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Perspective

LaGomiCs—Lagomorph Genomics Consortium: An International Collaborative Effort for Sequencing the Genomes of an Entire Mammalian Order

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Abstract

The order Lagomorpha comprises about 90 living species, divided in 2 families: the pikas (Family Ochotonidae), and the rabbits, hares, and jackrabbits (Family Leporidae). Lagomorphs are important economically and scientifically as major human food resources, valued game species, pests of agricultural significance, model laboratory animals, and key elements in food webs. A quarter of the lagomorph species are listed as threatened. They are native to all continents except Antarctica, and occur up to 5000 m above sea level, from the equator to the Arctic, spanning a wide range of environmental conditions. The order has notable taxonomic problems presenting significant difficulties for defining a species due to broad phenotypic variation, overlap of morphological characteristics, and relatively recent speciation events. At present, only the genomes of 2 species, the European rabbit (*Oryctolagus cuniculus*) and American pika (*Ochotona princeps*) have been sequenced and assembled. Starting from a paucity of genome information, the main scientific aim of the *La*gomorph *Geno*mic *Con*sortium (LaGomiCs), born from a cooperative initiative of the European COST Action "A Collaborative European Network on Rabbit Genome Biology—RGB-Net" and the World Lagomorph Society (WLS), is to provide an international framework for the sequencing of the genome of all extant and selected extinct lagomorphs. Sequencing the genomes of an entire order will provide a large amount of information to address biological problems not

only related to lagomorphs but also to all mammals. We present current and planned sequencing programs and outline the final objective of LaGomiCs possible through broad international collaboration.

Lagomorphs are such a distinct lineage of mammals that the very word "lagomorph" is a circular reference meaning "hare-shaped". Ancestral lagomorphs evolved following the Cretaceous-Paleogene (K-Pg) boundary 53 million years ago and are in the same major mammalian clade as rodents and primates (Euarchontoglires; O'Leary et al. 2013).

The order comprises 91 living species divided into 2 families (Figure 1; Hoffmann and Smith 2005; Alves and Hacklander 2008): 1) the pikas (Family Ochotonidae) and 2) the rabbits, hares and jackrabbits (Family Leporidae). However, the systematics of the order is unclear and currently under review by the IUCN Species Survival Commission (SSC) Global Mammal Assessment and Lagomorph Specialist Group (LSG).

The Ochotonidae comprises 30 species of small (70–300 g) egg-shaped mammals with distinct rounded ears and no visible tail. Most of these species are vocal. There are 2 major groups of pikas (Smith 1988; Smith et al. 1990; Lissovsky 2014): 1) those that live in rocks or talus have low reproductive rates and are generally long-lived; are territorial either as individuals or pairs and have extremely low rates of social interaction; and 2) those that live in meadow or steppe habitat and burrow, have high reproductive rates, and are generally short-lived; these species form extended families and are highly social.

The family Leporidae comprises 32 species of hares (genus *Lepus*) and 29 species of rabbits (Figure 1). The hares are the largest lagomorphs (2–5 kg), having long ears and hind legs and the rabbits include a range of monotypic genera (Verde Arregoitia et al. 2015), some of which are Evolutionary Distinct and Globally Endangered (EDGE) species. The rabbits also have somewhat long ears, but not as long as the hares, and present a more rounded body type. Other rabbits include a variety of unique forms found around the world: for example, the Riverine rabbit (*Bunolagus monticularis*) in South Africa; the hispid hare (*Caprolagus hispidus*) of the Terai region of

India and Nepal; the black Amami Island rabbit (*Pentalagus furnessi*) that occupies isolated islands in the far south of Japan; the Annamite striped rabbit (*Nesolagus timminsi*) of southeast Asia; and the Volcano rabbit (*Romerolagus diazi*) that lives at high elevations on volcanoes surrounding Mexico City. The only domesticated species of the order, the European rabbit (*Oryctolagus cuniculus*), is the most widespread species globally due to its use by humans (e.g., for meat and fur production, for its role as a biomedical model, and for many biotechnology applications). This species also has the most within-species phenotypic diversity due to artificial selection by humans. There are wild forms (mainly on its native region, the Iberian Peninsula, and southern France) as well as repeated human-mediated dispersal of domesticated rabbits into the wild, in particular on many islands and continents, including Australia and New Zealand, often with devastating consequences for native flora and fauna.

Relevance and Special Features of the Order Lagomorpha

Ecosystem Services, Conservation, and Biodiversity

Lagomorphs are herbivores playing crucial roles in ecosystems due to their medium size and their position in the food chain as prey species. Nevertheless, their diversity in terms of size, behavior, abundance, and reproductive capacity, among other characteristics, allows them to provide varying ecosystem functions and services. Some species are rare, occur in low number with minor relevance to the ecosystem as a whole, but have intrinsic conservation value as rare or vulnerable species that are often evolutionarily distinct.

The order Lagomorpha includes some of the most endangered species of all mammals. Roughly 25% of all extant lagomorphs are listed in a threatened category on the IUCN Red List (Smith 2008). Others, however, are very important as game species or key prey for predators. For example, the European brown hare (*Lepus europaeus*) and the Iberian hare (*L. granatensis*) are 2 major game species in Europe and produce large quantities of healthy game meat. In fact, leporids throughout the world serve as game or subsistence meat. Hares also benefit from human-altered environments and occur in agricultural fields as well as in urban areas.

Some lagomorphs are considered keystone species, acting as main drivers of an ecosystem that create opportunities for many other species, some of which are valuable resources for humans (Delibes-Mateos et al. 2011). For example, the European rabbit is a keystone species in the Mediterranean ecosystem in southern Europe, an area defined as a 'global biodiversity hotspots' (Myers et al. 2000; Delibes-Mateos et al. 2008). This species is the main prey of the Iberian lynx (*Lynx pardinus*), the world's rarest felid, and 20 other carnivores in the Mediterranean region. Even outside their native range, such as in Sweden, the European rabbit has a recognized importance for threatened plant and insect species (Larsson 2006). The snowshoe hare is another example of a lagomorph as a
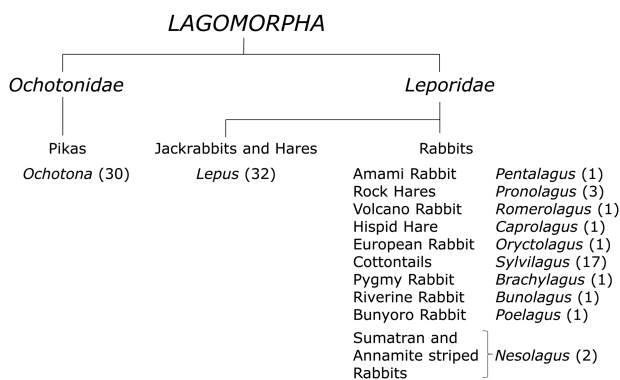

LAGOMORPHA

Ochotonidae — Leporidae

Pikas	Jackrabbits and Hares	Rabbits	
Ochotona (30)	*Lepus* (32)	Amami Rabbit	*Pentalagus* (1)
		Rock Hares	*Pronolagus* (3)
		Volcano Rabbit	*Romerolagus* (1)
		Hispid Hare	*Caprolagus* (1)
		European Rabbit	*Oryctolagus* (1)
		Cottontails	*Sylvilagus* (17)
		Pygmy Rabbit	*Brachylagus* (1)
		Riverine Rabbit	*Bunolagus* (1)
		Bunyoro Rabbit	*Poelagus* (1)
		Sumatran and Annamite striped Rabbits	*Nesolagus* (2)

Figure 1. Overview of systematics of the order Lagomorpha (number in parenthesis indicates the number of species within the genera; Hoffmann and Smith 2005; Alves and Hacklander 2008).

keystone species, with the 10-year cycle that dominates 5 000 000 km² of northern forests in North America and includes a host of avian predators, mammalian predators, and secondary prey fluctuating in step with the hare population (Krebs et al. 2001).

Pikas are temperate to cold-temperature specialists living primarily at high elevations and/or high latitudes. In contrast to fossil species, extant species are specialists living in extreme environments and are largely heat intolerant (MacArthur and Wang 1973, MacArthur and Wang 1974; Smith 1974). As a consequence, they are unable to disperse through warmer environments. Their distribution directly reflects the climatic status of an ecosystem. Studies on the American pika (*Ochotona princeps*) confirm that their temperature sensitivity makes them among the first species to respond to changes in climate, highlighting them as excellent indicators of global warming (Smith et al. 2004; Ray et al. 2012). Genetic evidence has revealed both highly restricted dispersal and putative signatures of selection in response to rapidly changing environments (Henry et al. 2012; Henry and Russello 2013; Lemay et al. 2013).

Species Distribution Modelling using climatic, topographical, and habitat variables for all lagomorph species under past and current climate scenarios and projected into future climatic conditions suggest more than two-thirds of lagomorph species will be impacted (Leach et al. 2015). Thirty-six lagomorph species are predicted to experience range loss, 48 poleward movements, and 51 elevational increases. Thirty-five species are predicted to undergo poleward movements or elevational increases *and* range declines. Small-bodied species (predominately pikas) are more likely to exhibit range contractions and upward elevational shifts but little poleward movement, whilst fecund species are most likely to shift latitudinally. The average poleward shift for the order Lagomorpha has been estimated at 1.1° with an elevational upward shift of 165 m. These are much greater changes than those calculated in a meta-analysis collating information on a wide variety of taxonomic groups (Parmesan and Yohe 2003). This vulnerability to climate change makes the Lagomorpha an ideal order to study not only because they are potentially at the greatest threat of extinction, but also because they will be an effective indicator species displaying changes that we may expect to see in less vulnerable groups as local temperatures increase.

Local Adaptation

Lagomorphs are adapted to a broad range of environments and thus have been able to achieve a worldwide distribution. In contrast to pikas that are cold-temperature and hypoxia specialists, hares have a wide range of remarkable adaptations permitting them to occupy habitats from extremely arid conditions to Arctic tundra. For example, some boreal species (*L. americanus* and *L. timidus* among others) undergo seasonal coat colour change from a brown summer morph to a white winter pelage in adaptation to seasonal snowpack conditions, a trait with limited phenotypic plasticity (Zimova et al. 2014). However, global warming may endanger the survival of these species (Mills et al. 2013). As a consequence, many boreal lagomorph species may act as important markers of climate change because of mismatching between the coat color and the timing of snowfall in the autumn and snowmelt in the spring (Mills et al. 2013). *Lepus timidus* is particularly well adapted to cold habitats, and mitogenomic analyses suggest that mitochondrial DNA (mtDNA) may have played a role in this adaptation, with several instances of positive selection identified in genes encoding proteins of the oxidative phosphorylation chain (Melo-Ferreira et al. 2014c). Other species

are well adapted to hot climates (e.g. the long eared *L. californicus* and *L. callotis*). Additional ecological features contribute to make lagomorphs of particular relevance for the study of diurnal, crepuscular or nocturnal habits, quick movements, reproduction features, parental care, behavior, and disease resistance among other biological features. These considerations make lagomorphs invaluable models to study the architecture of adaptive evolution and the nature of ecological specialization.

Phylogenetics, Speciation, and Hybridization

Pikas, hares, and cottontails are notoriously difficult groups in terms of taxonomic classifications and definition of species, mainly due to broad phenotypic variation within taxa, overlap of morphological characters across taxa, and the relatively recent speciation events in some genera. Molecular markers, initially mtDNA and more recently multi-locus approaches have been used to disentangle questions related to phylogenetics and classification within the different groups (Matthee et al. 2004; Lanier and Olson 2009; Melo-Ferreira et al. 2012, Melo-Ferreira et al. 2015). However, these studies have often uncovered extensive sharing of genetic variation among species, complicating phylogenetic inference based on a comparatively small number of loci. These findings are largely influenced by the retention of ancestral polymorphisms and incomplete lineage sorting across the history of the group, but also, in some cases, due to secondary introgression across historical or current hybrid zones (Thulin et al. 2006; Melo-Ferreira et al. 2005, Melo-Ferreira et al. 2012, Melo-Ferreira et al. 2014a, Melo-Ferreira et al. 2014b). Indeed, several lagomorph systems have become textbook models for speciation and hybridization studies (e.g., *O. cuniculus*, Carneiro et al. 2013, Carneiro et al. 2014a, Carneiro et al. 2014b; *L. europaeus/timidus/granatensis*, Alves et al. 2008, Melo-Ferreira et al. 2011, 2012; *S. transitionalis/obscures/floridanus*, Litvaitis et al. 2008). At this point, more data and studies are needed to better resolve lagomorph systematics and evolution.

Palaeontology and Ancient DNA

Extant and extinct species provide complementary information on the biological diversity and evolutionary mechanisms that have occurred and are still occurring within the order Lagomorpha (Ge et al. 2013). Among European taxa, the relatively recent extinction of *Prolagus* is of particular interest for palaeobiogeographical and biochronological research. *Prolagus* is one of several ochotonids that populated Europe during the last 25 millions of years, and was remarkable due to its longevity, extraordinary abundance, and geographical distribution (López Martínez 2001). There is sufficient archaeological evidence (Vigne et al. 1981; Angelone et al. 2008) as well as testimonies from historians (e.g. Polybius) that *Prolagus* survived in Corsica and Sardinia until the classical epoch. The availability of archeological specimens attributed to *Prolagus sardus* can provide genomic information from this extinct species. Moreover, genetic analyses of ancient specimens of *L. europaeus* and *L. timidus* would allow a better understanding of the adaptive process that led to high altitude specialization of the latter. This information will assist in predicting the possible evolutionary responses to climate change, providing an important evolutionary context for the contemporary management of lagomorph species.

Diseases

Lagomorphs are carriers of pathogens with zoonotic potential (e.g., Tularemia) or may act as reservoirs and/or asymptomatic hosts and

carriers of new, emerging and/or potential zoonotic infections and infestations (i.e., Lyme borreliosis, Crimean-Congo haemorrhagic fever, tick borne encephalitis, paratubercolosis, etc.). The role of different lagomorphs in Europe including the native *Lepus* spp. and *O. cuniculus* as well as the imported species such as *Sylvilagus floridanus*, also may be linked to the presence of new vectors (mosquitoes, sand-flies, ticks, etc.) and to changing climate conditions or different ways of dissemination and diffusion of pathogens that on the whole have determined new epidemiological patterns. In particular, rabbits may act as sentinels of zoonotic infections because they are reared in industrial units for meat production as well as in small rural operations, used as laboratory animals and kept as pets, all the while being present simultaneously in the wild (Shaughnessy et al. 2013).

There is evidence from the literature that the susceptibility to diseases, especially viral diseases such as European brown hare syndrome, rabbit hemorrhagic disease, and myxomatosis, may be linked to genetic factors (Nyström et al. 2011; Abrantes et al. 2012; Kerr 2012; Lopes et al. 2014a) that are likely different between breeds/populations and even differentially expressed at an individual level. In particular, the study of cell receptors for pathogens may lead to improvements in our understanding of pathogenesis of infections including to what extent affected hosts develop innate and adaptive immunity that confers resistance to diseases. A better understanding of the processes involved (including interactions with other factors and organisms) should provide new insights into disease control. These studies are particularly important since there are several examples of viral species jump. Indeed, it has been shown that *Sylvilagus* is susceptible to European brown hare syndrome and *Lepus granatensis*, *L. capensis* and *L. corsicanus* are susceptible to rabbit hemorrhagic diseases (Puggioni et al. 2013; Camarda et al. 2014; Lopes et al. 2014b; Lavazza et al. 2015). Furthermore, direct epidemiological insights may result from the biological similarities between lagomorph and human diseases.

Rabbits and hares, which are present in the wild as large populations, offer the opportunity to perform large-scale analyses of natural ecosystems and to repeat them at regular intervals to follow the dynamics of the host and parasite genotypes under selection and co-evolution. Specifically, the role of density and frequency-dependent selection in host parasite co-evolution may be addressed, for example, in *S. floridanus*. This species facilitated the spread of exotic nematodes and one exotic flea in the population of autochthonous lagomorphs in Europe (Meneguz and Tizzani 2002; Tizzani et al. 2011). These imported parasites offer a robust epidemiological model to study the effects of pathogens in non-coevolved hosts.

Animal Models

The European rabbit is one of the most used experimental animals for biomedical research, particularly as a widely-used bioreactor for the production of polyclonal and monoclonal antibodies. Studies of the rabbit immune system have greatly contributed to our knowledge of the structure, function, and regulation of antibodies (reviewed in Pinheiro et al. 2011). However, many unique physiological features of the rabbit (like human but unlike rodents) have also made it an excellent species for investigating a number of aspects of human diseases such as cardiovascular disease, atherosclerosis, respiratory disease, immune-related diseases, osteoarthritis, ocular research, and Alzheimer's disease and reproductive physiology (Fan et al. 2015). Because of their short life spans, short gestation periods, high numbers of progeny, low cost, and availability of genomic and proteomic information, rabbits usually serve to bridge the gap between smaller

rodents (mice and rats) and larger animals (dogs and monkeys) and play an important role in many translational research activities such as pre-clinical testing of drugs and diagnostic methods for patients. One of the best contributions made by rabbits in the history is the discovery of statin, the most potent lipid-lowering drug which is currently prescribed for more than 30 million cardiovascular disease patients in the world. From the point of view of therapeutic development, it has become evident that many human diseases cannot be properly investigated by rodents such as psychological stress (Nalivaiko 2011) and sepsis (Seok et al. 2013). Many clinical trials failed possibly because the initial concepts were mainly based on mouse studies. Therefore, rabbit serves as an alternative animal model for human diseases to solve those specific questions, which cannot be conducted by rodents. Transgenic rabbits along with the advent of knock-out rabbits will pave a novel way for the development of both therapeutic and diagnostic strategies in the future (Fan and Watanabe 2003; Yang et al. 2014).

Thus, comparative analysis of the rabbit genome with other Lagomorphs will provide an informative context for the rabbit genome and will increase the utility of the rabbit as a biological model. The study of epigenetic changes in regulatory genes may reveal a major way that animals adapt to environmental challenges (Zhang and Meaney 2010) and lagomorph genome sequencing could identify comparable critical genes and their promoters in rodents, primates, and lagomorphs.

Pikas are considered interesting models to study biological mechanisms related to hypoxia from different perspectives. Although pikas are associated with cold environments, not all species evolved at high elevations. Identifying genes responsible for hypoxia tolerance will reveal if lower elevation species may be limited in their ability to shift their ranges in response to climate change due to a lack of hypoxia adaptations. In addition, comparative analyses within the genus *Ochotona* will reveal information about adaptation and resistance to hypoxia with potential applications to the biomedical field.

Pikas also constitute a potential mammalian model for investigating biotic responses to climate change. The latitudinal and elevational distributions of the American pika, in particular, provide interesting opportunities for testing theoretical predictions associated with the roles of historical processes and contemporary forces in shaping genome-wide patterns of neutral and adaptive genetic variation.

Status of Lagomorph Genomics

The genomes of 2 lagomorph species, the European rabbit and American pika, have been preliminarily sequenced and assembled by the Broad Institute in the framework of the Mammalian Genome Project (Lindblad-Toh et al. 2011). The European rabbit genome has been subsequently improved and used to infer information about the domestication process of this species (Carneiro et al. 2014b). These assemblies are publicly available in the ENSEMBL and NCBI databases. The first release of the low-coverage 1.93× assembly (OchPri2.0, http://www.ensembl.org/Ochotona_princeps/Info/Index) of the *O. princeps* genome has been recently improved by the Broad Institute (OchPri3.0). The European rabbit genome (second assembly: OryCun2, GCA_000003625.1) was sequenced to a 6.51-fold coverage (~7×). Approximately 82% of its 2.74 Gb has been anchored to chromosomes (http://www.ensembl.org/Oryctolagus_cuniculus/Info/Index). For the Ochotonidae 273,670 sequences are already deposited in GenBank and EMBL databases (20 Sept. 2015), most of which

(99.47%) are from *O. princeps* whose genome still includes many unassembled scaffolds. The Leporidae accounts for 2 259 925 entries (20 Sept. 2015), most of which (99.54%) are from *O. cuniculus*.

Transcriptome data have so far been reported for just a few species. For example, in the American pika RNA-seq data have been obtained for animals from high and low elevation sites (Lemay et al. 2013). Also, RNA-seq data from *Lepus granatensis* has been included in a study about the efficacy of natural selection in vertebrates and invertebrates (Gayral et al. 2013). A microarray designed for *O. cuniculus* was used to quantify transcript abundance in snowshoe hares (Lavergne et al. 2014).

Collectively, however, these data indicate that genomic information in the Lagomorpha is still in its infancy, despite the large number of biological questions that can be addressed by research efforts in this field.

The Lagomorph Genomics Consortium (LaGomiCs)

Overview and Aims

Sequencing the genomes of all extant species of a mammalian order will provide a unique opportunity to address a large number of biological problems not only related to lagomorphs but also related to all mammals. Indeed, it is currently one of the few orders that have a relatively small number of species that would feasibly permit comprehensive genome sequencing. As outlined, lagomorphs possess many advantageous characteristics to address a broad array of biologically significant topics. A comparative genomic analysis will provide basic information on genome evolution and possibilities to analyze the detailed properties differentiating a unique mammalian order. These data will potentially elucidate features of the ancient lagomorph genome. In addition, sequencing genomes of this order will establish sequence-based rules to clarify the complex systematics within the Lagomorpha providing information for conservation prioritization.

The Lagomorph Genomics Consortium (LaGomiCs; Figure 2) was born from a cooperative initiative of the European COST Action TD1101, "A Collaborative European Network on Rabbit Genome Biology – RGB-Net" 2011–15 (http://www.cost.eu/COST_Actions/bmbs/TD1101; http://www.biocomp.unibo.it/rabbit/) and the World Lagomorph Society (WLS; http://www.world-lagomorphsociety.org/).

Its main scientific aim is to provide an international research framework whose final objective is the sequencing of the genomes of all extant and selected extinct lagomorph species over the next 5 years. Initially, a priority list of proposed species to be sequenced

Figure 2. Official logo of the Lagomorph Genomics (LaGomiCs) Consortium.

has been prepared (Table 1) considering the availability of samples, research priorities, and key questions attached to the proposed species. This list covers the whole Order and includes endangered species, species with taxonomic problems, and the most representative species of the different taxa.

The main political aim of LaGomiCs is to coordinate research efforts to avoid the duplication of activities, facilitate the exchange of data and analyses, and efficiently maximize the scientific impact of these genomic resources. LaGomiCs facilitates the coordination of scientists with overlapping biological interests focused on lagomorph species and welcomes the further development of research questions based on the interest of additional contributors.

Strategic Issues

The sequenced genomes of the European rabbit and American pika will provide key resources to facilitate broad-scale genomic research projects of lagomorph species. To this aim, current or future projects are expected to produce next generation sequencing (NGS) data from additional lagomorphs. Table 2 shows a list of sequencing programs in species of this order, partially overlapping the priority list (Table 1). These programs confirm the emerging interests of the lagomorph research community in developing the resources necessary to address biological questions from a genomic perspective.

The cooperation of national and international funding agencies as well as coordinated activities among research groups will ensure that the necessary resources to achieve the scientific aim of the LaGomiCs are secured. RGB-Net and the WLS will provide the strategic framework to carry out cooperative activities, exploit common interests, prepare research programs, and apply to research agencies. LaGomiCs will follow protocols and methodologies already proposed by the 10K Genome Project, considering the strategic and key issues of sampling and storage of biological material needed for the project and all other steps that should be completed to reach its goal (Genome 10K Community of Scientists 2009; Koepfli et al. 2015).

Sampling and Storage of Biological Material

Sequencing the genome of all lagomorph species requires an important preliminary step of collection of biological samples. Sampling will be coordinated by LaGomiCs according to specific standards and rules that all contributing scientists should follow. Samples will be registered in the Biosamples database, which is a freely available service provided by EMBL-EBI (http://www.ebi.ac.uk/biosamples/index.html). The purpose of the Biosamples database is to provide a central point of access to information about any biological sample, which may then be referenced by other external databases (e.g. the ENA database).

An example of a Biosamples record can be seen here: http://www.ebi.ac.uk/biosamples/sample/SAMEA1145802

> Sample Accession: SAMEA1145802
> Name: source GSM570796 1
> Sample Description:
> Organism: *Oryctolagus cuniculus*
> characteristic[cell type]: stomach cells
> characteristic[strain]: Dutch rabbit
> comment[Sample_source_name]: rabbit stomach cell
> comment[Sample_description]: Gene expression in rabbit stomach cells

The 'Sample Accession' is a stable identifier, which will be assigned to a sample when it is registered to the database. This accession will

Table 1. Priority list for sequencing lagomorph species. This is an updated list based on the documents produced during the First LaGomiCs meeting held in Bologna (Italy) on the 18–19 February 2013

Species	Sample providers	Contacts/proposers	Estimated availability (year)
Lepus timidus	Paulo C Alves, Ettore Randi, Cristiano Vernesi, Carl-Gustaf Thulin, Neil Reid	Paulo C Alves, J Melo-Ferreira, Ettore Randi, Cristiano Vernesi, Luca Fontanesi	2016
Lepus europaeus	Paulo C Alves, Ettore Randi, Cristiano Vernesi, Carl-Gustaf Thulin, Zissis Mamuris, Mihajla Djan	Paulo C Alves, José Melo-Ferreira Ettore Randi, Cristiano Vernesi, Luca Fontanesi	2016
Lepus corsicanus	Ettore Randi, Cristiano Vernesi, Luca Fontanesi	Ettore Randi, Cristiano Vernesi, Luca Fontanesi. José Melo-Ferreira	2016
Lepus castroviejoi	Paulo C. Alves	José Melo-Ferreira	—
Lepus capensis	Paulo C. Alves	—	—
Lepus saxatiliis	Paulo C. Alves	—	—
Lepus granatensis	Paulo C. Alves	Paulo C. Alves, J Melo-Ferreira	—
Lepus tolai	—	—	—
Lepus nigricolis	—	—	—
Lepus coreanus	Hung Sun Koh	—	—
Lepus callotis	—	—	—
Lepus flavigularis	—	—	—
Lepus insularis	—	—	—
Lepus californicus	—	—	—
Lepus townsendii	Paulo C. Alves	Paulo C. Alves, J Melo-Ferreira	—
Lepus americanus	Paulo C. Alves	Paulo C. Alves, J Melo-Ferreira	—
Lepus othus	—	—	—
Lepus articus	—	—	—
Sylvilagus floridanus	—	Thomas McGreevy Jr. Adrienne Kovach, Anthony Tur, Thomas Husband, Tim King	—
Sylvilagus obscurus	—	Thomas McGreevy Jr., Adrienne Kovach, Anthony Tur, Thomas Husband, Tim King	—
Sylvilagus transitionalis	—	Thomas McGreevy Jr., Adrienne Kovach, Anthony Tur, Thomas Husband, Tim King	—
Sylvilagus audubonii	—	—	—
Sylvilagus cunicularius	—	—	—
Sylvilagus brasiliensis	—	—	—
Sylvilagus mansuetus	—	—	—
	—	—	—
Sylvilagus nuttallii	—	—	—
Ochotona curzoniae	—	—	—
Ochotona macrotis	Katherine Solari	—	—
Ochotona princeps (>7x)	—	—	—
Ochotona thibetana	—	—	—
Prolagus sardus (Ext, bones)	Chiara Angelone	Luca Fontanesi, Cristiano Vernesi	2016
Romerolagus diazi	Fernando A. Cervantes	Luca Fontanesi, Paulo C. Alves	2016–17
Pronolagus rupestris	Terrence J. Robinson	Luca Fontanesi	2016–17
Bunolagus monticularis	—	—	—
Pentalagus furnessi	—	—	—

Table 2. List of ongoing or planned sequencing programs in lagomorph species

Species	Status/comments	NGS/sequencing strategy/depth	Biological questions/projects	Principal investigators/contacts	Partners
Ochotona macrotis	Ongoing	Transcriptome/Illumina	Adaptation/climate change	Katie Solari, Liz Hadly, Uma Ramakrishnan	—
Ochotona princeps	Completed (7×)	Genome/Sanger/Illumina	Mammalian Genome Project	Kerstin Lindblad-Toh, Federica Di Palma	—
Ochotona princeps	Ongoing	Transcriptome/454	Conservation/climate change	Michael Russello	—
Ochotona princeps	Ongoing	Genome/Illumina	Conservation/climate change	Michael Russello	—
Lepus americanus	Ongoing (20×)	Genome/RNA-seq/Illumina	Adaptation/conservation/climate change	Paulo C. Alves, José Melo-Ferreira,	Jeffrey M. Good, L. Scott Mills
Lepus americanus	Ongoing	All exome/Illumina	Adaptation/Conservation/Climate change	Jeffrey M. Good, Paulo C. Alves, José Melo-Ferreira,	L. Scott Mills
Lepus americanus	Completed (10×)	Genome/Illumina	Rabbit domestication project	Leif Andersson	CIBIO, Miguel Carneiro, Nuno Ferrand, Paulo C. Alves
Lepus castroviejoi	Planned	Genome/Illumina	Reticulate evolution/speciation/adaptation	José Melo-Ferreira, Paulo C. Alves, Pierre Boursot	—
Lepus corsicanus	Planned	Genome/Illumina	Speciation/conservation	Ettore Randi, Cristiano Vernesi, Luca Fontanesi	—
Lepus corsicanus	Planned	Genome/Illumina	Reticulate evolution/speciation/adaptation	José Melo-Ferreira, Paulo C. Alves, Pierre Boursot	—
Lepus europaeus	Ongoing (15×, 10 specimens)	Genome/Illumina	Reticulate evolution/speciation/adaptation	José Melo-Ferreira, Paulo C. Alves, Pierre Boursot	—
Lepus europaeus	Planned	Genome/Illumina + Ion Proton	Conservation/climate change	Cristiano Vernesi, Ettore Randi, Luca Fontanesi	—
Lepus europaeus	completed	RNA-seq of heart and liver of 6 hares (2 from Greece, 2 from Cyprus, 2 from Poland)	—	Zissis Mamuris	—
Lepus granatensis	Ongoing (20×, 10 specimens)	Genome/Illumina	Reticulate evolution/speciation/adaptation	José Melo-Ferreira, Paulo C. Alves, Pierre Boursot	—
Lepus timidus	Planned	Genome/Illumina	Conservation/climate change	Cristiano Vernesi, Ettore Randi, Luca Fontanesi	—
Lepus timidus	Ongoing 20×, 4 specimens, including a reference genome (60×)	Genome/Illumina	Reticulate evolution/speciation/adaptation	José Melo-Ferreira, Paulo C. Alves, Pierre Boursot,	Jeffrey M. Good, L. Scott Mills

Table 2. *Continued*

Species	Status/comments	NGS/sequencing strategy/depth	Biological questions/projects	Principal investigators/contacts	Partners
Lepus timidus hibernicus	Planned	Genome/Illumina	Determine levels of interspecific introgression with *L. europaeus*	Neil Reid	Paulo C. Alves
Lepus townsendii	Planned	Genome/Illumina	Reticulate evolution/speciation/adaptation	José Melo-Ferreira, Paulo C. Alves,	L. Scott Mills
Oryctolagus cuniculus	Completed (7×)	Genome/Sanger/Illumina	Mammalian Genome Project/ Rabbit domestication project	Leif Andersson, Kerstin Lindblad-Toh, Federica Di Palma	CIBIO, Miguel Carneiro, Nuno Ferrand
Poelagus marjorita	Planned	Genome/Illumina	Reticulate evolution/speciation/adaptation	José Melo-Ferreira, Paulo C. Alves, N Ferrand	—
Sylvilagus floridanus	Ongoing	Genome/Illumina	Conservation	Thomas McGreevy Jr. Adrienne Kovach, Anthony Tur, Thomas Husband, Tim King	—
Sylvilagus obscurus	Ongoing	Genome/Illumina	Conservation	Thomas McGreevy Jr, Adrienne Kovach, Anthony Tur, Thomas Husband, Tim King	—
Sylvilagus transitionalis	Ongoing	Genome/Illumina	Conservation	Thomas McGreevy Jr, Adrienne Kovach, Anthony Tur, Thomas Husband, Tim King	—

then be used within the consortium to track samples at every stage of analysis. Following the accession, the record consists of a set of attributes composed of key/value pairs. The choice of attributes is flexible as it is possible to annotate samples according to information available (e.g., cell type, sex, location, age, health, and a textual description of the sample).

Furthermore, Biosamples records may be collected together into one or more "groups", which also may have attributes, and are identified by a group accession identifier (e.g. http://www.ebi.ac.uk/biosamples/group/SAMEG64149). Creating a LaGomiCs group for the consortium will allow easy access to the complete set of Biosamples associated with the project.

In addition to using the Biosamples resource, the consortium also will hold its own central record of samples held by consortium members. The central record will reference Biosamples accession identifications, and can track other consortium-relevant information, such as whether the sample may be shared by the owner with consortium members.

Sequencing Approaches and Data Analysis

Next generation sequencing technologies will provide cost effective approaches that can produce sufficiently reliable sequence data useful for assembling mammalian genomes. Methods for sequencing and assembling mammalian genomes with NGS data are reasonably well established, although still challenging (Nagarajan and Pop 2013). Generation and analysis of genome sequences will be most efficient if, to the extent possible, the methods are uniform across all species.

For each species, a minimum number of sequences should be produced to build a *de novo* assembly independent from other species. Species in the lagomorph clade are sufficiently divergent so that it is inappropriate to use inter-species sequence alignment or any low-coverage sequencing strategy.

In addition, the sequencing strategy should be tailored for the specific assembly tool to be used in the next downstream steps. The Allpaths-LG assembler is currently a forerunner in successfully producing mammalian genomes from NGS reads (Gnerre et al. 2011), and should be used preferentially for the LaGomiCs project. Allpaths-LG requires the following sequencing data as its input:

1. Paired end Illumina sequencing of 180 bp fragments to a ~45× depth of coverage across the genome.
2. Mate pair Illumina sequencing of multiple libraries with long inserts in the range 3–8 kb, totalling to a ~45× depth of coverage.

The genome assemblies produced from the Allpaths-LG strategy will be sufficient for most downstream analyses. However, assemblies could optionally be improved for key lagomorph species by sequencing of fosmid libraries (Gnerre et al. 2011) or using optical mapping data (Dong et al. 2013) and other sequencing approaches and technologies allowing the sequencing of long reads (Pendleton et al. 2015). RNA-seq data will be obtained from tissue samples from key species and used to facilitate annotation of assembled genomes.

The likely advent of new sequencing and assembly technologies during the course of this project will facilitate higher quality assemblies and reduce the necessary effort. Once the genome assemblies have been generated, a host of additional analyses can be addressed including genome annotation, evolution, and speciation. Having genomic resources for an entire order of species will support novel analytical approaches to address ecological and evolutionary questions.

Indepth Characterization of the Transcriptome for Genome Annotation

To identify all poly-adenylated transcripts in a genome, a variety of adult, embryonic, and developmental tissues (10–15 tissues; e.g. blood, brain, heart, kidney, liver, lung, ovary, skeletal muscle, and skin), from multiple individuals/species will be collected and used for RNA-Seq and transcriptome assembly. Initial target species will be those most easily accessible for the collection of fresh tissues for RNA storage and isolation. The resulting transcriptome will be rich in alternative isoforms and yield an accurate picture of tissue distribution for both coding and noncoding transcripts.

RNA-Seq libraries from each tissue will be constructed using a strand-specific dUTP protocol as described by Levin et al. (2010). Sequencing can be carried out on machines/platforms yielding a minimum of 50 Million 101 bp pair-end reads per tissue. PolyA isolation step will be performed to maximize the breadth of protein-coding transcripts sequenced (Di Palma, personal communications). Strand specificity is useful for all RNA-sequencing and because it allows the determination of the orientation of transcripts it is particularly important for RNA sequencing used in annotation.

Reads will be aligned to the genome using a gene annotation system (Curwen et al. 2004). Protein-coding gene models will be annotated by combining alignments of UniProt (UniProt Consortium 2013) vertebrate/mammalian protein sequences and RNAseq data. In parallel, reads from each tissue will also be assembled separately using Trinity *de novo* transcriptome assembler (Grabherr et al. 2011). As Trinity uses no reference genome for the assembly process, it will serve as a validation step for the transcriptome. The annotation will be further expanded (as needed) by syntenically mapping using the species Ensembl gene set and the protein coding genes from the expanded Rabbit annotation (Ensembl/Broad Institute) with the synteny aligner Satsuma (Grabherr et al. 2010) and a specially developed local aligner (Broad/Uppsala, Di Palma personal communications). This will further characterize noncoding transcripts, missed protein coding genes, and alternative isoforms as well as antisense transcripts.

A Database of Well-Annotated Genes of the Lagomorpha

After the RNA-seq based genome annotation, the next step will be to provide annotation of protein functions of the new genomes. Automated annotation will be obtained using the University of Bologna Biocomputing Group annotation systems. This method is based on a large-scale genome cross comparison and a non-hierarchical clustering procedure characterized by a metric that after statistical validation transfers knowledge within a set of sequences in a cluster that includes homologous and orthologous genes (Piovesan et al. 2011; Radivojac et al. 2013). The annotation system will be specifically implemented as to contain all lagomorph gene sequences with links to all the available information on model animals making the structural location of the different variants among species apparent.

Data Storage, Ownership, and Release Policies

Genomics as a field has benefited tremendously from pre-publication release of sequence data and has a long history of developing forward-looking community-based policies in this area including the 1996 Bermuda Agreement, the 2003 Fort Lauderdale Agreement, and the 2009 Toronto Statement. These approaches have allowed for the field to expand quickly and have driven many discoveries.

LaGomiCs will follow these strategic release policies providing beneficial information to the lagomorph scientific community.

LaGomiCs will use the European Nucleotide Archive (ENA) to deposit its nucleotide sequence data (http://www.ebi.ac.uk/ena/about/about). ENA is a service provided by EMBL-EBI, which both stores nucleotide sequence data and records information relating to the experimental workflow that produced the sequence data. Submitting sequence data to the ENA is a central and mandatory step of a research project, as it satisfies the rules set by scientific publishers to make research data freely available to the scientific community. Sequences submitted at an early stage to the ENA databases by the LaGomiCs consortium may be held privately until a nominal publication date, after which it would be made freely available. Specifically, LaGomiCs will use the sequence read archive to deposit its raw sequencing data and EMBL-Bank to deposit assembled sequences of the lagomorph genomes.

In addition, a UCSC Track Data Hub will be made available for the community through a hosting institution. Track hubs are web-accessible directories of genomic data that can be viewed on the UCSC Genome Browser alongside native annotation tracks. The data underlying the tracks in the hub would reside on the selected institute's servers. The tracks would be both publicly available and linked through both the institute's website and the UCSC Genome Browser.

Additionally, a Memorandum of Understanding (MoU) will be produced to regulate the sharing and storage of samples, the sharing and use of unpublished data and resources, including publication policy, following standards already proposed in genomic projects and open access policies.

A Portal for Lagomorph Genomic Resources and International Collaborations

LaGomiCs has established a website to house all useful content and contacts (http://biocomp.unibo.it/lagomics/) to foster the growth of the lagomorph research community interested in lagomorph genomics and to provide a virtual meeting point to share information and initiatives. LaGomiCs also has established a collaborative connection with the Genome 10K project with the specific intention to coordinate sequencing initiatives to avoid duplication of efforts and to increase the transparency of the need for genomic resources in lagomorph species.

Science Outreach and Education

Lagomorphs are well-known species, both from a historical and contemporary perspective. Several species are present in folklore and mythology from different countries while others are popular in literature or as cartoon figures. They also are charismatic creatures, easily creating empathy with both adults and children. Such characteristics make lagomorphs excellent vehicles for science communication and education. For example, American pikas are at the center of multiple citizen science programs at the regional (e.g. North Cascades National Park, WA, USA) and global (e.g. Worldwide Pika Project; http://www.adventurescience.org/pika.html) scales. Moreover, they are a focal species for ScienceLIVE (http://www.science-live.org/), an organization that facilitates understanding of science by connecting field researchers to the general public. Knowledge generated within the framework of this project can thus be used to contribute to the public understanding of science, from basic biological disciplines to more complex or sensitive topics.

Conclusions

The sequencing of the genome of all lagomorphs is particularly challenging due to the difficulties in organizing appropriate sampling and coordinating data production and data analysis. However, we are able to take advantage of what has already been done in this field by several groups and what specific local projects will obtain in the future. An international framework is needed to collect and combine efforts that, on the whole, will obtain the sequencing of the genome of all species of an entire mammalian order. Different expertises are needed to fully exploit sequence-derived information that might address biological problems not only related to lagomorphs but also to all mammals. The LaGomiCs is open to all contributors who are willing to share their experience, expertise, specialization, and resources in all fields relevant for reaching the final aim.

Acknowledgments

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