ancestry were probably brought in Southern Europe by the Silk Road route (~200 bc to 1,720 ce) connecting Asia to the Mediterranean sea (and stopping in Italy), in agreement with the decreasing gradient of indicine ancestry observed from Sicily to Italy mainland and Corsica. Overall, due to their various origins, Mediterranean cattle breeds represent attractive models to further assess the relative contribution of the major bovine ancestries in climate adaptation.

BOVITA Project Autofunded - Genomic Adaptation of Local Cattle Breeds in the Alpine Massif (Ciampolini *et al.* 2019. ASPA 23rd Congress, Sorrento, June 11-14. Book of abstracts ISSN: 1828-051X, Communication code 0039, *https://www.tandfonline.com/tjas*. – Understanding adaptive ability of livestock is a key factor in the context of global climate change and become a crucial focus in conservation and management for a sustainable farming in a changing environment. One way to better understand adaptation abilities is to identify genes underlying adaptation phenotypes. This goal can be achieved by genetically characterizing livestock species and detecting footprints of selection in the animal genome. The recent availability of genome-wide SNP panels allows providing background information concerning genome structure in domestic animals, opening new perspectives to livestock genetics. In order to investigate the genetic regions with a potential adaptive role, we investigate 24 cattle breeds, (high productive specialized breeds and several autochthonous populations) reared in six different neighboring nations from East to West Alpine Massif (Fig. 2). Several analyses have been performed to detect footprints of selection and genomic regions associated with climate variables. More precisely, using breed GPS coordinates, four climatic variables (annual mean temperature, annual precipitation, annual mean radiation, and annual mean moisture index) were extracted from the Climond database (Fig. 2). In order to identify footprints of selection, a whole genome scan for adaptive differentiation were performed using Bovine 50K SNP chip genotyping data with the XtX model implemented in the BAYPASS software. In addition, across-population whole genome scans for association with the population-specific climatic variables were performed using the AUX model. Footprints of selection were detected on BTA6 and BTA18 pointing out several candidate genes (*i.e.,* LCORL, PDGFRA, KDR and SPG7); moreover different genomic regions (on BTA 6, 10, 19 and 20) were associated with annual mean radiation. Ongoing analyses will specify candidate regions and genes involved in local adaptation in the Alpine massif.
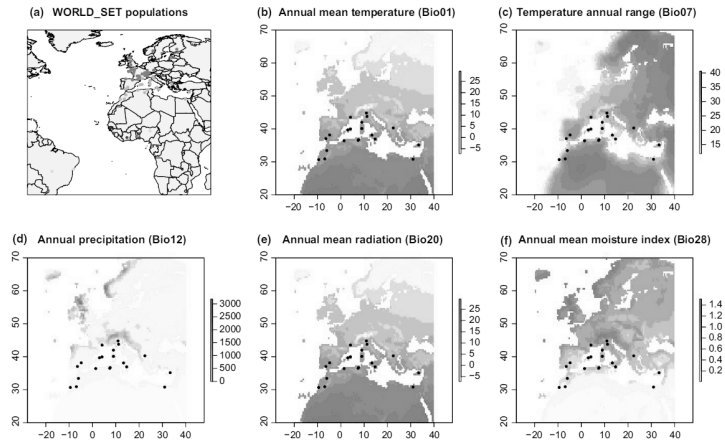
Fig. 1. Cattle breed location and geographic pattern of the annual mean values of the five main climatic variables. (a) Location of the 62 breeds of the WORLD-Set, including the 21 breeds of the MED-Set. EUT, AFT, ZEB are indicated in red, blue, green, respectively, and the breeds located in the Mediterranean area (from the MED-Set), in orange. ANA and TUR hybrids breeds, not considered in the MED-Set are in black. (b) to (f) Geographic pattern of the annual mean temperature (b), the temperature annual range (c), the annual precipitation (d), the annual mean radiation (e) and the annual mean moisture index (f), with the location of Mediterranean breeds (MED-Set) [Colour figure can be viewed at wileyonlinelibrary.com]. From Flori, L., Moazami-Goudarzi, L., Alary, V. *et al.* Contributor: Roberta Ciampolini. Molecular Ecology 2019, 28: 1009-1029. © 2019 John Wiley & Sons Ltd. *https://doi.org/10.1111/mec.15004.*
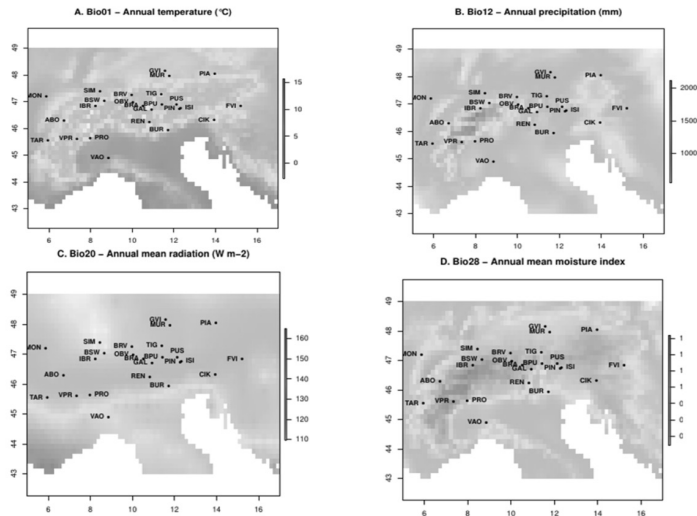
Fig. 2. Cattle Breeds and Climatic Covariates. BOVITA Project Autofunded - Genomic Adaptation of Local Cattle Breeds in the Alpine Massif (Ciampolini *et al.* 2019. ASPA 23rd Congress, Sorrento, June 11-14. Book of abstracts ISSN: 1828-051X, Communication code 0039, *https://www.tandfonline.com/tjas*).

REFERENCES

Alston, C.L., Howard C., Oláhova M. *et al.* (2016). A recurrent mitochondrial p.Trp 22Arg NDUFB3 variant causes a distinctive facial appearance, short stature and a mild biochemical and clinical phenotype. Journal of Medical Genetics 53: 634-641.

Arévalo M.T., Navarro A., Arico C.D. *et al.* (2014). Targeted silencing of anthrax toxin receptors protects against anthrax toxins. Journal of Biological Chemistry 289: 15730-15738.

Bruford M.W., Ginja C., Hoffmann I. *et al.* (2015). Prospects and challenges for the conservation of farm animal genomic resources, 2015-2025. Frontiers in Genetics 6: 314.

Ciampolini R., Flori L., Moazami-Goudarzi K. *et al.* (2019). Communication code O039 - ASPA 23rd Congress, Sorrento, June 11-14, 2019 Book of Abstracts.

Ding R., Quan J., Yang M. *et al.* (2017). Genome-wide association analysis reveals genetic loci and candidate genes for feeding behavior and eating efficiency in Duroc boars. PLoS One 12, e0183244.

Dominguez S.L., Hegde G., Hanson J.E. *et al.* (2018). Antibody-mediated stabilization of NRG1 induces behavioral and electrophysiological alterations in adult mice. Scientific Reports 8, 8239. *https://doi.org/10.1038/s41598-018-26492-4*.

D'Angelo F., Ciani E., Sevi A. *et al.* (2006). The genetic variability of the podolica cattle breed from the Gargano Area. Preliminary results. Italian Journal of Animal Science 5: 79-85.

Felius M., Beerling M.L., Buchanan D.S. *et al.* (2014). On the history of cattle genetic resources. Diversity 6: 705-750.

Flori L., Thevenon S., Dayo G.K. *et al.* (2014). Adaptive admixture in the West African bovine hybrid zone: Insight from the Borgou population. Molecular Ecology 23: 3241-3257.

Flori L., Moazami-Goudarzi K., Alary V. *et al.* (2019). A genomic map of climate adaptation in Mediterranean cattle breeds. Contributor: Roberta Ciampolini. Molecular Ecology 28: 1009-1029. © 2019 John Wiley & Sons Ltd, *https://doi.org/10.1111/mec.15004*.

Frachon L., Bartoli C., Carrère S. *et al.* (2018). A genomic map of climate adaptation in *Arabidopsis thaliana* at a micro-geographic scale. Frontiers in Plant Science 9: 967.

Franks S.J., Hoffmann A.A. (2012). Genetics of climate change adaptation. Annual Review of Genetics 46: 185-208.

Gaillard D., Bowles S.G., Salcedo E. *et al.* (2017). ß-catenin is required for taste bud cell renewal and behavioral taste perception in adult mice. PLoS Genetics 13, e1006990.

Gao Y., Gautier M., Ding X. *et al.* (2017). Species composition and environmental adaptation of indigenous Chinese cattle. Scientific Reports 7, 16196.

Gautier M. (2015). Genome-wide scan for adaptive divergence and association with population-specific covariates. Genetics 201: 1555-1579.

Gautier M., Faraut T., Moazami-Goudarzi K. *et al.* (2007). Genetic and haplotypic structure in 14 European and African cattle breeds. Genetics 177: 1059-1070.

Gautier M., Laloë D., Moazami-Goudarzi K. (2010). Insights into the genetic history of French cattle from dense SNP data on 47 worldwide breeds. PLoS One 5, e13038.

Gautier M., Hocking T.D., Foulley J.L. (2010). A Bayesian outlier criterion to detect SNPs under selection in large data sets. PLoS One 5, e11913.

Guenther C.A., Tasic B., Luo L. *et al.* (2014). A molecular basis for classic blond hair color in Europeans. Nature Genetics 46: 748-752.

Ho S., Larson G., Edwards C.J. *et al.* (2008). Correlating Bayesian date estimates with climatic events and domestication using a bovine case study. Biology Letters 4: 370-374.

Loftus R.T., MacHugh D.E., Bradley D.G. *et al.* (1994). Evidence for two independent domestications of cattle. Proceedings of the National Academy of Sciences 91: 2757-2761.

Lv F.H., Agha S., Kantanen J. *et al.* (2014). Adaptations to climate-mediated selective pressures in sheep. Molecular Biology and Evolution 31: 3324-3343.

Mirkena T., Duguma G., Haile A. *et al.* (2010). Genetics of adaptation in domestic farm animals: A review. Livestock Science 132: 1-12.

Pausch H., Wang X., Jung S. *et al.* (2012). Identification of QTL for UV-protective eye area pigmentation in cattle by progeny phenotyping and genome-wide association analysis. PLoS One 7, e36346.

Peel M.C., Finlayson B. L., McMahon T. (2007). Updated world map of the Köppen-Geiger climate classification. Hydrology and Earth System Sciences 11: 1633-1644.

Schwartz M. (2009). Dr. Jekyll and Mr. Hyde: A short history of anthrax. Molecular Aspects of Medicine 30: 347-355.

Segnalini M., Bernabucci U., Vitali A. *et al.* (2013). Temperature humidity index scenarios in the Mediterranean basin. International Journal of Biometeorology 57: 451-458.

Stock F., Gifford-Gonzalez D. (2013). Genetics and African cattle domestication. African Archaeological Review 30: 51-72.