

**P1****Using genetic non-invasive sampling to assess the current distribution, trends and conservation of the Broom hare, *Lepus castroviejo***

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The Broom hare (*Lepus castroviejo* Palacios 1976) is an Iberian endemism, with a distribution restricted to the Cantabrian Mountains (Northwest of Spain). This leporid species is listed in the IUCN Red List as Vulnerable. Nevertheless, its current geographical distribution, population size and several aspects of its ecology, behavior, diseases, physiology, reproduction and genetic diversity are poorly known, making the conservation efforts difficult to implement. In this work, we aim to assess the current geographical distribution and evaluate the population trends. Presence/absence data was obtained using genetic non-invasive sampling, namely detection of pellets, and subsequent identification using mitochondrial and nuclear DNA markers. Hunting bags and tissue samples from hunted specimens were also gathered from hunting associations. A set of 17 microsatellites was used to assess putative hybridization with the parapatric species, Iberian hare, *Lepus granatensis*, and brown hare, *Lepus europaeus*, and to test the applicability of mark-recapture methods to estimate population size based on individual genetic identification. This work was kindly funded by Mr Klaus Tamm, through the World Lagomorph Society.

**P2****Vegetation characterizes and predator type influence on western lagomorphs escape strategies?**

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Lagomorphs are important prey species and have evolved many strategies to avoid predation. Many of the strategies correspond to specific land use types. For example, cotton tail ricochet movement corresponds with vegetation that will act as cover. While Black tail jackrabbits use grasslands to escape from mammal predators and use shrubby refuges to escape from avian predators. The escape mechanisms are well honed from many years of evolutionary pressure but with land use change and habitat destruction has left many of these lagomorphs without their normal habitats and needs. We have been studying various lagomorphs' resources selection and predator avoidance behaviors to better understand what they need for refuge and escape cover. This becomes even more important when you incorporate different predator types. For example, we found that when blacktail jackrabbit are being chased by a canid predator it stays in the open and runs in a long oval, often circling back to near the start of the chase. However, when an avian predator chases a black tailed jackrabbit and gets close to the jackrabbit, they use cover like sage to slow the predator and break visual contact and often run perpendicular to the original chase. This is a very important project to better understand how to manage for lagomorphs, which will allow wildlife managers to better restore food web dynamics. For example, vegetation is not as important for desert cottontail as refuge opportunities. Whereas mountain cottontail requires specific vegetation types. Some important management could include repopulating areas or reconnecting habitat types. The knowledge from this project will be instrumental for restoration of lagomorph populations.

**P3****An annotated de novo draft genome of the mountain hare (*Lepus timidus*)**

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Generating genomic resources for Lagomorphs provides important tools to study relevant questions related with the biology and evolution of these organisms. Here, we report the first draft reference genome for a hare species, the mountain hare (*Lepus timidus*). The hare genome was *de novo* assembled using Illumina overlapping paired-end and mate pair reads, following the ALLPATHS-LG protocol. We then relied on synteny between the hare and European rabbit (*Oryctolagus cuniculus*) chromosomes to order and place the hare scaffolds onto putative chromosomes. Among the 4,205 resulting super-scaffolds, 22 have direct correspondence to the rabbit chromosomes and represent 83% of the genome. Gene annotation using *ab-initio* predictions, homology search and transcriptome data identified 24,578 protein coding genes. Of these, 683 were solely derived from hare transcriptome data and thus might be hare specific. Since previous genomic studies on hares have relied on the use of the rabbit reference genome, we tested whether using the new hare genome assembly impacts downstream genomic analyses. Mapping whole-genome re-sequencing data of two hare species to the rabbit or the new hare genome assembly resulted in comparable inferences of historical demography profiles, suggesting similar performance of the alternative reference-based strategies. These results show that the chromosome-scale hare draft genome is a valuable new resource to discover and investigate hare-specific variation, which can be used for chromosome-wide analyses and genome scans in hares.

**P4****Taking genomic research from the university to high school: Hare immune related gene polymorphism repertoire**

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Science education provides students with valuable skills on critical thinking, which is fundamental for their education and life. Bringing science to students is an efficient way of promoting science literacy, while providing a clear understanding on current avenues of research. Here we present a scientific work on genomics that has been developed with a local high school community. This project replicates the work performed in a conventional research project, from its critical conception to the final public release of the results. This year's project was focused on understanding the importance of genetic variability for species' survival and adaptation to novel environments. We take advantage of public hare (*Lepus* spp.) whole genome data to produce datasets of candidate genes to confer resistance to diseases (reviewed in several specialized articles). We analyse genetic variation on these genes across individuals, and interpret the results in the framework of several epidemic diseases that have been strongly affecting hare population in Europe.

**P5****Brown hare farming in Serbia, SWOT analysis after 2 years of experience**

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An experimental farm for brown hare (*Lepus europaeus*) breeding was formed outside the settlement, not far from Zrenjanin town in the Vojvodina, northern province of the Republic of Serbia. The farm was organized for research purposes, with the aim of serving as a reproductive center of the parent farm. In addition to the research aspect, the function of the farm was related to the need to supply other farms or direct reintroduction of brown hare into the hunting ground. During its operation, the farm went through various phases and faced many challenges, which led to the following: registration and obtaining a work permit, production and commissioning of breeding equipment, maintenance of hare and equipment, food quality, emergency events on the farm, safe transportation and hare introduction. The strength of the farm is reflected in: legally established brown hare breeding farm ground, constant monitoring, good equipment, environment and position of the farm and innovative approach. Weaknesses are reflected in the specific conditions on the farm, where specific behavior of workers during daily operations on the farm is necessary. Problems due to sudden changes in behavior, poor diet or illness can lead to the mass death of brown hares. The opportunity for a brown hare farm can refer to the following: Legal distribution in hunting grounds, no adequate competition, cooperation with scientific research institutions. Threats that can be a generator of potential problems: achieving productivity that enables profitability. In addition, there is an increase in breeding costs (food prices), replacement of maintenance workers and undefined veterinary regulations. High costs can lead to an increase in the selling price of hares, which can lead to a drop in demand.

**P6****Camera-trap distance sampling for hare density and abundance estimation**

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The Irish hare (*Lepus timidus hibernicus*) as an endemic sub-species of the mountain hare is listed under the EC Habitats & Species Directive (92/43/EEC) that requires that member states regularly undertake national conservation assessments of its status. During winter 2018/19, we deployed 596 camera traps for 106,026 survey hours (equivalent to approximately 12 years of continuous observation) in 44 x 1km<sup>2</sup> squares selected throughout Ireland to be statistically representative of the country's overall habitat composition. A total of 253 Irish hare were detected in 85% of squares suggesting a highly widespread common distribution. Hare activity was bimodal and crepuscular peak at dawn and dusk. Adapting methods from Caravaggi *et al.* (2016) for estimating target animal distance from the camera lens and Howe *et al.* (2017) for fitting a Distance Sampling model to camera trap data, our optimal model assumed a hazard-rate Detection Function estimating *ca.* 3 hares/km<sup>2</sup>. These results suggest the population remains stable since the previous National survey in 2006/07 but at comparatively low densities. A review of potential threats and pressures, highlighted three concerns: i) agriculture, including intensification, mowing and cutting of grassland and habitat restructuring, ii) illegal poaching and iii) disease, most notably the recent discovery of rabbit haemorrhagic disease virus (RHDV2) in the Irish hare for the first time during July 2019.

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**P7**

Comparing behaviour and ecological preferences among sympatric hares in France

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Three species of hares are present in continental France: the European hare (*Lepus europaeus*) and the mountain hare (*L. timidus*) which are indigenous, and also the Iberian hare (*L. granatensis*) which was introduced from the years 1970 in some Mediterranean localities. There is a transition zone in the Alps between the European hare and the mountain hare, and another one in the Mediterranean area between the European hare and the Iberian hare. The project aims at comparing behaviour and ecological preferences between sympatric hares in these transition zones, in relation to local microclimates, in order to better understand, which ecological factors shape the distribution of these species.

**P8**

## Diet preferences of the Alpine mountain hare (*Lepus timidus varronis*) and the influence of plant identification level and landscape plant diversity on their detectability

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Diet preferences are important in understanding the ecology and evolution of populations. Diet preferences of Alpine mountain hares (*Lepus timidus varronis*) are so far unknown. To determine diet preferences, usually the lowest taxonomic rank i.e. species level is used. However, it is questionable whether detectability of diet preferences varies with respect to the taxonomy rank of plant identification or with the extent of plant species diversity of landscapes. We studied diet preferences of the Alpine mountain hare by analyzing the botanical composition of fecal pellets and comparing the results with the food plants available. Alpine mountain hares preferred ligneous plants, whereas grasses were neutrally or negatively selected. Identification at the genus level revealed the most information in terms of diet preferences, whereas only little information provided the plant identification at the species level. The hares expressed more selections in the homogenous than in the diverse habitat. Hence, for herbivorous mammals less diet selection might be necessary in a diverse habitat. Accordingly, the detection of diet preferences may be more difficult in landscapes with high plant diversity.



**P9**Estimating sustainable harvest rates for European hare (*Lepus europaeus*) populations

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Hunting quotas are used to manage populations of game species to ensure sustainable exploitation. However, unpredictable climatic events may interact with hunting. We established a population model for European hares (*Lepus europaeus*) in Lower Austria. We compared the sustainability of voluntary quotas used by hunters and derived from hare-specific guidelines, actual numbers of hares shot, and our recommended quotas for hares derived from climate and population modeling. We used population modelling based on vital rates and densities to adjust our recommended quotas to achieve sustainable harvest. Survival of Age Classes 1 and 3 had the highest impact on the population growth rate. Population viability analysis showed that a recommended quota with a harvest rate of 10% was sustainable for population densities of 45 hares/km<sup>2</sup>, and that the threshold for hunting should be raised from 10 hares/km<sup>2</sup>, so that hare populations with < 15 hares/km<sup>2</sup> are not hunted. The recommended quota outperformed the voluntary hunting quota, since more hares could be harvested sustainably. Age Class 1 survival was strongly linked with weather: a single year with unfavorable weather conditions (low precipitation) negatively affected population densities. Game species, including the European hare, face increasingly frequent weather extremes due to climate change, so hunting quotas need to be sensitive to frequent population fluctuations.

**P10****Different cause-specific mortality rate but similar juvenile survival in two populations of European hares in France**

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Understanding populations' fluctuations over time and space is critical to manage wildlife species especially for harvested species that need realistic management plan. The European hare (*Lepus europaeus*) is a common game species in Europe. However, a decrease in the hunting bag has been shown since 1960s, likely due to habitat change and loss of cover. In France, some decrease seems to occur likely related to breeding success but we lack information about demographic parameters, in particular for yearling. We took advantage of two telemetry surveys carried out on two different populations in Central France, Maves and Chareil away from 200 km, in both mid-1990 and mid-2000 respectively, to estimate first-year survival rate and to investigate cause-specific mortality rates of young hares. Despite an age effect, we found that monthly survival rates of young individuals were similar between sites/periods. Nonetheless, we found different proportions in the causes of death. Predation mortality was much more important in Chareil than in Maves, while an opposite relation was found for collision likely explained by sites' characteristics (landscape structure and predator densities). During the hunting season, hunting mortality was the more important source of death. We found no temporal difference in non-hunting cause-specific mortality rates, suggesting that hunting mortality may be an additive source of mortality. Our study suggests that, despite in different place and at different period, hare yearling survival remains the same in a context of difficulty for the species, suggesting that productivity may be the critical demographic parameter. Also, despite deeper analysis are required, our analysis suggest that hunting is still the most relevant lever to manage hare populations.

**P11****Where did the leveret go? Proposition of a study to identify potential causes of loss and impact on the functioning of farmland ecosystem**

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Predation is one of the most important interspecific relationships, structuring the functioning of ecosystems. Several trophic levels are involved, and any change at any level can have critical issues for other species. In particular, any change in small mammals can have detrimental effect for birds and mammals on the same trophic levels or above. Farmlands have experienced a loss of biodiversity, with a decrease for all taxa, including several birds and mammals prey species. The main reasons involve change in the habitat due to intensification of agriculture over several decades. Some indicators however suggest that several predator species have experienced increase over the same period, especially generalist and/or opportunistic species. In France, evolution of the structure of habitat might have negative impact on rodents, and decrease in rabbits and partridges' species have been documented. We thus propose to study trophic relationships, focusing on the European hares, perhaps the last alternative species still not decreasing in France. This species is very common in France and an alternative prey for lots of predators. Despite a relative stability of the population, we found a decrease in breeding success of the species over the last decade, probably resulting from a decreasing in leveret survival. The main hypothesis is a higher vulnerability to predation related to both landscape and agricultural practices changes in farmlands. The first step of our programme is to identify litters' place and to evaluate potential sources of mortality, based on a GPS survey of does. We equipped 9 does with GPS collars in early February, with fixes during suckling time. We hope to present preliminary results and further steps of our study.

**P12****Distribution range and ecological niches of Leporids in Africa and Near East**

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Assessment and conservation planning rely heavily on a clarified delineation of species or other taxonomic units relevant for conservation. Unfortunately, there is still a profound lack of knowledge concerning the systematics and distribution of leporids in Africa and Near East, where currently seven *Lepus* species (*L. capensis sensu lato*, *L. europaeus*, *L. fagani*, *L. habessinicus*, *L. saxatilis*, *L. starcki*, *L. victoriae*), four *Pronolagus* species (*P. crassicaudatus*, *P. randensis*, *P. ruprestris*, *P. saundersiae*), as well as *Poelagus marjorita* and *Bunolagus monticularis* are recognized by the IUCN. Particularly the widespread cape hare (*L. capensis s.l.*), shows a strong population structure, pronounced genetic and morphological variation as well as uncertain relationships to other taxa. While phylogeographic studies provide a valuable tool to resolve such diversification patterns and give phylogenetic insights, they are limited by the availability of samples across regions. Here, we review the distribution of African leporids, and present distribution maps based on georeferenced museum specimens and observational data. Therefore, we built a data base, comprising occurrence data from a wide range of sources, including museum collections, literature resources and online citizen science databases. Our work provides a basis for coordination and prioritization of future phylogeographic studies as we summarize comprehensive information on availability of representative specimens across geographic regions. Furthermore, by combining occurrence data with environmental variables, we attempt to model the potential distribution of the species and examine possible differences in ecological niches to check them for plausibility regarding current systematics. Ultimately, our work highlights knowledge gaps that should be urgently filled, and contributes to the clarification of the geographical distribution and environmental niches of African leporids, which is key for defining appropriate conservation management measures.

**P13****Effect of intraspecific interactions on space partitioning of European rabbit *Oryctolagus cuniculus***

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Social interactions in group-living species such as European rabbit (*Oryctolagus cuniculus*) may play a major role in space partitioning. Rabbits interact with each other on different ways within and between groups which may affect individual space use, especially if territoriality occurred in high population density. We aim to assess the effects of neighbours' presence on the spatial and temporal arrangement of individual utilisation distribution (UD). 31 European rabbits were monitored combining GPS collars and VHF radio-tracking in western France during several months between 2017 and 2021. We used 14177 GPS and 1045 VHF locations to estimate individual home ranges using Kernel density estimates (95% and 50%) and local convex hull (LoCoH). We calculated different spatial and temporal UD overlap metrics. Using a null model approach commonly used in ecology, we tested the effect of the presence of neighbors on the shape and distribution of individual UD. The estimated mean home range sizes are 2.5 ha (Kernel95% – 95% CI: 0.9-5.3 ha) and 1.6 ha (LoCoH – 95% CI: 0.6-2.9 ha). An individual can move up to 219 m away from its warren on average (max distance - 95% CI: 126-352 m). The overlap rate of UD (area and volume) varies with the sex and the potential social distance between individuals (i.e. individuals using a common burrow). In addition, male individual UD display evidence of neighbor avoidance with shapes fitting into each other. Our primary results support the hypothesis of a strong space partitioning within the studied population based on social constraints.

**P14****Entangled in introgression and phylogenetic complexity: Taxonomy of the genus *Lepus* in Western and Central Asia inferred from integrative approaches**

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Taxonomy and phylogenetic relationships between species of the genus *Lepus* in Western and Central Asia have long been debated. Lack of diagnostic morphological characters between species, intra-species varieties in morphological traits, hybridization, and mosaic evolution of different parts of the genome made critical controversies over the taxonomy of the genus *Lepus* especially in the region of sympatry e.g., Middle East. Applying integrative approaches, phylogenetic relationships between different taxa of hares (genus *Lepus*) from Western and Central Asia were addressed. The taxonomic status of hares in Western and Central Asia was revised using morphological, morphometric, ecological niche modeling, and molecular approaches. The results indicated that the genus *Lepus* includes four different species, *Lepus tolai* (Russia and China), *L. arabicus* (Arabian Peninsula and Iraq), *L. tibetanus* (being restricted to northeastern Iran, southeast of the Caspian Sea to Pakistan and Afghanistan), and *L. europaeus* (occurs in the Levant, Asia minor to the western parts of Iran). The hares over the central and eastern parts of Iran, which are morphologically similar to *L. tibetanus*, clustered with *L. europaeus* in the tree based on the mitochondrial *cyt b*, but with *L. tibetanus* in the phylogenetic tree based on five nuclear markers. In the extensive region of apparent introgression between *L. tibetanus* and *L. europaeus* in Western Asia, Ecological Niche Modeling (ENM) suggests that suitable habitat for *L. europaeus* is available in some parts. Nevertheless, these regions are occupied by the presumed hybrid populations between *L. tibetanus* and *L. europaeus*, suggesting that parental species may be excluded from these areas because of competitive exclusion and selective advantages of hybrids.

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**P15**

European rabbit *Oryctolagus cuniculus* and hare *Lepus europaeus* are vulnerable on Dutch Red List

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In the new 2021 edition of the Red List of Threatened Mammals of the Netherlands, the European rabbit and European hare were included as "Vulnerable". The Dutch Red List methodology is based on current distribution and population size and changes thereof compared to 1950. Both species are still relatively widespread, but have declined in numbers significantly since the 1950. On our poster, we present these numbers and trends, and possible causes for the Red List status. Concluding, we compare the status on the Dutch national list with their status in neighbouring countries.

**P16****Lack of recovery, RHD infection rates, food quality and genetic diversity of rabbits *Oryctolagus cuniculus* in the Dutch coastal dunes**

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The coastal dunes of The Netherlands Europe used to be known for the high rabbit density. The vegetation offered food of high quality, and the sandy hills offered a good opportunity for digging burrows. But the populations do not seem able to recover from the outbreaks of RHD and RHD2. We studied potential causes of this lack of recovery, focusing on RHD-infections, genetic diversity and possible inbreeding, food and habitat quality. We did this by sampling rabbits at 25 sites with different densities and trends, describing the habitat and collecting fecal pellets for food quality estimation. All but one population showed presence of RHD-V2. Populations differed from each other genetically, but showed no signs of inbreeding despite being isolated and in low densities ever since the decimation by RHD over 30 years ago. Trace elements phosphorus and calcium levels showed a correlation to the population density. Habitat quality, expressed as openness, cover, presence of burrows, sufficient foraging varied among sites but was not clearly related to density. The big challenge of the study was finding populations of high density. Nearly all locations had low densities and few showed a recovery to older densities, which might explain the lack of clear causality in the factor study. Based on the results and literature, a number of 'no regret' conservation interventions were formulated.



**P17****Highest densities of mountain hare (*Lepus timidus*) associated with ecologically restored bog but not grouse moorland management**

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Over the last 20 years, ecological restoration of degraded habitats has become common in conservation practice. Mountain hares (*Lepus timidus scoticus*) were surveyed during 2017-2021 using 830km of line transects in the Peak District National Park, England. Historically degraded bog areas were previously reported having low hare numbers. Following bog restoration we found hare densities of 32.6 (95%CI: 25.2–42.2) individuals km<sup>-2</sup>, notably higher than neighbouring degraded (unrestored) bog with 24.4 (20.6–29.0) hares km<sup>-2</sup>. Hare density on restored peatland was 2.7 times higher than on bogs managed for grouse shooting at 12.2 (9.4–15.8) hares km<sup>-2</sup> and 3.3 times higher than on heather moorland managed for grouse shooting at 10.0 (6.1 to 16.6) hares km<sup>-2</sup>. Yearly estimates varied most on habitats managed for grouse, perhaps indicative of the impact of habitat management e.g. heather burning and/or hare culling to control tick-borne louping ill virus in gamebirds. Acid grassland used for sheep farming had a similar density to grouse moorland at 11.8 (7.3–19.2) hares km<sup>-2</sup>. Unmanaged dwarf shrub heath had the lowest density at 4.8 (2.6-8.8) hares km<sup>-2</sup>. Hare populations are characterised by significant yearly fluctuations, those in the study area increasing by 60% between 2017 and 2018 before declining by ca. 14% by 2020 and remaining stable to 2021. During an earlier survey in 2002, total abundance throughout the Peak District National Park was estimated at 3,361 (2,431-4,612) hares. The present study estimated 3,562 (2,291-5,624) hares suggesting a stable population over the last two decades despite fluctuations likely influenced by weather and anthropogenic factors. Mountain hares in the Peak District favoured bog habitats and were associated with restored peatland habitat. Wildlife management should be cognisant of hare density variation between habitats which may have implications for local extinction risk.

**P18****Influence of weather conditions and age on burrow use of European rabbits during winter**

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The European rabbit (*Oryctolagus cuniculus*) is one of the few lagomorph species building burrows. In this species, these complex structures are used by females for reproduction and by all animals of the social group as a refuge from predation and adverse weather conditions. In our study on a 2-ha field enclosure population in a temperate zone habitat, we tested how different weather factors affected the burrow use of subadult and adult individuals. In particular, we studied the influence of weather conditions on the probability to find the animals inside the burrow during the morning hours, when European rabbits show a peak in activity. Morning burrow presence was recorded during daily check walks from late autumn to late winter, and data from 3 winter seasons were analyzed. Hourly weather data were obtained from a nearby meteorological station. Results showed that, for adults and subadults, morning burrow presence increased with the amount of precipitation. This association was more pronounced as the ambient temperature decreased. It is well known that precipitation occurring together with low ambient temperatures impose particularly unfavorable environmental conditions, under which the rabbits in our study were more likely to remain in the burrows. In addition, in subadults, higher probabilities of burrow presence were found to be driven only by low ambient temperatures independently of precipitation, a pattern which was absent in adults. As subadults, i.e. animals born during the previous reproductive season, are still notably smaller than adults during the winter, we suggest that the increased burrow use of animals of the former age class during colder winter days was due to their higher susceptibility to heat loss.

**P19****Evaluation of the effectiveness of measures to prevent damage to agriculture by the European rabbit in its native range**

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The European rabbit (*Oryctolagus cuniculus*) is an endangered species native to the Iberian Peninsula. Lately, it has caused great economic losses in the Iberian agricultural crops. The effectiveness of various damage prevention measures was evaluated within the framework of "G. O. Preveco" project. Sixty-three treatment plots were selected where perimeter fencing, raptor perches, inhibitor products, sound repellents and perimeter planting were implemented as preventive measures. Twenty-six plots were selected as control. Damage caused by affection (DA), production loss (PL) and rabbit abundance (RA) was estimated in all of them. Economic balance (measures cost vs revenues due to damage reduction) was estimated after one year and three years of measures implementation. Previous values of revenues were considered depending on the crops. A mean comparison analysis was carried out between the damage values obtained in the treatment plots and the control plots. The effect of RA on the success or failure of prevention measures was analyzed using linear models. The measures that significantly reduced DA and PL were inhibitor products (Anova A:F=14.881, p=0.0006; P:F=7.35, p=0.013), perimeter fences (K-W, A: z=4.163, p=0.0014; P: z=3.285, p=0.04) and sound repellents (Anova, A:F=7.87, p=0.008; P:F=1.999, p=0.053, marginally significant). Raptor perches and perimeter planting did not significantly reduce the estimated parameters. Positive and statistically significant relationship was identified between DA and PL with RA in the plots treated with measures which did not reduce damage (perches and perimeter planting). No such relationship was found for control plots or those with measures that reduced damage. Inhibitor products were the only measure with a positive economic balance in the first year, while at three years, inhibitors, fencing, repellents, and roosts showed a positive economic balance. Although more studies are needed, these results showed that it is possible to prevent rabbit damage in agriculture in a cost-effective way.

**P20****Analysis of the efficacy of artificial warrens for the improvement of rabbit populations (*Oryctolagus cuniculus*)**

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The rabbit is a keystone species in Mediterranean systems. Rabbit densities have reduced drastically, mainly due to habitat fragmentation or viral diseases. Strategies for the conservation of the multiple species that feed on rabbits, such as the Iberian lynx, should include actions to improve the abundance of their main prey species, such as the construction of rabbit warrens. In the different LIFE projects, different models of warrens have been used for the actions to improve rabbit abundance. To optimize these actions, WWF designed a new model of warren that was more durable, resistant and cheaper, and that allows its construction in various sizes. We installed warrens of 2, 4 and 6 nesting chambers of this new model in privately owned lands of Ciudad Real. We assessed the efficacy of these warrens by evaluating the number of rabbits per warren and the number of warrens used as a function of different variables, including the size of the warren. For this purpose, data from 175 warrens were used. The results suggest that the largest warrens in this study are used more and have more rabbits. The medium-sized warrens are used less, and the 2-chambers warrens have practically no use. The slope has a negative effect, as well as the distance to the nearest warren. No effect of rabbit density, habitat and orientation variables was found, probably due to homogeneity in the warrens analyzed. We recommend building warrens of 6 nesting chambers in locations as flat as possible (slopes <10%) and with short distances between warrens (10-50 meters), avoiding building isolated warrens. The construction of 2-chambers warrens is not recommended. Although the results are not conclusive for the effect of habitat variables, we recommend avoiding locating the warrens in areas with no grass cover, as this is necessary as a food source for rabbits.

**P21****New insights into interspecific competition within the genus *Lepus* in the Alps**

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As an ice age relict, the Alpine mountain hare (*Lepus timidus varronis*) is particularly affected by ongoing climatic changes. In parallel, the European hare (*Lepus europaeus*) is expanding its range towards higher altitudes. Competition for scarce resources and hybridization between the closely related species are the consequences in areas, where both leporids live in sympatry. We hypothesized that mountain hares can be found at higher altitudes, especially when European hares share the habitat with them. Furthermore, we assumed that introgression rate was higher in areas of sympatry. To test our hypotheses we studied the distribution and introgression of both hare species in four study sites in the province of South Tyrol using non-invasive faecal pellet sampling and genetic analysis. Introgression could only be detected in areas where both species were present, with a proportion of introgression ranging between 5 % and 14 %. The distribution of hare species across the altitudinal range did not show a clear picture. In fact, in one area mountain hares were found at lower altitudes (forests) than European hares (above the tree line). The latter species was found on alpine meadows up to 2,430 m a.s.l., even in winter.

**P22****Non-lethal impacts of hunting on mountain hares**

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Direct effects of hunting on wildlife populations has been thoroughly studied, however there is an increasing focus on the non-lethal effects of hunting on individuals. In order for the cultural heritage of hunting to prevail, we need to ensure ethical hunting practices. In several European countries, including Norway, mountain hares are hunted using hounds. The hound will chase the hare either until the hare is shot, the hound loses track of the hare, or the dog owner decides it is time to end the hunt. Alternative ways of hunting hares is by walk-up hunting using a rifle at longer distances or shooting the hare as it flushes using a shotgun. In this study we investigate the non-lethal impacts of hunting on mountain hare stress hormone (glucocorticoid metabolite) levels. We utilize two approaches; 1) Comparing blood stress hormone levels of individual hares shot using hounds, versus hares shot not using hounds. 2) Comparing fecal stress hormone levels in local hare populations before and after hunting events using hounds. Hares were sampled from September to March of 2020/21 and 2021/22. Lab analysis is to be carried out in August-September of 2022

**P23****Optimisation of data/bio-loggers in behaviour monitoring, survival assessment and death cause identification – A case study: reinforcement operations of the European hare (*Lepus europaeus*)**

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Assessing behaviour, survival and the causes of death of a targeted species in in-situ conservation is not only critical, but also challenging. To solve these issues, the use of data/bio-logger (e.g. GPS, accelerometer) is increasing. However, error margin of location data is usually not well documented and the cause of death may not be well interpreted. A GPS collar was specifically designed to fit a lagomorph species, *Lepus europaeus*. With regard to ethical issues, all size, weight and shape of both the collar and the antenna were *Lepus*-adapted by testing several material strength tests on captive individuals. First, we assessed GPS positioning accuracy in link to both habitat characteristics (e.g. open field vs forest) and device parameters (satellite number, acquisition time, HDOP); we put GPS collars in different spots of a study area and then computed the distance between location data and the true location. Second, we assessed both survival and the causes of death of the released animals by developing a death alert system, using tri-axial accelerometers; we first fitted captive hares in an acclimatization area and then compared both their activity and inactivity thresholds at different time of day. The effectiveness of our optimisations was corroborated on 74 animals, fitted with the data/bio-loggers and released in the study area. GPS positioning accuracy – As vegetation densities decrease, GPS positioning accuracy increases. High number of satellites received combined to low acquisition time and low HDOP values are linked to highly accurate locations (10m or less). Survival and death causes – Death alert is activated when activity values from all 3-axis of the accelerometer are below a defined threshold during several consecutive time intervals. Such optimisations are essential in wildlife conservation programs. We expect an increasing interest of ecologists in confronting these issues.

**P24****Pathogens in Dutch brown hares**

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The Dutch Wildlife Health Centre (DWHC) performs post-mortem examination of wildlife in the Netherlands. Between 2009-2021 DWHC executed a complete necropsy on 529 European brown hares (*Lepus europaeus*), 35 animals were not or incompletely investigated due to autolysis. Most hares were found dead, others were euthanized or shot during the hunting season (October 15th until December 31st) because of signs of disease or abnormal behaviour. Complete necropsy of animals consists of macroscopical and microscopical examination including additional bacteriological and viral investigation when needed and possible. All submitted hares (n= 327) between 2011 up to and including 2017 were tested for the zoonotic bacterium *Francisella tularensis*, causing tularaemia. The most frequent pathogens found were the bacteria *Yersinia pseudotuberculosis* (61/529) and *Francisella tularensis* (52/529), closely related Lagoviruses, European brown hare syndrome virus (EHBSV) and rabbit hemorrhagic disease virus type 2 (RHDV-2) (36/529), and protozoa *Eimeria spp.* (89/529 contributory cause or cause of death), causing coccidiosis. Mild coccidiosis was determined in 54/529 hares. *Yersinia* was found in almost all years (10/13) and the proportion of positive hares to hares examined varied between 0 and 32%. In 2013 presence of *Francisella tularensis* was proven, this being the first case in the Netherlands after 60 years. From 2013 up to and including 2021, the percentages of hares with tularaemia lies between 4 and 18%. Until 2016 sporadic cases of EBHSV were detected (1–8 %). In December 2016 the first case of RHDV-2 was confirmed. In 2017 no Lagovirus was found in the examined hares, but in 2018 and 2019 respectively 27% and 19% of the examined hares died of a Lagovirus (EBHVS and/or RHDV-2) infection. In all years hares suffered from coccidiosis.



**P25****Phylogeography and phylogenetic relationships of African leporids**

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Despite Africa hosting a quarter of global biodiversity, knowledge on leporid species is very limited. In particular, the evolutionary history, phylogeography and phylogenetic relationships of African leporids are poorly known. Clarifying these aspects is therefore crucial for implementing appropriate conservation measures. Here, we assessed the phylogenetic relationships and phylogeographic patterns of two important genera of African leporids: hares (*Lepus*) and rockhares (*Pronolagus*). The taxonomy of African hare species, namely *Lepus capensis*, *L. saxatilis* and *L. victoriae*, is controversial, mostly due to the similarity of morphological characters. Moreover, the genetic data available for *L. saxatilis* and *L. victoriae* is limited, and the distribution of both species is still unclear. There are four species of Rockhares all endemic to southern Africa, and like the African hares little is known about their genetics. Namely, Jameson's red rock hares (*Pronolagus randensis*) have allopatric distributions, and there is evidence for the occurrence of different ecotypes. A striking example occurs of Angola where two subspecies adapted to different altitudes, have been suggested but never investigated genetically. By taking advantage of a wide range of specimens collected over the last century in museums worldwide, we used a target sequencing approach to generate mitogenomes over 100 samples from three African hare species (*L. capensis*, *L. saxatilis* and *L. victoriae*) and 20 rockhare species, including the two described ecotypes of *P. randensis* in Angola. We believe that this work will contribute to a better understanding of the taxonomy and geographical distribution of these species and ultimately the evolutionary history of the African leporids, which are crucial for supporting conservation measures.

**P26****Playback of alarm calls affects foraging time of conspecific hares, yet not their nutrient intake**

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Antipredator responses can affect nutrient intake, which could lead to nutritional deficits and eventually lead to a reduced birth- and survival rate. However, most of the small herbivores are nocturnal or crepuscular and therefore nutrient intake is very difficult to observe or measure in the field, particularly when in cover. Our objective was to experimentally assess the effect of a predator-prey encounter on the nutrient intake of a cryptic herbivore, the European hare (*Lepus europaeus*). To simulate an encounter, we used playback experiments with three different treatments 1) playback of a conspecific alarm call, 2) playback of sheep (control sound), and 3) no sound. We used accelerometers to study antipredator behaviour during the circadian rhythm, while GPS was used to study habitat use. To understand the effect of the antipredator response on nutrient intake, we investigated its effect on the time spent costly vigilant, the vegetation height in which the hares were present, and the time spent foraging. Nutrient intake was calculated by multiplying the time spent foraging by the phosphorus concentration in the forage. As a response to conspecific alarm calls, 1) hares did not increase their time spent costly vigilant, 2) hares did not spend more time in tall vegetation, but 3) increased their time spent foraging. Still, the nutrient intake of hares was not affected. Therefore, the antipredator behaviour of the European hare as a response to playback of conspecific alarm calls did not come with a nutritional cost. Possibly, hares spent more time foraging with an increased predation risk, because hares are non-central place foragers and therefore missed opportunity costs for foraging was high. However, differences in nutrient concentration nullified the effect of increased foraging on the daily nutrient intake.

**P27****Positive selection and climate effects on the MHC-DQA and MHC-DQB genes in brown hares (*Lepus europaeus*) from Europe and the Middle East**

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Brown hares are widely distributed in a variety of different habitats supposedly representing heterogeneous pathogenic landscapes. Locally or regionally varying climate may impose differences in distribution and richness of pathogen niches and/or vectors. Hence, immunogenetic variability is expected to vary across larger regions due to local or regional adaptation following selective responses to varying pathogenic pressures. Here, we examined the spatial distribution of MHC-DQA and MHC-DQB genes in 251 individuals from Europe, the Anatolian Peninsula, and Israel, and tested for positive selection and effects of climatic parameters on the spatial distribution of protein variants encoded by those genes. Specifically, we sequenced the exon 2 of the MHC-DQA and MHC-DQB genes and obtained individual genotypes by phasing. We calculated genetic diversity indices and tested for selection signals using several approaches, also by accounting for possible recombination effects. We used multinomial regression models and model averaging to test for effects of locality-specific bioclimatic variables, downloaded from the WorldClim database, on the occurrence of the most frequent protein variants detected. We found a higher number of protein variants for MHC-DQB exon 2 (35) than for MHC-DQA exon 2 (27). Our recombination tests indicated a higher importance of recombinations than point mutations for the evolution of MHC-DQB exon 2 and the opposite for MHC-DQA exon 2. In addition to various significant selection signals, our multinomial regression models revealed significant climatic effects on the spatial distribution of the most frequent protein variants, independently from latitude and longitude. The model results also indicated that individuals homozygous or heterozygous for certain protein variants were favored under specific climatic conditions, particularly at lower precipitation, especially during the wet period of the year. Apparently, positive selection, recombination, and climatic adaptations – supposedly reflecting pathogenic landscape variation – have shaped the spatial distribution of variability at the studied MHC genes.

**P28****RHDV2 is likely to cross the species barrier in mammals: investigations of virus infection in wild species in France**

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Rabbit haemorrhagic disease (RHD) affects both wild and domestic European rabbits (*Oryctolagus cuniculus*). This lethal disease is caused by a lagovirus (*Caliciviridae*), the RHDV (genotype GI.1). A new genotype, GI.2, commonly called RHDV2, emerged in 2010 in European rabbits. It quickly spread in Europe and throughout the world and display a broad host range. Indeed, in addition to its initial host, RHDV2 infects several hare (*Lepus* spp.) and rabbit (*Sylvilagus* spp.) species in Europe and northern America. RHDV2 also may infect non-lagomorph species such as Alpine musk deer (*Moschus chrysogaster*) or Eurasian badger (*Meles meles*) showing its strong ability to cross species barriers, even between distant species such as mammals. We detected by RT-PCR a case of RHDV2 infection in the liver of a badger collected by the SAGIR network in 2021. However, the state of the carcass did not allow to perform histological analysis to confirm that RHDV2 infection was actually the cause of the death. To go further in the description of RHDV2 in badgers in France, and also to detect putative infections of other wild species by this virus, we will seek to detect RHDV2 infections 1) in animals analyzed by the SAGIR network and showing evocative lesions of RHDV2 with no other cause of mortality identified and 2) systematically in a sample of carcasses analyzed by SAGIR. To do so, we plan to take advantage of epidemiological studies involving badgers which would provide at least 200 analyzable individuals. Indeed, SAGIR network is specialized in the surveillance of diseases of birds and wild terrestrial mammals, and by giving access to many species and carcasses at a national scale it could expand the panel of studied species on RHDV2 transmission.

**P29****Sibling differences in early body mass of juvenile European rabbits modulate within-litter covariance of allele-specific MHC heterozygosity, coccidian endoparasite load and growth**

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Although littermates in altricial mammals usually experience highly similar environmental conditions during early life, considerable differences in growth and health can emerge among them. In a study on juveniles of a European rabbit (*Oryctolagus cuniculus*) population with low MHC polymorphism, we tested whether litter sibling differences in endoparasitic coccidia load and body mass at the end of the vegetation period were associated with differences in starting body mass and in immune-genetic (MHC class II DRB) constitution. We hypothesized that siblings with a relatively lighter starting mass might be more susceptible to endoparasite infections than their litter mates and thus, negative effects of a more unfavourable MHC constitution might be particularly pronounced in such individuals. Within-litter comparisons revealed that animals with a heavier starting mass and with a lower load of faecal hepatic coccidian oocysts reached a relatively heavier body mass in autumn, at an age of 5–8 months. Furthermore, there was evidence for an allele-specific heterozygote advantage, as animals with heterozygous combinations of the allele Orcu-DRB\*4 had relatively lower hepatic coccidia loads than their homozygous littermates. Consistent with our hypothesis, significantly higher hepatic coccidia loads and lower autumn body masses in homozygous compared to heterozygous individuals were only evident in initially lighter siblings, suggesting synergistic effects between an unfavourable MHC constitution and a low starting mass. This might lead to notable differences in fitness among litter siblings, as a low body mass and a high endoparasite burden are key factors limiting young rabbits' survival during winter.

**P30****Age-class specific trade-off between litter size and offspring body mass in the European rabbit**Lucille Dumontier<sup>1</sup>, Guénaél Cabanes<sup>2,1</sup>, Heiko G. Rödel<sup>1,3</sup>

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Young, first-season breeders frequently show a lower reproductive performance than older mothers. Our long-term study on a field enclosure population shows that this phenomenon is also apparent in the European rabbit (*Oryctolagus cuniculus*), for example with respect to offspring body mass, an important predictor of early offspring survival. Generally, offspring body mass was negatively correlated with litter size. However, one-year-old mothers showed significantly lower litter size-dependent offspring masses compared to older ones, possibly due to the lower body masses and typically lower social rank positions in females of the former maternal age class. Interestingly, litter sizes of one-year-old and older mothers did not differ notably within the different reproductive cycles along the breeding season. This indicates an age class-dependent shift in the trade-off between litter size and offspring mass towards a larger litter size in one-year-old females, thus reaching similar litter sizes than older females at the cost of their offspring masses. We explored the potential fitness consequences of this age class-dependent reproductive strategy by an individual-based model, using parameter estimates taken from published information on survival and reproduction of European rabbits. This model had been previously validated to adequately project the observed weather- and density-dependent population dynamics of the study population. We simulated the litter size-dependent and offspring body mass-dependent reproductive success of females, quantified as the number of offspring surviving to the onset of the next breeding season. Analyses of these simulations under different weather conditions indicated that in one-year-old females, an increased litter size resulted in a payoff in reproductive success, thus exceeding the associated decrease in reproductive success due to the lower body masses in offspring of such larger litters. These results might help explaining the evolution of the age class-dependent trade-off between litter size and offspring mass observed in the European rabbit.

**P31****Spatiotemporal monitoring of myxomatosis in European wild rabbit (*Oryctolagus cuniculus*) in Spanish Mediterranean ecosystems**

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An epidemiological surveillance program was carried out to determine the seroprevalence to myxoma virus (MYXV), spatiotemporal patterns and risk factors associated to MYXV circulation in wild rabbit (*Oryctolagus cuniculus*) in Spanish Mediterranean ecosystems. A total of 2,376 animals were sampled in 136 hunting estates from Andalusia (Southern Spain) during four study periods: 2009-2012 (P1), 2012-2015 (P2), 2015-2018 (P3) and 2018-2021 (P4). Antibodies against MYXV were found in 59.9% (1,424/2,376; 95%CI: 58.0-61.9) wild rabbits using a commercial indirect ELISA. At least one seropositive animal was detected in 131 (96.3%) out of the 136 game estates sampled. Five statistically significant spatiotemporal clusters of high seroprevalence were identified using a Bernoulli model; one in P2 and four in P3. The generalized linear mixed model analysis identified season (autumn), age (adult and juvenile), outbreaks of myxomatosis during the month previous to the sampling, mean annual temperature and seropositivity to rabbit haemorrhagic disease virus as risk factors potentially associated with MYXV exposure. The results indicate a high exposure, widespread but non-homogeneous distribution and endemic circulation of MYXV in wild rabbit populations in southern Spain during the last decade. The present study highlights the importance of long-term surveillance for a better understanding of the epidemiology of MYXV in wild lagomorphs.

**P32****Study of the effects of agricultural management on sperm quality parameters of wild rabbit and hares**

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The intensification of the agricultural system during the 20th century has caused an important homogenization of the landscape due to land consolidation and introduction of pesticides and chemical fertilizers to the environment. This fact has been related to the loss of biodiversity. Concerning pesticides, there are important shortcomings in the knowledge of secondary or sublethal effects on wildlife. In order to clarify if pesticides are responsible for wildlife reproductive impairment in agrosystems, male reproductive parameters of the European rabbit (*Oryctolagus cuniculus*) and the Iberian hare (*Lepus granatensis*) were studied between areas with different agricultural management. Specifically, testes of hunted rabbits and hares were collected from pesticide-treated (n=6 and 23, respectively) and pesticide-free (n=16 and 7, respectively) areas during the 2021/22 hunting season in the southwestern Spain (Extremadura region). Testes and epididymis, obtained on the day of capture, were weighted and conserved at 5°C until processed the following day (12h). Total cauda epididymal spermatozoa were counted, and the percentage of morpho-anomalies determined by eosin/nigrosine stain. A battery of sperm parameters was determined by flow cytometry. Motility parameters were determined, only in hares, by a computer assisted semen analysis. Preliminary results showed differences between hares from pesticide-free and pesticide-treated areas in the weight of the testes (17.22±2.11g vs 21.87±0.87g, respectively) and epididymis (1.06±0.10g vs 1.77±0.17g, respectively). However, although not significant, hares from pesticide-treated areas showed worst motility parameters than from pesticide-free areas, such a higher percentage of immotile spermatozoa (53.54±6.14% vs 37.16±10.41%, respectively) and lower percentage of spermatozoa displaying progressive motility (29.22±3.79% vs 36.04±6.96%, respectively). Differences were not detected in rabbits. The results suggest that management based on the use of pesticides may have a negative effect on the reproduction, and therefore be a key modulating factor in the population dynamics of species associated with agrosystems.



**P33****Effect of myxomatosis on reproductive parameters of wild adult male hares**

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In 2018, the first outbreak of myxomatosis, an infectious disease caused by myxoma virus, was described in Iberian hares (*Lepus granatensis*) in southern Spain. Since then, myxomatosis has threatened hare populations throughout the Iberian Peninsula. Infected animals show severe vascular changes, which are usually represented externally by blepharitis. In this context, this study analyses for the first time the effect of myxomatosis on sperm quality in Iberian hares. For that, a total of 42 adult male hares were sampled during the 2021/22 hunting season in the southwestern Spain (Extremadura region). Animals were grouped into two categories according to the presence or absence of symptoms compatible with myxomatosis (n=13 and 29, respectively). Testes and epididymis, were weighted and the sperm concentration and the percentage of morpho-anomalies determined. Sperm parameters were determined by flow cytometry such as viability, spontaneous acrosome reaction, plasma membrane disorganization and mitochondria membrane potential. Motility parameters were determined by a computer assisted semen analysis. Statistical analyses were performed using an unpaired t-test. Preliminary results showed differences between hares with and without myxomatosis symptoms in testicular weight ( $12.78 \pm 1.27$ g vs  $20.91 \pm 0.87$ g) as well as in the total number of spermatozoa ( $376 \times 106 \pm 156$  vs  $517 \times 106 \pm 119$ ), being significantly lower in the myxomatosis group. The epididymis weight ( $1.606 \pm 0.14$ g vs  $2.21 \pm 0.29$ g) and the percentage of spermatozoa showing morpho-anomalies ( $16.97 \pm 3.19\%$  vs  $39.25 \pm 9.05\%$ ) were significantly higher in the myxomatosis group. Hares with myxomatosis tended to show worst motility parameters, such as a higher percentage of immotile (presence vs absence;  $56.72 \pm 9.21\%$  vs  $49.59 \pm 5.37\%$ ) spermatozoa and a lower percentage of spermatozoa displaying progressive motility ( $20.71 \pm 5.45\%$  vs  $31.13 \pm 3.33\%$ ), although they were not statistically different from the group without myxomatosis. Regarding the sperm parameters determined by flow cytometry, statistically significant differences between the groups not were found.

**P34****Studying home range and spatial behaviour of European rabbit using GPS collars**

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The European rabbit, *Oryctolagus cuniculus*, a game species that was abundant in the past in France is decreasing since the 1950s. Despite this decline, a wildlife management conflict arose between hunters and farmers due to damages caused by the species to crops (cereals, vineyards, olive groves). The actual challenge is to combine a sustainable management of the species while limiting the risk of agricultural damages that it can cause. For this, the knowledge of the species behavior and of the size of its home range is essential. For a few years, the existence of light GPS collars suitable to the European rabbit allows to study these questions by obtaining accurate location data, without disturbance due to radio-tracking with an operator. We aimed to study the spatial behavior as well as the habitat use of wild rabbits in agricultural areas. During three years, we monitored 18 European rabbits equipped with GPS collars for several months in a rural area in the south of France. This allowed us to estimate their home range at night when wild rabbits are active. Here we present novel descriptive data about European rabbit spatial and territorial behavior. In particular, we compare home ranges of individuals between sexes and look at their evolution during successive months.

**P35****The legacy of recurrent introgression during the radiation of hares**

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Comparative studies on radiating species often reveal a complex history of divergence involving many instances of gene flow among extant species. However, few studies have evaluated the extent and long-term impact of introgression in the phylogenetic context of a radiation. Hares (*Lepus* spp.) represent a widespread mammalian radiation of 32 extant species adapted to contrasting habitats and undergoing recurrent admixture. To assess the importance of recurrent hybridization during the diversification of hares, we use whole-exome data from 15 hare species representing the global distribution of the genus. We inferred species relationships and divergence times with a coalescent framework. We detect extensive allele sharing among species, reflecting extensive incomplete lineage sorting and temporally layered events of hybridization. Our phylogenetic analyses reveal that recurrent introgression occurred at all stages along the *Lepus* radiation, including recent admixture among extant species but also ancient introgression among the early lineages of the genus. We show that one such ancient hybridization event has resulted in the sharing of adaptive alleles among northern hemisphere species that may be relevant for adaptation in highly seasonal environments, as the alleles belong to genes involved in circadian rhythm regulation, pigmentation, and thermoregulation. This work illustrates how the genetic legacy of ancestral hybridization may persist across a radiation, leaving a long-lasting signature of shared genetic variation that may contribute to adaptation.

**P36**

The number of B and T lymphocytes, including their subpopulations, in rabbits experimentally infected with GI.1a-RHDVa virus (*Lagovirus europaeus* / GI.1a)

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The immune response in rabbits infected with GI.1a-RHDVa virus is poorly understood (Vet Res Commun 46, 233–242, 2022. <https://doi.org/10.1007/s11259-021-09851-x>), although it is the immunology indicators that determine the susceptibility of mammals, including animals to viral infections, including *Lagovirus europaeus* / GI.1 infection, and possibly it also determines the more intense spread of the GI.1a-RHDVa virus in the natural environment as compared to the GI.1-RHDV virus. The study assessed the dynamics of B (CD19+) and T (CD5+) lymphocytes, including the subpopulation of T lymphocytes – T (CD4+), T (CD8+) and T (CD25+) in peripheral blood in experimentally intramuscularly infected rabbits with GI.1a-RHDVa virus - Dutch strain NL-2 with positive haemagglutination (HA +), were carried out on 20 Polish hybrid rabbits (10 infected and 10 control rabbits). Peripheral blood lymphocytes of rabbits were determined on flow cytometer against Biokom (Bio-Rad) rabbit monoclonal antibodies at 0, 4, 8, 12, 24, 36 and 48 hours as the mortality the infected animals at 24 and 36 hours were both 40% and at 48 hours 20%.

In the study was an increase in the number of B (CD19+) lymphocytes at 36 and 48 hours and T (CD5+) lymphocytes and their subpopulation with the CD4 + receptor at 8, 12, 24, 36, 48 hours, as well as an increase in the number of lymphocyte subpopulations were recorded T (CD25+) at 12, 24, 36 and 48 hours after infection. Research has also shown a decrease in the number of T lymphocytes (CD8+) subpopulations at 8, 12, 24, 36 and 48 hours after infection. Relating changes in the number of tested lymphocytes to the mortality of infected rabbits, it can be cautiously concluded that infection of rabbits with the tested virus, at the beginning of the infection, is associated with T lymphocytes, and later with both T and B lymphocytes.

**P37**

The occurrence of two heavy metals (lead and cadmium) in the liver of brown hare (*Lepus europaeus*) from the three location in the region with the intensive agriculture production in Vojvodina

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Heavy metals such as lead (Pb) and cadmium (Cd) are possible contaminants of water, soil and air, as the results of the human activities in agriculture, or industry and they can be found in the liver or kidney of animals as indicators of pollution.

Vojvodina, the north part of Republic of Serbia is a region with a very intensive agricultural production, especially crop production. The brown hare is well adapted and numerous in Vojvodina, Serbia, and as an expressive herbivore, presents a good choice as a biomonitor for the occurrence of lead and cadmium in the chosen biotope.

In total, 60 samples of hare liver taken immediately after the harvesting were analyzed. The samples were collected during hunting season, in three regions in Vojvodina province (Srem, Banat and Bačka). One location in each region was chosen and from each location 20 samples of hare's liver were collected. The average concentration of lead in all samples of liver was 851.43 µg/kg of wet tissue. There was no significant difference in the concentration of lead in samples from the three locations. Maximum amount of lead found in the samples was 2302 µg/kg of wet tissue. The concentration of cadmium in all samples was in the range 12.90-1183.00 µg/kg of wet tissue. There was a statistically significant difference between the average concentrations of cadmium depending on the location. The average concentrations of cadmium in the location I, II and III were 65.57, 205.91 and 413.99 µg/kg of wet tissue.

**P38**

The pattern of maternal behavior in the New World Mexican cottontail rabbit *Silvilagus cunicularius* in comparison with the Old World European rabbit *Oryctolagus cuniculus*

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Maternal behavior has been well studied in the European rabbit *Oryctolagus cuniculus*, both in nature and in this species' domesticated form in the laboratory. Studies consistently report a pattern of unusually limited maternal care. Shortly before giving birth, mothers dig a separate nursery burrow or chamber which they line with dry grass and fur pulled from their flanks and ventrum. Immediately after giving birth, they leave the altricial young, close the entrance to the burrow, and only return once approximately every 24 hours to nurse the pups for a few brief minutes. The pups start to leave the nursery burrow when around 15 to 20 days old, and if the mother is pregnant with a further litter, are abruptly weaned when about 25 days old before the birth of the next litter. Relatively little is known about maternal behavior in other rabbit species. We therefore asked whether mothers of the endemic Mexican cottontail rabbit *Silvilagus cunicularius*, would show a pattern of maternal care similar to that of the European rabbit. Observations over several years of wild caught females and their captive-bred daughters kept in small breeding groups in enclosures within the natural habitat of the animals at the field station Estación Científica La Malinche in the Malintzi National Park, central Mexico, showed close parallels with the European rabbit; in construction and lining of the nursery burrow, in the pattern of brief, once-daily nursing, and in the abrupt weaning of the young. We therefore conclude that the unusual pattern of “absentee” mothering and associated behaviors such as burrow and nest building characteristic of the European rabbit, represent a highly conserved pattern of maternal care apparently retained over millennia.

**P39****The rabbit as an animal model for human disease: is it better than mice?**

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The European rabbit (*Oryctolagus cuniculus*) was the first animal model used to understand human diseases like rabies and syphilis. Nowadays, the rabbit is still used to study several human infectious diseases including syphilis, HIV and papillomavirus, as well as non-infectious conditions as intestinal immunity, lupus and cancer. However, due to several mainly practical reasons, it has been replaced as an animal model by mice (*Mus musculus*). The rabbit and mouse share a recent common ancestor and are classified in the superorder Glires which arose at approximately 82 million years ago (mya). These species diverged from the Primates' ancestor at around 92 million years ago and, as such, one expects the rabbit-human and mouse-human genetic distances to be very similar. To evaluate this hypothesis, we aligned and calculated the genetic distances for 12000 genes from human, rabbit and mouse using the Ensembl database. The results we obtained show that the rabbit-human genetic distance is lower than the mouse-human genetic distance for 90% of these genes. These results can be explained by the increase of mutation rates in the mouse lineage suggested by some authors and clearly show that, at the nucleotide and amino acid similarity, the European rabbit is a better model to study human diseases than the mouse.

**P40****Government response to RHDV2 at the wildlife-domestic animal interface in the United States**Elizabeth Pienaar, Hannah Shapiro*University of Georgia, USA*Email contact: [Elizabeth.Pienaar@uga.edu](mailto:Elizabeth.Pienaar@uga.edu)

The management of diseases at the wildlife-domestic animal interface requires collaboration between government agencies and diverse stakeholder groups. In the United States, one of the most recent disease threats to emerge at the wildlife-domestic animal interface is rabbit hemorrhagic disease virus 2 (RHDV2). RHDV2 is a highly contagious virus that infects wild and domestic rabbits and hares (lagomorphs). Since its emergence in France in 2010, RHDV2 has spread to multiple continents due to human-mediated movements of lagomorphs. This virus has resulted in population declines of wild lagomorphs, with associated biodiversity and hunting impacts, as well as economic losses for commercial rabbit industries. In March 2020, RHDV2 was confirmed in wild and domestic lagomorphs in New Mexico and has since been confirmed in 18 states and Mexico. To understand how government agencies have responded to RHDV2 emergence in the US, we conducted surveys of animal health personnel at 95 state wildlife and agricultural agencies, thereby accounting for all 50 states. We obtained further detailed information by conducting follow-up in-depth interviews with agency personnel. Agencies have primarily responded to RHDV2 through disease investigations of potential RHDV2 cases, vaccinations, and education and outreach with the public and stakeholder groups. However, agencies' inconsistent jurisdiction over lagomorph populations and industries and limited knowledge of human-mediated movement of rabbits have hindered management efforts. Agencies should engage in collaborative, proactive interagency disease management and improved outreach that targets diverse stakeholder groups to control the rapid spread of RHDV2 to new areas.



**P41****Falconer support for interventions to mitigate the spread of rabbit hemorrhagic disease virus 2 (RHDV2) across the United States**Elizabeth Pienaar, Hannah Shapiro*University of Georgia, USA*Email contact: [Elizabeth.Pienaar@uga.edu](mailto:Elizabeth.Pienaar@uga.edu)

Understanding stakeholders' perceptions of wildlife diseases is essential, as the success of management strategies to mitigate pathogen transmission often depend on stakeholder support and engagement. In the United States (U.S.), wildlife agencies rely on stakeholder groups to report RHDV2-related mortalities and engage in voluntary biosecurity measures designed to reduce the risk of RHDV2 spread. We conducted research in the U.S. on how falconers' risk perceptions, knowledge of RHDV2, trust in government, hunting behaviors, and demographic characteristics influenced their willingness to engage in voluntary biosecurity measures and support for agency-led management actions. From April 2021 – March 2022, we surveyed 457 falconers across the U.S. Falconers are an important stakeholder group in the management of lagomorph diseases, as they hunt more frequently than other small game hunters. We found that falconers were willing to engage in all the biosecurity measures, including reporting suspicious rabbit deaths (89.1%), removing inedible parts of rabbit carcasses from the field (81.0%), and sanitizing hunting/falconry equipment (79.4%). Respondents were less supportive of hypothetical agency-led management actions, including restricting rabbit hunting in areas with endangered lagomorphs (59.3%) and banning the transport of rabbits from areas with RHDV2 (55.6%) or until the U.S. starts producing a RHDV2 vaccine (46.6%). Falconers' willingness to engage in these behaviors and support for regulatory measures were positively correlated with the importance they placed on biosecurity, risk perceptions, and trust in government. Outreach efforts should focus on communicating the risks RHDV2 poses to hunting and falconry and how engaging in specific biosecurity behaviors can lower the risk of RHDV2 spread. Additionally, wildlife agencies should continue to build trust with falconry groups, as falconers can provide important information on changes in lagomorph populations and disease outbreaks.

**P42****A new HaCV-EBHSV recombinant is circulating in European brown hares from Spain**

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Lagoviruses are highly lethal hepatic RNA viruses that cause disease outbreaks in populations of European rabbit (*Oryctolagus cuniculus*) and European brown hare (*Lepus europaeus*). While the former is mainly affected by rabbit hemorrhagic disease virus (RHDV; *Lagovirus europaeus*/GI), outbreaks in the latter are usually associated with European brown hare syndrome virus (EBHSV; *Lagovirus europaeus*/GII). EBHSV causes European brown hare syndrome (EBHS), first reported in Sweden in 1980 and later in several European countries. Non-pathogenic viruses also circulate in European brown hare populations, named hare caliciviruses (HaCV), whose role in EBHS epidemiology is yet unknown. A very common phenomenon in lagoviruses is the occurrence of recombination, with several events described at the boundary between the polymerase and the capsid protein, as well as at the p16/p23 boundary. Interestingly, these events were always reported in GI viruses and never in the GII genogroup. Following an EBHS outbreak in Catalonia in 2020/2021, complete EBHSV coding sequences were recovered from the livers of death European brown hares. A genome-walking approach was employed using primers designed in conserved regions of the EBHSV genome. Visual inspection of eight of the obtained sequences detected an incongruence that was further confirmed by RDP and phylogenetic analysis. A Blast analysis revealed 84% identity with a French HaCV strain in the fragment ~1-1900 and 98% identity with a German EBHSV strain for the rest of the genome. Contrarily to the majority of the recombination breakpoints detected in lagoviruses, this breakpoint is located within a protein rather than at the boundary between proteins. This is the first report of a recombination event in GII lagoviruses. The new recombinant is associated with mortalities in European brown hares and circulates alongside with the non-recombinant EBHSV. The impact in virulence and transmission of this non-pathogenic insertion still needs to be assessed.

**P43****What's in a nest? Contribution of mother's feces and of nest hay to pup growth and survival in the European rabbit *Oryctolagus cuniculus***

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The European rabbit *Oryctolagus cuniculus*, like various mammals, shows coprophagia (ingestion of feces). Benefits include the assimilation of nutrients present in the feces and synthesis of hormones and neurotransmitters by gut microbiota. In nature and in the lab, after giving birth in a grass-lined nest, rabbit mothers leave the young, returning once a day to briefly nurse. We previously observed that mothers also deposit fecal pellets in the nest. In a systematic study of this, we recorded the pre-weaning behavior of mothers and young of 14 litters. We found that mothers start to deposit fecal pellets in the nest 3–5 days before parturition and cease between postnatal days (PD) 11–13. From PD 13–14, pups begin to nibble the pellets and nest hay, which largely disappear by the time the pups leave the nest around PD 20 and shortly before weaning around PD 25. The pattern of pellet deposition was similar in primiparous and multiparous females (n = 7/group), suggesting this to be largely independent of maternal experience. The presence of plant fiber in the fecal pellets of pups with access to the mother's fecal pellets and nest hay but not in the pellets of pups denied such access, confirms the ingestion by pups of such nest contents before weaning. We are presently examining the possible short- and long-term effects of the presence or absence of fecal pellets and hay in the nest on the transition to solid food and on the survival and physical development of the young. Grant from CONACyT 782924 to RBM.

**P44****Elucidating European rabbit gut microbiome composition to infer individual susceptibility to rabbit haemorrhagic disease in wild populations**

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*Lagovirus europaeus*/GI.2 (henceforth GI.2) is the causative agent of rabbit haemorrhagic disease (RHD), a lethal and emerging infectious disease in several species of lagomorphs. Applied methodologies to explore the underlying susceptibility mechanisms of host species to fatal infection can provide insights on diseases dynamics, and lead to the development of more effective and ecologically sound treatments to minimize lethality. Host susceptibility to a variety of diseases have explicit or purported links to their gut microbiome. Here, we studied the variation in gut microbiota (hard faecal pellets) in a wild population of European rabbits before, during and after the RHD outbreak. From each individual, we collected samples of liver, blood and fresh faecal pellets to determine the presence of antigen, antibodies and gut microbiota composition, respectively. For antigen detection, we carried out RNA extraction from liver samples, cDNA synthesis and RT-qPCR amplification of short conserved region of GI.2. Antibodies against GI.1 and GI.2 were detected using an indirect ELISA. From faecal pellets, we carried out DNA extraction, PCR amplification of the 16S rRNA bacterial gene, and Illumina MiSeq high-speed multiplexing sequencing. Raw genetic sequences were processed computationally and were taxonomically identified against a reference bacterial genetic database. Correlational analysis for the relative abundance of bacterial taxa and hypothesis contrast were carried out to unveil significant differences in the gut microbiome composition among the different serological and antigen profiles. A specific taxonomic indicator index based on the relative abundance of selected species of Bacteroidales vs. Firmicutes revealed significant differences between dead and resistant individuals. This result strongly suggests that a relationship exists between the individual gut microbiome and RHD susceptibility and expands the frontiers of lagomorph epidemiological research that can potentially lead to new biotherapeutic treatments or population biocontrol management. Yet, the complete nature of this relationship remains to be determined in further studies.

**P45****Increasing diversity of circulating lagoviruses in Australia and New Zealand – implications for rabbit management**Robyn Hall <sup>1,2</sup>, Janine Duckworth <sup>3</sup>, Tanja Strive <sup>1,2</sup><sup>1</sup> CSIRO Health & Biosecurity, Acton, ACT 2601 Australia ; <sup>2</sup> Centre for Invasive Species Solutions, Bruce, ACT 2617 Australia ; <sup>3</sup> Manaaki Whenua – Landcare Research, Lincoln 7608, New ZealandEmail contact: [Tanja.Strive@csiro.au](mailto:Tanja.Strive@csiro.au)

Recent years have seen a drastic increase in lagovirus diversity in Australia: RHDV1 (strain v-351) and the antigenic variant RHDVa-K5 were deliberately released nationwide as biocontrols in 1996 and 2017, respectively. In addition, two exotic incursions, RHDV2 (in 2015) and RHDVa-Aus (in 2014) were detected, neither of which were deliberately released. Following its arrival in Australia, RHDV2 spread across the continent within 18 months, leading to an average reduction of wild rabbit populations by 60% across the continent. In New Zealand, RHDV1 v-351 and RHDVa-K5 were also released, in 1997 and 2018, with the difference that the first release was not endorsed by the New Zealand government. In 2017, RHDV2 also arrived in New Zealand. Interestingly and unexpectedly, the NZ RHDV2 was identified as a GI.3P-GI.2, distinct from the Australian GI.1bP-GI.2, suggesting Europe as the most likely origin of this incursion. Adding to this increasing diversity, novel non-pathogenic lagoviruses have recently been identified in New Zealand (rabbits) and Australia (rabbits and hares). Additionally, virulent recombinants between RHDV2 and other, mostly non-pathogenic, lagoviruses have arisen at least six times independently and locally replaced previous RHDV2s, although the mechanisms of their epidemiological fitness advantage are not yet understood. The genetic diversity evident among these viruses suggests that the Lagoviridae family remains undersampled, possibly with a substantial number of non-pathogenic viruses yet to be discovered. Exploratory metatranscriptomics approaches are a powerful tool to help close these knowledge gaps and better understand the true diversity and evolution of this important virus family. As virulent lagoviruses are used for biological control to manage invasive rabbits in both Australia and New Zealand, it is of particular importance to continue systematic monitoring programs to better understand the spread, distribution and possible interactions of these viruses, and any effects this may have on current and future rabbit management.

**P46****How 20 years of RHDV1 and myxoma virus followed by 3 years of RHDV2 have impacted the Turretfield rabbit population**

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The long-term Turretfield (South Australia) rabbit epidemiological monitoring project started in October 1996 with release of RHDV1 (GI.1) on the site. This small and largely isolated population of rabbits has been regularly monitored ever since. Utilising the *Known To Be Alive* technique, rabbit numbers have fluctuated markedly, driven by seasonal conditions and the limiting impacts of RHDV1 and the myxoma virus. From 1996 to 2005 RHD outbreaks occurred in spring on a largely biannual cycle. However, from spring 2006 to 2015, outbreaks became an annual event associated with an apparent cyclical recovery in the population, until the arrival of RHDV2 (GI.2) in autumn (May) of 2016. This new genotype was first detected at Turretfield in only three dead tagged rabbits, all previously recorded with RHDV1 antibodies, suggesting RHDV2 had only a minimal initial impact. The next RHDV2 outbreak was detected in winter 2017, during which 17 carcasses were located and all but one tested positive for RHDV2. Many of these rabbits had RHDV1 antibodies. In the 12–18 months following first detection of RHDV2 the Turretfield rabbit population had an 80% decline in numbers. The earlier timing of the RHDV2 outbreaks has in some years been in concert with significant impacts by the myxoma virus killing cohorts of juveniles and some adults. An assessment of mortality from myxomatosis during the period of RHDV2 from 2016 to 2018 shows that myxomatosis had an increased negative impact on rabbit survival. Myxomatosis was active throughout the year before RHDV2 reached the site in 2016, but became concentrated in spring in the following two years. As a result, the age at which most rabbits were infected by myxomatosis and their survival rates were greatly reduced. Since the arrival of RHDV2 the Turretfield population has fluctuated at a much lower level.

**P47****Number of daily load cycles positively influences Haversian remodeling in rabbit jaws**

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Haversian bone remodeling occurs, in part, as a response to microdamage accumulation in more mature bone, serving to repair and maintain the skeleton's mechanical integrity. The link between secondary osteon densities (i.e., products of remodeling) and behaviors that engender loads within primate limb bones and jaws have suggested that the number of daily load cycles, rather than peak load magnitude, affects the degree to which bone is remodeled. Although the bony response to higher bite forces in the mammalian skull is well known, osteogenesis due to protracted chewing (=cyclical loading) is poorly understood. This is surprising as cyclical loading is oft-invoked to explain robusticity of masticatory structures in living and fossil mammals. Here, we test this hypothesis using 30 male white rabbits (*Oryctolagus cuniculus*) raised for 48 weeks from weaning (4 weeks old) on one of two diets. All rabbits were fed rabbit pellets, but half also ate hay daily (n=15 control & n=15 over-use subjects). Hay is tougher and stiffer (E=3336MPa, R=2760Jm<sup>-2</sup>) than pellets (E=29MPa, R=1031Jm<sup>-2</sup>), requiring greater chewing investment and correspondingly increased chewing duration to process. It does not, however, substantially alter peak mandibular bone strain versus pellet-only mastication. Thus, rabbits raised on hay experienced more daily jaw-loading events (i.e., elevated cyclical loading), with no difference in load magnitude between experimental diets. To compare mandibular remodeling between treatments, osteon population density (OPD) and percent Haversian bone (%HAV) were measured from 100µm coronal sections of the right mandibular corpus. Mann-Whitney U-tests revealed significant differences in OPD (P=0.14) and %HAV (P=0.003), with higher OPD and %HAV in the over-use cohort. This supports the hypothesis that remodeling activity is largely determined by the degree of cyclical loading, not load magnitude. This finding should be heeded by researchers aiming to infer load history from skeletal elements in comparative, bioarchaeological and paleontological contexts.

**P48****Picking leporid noses: turbinal morphology in *Nesolagus timminsi* and *Pentalagus furnessi***Irina Ruf

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The turbinal skeleton, that supports the respiratory and olfactory epithelium of the mammalian nose, provides phylogenetic as well as morphofunctional information. Systematic relevant turbinal characters have already been described for Lagomorpha although not covering all living genera. For the first time, the turbinal skeleton of the two enigmatic extant leporids *Nesolagus timminsi* and *Pentalagus furnessi* is described based on  $\mu$ CT scans and virtual 3D reconstructions. In general, the turbinal skeleton of *Nesolagus* (N=1) and *Pentalagus* (N=2) resembles the pattern and characters observed in other Leporidae. The maxilloturbinal is highly dendritic, the nasoturbinal forms a straight lamella connected to the crista semicircularis, and the frontoturbinal recess houses two frontoturbinals and one interturbinal between them. Both species have three ethmoturbinals and one interturbinal between ethmoturbinal I and II. All these turbinals are scrolled and in most parts double-scrolled in cross-section. In this regard *Nesolagus* and *Pentalagus* resemble the pattern observed in many leporid genera (*Pronolagus*, *Oryctolagus*, *Sylvilagus*, *Lepus*). The overall proportions of the turbinal skeleton of *Pentalagus* compares to that of its sister-taxon *Caprolagus hispidus*. However, the latter has four ethmoturbinals. The interturbinal of the frontoturbinal recess in *Pentalagus* is significantly small and simple shaped. This can be regarded as an apomorphic character. Both species show an additional small and short interturbinal in the frontoturbinal recess but with varying patterns. On the left side *Nesolagus* has another small interturbinal between ethmoturbinal I and the interturbinal of the ethmoturbinal recess. Intraspecific and interspecific varying additional interturbinals have been already described in *Oryctolagus* and some *Sylvilagus* and *Lepus* species. These new observations contribute to our knowledge of turbinal skeleton morphology in Lagomorpha adding so far undescribed species.



**P49****Habitat connectivity of *Lepus europaeus* in the Canton of Geneva, Switzerland**

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Given the decline of hare populations in Europe since the 1960s, mainly due to intensification of agricultural practices, few studies have addressed habitat selection of hares, and even fewer have addressed their movement needs (i.e., dispersal and mate finding). However, knowledge of functional connectivity is essential to inform landscape planning aimed at reversing this negative trend. We studied five radio-collared European hares for six months in an agricultural area in the Geneva countryside to investigate habitat selection and identify barriers to movements of this species. In this regard, two methods were studied, one using eigenanalysis of selection ratios analysis, and the other based on a resource selection function. Our results showed that Biodiversity Promotion Surfaces, as well as the proximity to forest, are preferred by hares during activity periods. Urban areas and roads with high traffic were identified as barriers to hare movement. Based on this information, a habitat suitability map was created. Suitability values were then converted to resistance values using a negative exponential function. In this way, two connectivity maps at the scale of the study area were modeled using Circuitscape software and then extrapolated to the entire Canton of Geneva. The results of the modeling allowed us to identify the potential European hare flows crossing the canton. This can serve as a basis for decision makers to better plan for the conservation of the European hare, as well as other agricultural species.