

# Proceedings



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**5<sup>th</sup> World Lagomorph Conference**

California State University Stanislaus

Turlock, California July 11-15, 2016



# 5<sup>th</sup> World Lagomorph Conference

## Proceedings

Hosted by



CALIFORNIA STATE UNIVERSITY  
**Stanislaus**

Turlock, California  
July 11-15, 2016

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July 2016

## WELCOME

Dear Colleagues,

We welcome you to the 5<sup>th</sup> World Lagomorph Conference, a conference sponsored by the World Lagomorph Society and the IUCN Species Survival Commission Lagomorph Specialist Group that is held every four years for researchers on wild hares, rabbits, and pikas.

The 5<sup>th</sup> World Lagomorph Conference is hosted by California State University, Stanislaus, where the College of Science and the Endangered Species Recovery Program took the lead on its organization. This is the fifth time that lagomorph scientists from around the world have come together to exchange ideas and discuss current and future trends in numerous fields of lagomorph biology, conservation, and management. This is the first time the conference is being held in the United States of America. Previous conferences were held in Canada (Ontario, 1985), Portugal (Vairão, 2004), Mexico (Morelia, 2008), and Austria (Vienna, 2012).

We are happy to welcome colleagues from 23 countries to Turlock. We have general sessions on “Lagomorphs and Climate Change,” “Ecology, Behavior, Management, and Conservation,” “Evolution,” “Morphology and Physiology,” and “Diseases.” We are hosting two formal workshops, “Lagomorph Systematics and LaGomiCs” and “Population Trends and Management of Lagomorphs in Western North America.” However, informal workshops, meetings, and roundtables can also be easily accommodated. The coffee break, lunch, and poster display areas are conveniently located, and posters will be displayed for most of the conference. So, the 5<sup>th</sup> World Lagomorph Conference offers not only opportunities for an update on current research on lagomorphs, but also many opportunities to network and interact with other researchers. Those opportunities include but are not limited to the social program: Icebreaker Party at our Faculty Development Center (Monday evening), Lagomorph Art display by our Biological Illustration class at the Stanislaus State Art Space on Main in Downtown Turlock (Tuesday evening), the Western BBQ at the Faculty Development Center (Thursday evening), as well as an optional post-conference excursion to Yosemite Valley (all day Friday).

Along with our team here on campus, it is a pleasure to host you in Turlock. We hope you enjoy your stay and that you have a wonderful conference.

Patrick Kelly, California State University Stanislaus  
Andrew Smith, Arizona State University

Organizers of the 5<sup>th</sup> World Lagomorph Conference

Dear Participants in the 5<sup>th</sup> World Lagomorph Conference,

The World Lagomorph Society (WLS) was established in 2006, after the 2<sup>nd</sup> World Lagomorph Conference (WLC) was held in Portugal (2004). This society was inspired by the need to improve cooperation and collaboration among lagomorph researchers, to spread existing information on lagomorphs, and to promote greater collaboration.

Thus, some of the main aims of the WLS are to act as a channel for communication between people interested in lagomorphs, to support the organization of scientific and technical meetings, to spread published information and to publish specific bulletins. The WLS has helped with the organization of previous WLCs and has supported the participation of some lagomorph researchers through travel grants to attend WLCs, including this one. We believe that the WLS can significantly increase lagomorph research and improve our knowledge on several species that remain poorly understood. We also can contribute to the conservation of some endangered lagomorph species and improve the tools for managing those species that are considered game or problematic species.

The success of the society depends on your participation. Therefore, we ask that you join the WLS and make our dream real.

Paulo C. Alves  
President of the World Lagomorph Society

Dear Lagomorph Researchers,

The IUCN Species Survival Commission Lagomorph Specialist Group is pleased to help sponsor the 5<sup>th</sup> World Lagomorph Conference. Lagomorphs constitute a unique lineage of mammals; they contribute greatly to the functioning of ecosystems and when harvested sustainably improve the welfare of people. Unfortunately, a very high percentage of lagomorph species are also among the rarest of all mammals and are threatened with extinction. It is thus important for researchers, conservationists and managers who work on lagomorphs to meet and share their experiences and thoughts. I have had the privilege to attend all four prior World Lagomorph Conferences, and applaud the hard work of the local organizing committee for ensuring that the tradition of lagomorph biology continues.

With Best Wishes,

Andrew Smith  
Chair, IUCN/SSC Lagomorph Specialist Group

## Contents

Venues .....	7
Schedule at a glance .....	8
Program .....	9
Plenary and Special Presentations .....	15
Oral Presentations.....	19
Poster Presentations .....	68

## VENUES

Please be aware of different venues. All social events will not be held on campus and venues are subject to change. The numbers in parentheses refer to the campus map below.

- On Campus Housing: Village III (38A)
- Free Parking: Parking Lot 7
- Sessions and Workshops: Nora and Hashem Naraghi Hall of Science (35) & Science 1 (9)
- Icebreaker & Lunches: John Stuart Rogers Faculty Development Center (30)
- Breakfasts: Warrior Grill (12)
- Student Art Exhibit: Art Space on Main, Downtown Turlock (shuttle provided)
- Western BBQ: John Stuart Rogers Faculty Development Center (30)



<https://www.csustan.edu/campus-maps>



## SCHEDULE AT A GLANCE

<b>Monday, July 11</b>		
19:00 – 21:00	Icebreaker Party	John Stuart Rogers Faculty Development Center, Room 118
<b>Tuesday, July 12</b>		
8:00 – 8:30	Opening and Welcome Address	Naraghi Hall of Science, Room 101
8:30 – 9:30	Plenary Lecture	Naraghi Hall of Science, Room 101
	<i>Coffee Break &amp; Posters</i>	
10:00 – 11:45	Session A: Lagomorphs and Climate Change	Naraghi Hall of Science, Room 101
	<i>Lunch Break</i>	
13:30 – 15:00	Session B: Ecology, Behavior, Management, and Conservation I	Naraghi Hall of Science, Room 101
	<i>Coffee Break &amp; Posters</i>	
15:30 – 17:30	Session C: Ecology, Behavior, Management, and Conservation II	Naraghi Hall of Science, Room 101
19:00 – 21:00	Lagomorph Art by Stanislaus State Biology students	Stanislaus State Art Space on Main, Downtown Turlock
<b>Wednesday, July 13</b>		
8:15 – 8:30	Announcements	Naraghi Hall of Science, Room 101
8:30 – 9:30	Plenary Lecture	Naraghi Hall of Science, Room 101
	<i>Coffee Break &amp; Posters</i>	
10:00 – 12:00	Session D: Evolution I	Naraghi Hall of Science, Room 101
	<i>Lunch Break</i>	
13:30 – 15:00	Session E: Ecology, Behavior, Management, and Conservation III	Naraghi Hall of Science, Room 101
	<i>Coffee Break &amp; Posters</i>	
15:30 – 17:30	Workshop 1. Lagomorph Systematics and "LaGomiCs"	Naraghi Hall of Science, Room 101
15:30 – 17:30	Workshop 2 - Population Trends and Management of Lagomorphs in Western North America	Science 1, Room 135
17:30 – 18:30	General Assembly of the World Lagomorph Society (WLS)	Naraghi Hall of Science, Room 101
<b>Thursday, July 14</b>		
8:30 – 9:30	<b>Session F:</b> Morphology and Physiology	Naraghi Hall of Science, Room 101
9:30 – 11:00	Poster session with authors	Naraghi Hall of Science, 2 <sup>nd</sup> floor
11:00 – 12:00	<b>Session G:</b> Ecology, Behavior, Management, and Conservation IV	Naraghi Hall of Science, Room 101
	<i>Lunch Break</i>	
13:30 – 14:45	<b>Session H:</b> Diseases	Naraghi Hall of Science, Room 101
	<i>Coffee Break &amp; Posters</i>	
15:15 – 16:00	Special Presentation.	Naraghi Hall of Science, Room 101
16:00 – 17:00	Meeting of the IUCN/SSC Lagomorph Specialist Group	Naraghi Hall of Science, Room 101
18:00	Western BBQ, Group Photo, and Closing Ceremony	John Stuart Rogers Faculty Development Center, Room 118
<b>Friday, July 15</b>		
8:00 – 18:00	Post-conference excursion to Yosemite National Park	

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## PROGRAM

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### MONDAY, JULY 11

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#### John Stuart Rogers Faculty Development Center, Room 118

13:00                      *Registration Desk Open*  
 19:00    21:00        Icebreaker Party

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### TUESDAY, JULY 12

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#### Nora and Hashem Naraghi Hall of Science, Room 101

7:00                      *Registration Desk Open*  
 8:00    8:30            Opening with Welcome Address of WLC organizers; Dean, Provost, and University President; WLS President and LSG Chairman.  
  
***Plenary Lecture***  
 Chair: Patrick KELLY  
 8:30    9:30            Constance MILLAR: The American pika (*Ochotona princeps*): How does a small lagomorph encounter climate?  


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 9:30    10:00           *Coffee Break & Posters*

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#### ***Session A: Lagomorphs and Climate Change<sup>1</sup>***

*Chair: Klaus HACKLÄNDER*

10:00    10:15    A1    Alexander KUMAR: The cascading effects of circannual phenologies across trophic levels—how western larch affect the coat color molt in snowshoe hares  
 10:15    10:30    A2    Nishma DAHAL: Effects of climatic fluctuations on the evolution of Himalayan pikas  
 10:30    10:45    A3    Simen PEDERSEN: Climate change induced molting mismatch? Reduction in annual snow cover causes increased winter generalist predation in mountain hares  
 10:45    11:00    A4    Marketa ZIMOVA: High fitness costs of climate change-induced camouflage mismatch  
 11:00    11:15    A5    Neil REID: Macroecology of the Order Lagomorpha; implications for the impact of global climate change  
 11:15    11:30    A6    Scott MILLS: Lagging morphs of lagomorphs in winter  
 11:30    11:45    A7    Erik BEEVER: Does relative humidity or its derivatives predict a lagomorph species' occupancy and density better than temperature, habitat area, and vegetation across an ecoregion?  


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 12:00    13:30           *Lunch Break*

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<sup>1</sup> The sequence of presentations was modified from the original program as follows (first to last): Reid (A5), Mills (A6), Zimova (A4), Kumar (A1), Pedersen (A3), Dahal (A2), and Beaver (A7).

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**TUESDAY, JULY 12 (CONTINUED)**


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**Session B: Ecology, Behavior, Management, and Conservation I**

Chair: Andrew SMITH

- |       |       |    |   |
|-------|-------|----|---|
| 13:30 | 13:45 | B1 | Andrew SMITH: Behavioral ecology of American pikas ( <i>Ochotona princeps</i> ) at Mono Craters, California: Living on the edge                 |
| 13:45 | 14:00 | B2 | Katherine SOLARI: Hypoxia-induced plasticity in gene regulation: An experimental study of the Asian pika, <i>O. dauurica</i>                    |
| 14:00 | 14:15 | B3 | Weidong LI: Better understanding the Ili pika ( <i>Ochotona iliensis</i> ) through infrared technology  |
| 14:15 | 14:30 | B4 | Lisa SHIPLEY: Risky rabbits: Evaluating tradeoffs in food and predation risks by pygmy rabbits and mountain cottontails in the sagebrush-steppe |
| 14:30 | 14:45 | B5 | Charlotte MILLING: Seasonal behavioral thermoregulation of an arid land leporid   |
| 14:45 | 15:00 | B6 | Janet RACHLOW: Burrow use by pygmy rabbits: Dynamic patterns and ecological effects   |

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 15:00 15:30 *Coffee Break & Posters*


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**Session C: Ecology, Behavior, Management, and Conservation II**

Chair: Janet RACHLOW

- |       |       |    |   |
|-------|-------|----|---|
| 15:30 | 15:45 | C1 | Thomas MCGREEVY: Comparative population genomics of New England cottontail ( <i>Sylvilagus transitionalis</i> ) and eastern cottontail ( <i>S. floridanus</i> ) in the northeastern US                              |
| 15:45 | 16:00 | C2 | Jennifer RIPPERT: Genetic diversity and functional connectivity of the riparian brush rabbit: Implications for conservation of an endangered lagomorph  |
| 16:00 | 16:15 | C3 | Erik GANTENBEIN: Managing an endangered brush rabbit in the context of extreme weather events   |
| 16:15 | 16:30 | C4 | Mariana SOLORIO-DAMIÁN: Effect of direct signals from predators in activity patterns and physiological stress in the zacatuche rabbit ( <i>Romerolagus diazi</i> ) at the Chichinautzin Biological Corridor, Mexico |
| 16:30 | 16:45 | C5 | Oscar GONZÁLEZ: Bunchgrass ( <i>Muhlenbergia macorura</i> ) relevance in the ecology and conservation of the volcano rabbit ( <i>Romerolagus diazi</i> )  |
| 16:45 | 17:00 | C6 | David BROWN: History, status and population trends of cottontails and jackrabbits in the western United States  |
| 17:00 | 17:15 | C7 | Myles TRAPHAGEN: Loss of connectivity between United States and Mexico populations of the white-sided jackrabbit ( <i>Lepus callotis</i> )  |
| 17:15 | 17:30 | C8 | Matthew SIMES: Preliminary investigations of the spatial distribution of black-tailed jackrabbit ( <i>Lepus californicus</i> ) densities in the Mojave Desert ecoregion   |

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**Stanislaus State Art Space on Main, Downtown Turlock**

- |       |       |  |  |
|-------|-------|--|--|
| 19:00 | 21:00 |  | Lagomorph Art by Stan State Biology students. Appetizers and drinks will be served. This is also an opportunity to visit downtown restaurants. |
|-------|-------|--|--|

**WEDNESDAY, JULY 13**

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**Nora and Hashem Naraghi Hall of Science, Room 101**8:00 *Registration Desk Open*

8:15 Announcements

**Plenary Lecture***Chair: Andrew SMITH*8:30 9:30 José MELO-FERREIRA: Recent advances in understanding the evolution of lagomorphs

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9:30 10:00 *Coffee Break & Posters*

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**Session D: Evolution I***Chair: Luis RUEDAS*

- |       |       |    |   |
|-------|-------|----|---|
| 10:00 | 10:15 | D1 | Luis RUEDAS: Systematics and biogeography of South American cottontail rabbits (Mammalia, Lagomorpha, Leporidae: <i>Sylvilagus</i> )                                    |
| 10:15 | 10:30 | D2 | Yasin DEMİRBAŞ: Polymorphism of mitochondrial control region sequences in brown hares ( <i>Lepus europaeus</i> Pallas, 1778) from Turkey with different coat coloration |
| 10:30 | 10:45 | D3 | Rita CAMPOS: Lagomorphs as models for science education and outreach: Lessons from the evolutionary history of hares  |
| 10:45 | 11:00 | D4 | Paulo ALVES: Taxonomic, conservation and management implications of reticulate evolution in Lagomorphs  |
| 11:00 | 11:15 | D5 | Fernando SEIXAS: The genomics of historical introgression in Iberian hares  |
| 11:15 | 11:30 | D6 | Mafalda FERREIRA: Why do hares change color? Deciphering the genetic basis of seasonal coat color change using transcriptomics  |
| 11:30 | 11:45 | D7 | Andrey LISSOVSKY: Taxonomy of pikas ( <i>Ochotona</i> ) after the analysis of nuclear genes   |
| 11:45 | 12:00 | D8 | Narayan KOJU: A multilocus phylogeny of pikas ( <i>Ochotona</i> ) and its implications for systematics and gene flow  |
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- 12:00 13:30 *Lunch Break*

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**WEDNESDAY, JULY 13 (CONTINUED)**


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***Session E: Ecology, Behavior, Management, and Conservation III***
***Chair: Brian COOKE***

- |       |       |    |  |
|-------|-------|----|--|
| 13:30 | 13:45 | E1 | Brian COOKE: Introduced European rabbits in Australian ecosystems  |
| 13:45 | 14:00 | E2 | Philip STOTT: Old newspapers reveal the history of hare importations to Australia  |
| 14:00 | 14:15 | E3 | Martijn WETERINGS: Interspecific interactions between two sympatric species: Facilitation and competition between hare and rabbit in the context of predation risk |
| 14:15 | 14:30 | E4 | Stephanie SCHAI-BRAUN: Dietary preferences of the European hare ( <i>Lepus europaeus</i> ): A herbivore selecting its diet for a high fat content                  |
| 14:30 | 14:45 | E5 | Denise KARP: Prewaning survival in brown hare leverets ( <i>Lepus europaeus</i> )  |
| 14:45 | 15:00 | E6 | Fumio YAMADA: Genetic diversity analysis and successful restoration of the Amami rabbit on Amami-ohshima Island, Japan   |

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15:00 15:30 ***Coffee Break & Posters***


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**Nora and Hashem Naraghi Hall of Science, Room 101**

- |       |       |  |   |
|-------|-------|--|---|
| 15:30 | 17:30 |  | Workshop 1 - Lagomorph Systematics and "LaGomiCs" - Open Discussion<br><i>Leader: Paulo ALVES</i> |
|-------|-------|--|---|
- 

**Science 1, Room 135**

- |       |       |  |  |
|-------|-------|--|--|
| 15:30 | 17:30 |  | Workshop 2 - Population Trends and Management of Lagomorphs in Western North America<br><i>Leaders: David BROWN and Andrew SMITH</i> |
|-------|-------|--|--|
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**Nora and Hashem Naraghi Hall of Science, Room 101**

- |       |       |  |   |
|-------|-------|--|---|
| 17:30 | 18:30 |  | General Assembly of the World Lagomorph Society (WLS) |
|-------|-------|--|---|
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**THURSDAY, JULY 14**

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**Nora and Hashem Naraghi Hall of Science, Room 101**

8:00                      *Registration Desk Open*  
 8:15                      Announcements

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**Session F: Morphology and Physiology***Chair: Mark GROBNER*

8:30    8:45    F1    Irina RUF: The anterior process of the malleus in extant Lagomorpha and its systematic and functional implications  
 8:45    9:00    F2    Rafik BELABBAS: Effect of intra uterine crowding on fetal and placental development, irrigation, available uterine space per fetus and the characteristics of the uterine horns  
 9:00    9:15    F3    Brian KRAATZ: Geometric morphometric and the cranial anatomy of leporids  
 9:15    9:30    F4    Chavdar ZHELEV: European brown hare (*Lepus europaeus* Pall.) sperm quantity in testes from different seasons and ages from Bulgaria

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**Nora and Hashem Naraghi Hall of Science, 2<sup>nd</sup> Floor**

9:30    11:00                      Poster Session (with authors). *Coffee will be provided*

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**Session G: Ecology, Behavior, Management, and Conservation IV***Chair: Heiko RÖDEL*

11:00    11:15    G1    Jerome LETTY: Effect of an experimental reduction of red fox on brown hare population dynamics  
 11:15    11:30    G2    Irfan ALBAYRAK: Preliminary study on reproductive performance of brown hares (*Lepus europaeus* Pallas, 1778) in Turkey  
 11:30    11:45    G3    Klaus HACKLÄNDER: Estimating sustainable harvest rates in European hares using different population model approaches  
 11:45    12:00    G4    Heiko RÖDEL: Maternal nest building and perinatal offspring survival in the European rabbit

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12:00    13:30                      *Lunch Break*

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**THURSDAY, JULY 14 (CONTINUED)**


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**Session H: Diseases***Chair: Antonio LAVAZZA*

- |       |       |    |   |
|-------|-------|----|---|
| 13:30 | 13:45 | H1 | Jean-Sebastien GUITTON: Epidemiology of pulmonary protostrongyliasis in the European hare ( <i>Lepus europaeus</i> ) in France  |
| 13:45 | 14:00 | H2 | Isabel PACIOS-PALMA: Effects of myxoma virus and rabbit hemorrhagic disease virus on the physiological condition of wild European rabbits: Is blood biochemistry a useful monitoring tool?                                  |
| 14:00 | 14:15 | H3 | David PEACOCK: The source of Myxomatosis and RHDV/RHDV2: Could the Americas' leporids provide Australia's next rabbit biocontrol?   |
| 14:15 | 14:30 | H4 | Antonio LAVAZZA: Detection of the new emerging rabbit hemorrhagic disease type 2 virus (RHDV2) in European brown hares ( <i>Lepus europaeus</i> ) from Italy and Spain  |
| 14:30 | 14:45 | H5 | Ghislaine Le GALL- RECULÉ: Large scale detection of the rabbit haemorrhagic disease virus RHDV2 in European hare ( <i>Lepus europaeus</i> ) populations in France causing European brown hare syndrome EBHS-like outbreaks. |

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14:45 15:15 *Coffee Break*


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15:15 16:00 **Special Presentation**

Ken HICKMAN: Up close and personal with wild pygmies and pikas: How advances in camera trap technology are expanding wild lagomorph research capabilities

16:00 17:00 Meeting of the IUCN/SSC Lagomorph Specialist Group

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**John Stuart Rogers Faculty Development Center, Room 118**
18:00 Western BBQ, Group Photo, and Closing Ceremony

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**FRIDAY, JULY 15**


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8:00 18:00 **Post-conference excursion to Yosemite National Park**


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## **PLENARY AND SPECIAL PRESENTATIONS**



## **The American pika (*Ochotona princeps*): How does a small lagomorph encounter climate?**

CONSTANCE I. MILLAR

USDA Forest Service, Pacific Southwest Research Station, Albany, CA 94710, USA,  
cmillar@fs.fed.us

Despite their small stature, American pikas have gained a large reputation as an icon of climate vulnerability. Generally restricted to montane environments of western North America (WNA), pikas have high resting body temperatures that are within a few degrees of upper lethal level. They are poor thermoregulators and rely on their environment for thermal stability. Pikas are behaviorally crepuscular, have solitary habit with wide spacing between territories, and low reproductive capacity. Their broken-rock habitat (talus and related landforms) is patchily distributed, limiting population density to low levels under the best of circumstances. Patches are subject to repeat extirpation events, and rely on re-colonization for persistence. Individuals are philopatric to their natal talus, and have poor dispersal capacity (maximum 3 km; usually much less); if no territory is available, juveniles must disperse across open ground to new talus patches, which they attempt in late summer. These and other elements of life history have focused concern for persistence of American pikas under warming temperatures, and studies--especially in the Great Basin of southwestern US--have documented significant extirpations at warm margins.

Anticipating the response of small lagomorphs such as pikas to climate change requires knowledge of thermal conditions of their habitat during times of the day and year when individuals use them. I unpack components of pikas' physical habitat and habitat use, consider each relative to changing temperatures, and review the adaptive capacities of American pikas to future climates. In a recent multi-year study in Great Basin mountains, we measured diurnal and seasonal temperatures of free air and of six habitat components used by pikas: talus surface, talus matrix, haypile surface, talus below haypile, forage area, and dispersal area. Unique thermal regimes of taluses provide environments that are partially decoupled from free air: complex microclimatic processes within the coarse material of the talus include conduction, convection, chimney-flue circulation, and Balch circulation. The combined effect creates a buffered cool environment ("air-conditioning") in talus interiors in summer, even when talus surface and free air temperatures are very high, and a stable warm environment (near 0 °C) in winter, even when taluses are snow-free. Foraging grounds receive cold-air drainage from talus snouts in summer. Temperatures in the dispersal environment are often very hot in late summer, and may become key bottlenecks for patch recolonization under warming climates. These environments, however, are highly variable, and night temperatures are cool, affording potential dispersal by juveniles.

These and other conditions provide opportunities for pikas to adapt behaviorally to unfavorable surface air temperatures, and suggest that animals can accommodate a wider range of future climates than has been anticipated. I offer examples in the Great Basin where pika populations are persisting despite high air temperatures. Climate envelope models that use or model only surface air and do not include information on thermal components of pika habitat may lead to errant conclusions about the vulnerability of species. As a result of the unique elements of pikas' habitat and adaptive capacities, I propose that rather than icons of vulnerability, American pikas be explored as models of resilience under changing climates.

## Recent advances in understanding the evolution of lagomorphs

JOSÉ MELO-FERREIRA<sup>1,2</sup>

<sup>1</sup>CIBIO, Research Center in Biodiversity and Genetic Resources, InBIO Associate Laboratory, University of Porto, 4485-661 Vairao, Portugal

<sup>2</sup>Department of Biology, FCUP, Faculty of Sciences of the University of Porto, 4169-007 Porto, Portugal, Email: [jmeloferreira@cibio.up.pt](mailto:jmeloferreira@cibio.up.pt)

Lagomorphs are exceptional models to study relevant evolutionary processes. Also, understanding the evolution of the order is of fundamental importance to guide proper taxonomic classifications and conservation policies. Modern progresses in our capacity to collect massive amounts of genetic variation data have provided us with an unprecedented ability to understand complex evolutionary mechanisms in any species. I will go through some of the major recent advances in our understanding of the evolution of Lagomorphs in several different perspectives. I will particularly focus on studies that i) elucidate the nature of species formation and the importance of hybridization between the diverging close relatives, with examples from hares and rabbits, ii) reveal the genetic basis of relevant adaptive traits, such as seasonal coat color variation in hares, iii) infer the structure of rabbit domestication, and iv) clarify the phylogenetic relationships among Lagomorph species, such as leporids and ochotonids. I will further discuss how these advances contribute with resources that will allow expanding high resolution genomic studies to other species, and how this will help us come closer to understand in detail the evolution of Lagomorphs.

## Up close and personal with wild pygmies and pikas: How advances in camera trap technology are expanding wild lagomorph research capabilities

KEN HICKMAN

Independent Wildlife Researcher, San Carlos, CA 94070, USA, Email: ken@meer.net

While commercial digital trail cameras are fast becoming a standard tool in the arsenal of mammal researchers, in general, their use has been confined by their design to landscape scenes and meso- to mega-fauna at 5 meters, and not for detailed documenting of busy micro mammals, such as rodents and lagomorphs, at burrows, scurry zones and dens. Their focus, flash and field of view are fixed, and thus too limiting. They are, in essence, "Farsighted." But, for those unafraid of a soldering iron and wire, solutions do exist. The components for building custom camera traps are available, and allow camera selection and system design to overcome these commercial trail camera limitations, and get much more "up close and personal" with smaller species. In 2014, starting with techniques and homemade camera trap technology refined during studies of dusky-footed woodrats, (*Neotoma fuscipes*) at their stick houses, I began two long-term, high-quality camera trapping studies in Mono County, California on pygmy rabbits (*Brachylagus idahoensis*) at their burrows, and American pika (*Ochotona princeps*) at their talus-embedded haypiles. The goal was to remotely document details on behaviors, activity patterns, and sympatric species across multiple seasons in locations and ways not previously feasible with traditional commercial trail cameras, or even by field biologists with binoculars. The results are flowing and the higher quality tells the tale. New insights and detailed data on behaviors and patterns are being documented. The photos show that cam traps can be used to uniquely identify individual pygmies, pika and their predators and competitors, inventory species of foraged foods, and even document activity under the talus and snow in the dark of night and dead of winter. These studies, their results and methods show that camera traps are no longer just for bovines and big cats—low-cost, high-quality camera traps suitable for up-close, remote research of wild Lagomorphs can be readily built and deployed today.

## **ORAL PRESENTATIONS**

## **A1. The cascading effects of circannual phenologies across trophic levels - how western larch affect the coat color molt in snowshoe hares**

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Trophic level interactions can be affected by forest management in unanticipated ways, with profound effects on wildlife management. We examined a novel trophic interaction between two species of management interest. Western larch (*Larix occidentalis*) is a coniferous timber species of high commercial value that undergoes drastic annual phenologic changes, as it is a deciduous conifer that loses its needles in fall. This phenologic change in stand structure has the potential to affect another strongly interacting species, snowshoe hares (*Lepus americanus*), the primary prey for the federally threatened Canada lynx (*Lynx canadensis*). Hares also undergo a phenologic change annually molting from brown to white as means of camouflage against seasonal snow. Climate change is predicted to decrease the number of snow covered days potentially leading to an increase in white hares mismatched against a snowless background. The stand structure changes larch undergo might also influence snow cover providing a potential mechanism to link the larch needle phenology with hares. We quantified both the hare molt phenology and the larch needle phenology for 2 years in western Montana and examined covariates linked to the hare molt such as temperature and snow. We then examined the effect that larch stand structure might have on the hare molt possibly mediated by its effects on snow. We found that snow presence directly influences the molt as does the larch needle phenology. More snow was associated with whiter hares. Bare larch was also associated with whiter hares through its association with more snow. In addition, we found that hares associated with bare larch experienced a reduction in their mismatch by 10%. Together these findings serve as a novel example that the effects of climate change (e.g. temperature, snowpack duration) can have profound and unexpected indirect effects that cascade across phenologic changes at different trophic levels.

## A2. Effects of climatic fluctuations on the evolution of Himalayan pikas

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Pikas are highly adapted to cold and have limited dispersal ability. Consequently, they are strongly impacted by ongoing climatic change. We know that past climatic fluctuations have impacted the evolution, distribution and genetic diversity for many species. How might pikas have responded to past climatic change? We investigate this in a region particularly prone to the impacts of climatic change, the Himalaya. The Himalayan glaciers are melting twice as fast as the global average. This makes species in the high altitudes of the Himalaya particularly at risk of habitat loss. Unfortunately, distribution pattern of pikas in this region remain poorly known. We started by exploring phylogenetic relationship and distribution pattern of pikas in the Himalayan mountain range. We conducted transect based elevational sampling at eastern, central and western Himalaya and collected 438 fecal pellets and 105 tissue samples. All samples were subsequently identified using genetic distance method and phylogenetic approach. Fieldwork conducted over three years across the Himalayan mountain range allowed us to identify at least six species. Overall distribution pattern based on these locations revealed elevational segregation between closely related species belonging to the same subgenus, especially within younger species within subgenus *Ochotona*. We use this information on elevational distributions in conjuncture with climate models to predict how these same species may have responded to the climatic fluctuations. We then generated genome-wide SNP data to test our predictions on differential species response to recent climatic fluctuations. Analyses are ongoing, and we will present preliminary results on climatic models and genetics-inferred response to climatic change in this incredibly biodiverse and understudied region.

### **A3. Climate change induced molting mismatch? Reduction in annual snow cover causes increased winter generalist predation in mountain hares**

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In northern latitudes, species that have adapted to snow cover by molting and changing pelage color will be particularly vulnerable to human induced climate change, as predation levels may increase due to a mismatch with background coloration. Here, we investigated the relationship between abundances of mountain hare (*Lepus timidus*) and mammalian generalist predators (red fox (*Vulpes vulpes*) and pine marten (*Martes martes*)), and the duration of snow cover in winter. We analyzed 12 years of data from 622 snowtracking index lines in Southeast Norway. Hare abundance was positively related to generalist predator abundance, probably due to a combined numerical- and functional response of the predators. Hare abundance was negatively associated with a short duration of snow cover and this effect was stronger in areas with high predator abundance. Hence, we demonstrate a causal link between hare abundance, predation and snow cover. We expect declining hare densities in the future caused by interactive effects of reduced snow cover, increasing generalist predator densities and the hares limited ability to respond to rapid environmental changes through phenotypic plasticity or natural selection.

## A4. High fitness costs of climate change-induced camouflage mismatch

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Decreasing snow cover duration is one of the strongest signals of climate change across the temperate zone of the Northern hemisphere. The resulting later onset of snow in the fall and earlier loss of snow in the spring represents a new, potentially severe stressor for at least 18 color molting species and their respective communities. Snowshoe hares (*Lepus americanus*), an important prey species for many forest carnivores, molt from brown to white in winter to match their background and avoid detection from predators. The mistiming between ground snow cover presence and photoperiod-induced color molts results in camouflage mismatch during spring and fall. We previously found minimal plasticity in either seasonal color molt phenology to track seasonal snowpack or anti-predatory behaviors to minimize color mismatch, implying a 4-8 fold increase in mismatch by the end of the century. Here, we investigated the cost of color mismatch and the potential to adapt to the increasing mismatch through evolution. We observed nearly 200 wild hares over three widely disparate snow years and two study sites in Montana, USA, and monitored their survival, coat color molt phenology and color mismatch weekly using radiotelemetry. First, we found high variation in phenology of coat color molts and consequently in color mismatch between individuals. Next, we detected high fitness costs of mismatch, with hares suffering 4- 7% lower weekly survival rates when color mismatched. Without adaptive changes in the molt phenology, these fitness costs coupled with decreasing duration of snow season were projected to decrease annual survival by 11% by mid-century and 23% by late-century. Such decreases in survival could be sufficient to cause hare populations to decline strongly towards extinction, with annual geometric growth rate decreasing by 11% (24%) by mid (late) century. We conclude that in order to allow the species persistence under climate change, evolutionary rescue must be promoted by the maintenance of large population sizes with adequate gene flow and reduction in anthropogenic stressors including climate change.



## **A5. Macroecology of the Order Lagomorpha; implications for the impact of global climate change**

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Expertly-validated Species Distribution Models (SDMs) and phylogenetically-controlled regression suggest that responses to climate change are related to species traits in the Lagomorpha. Climate change may impact more than two-thirds of lagomorphs by 2080, with leporids (rabbits, hares and jackrabbits) likely to undertake poleward shifts with little change in range extent, whilst pikas are likely to show shifts to higher altitudes associated with range declines. Model predictions based on abiotic variables may be misleading. Interspecific interactions are widely reported in the Order with closely related, large-bodied, similarly-sized species in agricultural landscapes or at high elevations likely to have competitive interactions. Hierarchical spatial models suggest that for some species biotic (mutualistic and competitive) interactions are important whilst network analyses have been used to quantify the strength of such biotic interactions to assess ecological network stability under future conditions.

## A6. Lagging morphs of lagomorphs in winter

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Several species of lagomorphs undergo seasonal coat color molt from brown in the summer to white in the winter to match the presence of seasonal snow cover. Like most circannual traits in vertebrates, the timing of the color molt is triggered by photoperiod. As the seasonal molt to white confronts reduced seasonal snow duration – one of the most prominent signals of anthropogenic climate change – this visual form of phenologic mismatch leaves white animals increasingly exposed against a brown snowless background. Work to date suggests a maladaptive value of seasonal camouflage mismatch, directly linking it to reduction in snow duration due to climate change. Importantly, species of lagomorphs (and other species) that undergo seasonal coat color change manifest three population-level phenotypes in winter in different parts of their range: brown, white, and polymorphic (with sympatric brown and white individuals). Most populations consists of individuals that are white in the winter, with the duration of the white coat generally tracking snow duration. In other regions, where seasonal snow is sporadic or absent, individuals retain a brown coat color year-round. The third population-level phenotype composed of polymorphic individuals, is found in zones of special interest because they may represent active areas of climate-mediated evolution. We will discuss a global phenology database of winter coat color phenotypes we are assembling for 18 vertebrate species that undergo seasonal color molt, including 5 lagomorph species. To date, our database includes >2,300 georeferenced specimens with winter coat color confirmed from museums, photos or observations. If climate induced natural selection is operating globally on the functional trait of seasonal coat color, then we expect convergence in winter phenotypes in the same regions across species. Further, if regions with polymorphic brown and white winter morphs are especially active zones of ongoing evolution in response to climate change, then we predict they will be characterized by variables associated with rapid change in snowpack. All of which leads to a question, for lagomorphs, of whether the various color morphs represent evolution in action or evolutionary time lags; the latter would, of course, result in lagging morphs of lagomorphs.

## A7. Does relative humidity or its derivatives predict a lagomorph species' occupancy and density better than temperature, habitat area, and vegetation across an ecoregion?

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**Background, questions, and methods:** Contemporary climate change represents a pervasive yet spatially variable disturbance for species; its effects have been predicted and often shown to be most pronounced among habitat-specialist, poorly dispersing, and physiologically challenged species. Although relative humidity (RH), which varies at several spatial resolutions, may affect species via a number of direct physiological and indirect mechanisms, to date field-measured RH and its derivatives have not been used to predict patterns in species occupancy and abundance. Using American pikas (*Ochotona princeps*) as our model organism to investigate local extinction dynamics and patterns of abundance, we performed re-surveys at locations of historical pika records across the hydrographic Great Basin, spanning NV, OR, and CA. At each site, we walked 50-m transects in talus patches using paired-observer distance sampling to quantify detectability and density. We documented evidence of occupancy based on pika sightings, vocalizations, fresh haypiles [current occupancy]; old pellets, old haypiles, or both [past occupancy]; or none of these [unoccupied]. In this lowest-precipitation region of the species' geographic range, we hypothesized that RH or its derivatives (vapor pressure deficit, dew point) would better predict ecoregional patterns of pika occupancy and abundance during 2012-2015 than would temperature-related aspects of climate, talus-habitat extent, or vegetation. We evaluated evidence in support of mechanism-based competing hypotheses using information theoretic approaches.

**Results and conclusions:** Across the geographic range of *O. princeps*, great disparities exist in both the species' distributional status and trend. When looking at means and frequencies of threshold exceedances of RH that relate to diverse mechanisms by which climate may influence mountain species' distribution, we found great difference between conditions at pika-persistent compared to pika-extirpated sites. During 2012-2013, RH at pika-persistent sites averaged 87%, whereas extirpated sites averaged 64% ( $F = 234.2$ ,  $p = 0.0006$ ). At those same sensors during that period, although temperature averaged just over 4 C higher at the extirpated sites, the difference was much smaller ( $F = 22.5$ ,  $p = 0.018$ ). Across all years, sites of persistence had RH>85% significantly more often than did sites of extirpation, very likely reflecting differences in duration of snowpack between the two types of sites. We expect that results of the information theoretic analyses will identify which of the RH-related measures has the best ability to explain how climate is influencing species distribution and abundance, and in which seasonal and geographic contexts its influence surpasses that of other potential determinants (temperature measures, talus-habitat area, vegetative measures). Collectively, our results illustrate the nuance and complexity with which species are responding to climate change. Understanding underlying mechanisms of ecological response to contemporary climate change is critical to inform climate-adaptation conservation and management efforts.

## **B1. Behavioral ecology of American pikas (*Ochotona princeps*) at mono craters, California: Living on the edge**

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The behavioral ecology of the American pika (*Ochotona princeps*) was investigated at a relatively hot south-facing low-elevation site in the Mono Craters, California, a habitat quite different from the upper montane regions more typically inhabited by this species and where most prior investigations have been conducted. Mono Craters pikas exhibited a behavioral profile that contrasted significantly with that of pikas found in upper montane regions. Mono Craters pikas were less surface active than pikas in studies at high elevation sites, although their rate of short call vocalizations was similar. Mono Craters pikas did not exhibit typical foraging behavior: they were observed to feed and collect hay at significantly reduced rates, and did not construct large central place haypiles. Social behaviors (conspecific aggression, social tolerance, avoidance) were infrequent compared with prior studies in upper montane environments. The Mono Craters site appears to be one of the warmest localities in which pikas have been observed. Recorded talus surface temperatures consistently exceeded 30° C, and temperatures > 40° C were commonly recorded. In contrast, temperatures measured in the matrix of the talus were consistently cooler, and the apparent insulating effect of talus, as measured by the difference between surface and matrix temperatures, was typically most pronounced on the hottest days. Although pika activity was most frequent in early morning, late afternoon, and at night, pikas were also active during the hottest part of the day, presumably because of their ability to behaviorally thermoregulate by retreating into the cooler talus matrix. Data on populations of pikas which inhabit marginal sites can help us understand how pikas and other montane animals might respond in a world of climate change, so that we may more effectively plan for their conservation.

## **B2. Hypoxia-induced plasticity in gene regulation: An experimental study of the Asian pika, *O. dauurica***

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Limited oxygen at high elevation stresses aerobic metabolism. Alterations in gene expression is one mechanism used by species to tolerate hypoxic stress. The genus *Ochotona* (pikas) consists of 30 species with almost half of these occupying extremely hypoxic elevations of over 4000 m. Pikas play a vital role in these extreme environments, yet little is known about the mechanisms underlying hypoxia tolerance in these species. In order to investigate changes in gene expression in pikas due to changes in oxygen availability, we conducted an experimental study controlling for all environmental factors by using individuals from the only known captive pika population in the world (*O. dauurica*). We collected RNA-stabilized blood samples from 4 male pikas before and after exposing them to oxygen concentrations characteristic of 2000 m and 4000 m for 5 days while controlling all other variables. We have sequenced these samples on the Illumina platform and have created a *de novo* assembly of the transcriptome of this species. We are using this sequence data to identify differences in gene expression within an individual in order to gain understanding of the role of gene regulation in hypoxia tolerance. The degree to which expression patterns correlate with oxygen concentrations will inform the relative role that plasticity in gene expression may play in enabling this species to tolerate hypoxia. Higher plasticity in gene expression may also indicate flexibility within an individual to occupy vastly different elevations.

### **B3. Better understanding the Ili pika (*Ochotona iliensis*) through infrared technology**

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The Ili pika (*Ochotona iliensis*) is a rare rock-dwelling pika that occupies a restricted habitat in the Tian Shan of western China. In the 10 years following its description, there were only 27 verified sightings of the species. Following a hiatus of about 10 years, during which no investigations were conducted on the species, a comprehensive survey in 2002/2003 was conducted of all 14 sites previously known to have pikas - as well as suitable habitat surrounding these areas. No pikas were seen, and their characteristic sign (haypiles, scat piles) was absent from eight of the localities – including the type locality. Subsequent surveys in 2006 and 2010 also failed to locate any living pikas, and active pika sign was found at only five of the 14 locations. Finally, during our 2014 survey, two living Ili pikas were observed at Jipuk, the first to be seen in 20 years. These surveys indicated that the Ili pika is extremely rare and undergoing a precipitous decline. While finding living pikas has encouraged us, it also became clear that conventional field techniques such as relying on finding traces of activity from scats, urine stains, footprints in the snow, and haypiles in this high (2,800-4,100 m) remote habitat are insufficient to properly document the natural history of this species. Here I report preliminary results from our use of infrared technology (camera traps) placed to monitor the activities of Ili pikas. Data obtained from the use of camera traps has greatly expanded our knowledge of the species, as many images of the activities of the Ili pika have been recorded. While most pikas are generally assumed to be diurnally active, our infrared cameras document that Ili pikas are very active at night. We are accumulating activity data so as to analyze the circadian activity rhythm of the Ili pika. Early field observations indicate that Ili pikas occupy individual territories and that they rarely stay in one area for a long time, mostly wandering in a wide loop. They also appear to be asocial and less prone to utter vocalizations compared with other pika species. Thus understanding how individuals communicate becomes an issue. By using infrared cameras, especially those set up in the dens, we can monitor their activities and capture both videos and images for analysis. We have found that most Ili pikas sniff the traces of urine and scats left on stones, indicating that these form the basis for chemical communication to mark and expand a pika's territory, as well as to identify mates. We are also able to identify individuals through differences in their pelage coloration and size, enabling us for the first time to determine the number of individuals that can potentially interact. The camera traps also record potential predators; we have documented beech martens, ermines, red foxes, snow leopards and brown bears in the vicinity of Ili pikas. Taken together, these data will enable us to better manage and conserve this important endangered species.

## **B4. Risky rabbits: Evaluating tradeoffs in food and predation risks by pygmy rabbits and mountain cottontails in the sagebrush-steppe**

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When selecting food patches, herbivores face multiple risks imposed by their environment such as the risk of consuming food that does not meet their daily energy requirements, the risk of toxicity from plant secondary compounds, and the risk of predation. Because these risks operate simultaneously, animals must make tradeoffs between them when selecting food patches. To better understand how animals trade off food and predation risks, we manipulated both in a series of experiments with pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*), which often occupy the same sagebrush steppe landscapes and suffer high predation rates, but differ in their size, ability to digest fiber and detoxify toxins, and use of burrows. In 1461 experimental trials, 9-13 rabbits of each species were given a choice between two foraging patches that varied in the amount of fiber, toxins, level of exposure to predators, and distance from a burrow. Using the method of paired comparisons, we estimated and compared relative risks and tradeoffs both within and between species, and calculated the marginal rate of substitution of each type of risk for each other risk. Fiber, toxins, exposure to predators, and distance from a burrow all increased the risk of patches for pygmy rabbits, whereas only fiber and toxins did so for cottontails. In addition, the relative risk of toxins was lower, and the relative risk of distance was higher, for pygmy rabbits than cottontails. Pygmy rabbits traded off food quality for safety whereas cottontails traded off safety for food quality. This study provides a functional understanding of how the quality of food and predation risk influence habitat use by these rabbits, advancing our ability to assess habitat quality for lagomorphs and other small herbivorous mammals across landscapes. In addition, our models can be used to map landscapes of risks that guide management of imperiled habitats such as sagebrush-steppe.

## B5. Seasonal behavioral thermoregulation of an arid land leporid

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Expected changes in the global climate during the 21<sup>st</sup> century may have large implications for conservation of small mammals. Small mammals typically have limited dispersal abilities that may affect their ability to track long-term changes in climate. Pygmy rabbits (*Brachylagus idahoensis*) are small lagomorphs considered to be extremely vulnerable to extinction under climate change scenarios because of a predicted range contraction caused by increasing temperatures and their limited ability to disperse. Behavioral thermoregulation strategies such as the ability to identify and exploit thermal refugia and flexibility in the timing of daily activity could potentially buffer pygmy rabbits and other small endotherms against a changing global climate. Our objective was to quantify behavioral thermoregulation strategies used by pygmy rabbits during summer and winter. We hypothesized that individuals would exploit thermal refugia and alter activity patterns seasonally and daily as a function of the thermal environment. We radio-collared 52 pygmy rabbits to identify rest site selection during summer and winter and evaluated the influence of multiple thermal and security factors using conditional logistic regression and information theory. We also fitted 24 rabbits with accelerometers to quantify activity seasonally, during daily periods (dawn, day, dusk, and night), and in relation to daily extreme temperatures. During summer, rabbits selected rest sites that were cooler than unused sites and strongly avoided high levels of solar radiation. In winter, however, proximity to a burrow was the only significant variable affecting rest site selection. In both seasons, activity was characterized by high crepuscular peaks, but contrary to our expectations, it was not related to daily high or low temperatures. Sex and moon phase significantly influenced activity patterns, suggesting high levels of flexibility in timing of activity. These behaviors might provide thermoregulatory options that could buffer pygmy rabbits and other small mammals against a changing climate. Managing heterogeneity within landscapes to provide thermal refugia also may support long-term conservation of vulnerable mammals.



## **B6. Burrow use by pygmy rabbits: Dynamic patterns and ecological effects**

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Pygmy rabbits exhibit obligate burrowing behaviors that are unusual among North American leporids. Multiple residential burrow systems are inhabited year-round by individual rabbits that are neither gregarious nor territorial. Although home ranges can be large, pygmy rabbits tend to concentrate foraging and resting activity around burrow systems, which can remain occupied for long periods (>10 years). There are several ecological and conservation implications of this obligate burrowing behavior. First, ecosystem engineering activities (digging, defecation, and browsing) might influence habitat surrounding occupied burrow systems over time. Second, cumulative habitat changes might either enhance or decrease probability of continued burrow use. Third, occupancy of burrow systems could provide an index for monitoring changes in populations over time. Our objectives were to 1) quantify cumulative effects of rabbits on habitat surrounding burrow systems, 2) characterize long-term spatial and temporal patterns of burrow use, and 3) evaluate changes in densities of occupied burrows over time. We recorded locations and activity status of burrow systems at two study sites in eastcentral Idaho, USA, during 2002-2015, and we tracked occupancy of individual burrow systems through time. In 2014, we quantified soil and vegetation characteristics around burrow systems occupied by rabbits for 1 to 12 years. We expected that browsing would impact slow-growing sagebrush shrubs, but that digging and biodeposition would enhance soil nutrients, water infiltration, and potentially sagebrush reproduction. Density of active burrow systems ranged from 0.6/ha to >2.0/ha, and both density and proportion of burrows that were active differed between study sites and across years. Burrow systems were closely associated with mima mounds (i.e., areas of mounded micro-topography), and although distribution of mima mounds was significantly dispersed relative to random, the distributions of active burrow systems were clumped on the landscape. Pygmy rabbit occupancy had significant cumulative effects on vegetation properties that included reduced sagebrush canopy and live shrub cover. Soil properties were less influenced by duration of occupancy than by the localized spatial influence of burrowing. Two measures of sagebrush reproduction (seedling recruitment and inflorescence biomass) increased with duration of burrow occupancy. Although pygmy rabbits are inconspicuous and often occur at low densities, their dynamic patterns of burrow use suggest that they influence heterogeneity of sagebrush landscapes, and that loss of this species might have important consequences for other sagebrush-dependent wildlife.

## **C1. Comparative population genomics of New England cottontail (*Sylvilagus transitionalis*) and eastern cottontail (*S. floridanus*) in the northeastern US**

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The New England cottontail (*Sylvilagus transitionalis*) is a species of conservation concern and the focus of a multi-agency and multi-institutional effort to conserve the species. The drastic decline in the distribution of New England cottontail precipitated the establishment of a captive breeding program in 2011 at the Roger Williams Park Zoo in Providence, Rhode Island, which was subsequently expanded in 2015 to include the Queens Zoo in Corona, New York. Currently, New England cottontail from different geographic areas are being interbred; however, it is unknown if adaptive genetic differences exist in populations from different areas that should be maintained. We used a double-digest Restriction-site Associated DNA (ddRAD) approach to develop genomic markers to identify single nucleotide polymorphisms (SNPs). We have generated ddRAD libraries for 84 New England cottontail samples and we are currently processing an additional 100 samples. Adaptive SNPs will be identified using Bayesian methods to identify conservation units. Eastern cottontail (*S. floridanus*) is a non-native east of the Hudson River in the Northeastern US and potentially one of the reasons why New England cottontail have declined. Eastern cottontail could be competing with New England cottontail directly, indirectly, or interbreeding. To determine if New England cottontail and eastern cottontail have interbred in the wild we generated ddRAD libraries for 122 eastern cottontail samples and combined this data with 84 samples from New England cottontail to identify over 8,000 SNPs for both species. Initial principal component analyses show a clear separation between the two species; however, we will increase the sample size from each species and conduct simulation analyses to further test for hybridization. Eastern cottontail may be able to be used as a surrogate for New England cottontail gene flow if they show similar patterns. Thus, an understanding of eastern cottontail gene flow could help identify areas to establish new New England cottontail populations that would be less likely for eastern cottontail to infiltrate. Comparative population genomic structure analyses at the regional scale for both species shows a similar pattern of two major clusters; however, New England cottontail show a more clear pattern of finer population structure of six clusters. We will compare these results to a more local analysis of both species on Cape Cod, Massachusetts, to determine if similar environmental variables influence their respective gene flow. The results of our research will help inform management decisions for both captive and wild New England cottontail.

## C2. Genetic diversity and functional connectivity of the riparian brush rabbit: Implications for conservation of an endangered lagomorph

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The riparian brush rabbit (*Sylvilagus bachmani riparius*) is an endangered subspecies of the brush rabbit endemic to the riparian forests of California's Great Central Valley. Over the last 150 years, the Great Central Valley has experienced extensive habitat loss and fragmentation due to land use change. Remaining brush rabbit habitat in this region has been reduced to small, isolated patches. Over time, such reductions in habitat size and connectivity can impede gene flow among populations and accelerate genetic drift within populations, resulting in long-term evolutionary consequences. We used mtDNA sequencing and nuclear microsatellite variation to quantify population history and patterns of genetic subdivision among the five remaining *S.b. riparius* populations. Shared haplotypes across the current range of *S.b. riparius* suggest historic gene flow among populations. However, nuclear genetic variation supports two distinct genetic clusters within the subspecies, suggesting recent divergence within this taxon, consistent with the history of fragmentation across the region. Landscape based analyses of habitat suitability show limited opportunity for natural dispersal and connectivity across portions of the extant range, but provide guidance for critical areas of restoration. This study strongly supports the need for restoration of natural corridors of genetic connectivity across this highly altered landscape.

### **C3. Managing an endangered brush rabbit in the context of extreme weather events**

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One of only three (and one of only two naturally occurring) extant populations of the endangered riparian brush rabbit (*Sylvilagus bachmani riparius*, RBR) persists on the Oxbow Preserve in San Joaquin County, California. This population occurs on an 'oxbow' along the San Joaquin River, with an adjacent residential community contributing to an 'island effect' for this ~ 30-acre preserve. Owned and managed for conservation purposes for over a decade by a conservation organization (Center for Natural Lands Management, CNLM), the Preserve appears to be supporting a robust RBR population in spite of the limited amount of habitat, potential for predation by coyotes and domestic cats, and stress from proximity to human activity. Effective fencing, frequent patrols, and increasing protection through partnerships with regulatory agencies have reduced the incidence of trespass. Cameras installed onsite have also assisted in alerting CNLM to trespass events and perimeter weaknesses, as well as helping to document habitat use by the RBR population. However, the larger-scale impacts from extreme weather events are more challenging. Predictions of the effects of rapid climate change for near-coastal California vary but most include the expectation of more extreme weather events. The USFWS listing decision (1997) pointed to lack of refuge from flood events as a major threat to the species' persistence. The Oxbow RBR population is at risk both from extensive flooding and continued habitat degradation due to prolonged drought. Because the Oxbow Preserve lies within the floodplain, it is prone to near complete inundation during excessive river flows, leaving the rabbits with little effective cover on the higher elevation areas, such as the crown of the levee. Flooding is a natural occurrence and RBR are naturally adapted to respond. However, higher ground within the Preserve and adjacent property provides little to no protective cover in the event of a prolonged flood. The Preserve is surrounded by residential development and flood control levees, leaving the RBR vulnerable to predation during a flood event. CNLM management is focusing on promoting and restoring vegetation suitable as cover on higher elevations, (e.g., native *Rubus*, *Vitis* and *Rosa* spp.) that will provide protection from predation for RBR during future severe flood events. During recent periods of prolonged drought, there has been a general decline in health and vigor of riparian vegetation, which decreasing availability of food and cover for RBR. Impacts due to drought range from decreasing leaf cover for herbaceous and shrub plant species, and loss of mature overstory trees (i.e., *Populus*, *Quercus*, *Salix*), leading to an increase in annual weeds promoting catastrophic wild fire conditions. Habitat must be managed with the understanding that conditions do change. Preparing for future expected conditions by increasing high water refugia, enhancing and expanding healthy riparian vegetation, controlling invasive species and maintaining this refugia and enhanced habitat in perpetuity are ways that CNLM promotes perpetual protection of the RBR and other species at the Oxbow Preserve in the face of climate change.

## **C4. Effect of direct signals from predators in activity patterns and physiological stress in the zacatuche rabbit (*Romerolagus diazi*) at the Chichinautzin Biological Corridor, Mexico**

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Understanding how prey assess and respond to risks provides information on how the risk of predation affects the dynamics of populations. In this paper we use videotaping in a couplet experiment to determine if the zacatuche rabbit modifies its activity as a direct response to predation risk signals (coyote and bobcat excreta and urine). The initial sampling time and activity start time (latency) were used as a measure of risk perceived by zacatuches. In addition, we quantify metabolic cortisol levels in feces of animals that inhabit the sites used for behavioral experiments. Kaplan-Meier survival analyses indicated that zacatuches alter their patterns of activity in response to predation risk signals without differentiating between observation periods. It is possible that zacatuches avoid predation risk by altering their crepuscular activity; i.e., they prefer to leave later to engage in activities away from plant cover when predator sign is present than when it is absent. This behavior can also be linked to the presence and activity of the native predators. Analyses showed that both the mean and variance of fecal cortisol metabolite levels were significantly higher in animals exposed to odors than those that were not, suggesting that the perceived risk of predation has an effect on physiological stress. The next stage of our research will assess whether behavioral patterns such as surveillance, foraging, and social interactions are modified in response to predation risk.

NOTE: Mariana Solorio-Damián was unable to attend the conference. This work was not presented.

## **C5. Bunchgrass (*Muhlenbergia macroura*) relevance in the ecology and conservation of the volcano rabbit (*Romerolagus diazi*)**

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The volcano rabbit or zacatuche (*Romerolagus diazi*) is an endemic Mexican leporid, restricted to the central mountains of the Transvolcanic Belt and occurs in open pine forests with a dense cover of bunchgrass. Zacatuche depend on bunchgrass because it is their main food source, serves as shelter against the weather and predators, and it is used to burrow into or as material for burrows. The volcano rabbit habitat has been fragmented through land use changes, by burning and other factors. Habitat loss and fragmentation interrupts genetic exchange and reduces the vegetation at the landscape level. The International Union for the Conservation of Nature as well as the Zoological Society of London recommended that the main actions needed for *R. diazi* conservation must be habitat management and creation of habitat corridors to link isolated populations along with local law enforcement and education programs. Despite the fact that the importance of bunchgrass for the zacatuche dates since prehispanic times, little information is available on management prescriptions. Nevertheless, bunchgrass maintenance and management in captivity will support volcano rabbit conservation by generating stock plants to restore disturbed areas and to reconnect isolated zacatuche populations. The objective of this study was to establish a basic protocol of maintenance in captivity of *Muhlenbergia macroura* by creating a sustainable plantation in San Juan de Aragon Zoo in Mexico City. The biological material was obtained from Bosque Esmeralda ecoturistic park in Amecameca, Mexico state. The treatments were focused in the extraction, transplantation, irrigation and maintenance of this bunchgrass species. The development of this knowledge will help to maintain a *R. diazi* captive population and thus help *ex situ* research and conservation of this lagomorph. It will now allow us to have bunchgrasses for environmental management, to create habitat corridors, to maintain captive populations (to supply animals to be released in nature), and thereby ensure the preservation of ecological processes, ecosystem services, and biodiversity in one of the relict ecosystems in our country.

## **C6. History, status and population trends of cottontails and jackrabbits in the western United States**

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Cottontails (*Sylvilagus*) and jackrabbits (*Lepus*) are keystone prey species for mammalian and avian predators in western North America. The importance of leporids as a food base for golden eagles, and a concern that these animals may be declining, prompted us to review past population studies and evaluate state federal-aid reports on leporid survey and hunter success trends. Of the 6 states providing cottontail survey data, all showed declining population indices. Of the 12 states that collected cottontail hunt information, all but 2 (SD and WA) reported declining trends in hunt success. Information on jackrabbits was limited to CA, CO, NB and OK with jackrabbit hunt data in NV included with other leporids. Declines of >10% were reported for jackrabbit observations and/or hunt success in all states save OK. Populations of snowshoe hares, while shown to fluctuate greatly, exhibited no evidence of long-term changes in numbers based on survey and/or hunt success data except in WY. Based on these data both cottontail and jackrabbit numbers appear to have declined during the past 10 to 50 years with the largest decreases in CA, the Southwest, the Great Basin, and the central Plains. We attribute the reasons for this declining trend to changes in land use and habitat quality, to extended drought, and possibly to increased predation. We recommend habitat management measures to increase cottontail and jackrabbit survival rates to benefit leporid specialists such as the golden eagle.

## **C7. Loss of connectivity between United States and Mexico populations of the white-sided jackrabbit (*Lepus callotis*)**

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The white-sided jackrabbit (*Lepus callotis gaillardi*) is a nocturnal grassland obligate that occurs exclusively in Madrean Plains grassland east of the Sierra Madre from Durango, Mexico to its northern limit near Cloverdale, New Mexico. Formerly, this subspecies enjoyed an unbroken corridor between the U.S. and Mexico, but since the 1990s a documented population decline indicates that this connectivity may no longer exist. Woody shrub invasion into shrub-free grasslands, a habitat which the hare relies upon, has resulted in a fragmented archipelago of grassland habitat in a former sea of grass. Historic and recent survey locations of white-sided jackrabbit occurrence were used to build a path corridor model to determine if animals in the two countries have become isolated from each other. A vegetation classification model was built using Landsat thematic mapper imagery from 1984 and 2014 in order to quantify changes in habitat quality over a 30 year period. Shrub cover in the region since 1984 has increased by 304%, resulting in a 90% loss of habitat for the white-sided jackrabbit. This model suggests that a migration corridor no longer exists between the two countries, and that the US population is now separated from the nearest population in Mexico by about 100 kilometers. Currently, the US population consists of only about 65 animals that reside within a 5,838 hectare area of southwestern New Mexico. This population has declined in recent years due to non-point source habitat changes from pure grassland into Semidesert grassland and Chihuahuan desertscrub, but also from point-source impacts in the form of road kills by US Department of Homeland Security Border Patrol vehicles, which traverse the species' habitat during hours when the hare is most active. The resulting fragmentation of habitat and connectivity among populations, along with direct mortality, places the continued survival of the white-sided jackrabbit in doubt.



## **C8. Preliminary investigations of the spatial distribution of black-tailed jackrabbit (*Lepus californicus*) densities in the Mojave Desert ecoregion**

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Little is known about the density of Black-tailed jackrabbits across the Mojave Desert Eco-region. In response to growing concerns over the impacts of alternative energy development on the Golden Eagle, we initiated a study to survey and model Golden Eagle prey populations at a landscape scale to assess habitat quality and suitability in the Mojave Desert Ecoregion. Given the known relationship between jackrabbit abundance and the reproductive success of Golden Eagles, we focused on black-tailed jackrabbits (*Lepus californicus*). We conducted two years of nocturnal spotlight surveys at a series of stratified random transects to assess the availability of black-tailed jackrabbits and other prey species. In the process, we developed a protocol for a landscape level survey of leporids in a desert environment. Our surveys recorded animals at up to 160 m distant from the vehicle, although most detections were within 30-40 m of the transect line. We recorded specific spatial data for 155 jackrabbits in 695 km of transects during 2014, and 468 jackrabbits in 874 km of transects during 2015. We modeled spatial variability in jackrabbit population density across the landscape using climatic, vegetative, and geomorphic descriptors as eco-physiological variables. Our models produced a spatial map with heterogeneous densities of black-tailed jackrabbits across the Mojave Desert Ecoregion. Densities ranged from 0- to 40 animals/ km<sup>2</sup>. Higher density populations generally correlated positively with increasing elevation.

## D1. Systematics and biogeography of South American cottontail rabbits (Mammalia, Lagomorpha, Leporidae: *Sylvilagus*)

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A critical issue in the species currently derived from Linnaeus' 10<sup>th</sup> edition of the *Systema Naturae* is the lack of holotype material, which in many instances has led to taxonomic confusion and uncertainty, as well as an unstable taxonomy. In the particular case of the South American cottontail or Tapetí, and currently known as *Sylvilagus brasiliensis*, Linnaeus listed the type locality as "America Meridionali," that is: South America. As a result, *S. brasiliensis* acquired a vast widespread distribution in North and South America, over an area estimated as approximately  $1.09 \times 10^7$  Km<sup>2</sup>, and currently containing upwards of 37 named subspecies. Because the original description by Linnaeus was based on the posthumously published 1648 work of Georg Marcgraff, we restrict herein the type locality of *S. brasiliensis* to coastal Pernambuco State, near Recife, where Marcgraff largely was based. We select and describe a neotype from that area, specifically from a forest fragment in the Pernambuco Endemism Center of the Atlantic Forest Biome, located at 7°50'38.4"S, 35°6'7.3"W, elevation: ca. 137 m. Niche modeling suggests that *S. brasiliensis* as thus defined has a much more restricted range of 500–700 Km<sup>2</sup> with a narrow distributional band along northern Atlantic coastal Brazil. We compare the neotype of *S. brasiliensis* to holotype material of *S. andinus* and *S. tapetillus* and find sufficient distinction in characters and measurements between *S. brasiliensis*, *S. andinus*, and *S. tapetillus*, to retain the latter two as valid species level taxa. Molecular analyses based on limited sequences from the mitochondrial 12S rRNA and cytochrome-*b* genes support the distinction between *S. andinus* and *S. brasiliensis*. Genetic distances also support the lack of close relationship between the two taxa, with cytochrome-*b* showing a patristic distance between the two of 0.153, the largest interspecific distance among *Sylvilagus* species sampled. Our analysis of Lineages Through Time suggests that the vast unreported taxonomic diversity among Neotropical *Sylvilagus* is based on high speciation rates in the genus, combined with unusually low extinction rates. Episodes of explosive speciation in *Sylvilagus*, hypothesized to be ecologically driven, have been coeval with cooling events at the Miocene–Pliocene transition and following the Piacenzan Warm Period (Late Pliocene) into the Pleistocene; warm periods have in contrast slowed down speciation rates. The status of all named subspecies of *S. brasiliensis* will require careful scrutiny and comparison with the neotype to ascertain and establish species limits. Because of a lack of specimens of Neotropical *Sylvilagus*, our study suffers from a typological schema; however, our approach from first principles—examination of holotypes—not only serves to establish a more stable taxonomic framework for Neotropical *Sylvilagus*, but also further suggests that many of the existing taxa currently subsumed as subspecies within *Sylvilagus brasiliensis* likely are valid species in their own right.

## D2. Polymorphism of mitochondrial control region sequences in brown hares (*Lepus europaeus* Pallas, 1778) from Turkey with different coat coloration

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Turkey represents a biogeographic crossroads where possible long-distance gene flow from the Euro-Siberian, Irano-Turanian, and Saharo-Sindian faunal sub-regions may produce a rich admixture of phylogenetic lineages. In Turkish hares (*Lepus europaeus* Pallas, 1778) a number of coat colour variants have been described possibly reflecting the presence of distinct phylogenetic structuring. Initial mitochondrial d-loop sequence data indicated quite some diversity, but due to restricted geographical sampling no overall conclusions could be drawn. Here we analyzed sequence variation in the hypervariable part 1 of the mtd-loop region (476 bp) of 105 hares from different localities across Turkey to obtain a more comprehensive phylogeographical picture. We found 32 variable and 57 parsimony-informative sites which identified seventy haplotypes. Haplotype diversity (h) was 0.987, nucleotide diversity ( $\pi$ ) was 0.02707, and the average number of nucleotide differences (k) amounted to 12.8302. A maximum likelihood tree as well as analyses of molecular variance (AMOVA) revealed little (3.42%;  $p < 0.0001$ ) geographical partitioning of sequence variation among five regions. Partitioning of sequence variation due to coat colour variation (brownish vs. yellowish fur: 9.9%;  $p = 0.0098$ ; five coat colour classes: 7.41%;  $p < 0.0001$ ) was also relatively low. Concordantly, a median joining network of the currently analyzed Turkish haplotypes indicated a quite reticulate pattern without immediate phylogeographic signals. Our results indicate a high level of mtDNA admixture in Turkey and suggest that variation of coat colour phenotypes is to a large extent related to relatively recent local/regional environmental adaptations rather than to phylogenetic lineages. This accords to earlier findings of hares from Tunisia and Israel, which suggested little relevance of coat colours for phylogenetic assignment in the *Lepus capensis* (sensu lato)/*L. europaeus* complex.

### **D3. Lagomorphs as models for science education and outreach: Lessons from the evolutionary history of hares**

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Bridging the gap between academia and the society is a major challenge. But it is also a fundamental step for securing resources for research and to promote positive attitude changes towards science and science-based questions. Research on Lagomorphs touches several key aspects that can be used to narrow that gap. Furthermore, as highlighted in the recently published LaGomiCs White Paper, many Lagomorph species are familiar to the public, which facilitates the often difficult task of engaging the audience. In this work we discuss the potential of Lagomorphs for science education and outreach and use the evolutionary history of hares from the Iberian Peninsula as models to make complex evolutionary biology concepts accessible for different publics. Specifically, we will show how current research results on the evolutionary history of hares can be used to better understand evolution by natural selection and genetic drift, biogeography, climate change or the impact of hybridization on speciation and adaptation. We will also present two booklets about the evolution of hares, which build on a strong scientific component to communicate these topics and to propose educational hands-on activities that facilitate the understanding of the concepts.

## **D4. Taxonomic, conservation and management implications of reticulate evolution in Lagomorphs**

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Hybridization and gene exchange among species is a particularly common phenomenon in Lagomorphs, which suggests that it may have played and continue to play a major role in the evolution of the group. These observations raise a number of important related questions, namely on taxonomy and conservation. Additionally, when driven by anthropogenic pressures, introgressive hybridization may disrupt local adaptations, which highlights the importance of taking it into account while designing management and conservation practices. In this talk, we will revise the major forces driving the natural and anthropogenic hybridization on lagomorphs, innumerate examples in hares, rabbits and pikas and discuss how this should be taken into account in taxonomy, conservation and management.

## D5. The genomics of historical introgression in Iberian hares

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Introgressive hybridization between species is common in nature and can sometimes be massive. However, the causes and relevance of these phenomena in the evolutionary history of species is still far from being well understood. Up until recently, one major limitation was the fact that most studies could only be focused on a few loci, with a strong bias for the non-recombining mitochondrial DNA (mtDNA). With the advent of Next-Generation Sequencing we are now able to explore the genome-wide patterns of interspecific gene flow which can then be related with their functional content or genomic position and give us major insights into these phenomena. One prolific taxon for the study of introgression is genus *Lepus*, which is characterized by numerous cases of introgression, both past and ongoing. One striking case is the massive historical mtDNA introgression from the boreal mountain hare (*L. timidus*) into the northern populations of the Iberian hare (*L. granatensis*). This geographic structure and quasi-fixation of the introgressed variants in some populations contrasts with what was found for a few tested nuclear loci, which showed rare and geographically spread introgression. Such patterns evoke anecdotal byproducts of past hybridization, but a genome-wide survey could reveal regions of more massive introgression at some loci, eventually in relation to mtDNA massive introgression. Here we analyze complete genome sequences of 10 *L. granatensis* distributed all over Iberia, representing populations with and without introgressed mtDNA, 2 specimens of the donor species, *L. timidus*, and 1 outgroup (*L. americanus*). By applying phylogenetic (ABBA-BABA and evolutionary tree reconstruction) and distance-based (relative node depth) methods we pinpoint genomic regions introgressed from *L. timidus* into *L. granatensis*. We then characterize the genomic extent, frequency (sporadic or complete), geography and functional context of introgressed genomic segments. Particular attention is given to nuclear genes whose products interact with the mitogenome and its products, and thus may coevolve. Co-introgression of some of these genes with mtDNA would suggest a functional effect of genetic admixture, potentially adaptive. We also explore genes for which complete introgression was found, suggesting that natural selection may have caused the introgressed variants to sweep to fixation. We will thus present an unprecedented view of the role of introgression in shaping the evolutionary history of species.

## D6. Why do hares change color? Deciphering the genetic basis of seasonal coat color change using transcriptomics

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Hares (*Lepus* spp.) inhabit nearly all major ecotypes of the World, from the hot plains of the desert to the frozen landscapes of the arctic, each species displaying a set of remarkable adaptations to their specific environment. Boreal species of hares have developed the remarkable capacity to alternate between a white winter and a brown summer coat that allows them to remain cryptic in seasonally snow covered environments. However, the fitness of this adaptation is being threatened by shorter snow seasons that reveal white hares on a brown background in increasingly long periods. Revealing the molecular underpinnings of seasonal coat color change and alternate seasonal color phenotypes is therefore a valuable first step to understand the adaptive capacity of these boreal populations in a changing environment. In this work we address this question with transcriptomics by following gene expression changes along the molt of two species of hares. We sampled skin biopsies representing specific molt stages along the snowshoe hare (*L. americanus*) color changing spring molt and the mountain hare (*L. timidus*) color changing and non-color changing autumn molt. RNA was extracted and sequenced and the transcriptome assembled *de novo*. A differential expression analysis was then performed within and between species. This allowed us to describe the major expression waves along the molt cycle as well as identify specific gene pathways and functions activated in each molt stage, such as melanogenesis and circadian clock genes. With this work we provide a global picture of the molecular machinery behind seasonal coat color change and provide insight into the adaptive and evolutionary history of this remarkable adaptation.

## D7. Taxonomy of pikas (*Ochotona*) after the analysis of nuclear genes

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Taxonomic structure of *Ochotona* became more stable during the last 15 years. It is true especially concerning subgeneric structure, where molecular data converged with biogeography. Thus, there are four subgenera within *Ochotona* genus: *Ochotona* (burrowing species from Inner Asia), *Pika* (petrophilous species from the northern part of the genus distribution), *Lagotona* ("western" branch with one extant species *O. pusilla*) and *Conothoa* (species associated with the great Mountain chains in Asia). Notable advance was achieved at the species level too, with wide comparison of morphological and mitochondrial data. Several groups, however, display disagreement between these types of data or just data deficiency. Thus, *O. pallasii* and *O. alpina* were paraphyletic relatively *O. argentata* and *O. turuchanensis* respectively. The pair of species *O. erythrotis* and *O. gloveri* needs additional investigation due to high morphological similarity and scarce genetic information. The bigger part of disagreements falls into *Ochotona* subgenus. We studied MGF and PRKCI introns in order to solve some of these disagreements.

Paraphyly of *O. pallasii* relatively *O. argentata* was supported by nuclear genes. The level of genetic divergence between *O. pallasii* (Mongolia), *O. opaca* (Kazakhstan) and *O. argentata* corresponds to the species level.

Mitochondrial data in contrast to morphology showed identity of *O. nubrica* and *O. curzoniae*. Nuclear genes in our analysis differ these species well, though find them to be sister taxa. Thus the hypothesis about mitochondrial introgression in this pair was supported. Mitochondrial genes display very high variation within *O. thibetana*: this species includes at least 3 separate mitochondrial clades, which does not form well-supported monophyletic group. Nuclear genes support existence of these clades. Mitochondrial data conflict with morphology and nuclear genes in taxonomic position of taxon "morosa". Probable the latter is a separate species, which hybridize with *O. cansus*. It should be noted, that the level of variation within studied samples from *Ochotona* subgenus is notably lower than in other subgenera.

Another species, *O. syrinx* (former *O. huangensis*), which is morphologically similar to species from "thibetana" group, differ dramatically in both mitochondrial and nuclear genes. Actually this species should be separated in its own subgenus.

Interesting result was obtained for *O. turuchanensis*. This species turned out to be an internal branch within *O. alpina* in both mitochondrial and multilocus nuclear (using "species tree" approach) previous analyses. Analysing single loci, we found some alleles that is very different from alleles of *O. alpina*. Thus, probably the evolutionary history of *O. turuchanensis* is more complicated than simply a subspecies of Alpine pika.

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## D8. A multilocus phylogeny of pikas (*Ochotona*) and its implications for systematics and gene flow

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Systematics and evolution of pikas (genus *Ochotona*) have always been interesting because of the obscure phylogeny and incomplete taxon sampling of this taxon. In this study, 96-tissue samples from 11 valid species, three classified subgenera (*Pika*, *Ochotona* and *Conothoa*) from 27 locations were used to obtain multilocus sequences of 7031bp. Two mitochondrial (*CYT B* and *COI*) and five nuclear gene segments (*RAG1*, *RAG2*, *TTN*, *OXAIL* and *IL1RAPL1*) were sequenced. We analysed evolutionary histories using maximum likelihood (RAxML) and Bayesian analyses (BEAST) including coalescent-based approaches (\*BEAST) and test species boundaries using Bayesian *Posterior Probabilities* (BPP). Our study identified *O. syrinx* (*O. huangensis*) as a clade distinct from the other subgenera. Relationships among subgenera were not fully resolved, perhaps due to a rapid diversification during the middle Miocene (13.90 Ma [95%CI=9.91-17.53]), based on molecular divergence analyses. *O. thibetana* was found sister to *cansus-curzoniae* group (ML bootstraps [BS] =100 and Bayesian posterior probabilities [PP] =1) and *O. gloveri* sister to *O. forresti* (BS=96 and PP=1). Mitochondrial introgression from *O.cansus* to *O. curzoniae*, resulted in the elimination of *O. curzoniae* mtDNA by *O. cansus* was observed. We uncovered three cryptic species with strong posterior probabilities (BPP =1), each in a region of the Shaanxi (*O. sp. 1*), Sichuan (*O. sp. 2*) and Yunnan (*O. sp. 3*). Comprehensive morphological/morphometric analyses are needed for taxonomic revision. We recommend a subgeneric status for *O. syrinx* complex and propose the name of 'Huanghe', which means "Yellow River".

## E1. Introduced European rabbits in Australian ecosystems

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Following introduction in 1859, wild European rabbits (*Oryctolagus cuniculus*) caused serious economic damage in Australia, destroying crops and competing with livestock. Damage to native trees and shrubs also became a serious problem. Rabbits strip bark, often killing shrubs, and destroy seedlings, preventing regeneration. Changes in native pasture composition been quantified in relation to rabbit density; rabbits cause obvious changes at less than 1 rabbit/ha by removing the most palatable pasture species. At higher densities they promote introduced weeds and deplete vegetation cover. In some areas where pasture productivity is known, and rabbit density and food intake can be estimated, it is clear that rabbits can eat about half the pasture produced. How these changes specifically affect native fauna is less clear. Experimental work in national parks shows that where rabbits have been removed, or heavily controlled, large grazing herbivores such as kangaroos and wombats increased significantly. The release of rabbit haemorrhagic disease (RHD) saw similar increases in kangaroos as rabbits declined. Recently, large increases in the ranges of several species of rare small mammals have been reported. Indeed, the native hopping mouse *Notomys fuscus* has increased its distribution by an area the size of Portugal. The introduction of RHD, it is argued, has not only enabled restoration of suitable vegetation resources but also lowered the abundance of introduced foxes and cats that are considered major predators of small mammals. We can learn a great deal from these large-scale natural experiments and information has been gathered to help dispel several 'ecological myths'. For example, it has been argued by some ecologists that rabbits have become such a part of Australian ecosystems that removing them could spell disaster. However, all evidence suggests that the opposite is true: predators such as the wedge-tailed eagle (*Aquila audax*) now eat less rabbit but far more kangaroo and there has been no evidence of population decline of any raptors. Likewise, any prey-switching by introduced cats and foxes has been more than offset by the fall in numbers of these predators. We should not need to re-visit these ideas each time we consider introducing a new agent to help control rabbits

## E2. Old newspapers reveal the history of hare importations to Australia

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Trove, a project coordinated by the National Library of Australia, stores facsimiles of full-text back issues of newspapers and other historic documents and uses optical character recognition software to render the text into searchable form. Although confounded by smudged type, Trove is amenable to use as a tool for wildlife research. I used Trove to determine when hares were imported into Australia, where they were released, and the fates of the importations. I also used Trove, together with other sources of historical information, to determine the vegetation in which the hares were released. I used CLIMATCH to determine the extent of the match in climate between the origins and the destinations of the hares.

I found 41 attempts to import hares to Australia, 29 of which resulted in the landing of live hares. The c.70 hares that were sourced from England and five from Scotland highly likely were *Lepus europaeus* but the 11 hares sourced from Bengal, Ceylon, or Mauritius likely were *L. nigricollis*. Surviving hares from Britain were released at 14 locations resulting in the establishment of 11 populations of hares. Survival in captivity, but not release, of hares sourced from within the present distribution of *L. nigricollis* was detected.

There was a close climatic match between the locations where hares were known to have been released in Australia and the distribution of *L. europaeus* in Europe. However, sparse populations of hares occur at present in tropical areas of Australia where the climate match is low with Europe but moderately high with Bengal, Sri Lanka and Mauritius.

The vegetation at all release sites included grasses. The grasses were variably associated with open woodland and in one case each with forest or coastal saltmarsh. The grasses at all of the successful release sites were communities of mixed species that were mainly or dominantly C3 grasses. Two of the unsuccessful release sites were dominated by C4 grasses and one by a single C3 grass species. Native predators had been successfully suppressed at all but one release site; that site was close to dense forest and at that site two releases had failed to establish.

The grasses within the natural distribution of *L. nigricollis* are dominantly C4 species, as are the grasses within the tropical distribution of hares in Australia. Given that hares were imported to Australia from within the distribution of *L. nigricollis* and that the fates of these hares are not known, and given that the climate of areas further north matches that in Bengal and Ceylon, there is a need to determine whether *L. nigricollis*, or a hybrid, is present in Australia and to assess its pest potential.

Although the importation, release, and phoretic spread of hares in Australia was opposed by many small farmers, it was practiced and supported by wealthy landowners who were often parliamentarians and/or magistrates. They used their money, power, and influence to ensure that the hare would successfully establish in colonial Australia.

### **E3. Interspecific interactions between two sympatric species: Facilitation and competition between hare and rabbit in the context of predation risk**

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The geographic overlap between hare and rabbit is relatively recent. It has been suggested that both species co-exist by avoiding competition in spatial use. However, the spatial distribution of prey species is also determined by predators. During the behavioral response race prey trade-off food quality against predation risk. Interspecific interactions interfere with the food intake of the prey. To date, it is unclear which of these behavioral processes is most important in determining the spatial distribution of a prey species in a predator-multi-prey system. We tested the relative strength of (1) predation risk, (2) food quality and (3) interspecific interaction on prey species' patch use and the direction of their relationships. In this presentation we focus on the interspecific interactions between two sympatric prey species: the European hare (*Lepus europaeus*) and the European rabbit (*Oryctolagus cuniculus*). We applied a camera trap sensor network in various vegetation types that differed in predation risk.

Compared to vegetation types with a high nutrient concentration, rabbits spent more time in vegetation types with a low nutrient concentration, when hare spent more time in a patch. This suggests competitive exclusion of rabbits by hares, as both have a high degree of dietary overlap and avoided each other in time. Rabbits can sustain to graze on lower quality forage, because of their more efficient digestion compared to hares. Space use by wild rabbits could also be driven by antipredator behavior, because vegetation types with a on average low nutrient concentration were generally taller in mean herb height. It seems that apparent competition has increased the use of safer but lower quality patches by rabbits.

Although marginally significant, hares were probably facilitated by rabbits that created high quality habitats with a low mean herb height. Hares spent more time in patches with a lower mean herb height with increasing use of patches by rabbits. Rabbits are known to prevent biomass accumulation as important natural herbivores in coastal landscapes. Hares can thus benefit from rabbits in terms of an improved forage quality on grazed patches of lower mean herb height. They can also afford to be more selective, as they have larger home ranges and have access to a greater range of food resources. Subsequently, the benefit that hares have from rabbits could explain the lack of increase in hare population numbers as a reaction to a decrease in rabbit population numbers.

In the context of predation risk, our study shows that the patch use of hare and rabbit can be explained by their interspecific interactions.

## **E4. Dietary preferences of the European hare (*Lepus europaeus*): a herbivore selecting its diet for a high fat content**

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European hares (*Lepus europaeus*) of both sexes rely on fat reserves, particularly during the reproductive season. Therefore, hares should select dietary plants rich in fat and energy. However, hares also require essential polyunsaturated fatty acids (PUFA) such as linoleic acid (LA) and alpha-linolenic acid (ALA) to reproduce and survive. Although hares are able to absorb PUFA selectively in their gastrointestinal tract, it is unknown whether this mechanism is sufficient to guarantee PUFA supply. Thus, diet selection may involve a trade-off between a preference for energy versus a preference for crucial nutrients, namely PUFA. We examined the diet selection of European hares by analysing both the botanical and chemical composition of stomach contents and comparing these values with the food plants available in four seasons for three years in an arable area in Austria. We found that European hares selected their diet for high energy content (crude fat and crude protein), and avoided crude fibre. There was no evidence of a preference for plants rich in LA and ALA. We conclude that fat is the limiting resource for this herbivorous mammal, whereas levels of LA and ALA in forage are sufficiently high to meet daily requirements, especially since their uptake is enhanced by physiological mechanisms. European hares selected several plant taxa all year round, whereby preferences did not correlate to the plants crude fat, LA and ALA contents. Hence, European hares might not only select for plant taxa rich in crude fat, but also for high-fat parts of preferred plant species. As hares preferred weeds/grasses and various crop types while avoiding cereals, our results are in line with the suggestions to promote heterogeneous habitats with high crop diversity and set-asides to stop the decline of European hares across Europe. Moreover, farmers should be encouraged to sow set-aside and field strips in autumn. This would ensure that fresh food plants for European hares are available in winter when diet selection in this species is highly pronounced.

## **E5. Preweaning survival in brown hare leverets (*Lepus europaeus*)**

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Increased postnatal leveret mortality was identified as the main cause for the decline of brown hare (*Lepus europaeus*) populations in Switzerland. Even though, in terms of survival, the first few weeks in a brown hare's life are the most critical ones, we do not know in detail what happens during this stage. This might be because brown hare leverets behave very cryptically, which makes them difficult to study. Yet, it is critical to have a sound scientific knowledge about leveret mortality rates across different land-use types. Only then are we able to successfully and effectively mitigate factors promoting mortality and encourage measures increasing survival. We used thermal imaging and a specially trained sniffing dog to locate brown hare leverets. We radio-tagged 65 leverets in two different study sites in the Swiss lowlands, enabling us to monitor their spatial behaviour and activity levels. This information combined with mortality data was analysed as a function of different land-use types. Results show that survival varied according to different agricultural land-use types. This allows us to formulate practical measures for farmers intending to promote brown hares on their farmland.

## E6. Genetic diversity analysis and successful restoration of the Amami rabbit on Amami-ohshima Island, Japan

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The Amami rabbit, *Pentalagus furnessi*, is endemic to Amami-ohshima Island (712 km<sup>2</sup>) and Tokuno-shima Island (248km<sup>2</sup>) in southernmost Japan. On Amami-ohshima Island, about 30 small Indian mongooses were released to control the poisonous habu pit viper population at a public facility in a suburb of Naze City in 1979, but they have since expanded their distribution. Further, the mongoose did not become an effective predator of the habu pit viper population as expected. Instead, it became an invasive alien species and has had serious negative impacts on important native species on an island with a unique biota and high biodiversity. In 1974, before the introduction, the rabbit was distributed through almost the entire region of the island according to historical information. However, some of the regional disappearance of the rabbit included an area of mongoose distribution in a suburb of Naze City and an isolated population in a northern area of the island, as determined through our continuous pellet investigations between 1994 and 2003. However, as a consequence of the eradication project of the mongoose since 2005, remarkable success in restoring the rabbit in most of its habitat has been achieved, and the isolated population in the northern area is expected to be connected to the population in the southern area in a few years. To detect the influence of the population fragmentation, we investigated genetic structure of the rabbit populations on Amami-ohshima Island. We collected rabbit pellets from all over the island, sequenced the mtDNA control region and its 5'-flanking region (312 bp), and genotyped eight microsatellite DNA loci. The isolated population in the northern area had lower genetic diversity than the southern population, but genetic structure of the rabbit maintained the isolation-by-distance even though population decline would have caused low genetic diversity in the northern population. Therefore, when both the populations are connected to each other in the near future, gene flow between the populations will be reestablished. On the other hand, the Amami rabbit on Tokuno-shima Island is also isolated into two small populations, Mount Amagi in the north and Mount Inogawa in the south. The populations are separated by a large area of sugarcane fields and a large amount of paved roadways, which are probably a consequence of major agricultural development. Although our data on the distribution of the rabbits on Tokuno-shima Island is not as comprehensive as the rabbits on Amami-ohshima Island, we believe there is a genetic distance between the two populations on Tokuno-shima Island and that more genetic research on populations is necessary.

## F1. The anterior process of the malleus in extant Lagomorpha and its systematic and functional implications

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The anterior process of the malleus has a complicated evolutionary history as it is formed by the prearticular (gonial) and the ossified proximal Meckel's cartilage. Recent comparative ontogenetic studies show that in a number of placental mammals (especially Cetartiodactyla) the prearticular develops a prominent medial projection, the internal process (formerly described as 'ossiculum accessorium malleoli'). Functionally, the anterior process serves as anchor of the ear ossicle chain, and according to Fleischer the ear ossicles of *Oryctolagus cuniculus* therefore belong to the 'freely mobile type'. However, the extremely delicate anterior process almost inevitably brakes off when the ear ossicles are removed and the anterior articulation of the malleus is almost unknown.

Here we provide the first comparative description of complete mallei within extant Lagomorpha based on different ontogenetic stages. For this purpose, we studied histological serial sections of perinatal stages,  $\mu$ CT scans of adult cleaned skulls, and virtual 3D reconstructions of the malleus in 28 extant species of Leporidae and Ochotonidae.

Late fetal and early postnatal specimens of *Oryctolagus cuniculus* and *Lepus europaeus* reveal a distinct internal process of the prearticular that articulates with a groove at the underside of the tegmen tympani. This hinge joint allows a certain grade of mobility of the malleus, at least in juveniles. Adult leporids also show a delicate and thin internal process, but its inflated anterior portion is synostosed with the tegmen tympani; therefore, torsional movements of the malleus must be met by the elasticity of the thin pedicle of the internal process.

In a fetal *Ochotona* sp. the relatively long anterior process shows a very short internal process that does not contact the tegmen tympani at all. The anterior process of the malleus ends freely. During postnatal development the ochotonid ectotympanic bulla as well as the tegmen tympani becomes extensively pneumatized. The anterior process of the adults is very short and fused with a single trabecle of the tegmen tympani bony meshwork. Hence, considerable reorganisation of the anterior process must take place in perinatal stages of *Ochotona*, but because of lack of suitable material no details are known of this structural change.

Although the processus anterior of the malleus is synostotically connected with the tegmen tympani in all investigated adult lagomorphs, the mode of fusion is apparently not homologous in leporids and ochotonids. Based on comparison with the mallei in some rodents as outgroup, we assume that the middle ear of leporids in many regards is more plesiomorphic than that of ochotonids. The different patterns are clearly restricted to the family level and therefore can be interpreted as new autapomorphic groundplan features of Leporidae and Ochotonidae. Future studies on fossil Lagomorpha and of Rodentia are expected to further elucidate the ancestral morphotype of lagomorphs.

In contrast to previous assumptions, the malleus in Lagomorpha represents a highly modified 'transitional type' that still allows sufficient amplitudes during sound transmission by the thin and elastic bony pedicle of the internal process as observed in the adult stages.



## **F2. Effect of intra uterine crowding on fetal and placental development, irrigation, available uterine space per fetus and the characteristics of the uterine horns**

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The purpose of this work was to study in the rabbits of local Algerian population, the effect of intra uterine crowding on fetal and placental development, uterine horn characteristics and available uterine space per fetus. Thirty unilateral ovariectomized rabbit does were used in this experiment. The females were mated and sacrificed at 25 d of pregnancy. The right ovary and the uterine tract were collected, weighted, measured and the number of corpora lutea was recorded. The number of blood vessels reaching each implantation site was counted. The uterine horn was opened lengthwise and the position (oviduct, middle and cervix) and the status (live, dead or resorbed) of each fetus were noted. The fetuses and their placentas (fetal and maternal) were removed from the uterine horn, separated and weighted. Each fetus was dissected and the weight of its digestive tract, liver, brain and brown adipose tissue were recorded and its sex was noted. Finally, the weight and the length of the empty uterine horn were recorded. The traits measured on the fetuses were, available uterine space, placenta efficiency and intra uterine growth retardation. The ovulation rate, the weight of the ovary and the number of implanted embryos were double related to compensatory hypertrophy of the remaining ovary. The fetuses in oviduct position were heavier (+ 5%;  $P<0.05$ ), presented more brown adipose tissue (+ 22%;  $P<0.05$ ) and placentas weight (+19%, 0.05) than those in other positions. The fetuses in middle position presented higher value of placenta efficiency (8.15 vs 7.05 and 7.81 compared to those in oviduct and cervix positions respectively;  $P<0.05$ ). Available uterine space per fetus was significantly lower in the middle position compared to the other positions. There were no difference in the weight of maternal placenta and intra uterine growth retardation among the different positions within uterine horn ( $P>0,05$ ). All the traits measured in this study were influenced by the number of blood vessels reaching each implantation site and the best performances were noted for the fetuses receiving more than 6 blood vessels. However, no differences were found for all the traits measured between the fetuses male and female. The length and the weight of the empty and the full uterine horn were highly correlated to the number of implanted embryos ( $R= 0,78$  ;  $P<0,01$ ). The weight of fetuses were positively and significantly correlated to the available uterine space (0.68;  $P<0,001$ ) and to the development of fetal placenta ( $R = 0.70$ ;  $P<0,01$ ). The fetal position did not affect the proportion of dead fetuses. However, the fetuses with placenta receiving less than 2 blood vessels showed higher probability of death than those receiving more ( $P<0,05$ ). In conclusion, the intra uterine position and vascularization have influenced the development of the fetus and its placentas; however, at 25 d of pregnancy we did not find any difference between fetuses male and female for all the traits measured.

### **F3. Geometric morphometric and the cranial anatomy of leporids**

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The leporid cranium is a highly-transformed structure, and its shape has been recently shown to correlate with locomotor mode. In this study we use an expansive 3D data set derived from computed tomographic scans of nearly 200 crania of extant species to explore the functional significance of cranial shape evolution among leporids. We find dorsal cranial-arching strongly influences disparity among leporids; this trait is correlated to locomotor mode. Due to a limited fossil record, reconstructing the ancestral leporid cranium is difficult, but it is apparent that the highly-arched condition observed in many extant leporids has evolved multiple times. Further study suggests that morphological integration, particularly modular patterning, of the leporid cranium greatly influences overall patterns of dorsal arching.

## **F4. European brown hare (*Lepus europaeus* Pall.) sperm quantity in testes from different seasons and ages from Bulgaria**

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In order to be checked the sperm density, and the presence of spermatozoa in the epididymis of 315 testes from European brown hare of various ages (determined by dry eye lens weights) from Bulgaria, taken in inactive (January – September) and not active reproductive period (October – December), its tail of the epididymis has been cut. On the base of by slight microscope evaluation from the number (concentration) of spermatozoa were defined the quantity of sperm (Hubenov 1974). There were determinate 5 classes of sperm density (1 - no spermatozoa; 2 – single (very little) spermatozoa; 3 – sparse (moderately) sperm; 4 – medium thick (high) sperm; 5 – thick (massive) sperm). The quantity of spermatozoa was biggest from the beginning of the year until the end of August, after which followed a standstill from the beginning of September to the end of October. The testes activity was activated in November. Individuals up to six months of age had only the first and the second class of sperm, independently from the fact, that they were shot in June, July and August. In the age between seven and nine months, already there were individuals with class of sperm 4, which however, were born in the summer of the previous year from the year which they have been shot and follow the rhythm of the beginning of the active reproductive period from the end of the autumn, as it is in the adult hares (above 1 year old). The hares, born later in the season, i.e. after July, resemble to those, which were born in the beginning of the year (January – March), and show a strong growth of the reproductive tract at the age of 3 months. All hares, in age between ten and twelve months, had already reached the puberty and sexual maturity, and can participate in the reproduction (they had class of sperm from 3<sup>rd</sup> to 5<sup>th</sup>), independently from the fact, that they were born in the middle or in the end of the previous year from the year which they have been shot. The adult individuals had class of sperm 3, 4 and 5, during the whole reproductive period. Individuals born between May and June, which had reached the end of development of their body in the autumn, when the inactive reproduction period proceed, their puberty were postponed for several months, until the beginning of the next reproduction period. While for individuals, shot from October to December, with age up to six months, already had class of sperm 3. The young hares reach the puberty only in the reproductive period and reach sexual maturity at 2 - 3 months of age. The age at which the puberty is reached is depending on the date of birth. Individuals with age above 7 months were represented by all classes of sperm. For some of them, the reproductive period has not begun, and the testes were in standstill. Young hares (up to 1 year), during the whole year, were only 16 % with class of sperm 4 and 5. For adult hares, this percentage was double – 32 %. Young individuals, who had no spermatozoa or single ones, were 51 %, while for adult hares this percentage was 22 %. The average quantity of the spermatozoa was bigger for adults (46 %), than for young individuals (33 %).

## **G1. Effect of an experimental reduction of red fox on brown hare population dynamics**

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Predation may be a significant limiting factor for game population dynamics. In particular, red fox predation is often suspected to have a significant impact on brown hare demography.

We have assessed this impact by performing an experimental reduction of red fox abundance following a crossed control impact design on two adjacent and similar areas, Barrois and Sarce (around 200 km<sup>2</sup> each), during 6 years. From 2006 to 2008, intense red fox culling was applied in Barrois and basal culling in Sarce, and conversely from 2009 to 2011. Each year, we assessed red fox and brown hare population densities by distance sampling, and red fox culling rate and brown hare recruitment rate from hunting bag data. Intense culling revealed three-fold higher than basal culling. And, from similar initial red fox densities in Barrois and Sarce, around 1.5 fox/km<sup>2</sup>, intense culling during 3 consecutive years induced a decrease in red fox density of around 50% in both areas. We performed general linear modeling to assess the effect of red fox density and culling on brown hare demography. The reduction of fox density positively affected the recruitment rate of brown hare, likely through a better survival of young hares. Indeed, the proportion of young hares in the hunting bag, that was usually higher in Sarce than in Barrois, became higher in Barrois during the period of fox reduction. And this trend was reversed later when fox reduction was applied in Sarce. The increase of the proportion of young hares in the hunting bag was around 10%. However, at least at low density of hares, this gain had not a consistent clear effect on the following breeding population density of hares, possibly indicating some compensatory mortality of hares or a response depending on the predator-prey ratio.

## **G2. Preliminary study on reproductive performance of brown hares (*Lepus europaeus* Pallas, 1778) in Turkey**

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This study is based on 198 brown hare (*Lepus europaeus* Pallas, 1778) specimens obtained from climatically different regions of Turkey, and related field notes between 2006 and 2015 in Turkey. *L. europaeus* is one of the most important species as a food resource for carnivores and widely distributed in Turkey. However, biological data related to reproductive characteristics such as estrus, conception, birth period and litter size of does in Turkey are still limited. Data of reproductive performance exhibited normal distribution ( $P > 0.05$ ) in all regions. To test the effects of regional differences, an analysis of variance (ANOVA) was performed. Statistical analyses were carried out using PASW Statistics 18. Our results revealed that does from the Turkish Mediterranean had a significantly smaller mean litter size compared to Turkish Thrace and Central Anatolian ones. In addition, the mean litter size of does from the Black Sea region was larger than that in the Turkish Mediterranean, but this difference is not significant. Does inhabiting the continental climate region exhibit a short reproductive period between February and September, but those of the Mediterranean climate region have a small reproduction break in only November and ones in the Black Sea region have also a relatively small reproduction break in November and December. The breeding season of specimens from Turkish Thrace was shorter than that of the Black Sea and Turkish Mediterranean specimens, but longer than that of the Central Anatolian specimens. These results will contribute to an understanding of the reproductive performance of brown hare, *L. europaeus*.

### **G3. Estimating sustainable harvest rates in European hares using different population model approaches**

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Hunting, if sustainable, is an important conservation tool because the social and economic benefits derived from such use provide incentives for people to conserve them. To fulfill the demands of sustainable use hunters should base their harvest rates on sound population density estimates. We analysed age structure, reproductive performance, hunting bags and density trends of two hare populations in Lower Austria from 1999 to 2004 as well as climatic conditions in this area. First, a deterministic model of a generic population was constructed to model population growth and to conduct elasticity analysis. Secondly, indices of sustainable harvest rates were estimated without stochasticity and furthermore with added observed and modified stochasticity. Thirdly, the impact of two different Austrian hunting regimes on population dynamics were analysed and tested concerning sustainability. In terms of conservation, the simulations under a wider range of conditions, including unpredictable events, recommend only lower harvest rates of 10% and 15%, respectively in the long term. Changes in growth can happen suddenly and all the time and cannot be predicted on basis of former densities or survival rates. Therefore it is necessary to keep monitoring the population carefully every year to notice changes in population dynamics in real-time. Furthermore, trends in climatic conditions should be taken into consideration for setting hunting quotas since they can also function as predictors of survival rates and population growth. Results of this study show clearly that harvesting can only be sustainable in the long term if all factors affecting the population are taken into consideration and if hunting quota are adjusted continuously. We further suggest collecting long-term data on European hare concerning vital rates and densities but also on weather conditions, predation and disease-outbreaks, in order to stepwise adjust and validate the stochastic model for providing more precise simulations of sustainable harvest rates in the future.

## G4. Maternal nest building and perinatal offspring survival in the European rabbit

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Maternal care in altricial small mammals, including different species of rabbits, frequently involves the construction of a burrow or nest where mothers will give birth and raise their young. As burrow and nest building typically comprises a complex sequence of behaviours, delays in the timing of those behaviours and disturbances causing such delays might potentially interfere with mothers' successful reproduction. We investigated such purported effects in study of a fenced colony of European rabbits (*Oryctolagus cuniculus*) based on data from 11 years, where mothers gave birth in naturally constructed nursery burrows. We hypothesized that unusually late maternal burrow and nest building increases the occurrence of perinatal offspring mortality. Besides, we aimed to identify causes for the occurrence of such late burrowing and nest building behaviour. Most mothers dug their nursery burrow well before the last 24 h prior to parturition, and only built the grass nest or lined the nest with abdominal fur during the last day pre-partum. When mothers carried out all these tasks during the last 24 h pre-partum, the probability of perinatal litter mortality was significantly increased by around 25%. We suggest that social constraints might have directly affected mothers' patterns of burrow and nest building. Late building behaviour occurred with a significantly higher probability when females of the social group reproduced synchronously, i.e. when competition for breeding sites could be expected to be high. Our results suggest a pathway how mothers' social environment influences neonatal mortality, thus adding to our knowledge on drivers of maternal fitness.

# H1. Epidemiology of pulmonary protostrongyliasis in the European hare (*Lepus europaeus*) in France

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Pulmonary protostrongyliasis of hare is a parasitic disease caused by nematodes belonging to the genus *Protostrongylus* (Nematoda, Protostrongylidae). Between 2009 and 2012, two different worm species were morphologically characterized and identified by molecular biology in hares hunted or found dead in France: *Protostrongylus pulmonalis* (Frölich, 1802) and, for the first time in this country, *P. oryctolagi* (Baboš, 1955). One hare appeared to be co-parasitized by the two worm species.

We carried out a more specific study in south-eastern France to estimate the prevalence of pulmonary protostrongyliasis in hares and to identify intermediate hosts in two types of sites, mainly composed either of wine cultures or grasslands. *P. oryctolagi* was found in 55.3% of the 347 hunted hares which have been analyzed. Among these infested animals, 30% were severely affected, with visible lesions on more than half of lungs. The prevalence and parasite burden were higher for adults, which is consistent with an accumulative infestation of hares. Prevalence was much higher in wine cultures environment (around 80% for adults) than in grasslands (around 10% for adults). Even if not statistically significant, fecundity (number of placental scars) of highly infested females tended to be lower than others.

In order to understand the biological cycle of those parasites, we tried to identify their intermediate hosts. In total, 3315 terrestrial snails and 307 slugs were collected in the field in south-eastern France and analyzed to detect the presence of parasites. Identification of nematode parasites and snails were performed according to morphological and molecular approaches (D2 domain of the 28S rDNA for parasites; 18S and ITS-1 rDNA, COI and 16S mtDNA for snails). 18 snails were found positive for Protostrongylids larvae and haplotypes of the larvae corresponding to sequences of *P. oryctolagi* and *P. pulmonalis* were detected. Morphological and molecular identification of those molluscs revealed four different species: *Candidula gigaxii*, two different species of *Cerņuella* sp. and *Xeropicta derbentina*. All infested snails were collected in wine cultures and we noticed a positive relationship between the intensity of parasitic infestation in snail and hare populations.

The four snail species were also found in grasslands but their densities were lower than in wine cultures. That could partly explain the observed difference of prevalence in hares in those two habitats, even if the low parasite prevalence in snails suggest that intermediate host species may exist.

All together, and even if more studies are needed, those results significantly improve our understanding of the epidemiology of pulmonary protostrongyliasis in hare populations.



## H2. Effects of myxoma virus and rabbit hemorrhagic disease virus on the physiological condition of wild European rabbits: Is blood biochemistry a useful monitoring tool?

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Myxomatosis and rabbit hemorrhagic disease are the major viral diseases that affect the wild European rabbit (*Oryctolagus cuniculus*). These diseases arrived in Europe within the last decades and have caused wild rabbit populations to decline dramatically. Both viruses are currently considered to be endemic in the Iberian Peninsula; periodic outbreaks that strongly impact wild populations regularly occur. Myxoma virus (MV) and rabbit hemorrhagic disease virus (RHDV) alter the physiology of infected rabbits, resulting in physical deterioration. Consequently, the persistence and viability of natural populations are affected. The main goal of our study was to determine if blood biochemistry is correlated with serostatus in wild European rabbits. We carried out seven live-trapping sessions in three wild rabbit populations over a two-year period. Blood samples were collected to measure anti-MV and anti-RHDV antibody concentrations and to measure biochemical parameters related to organ function, protein metabolism, and nutritional status. Overall, we found no significant relationships between rabbit serostatus and biochemistry. Our main result was that rabbits that were seropositive for both MV and RHDV had low gamma glutamyltransferase concentrations. Given the robustness of our analyses, the lack of significant relationships may indicate that the biochemical parameters measured are poor proxies for serostatus. Another explanation is that wild rabbits might be producing attenuated physiological responses to these viruses because the latter are now enzootic in the study area.

### **H3. The source of myxomatosis and RHDV/RHDV2: Could the Americas' leporids provide Australia's next rabbit biocontrol?**

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Either directly or indirectly, the Americas feature as the primary source, and potential future source, of biological control agents for Australia's pest populations of *Oryctolagus cuniculus*. Myxomatosis, a largely benign disease in *Sylvilagus brasiliensis*, provided Australia in 1950 with its first biocontrol. Up to 2011 it is estimated this virus saved Australia's agricultural industries \$AUD53 billion. Highly pathogenic rabbit haemorrhagic disease virus (RHDV) became established in Australia in 1995 and, with myxomatosis, provided an additional \$AUD17 billion economic benefit. Recent research suggests that RHDV has also played a major role in landscape-scale recovery of native vegetation and 3 threatened mammal species. A new variant of RHDV, RHDV2 has become established in Australia in the past 12 months and is spreading rapidly – its impact is yet to be determined. There is now an argument that the RHDV, RHDVa and RHDV2 viruses may have emerged directly, or in recombination, from *Sylvilagus floridanus*. Leporid herpesvirus-4 emerged in 1990 in Alaska and Canada, infecting and killing *Oryctolagus cuniculus* in domestic and commercial rabbit farms. It has been hypothesised that the source of this virus may be from *Lepus americanus*. Finally, numerous large-scale introductions during the 1950s failed to establish *Oryctolagus cuniculus* in continental North America, despite extensive predator control being undertaken. There exists the possibility that these known pathogens, or additional undetected ones, were involved in the failures. Highlighting these failed introductions and pathogen jumps to *Oryctolagus cuniculus*, the leporids of the Americas are presented as the most likely source of additional biocontrols for Australia's pest populations of *Oryctolagus cuniculus*.

## H4. Detection of the new emerging rabbit hemorrhagic disease type 2 virus (RHDV2) in European brown hares (*Lepus europaeus*) from Italy and Spain

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Rabbit Hemorrhagic Disease virus (RHDV), a member of the genus Lagovirus, causes a fatal hepatitis (rabbit hemorrhagic disease = RHD) with a mortality of 80-95%. Since its first occurrence in Europe on 1986, RHDV has caused several outbreaks in wild and domestic rabbits, but never in European brown hares (*Lepus europaeus*) (EBH) and only once in two Iberian hares (*Lepus granatensis*). In 2010, a new RHDV-related virus, called RHDV2, emerged and rapidly spread in Europe, giving origin to extended epidemics, thanks to its specific antigenic profile that allowed RHDV2 to largely escape the immunity previously generated by RHDV in rabbit populations. During a 5-year spreading in Europe, RHDV2 was identified as cause of disease and mortality also in Cape hare (*Lepus capensis mediterraneus*) and Italian hare (*Lepus corsicanus*). Here we describe two distinct incidents of RHDV2 infection in EBH occurred in Italy and Spain in fall 2012 and spring 2014, respectively. One hare in North-Italy and two hares in Catalonia were found dead and the macro- and microscopic lesions found were highly suggestive of lagovirus infection. After necropsy, laboratory investigations were focused on diagnosis of EBHS. To better define the nature of the infection and confirm the initial diagnosis of lagovirus, two independent ELISAs, both based on specific MAbs produced against RHDV, RHDVa, RHDV2 and EBHSV were used to examine the livers of the three hares: i) the "typing ELISA" is a sandwich ELISA employing a panel of monoclonal antibodies (MAbs) able to identify the lagovirus species present in the samples (RHDV or EBHSV); ii) the "subtyping ELISA" based on a group of RHDV2 specific MAbs to specifically identify RHDV2. In addition the presence of EBHSV and RHDV2 RNAs was tested by RT-PCR using different sets of primers. In all three hares the identified viruses were characterized as RHDV2, genetically related to the other RHDV2 strains identified in Europe in the same areas and periods.

EBH is the most common hare species in Europe, often sharing the same living environments with rabbits. As consequence, EBH populations have been subjected to a high challenge by RHDV2 in the last years. The detection of just three hares affected by RHDV2 suggests that EBH is not a specific host of RHDV2 but, presumably it could be a spillover due to the high infection pressure of RHDV2 and the limited barriers existing between lagomorphs species. Thus, EBH may occasionally become infected with RHDV2, and also die showing typical EBHS-like lesions. To confirm that, a serological survey was conducted on hare sera and precisely 149 collected from North-Italy between 2007 and 2010 (pre RHDV2 period), 106 collected in the same period and areas in Spain where the two cases of RHDV2 in hares were identified, 154 and 253 collected from North-Italy respectively during 2012 and 2013. On the whole the seroprevalence for RHDV2 was very low with also generally low titres, thus suggesting that the RHDV2 infection was not widespread among hares. In conclusion, our findings contribute to improve the knowledge about the distribution and epidemiological characteristics of RHDV2.

## H5. Large scale detection of the rabbit haemorrhagic disease virus RHDV2 in European Hare (*Lepus europaeus*) populations in France causing European brown hare syndrome EBHS-like outbreaks

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Rabbit haemorrhagic disease (RHD) and European brown hare syndromes (EBHS) are two highly infectious and fatal viral diseases of European rabbits (*Oryctolagus cuniculus*) and both European and Mountain hares (*Lepus europaeus* and *Lepus timidus*), respectively. Their aetiological agents are two distinct lagoviruses (Family *Caliciviridae*). In 2010, a new genotype of RHDV, named RHDV2, with specific pathogenic, genetic and antigenic profiles, was identified in France. It has rapidly spread throughout European domestic and wild rabbit populations, then outside Europe. In France and in the Iberian Peninsula, RHDV2 has almost replaced the classical strains of RHDV. Moreover, it was shown to exhibit a broader host range by causing an EBHS-like disease in *Lepus capensis mediterraneus* and in *Lepus corsicanus*.

In France, a survey carried out in late 2010-mid 2011, while RHDV2 was emerging in rabbits, failed to detect this virus in European hares that died during an EBHS outbreak, suggesting that *Lepus europaeus* was not susceptible to RHDV2. However, recent increase in the number of negative EBHSV diagnostic results in hares showing clinical signs compatible with EBHS, lead us to look for RHDV2 in hares again.

We have carried out a retrospective survey by analysing the liver samples of 50 hares collected between 2013 and early 2015 by the SAGIR network and suspected to be dead of EBHS. They were screened for the presence of EBHSV using real-time RT-PCR then sequenced for genotyping following a RT-PCR that amplifies a part of the capsid protein (VP60) gene of both EBHSV and RHDV2. Complete VP60 sequences of EBHSV and RHDV2 were obtained for phylogenetic analyses following specific full-length gene PCR amplifications. We have also characterised the RHDV2 VP60 sequences from some infected rabbits collected during the same period.

Our results confirmed the occurrence of RHDV2 outbreaks in seven different hare populations located throughout France since November 2013. Phylogenetic analyses showed a strong epidemiological link between some RHDV2 outbreaks that occurred in both hare and rabbit populations.

In addition, we conducted an exhaustive molecular epidemiological study to estimate the prevalence of RHDV2-infected hares in 2015 using two real-time RT-PCRs for EBHS and RHDV2. We detected 70 cases of hares showing clinical signs compatible with EBHSV and infected by a lagovirus. Among them, 33 were infected by RHDV2 (47%) and 37 by EBHSV (53%).

Thus, our study confirms that RHDV2 can infect *Lepus europaeus* and induce severe lesions similar to those caused by EBHSV. This requires reconsidering the EBHS diagnostic tools. The high prevalence and the large geographic distribution of RHDV2 cases in hares suggest that this virus is becoming a major agent of EBHS-like disease in French hare populations and raises the question of the possible replacement of EBHSV by RHDV2. The enlargement of the host range of RHDV2 to *Lepus europaeus* may change the circulation pattern of the virus and consequently the epidemiology of the disease in sympatric hare and rabbit populations.

**POSTER PRESENTATIONS**  
(by first author)

## 1. Tenth anniversary of the monitoring of the management of the hare (*Lepus europaeus*) wildlife holding “Castello di Rascino” (Rieti, Italy)

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The Lazio hunting regional law envisions that in Italy there are different types of wildlife management for hunting purposes. Apart from the public and planned management, realized within the Hunting Territorial Areas (ATC), there is the private management. The latter can be exercised by Holiday farmhouse Companies allowing hunting (AATV) and/or the Wildlife Hunting Companies (AFV). For the AATVs is reserved 6% of the agroforestry-pastoral region, and they can be realized with business objectives in areas characterized by poor environmental value. For the AFVs is reserved 8% of the agro-forestry-pastoral regional territory, they should be realized in areas with high natural value and aimed at the gradual recovery of the ecological balance. The AFV have the dual aim of meeting the expectations of shareholding hunters and replenish self-sufficient population for the species of wildlife for which they were established. The Castello di Rascino AFV, located in the province of Rieti, was established in 2001 to manage Partridge and Hare. The first effect of the institution was limiting hunting access to members only, that imposed themselves to declare the hunted preys by immediately subjecting them to the supervision of the appointed wildlife technicians. The new control methods have produced a significant reduction in poaching. To increase opportunities for shelter and reproduction of the Hare, some inland areas were delimited within the AFV, in which hunting and dog training have been forbidden. The adoption of a pre-reproductive and post-reproductive monitoring program for the stocks it allows to keep track of the new dynamics of the lagomorph population. Also, the determination of the annual hunting amount is made considering the reproductive success and not in pre-reproductive period, as required by the competent authorities. The ongoing monitoring of the overall hunting is the guarantee that within the AFV is not shot down a greater number of preys than programmed. At any time of the hunting season, when the pre-set annual stock hunting is achieved, hunting is immediately stopped. Outside of the AFV the magnitude of the hunted preys is known only a few months after the end of the hunting season, when the number of slaughtered animals could be even higher than the target. To incentivize a culture tending to the gradual reduction of the killings, the AFV initiated a process of strengthening dog training. All this has led to a progressive reduction of the extent of repopulation, in accordance with the general technical-scientific ecommendations. These good practices conducted in the Castello di Rascino AFV resulted in a gradually increasing density of the Hare, which in a decade has grown from 1.3 to over 10heads/100ha, albeit with intermediate fluctuations. The recurring analysis of ear tags of the hunted animals shows a growing number of subjects born on site. To preserve the Hare on a large scale is beneficial that, like the AFV, also in the ATCs hunters are required to daily record the preys taken in the Authority's website, so that when the pre-established hunting stock is achieved, the hunt can be immediately stopped.

## 2. Monitoring of the European hare (*Lepus europaeus*) in the Natural Reserve of the Lungo and Ripasottile lakes (Rieti, Italy)

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In the Province of Rieti the *Lepus europaeus* is monitored for over a decade by the Hunting geographical areas (ATC Rieti1 and Rieti2) in the areas destined for hunting. The Lazio Regional Law 17/1995 (Art. 29, paragraph a) requires that in those territories the species status is annually updated according to the criteria included in the three-year management Plans. This allows us to quantify the amount of the hunting (which according to the Law 157/1992 has to be sustainable), and the perspective repopulation actions. To this end both the ATCs will have a similar monitoring system structured on sample areas covering at least 10% of the territories of their respective jurisdiction. In these areas the Spot Light Census (SLC) technique is applied. Therefore, the population dynamics of the species are potentially known in hunting areas and unknown in protected areas. In fact, apart from some sessions carried out to check the presence/absence of the Italic Hare (*Lepus corsicanus*), no protected area of the Province has ever adopted special monitoring programs for the species. These are the conditions which prompted the Natural Reserve of Lakes Lungo and Ripasottile (RNLLR) to enable a suitable five-year plan of the lagomorph monitoring on its territory. The goal is to keep under constant observation the dynamics of the species, also regarding the repopulation actions carried out in adjacent areas. It is not to be underestimated or to be excluded that there may be a migration of subjects from hunting areas to the protected area (assessed as the seasonal changes in the amount). In order to obtain comparable data with those of the hunting areas also in the RNLLR, the SLC technique is applied and the monitoring program provides that the detection sessions are carried out in pre-reproductive periods, post-reproductive periods, and subsequently to the repopulation activities carried by ATC. Given the fact that the RNLLR extends for about 3.000ha, that the SLC requires to inspect not less than 10% of the entire investigated territory and that the headlights used emit a light beam with a useful depth of 75m (resulting in a 150m buffer) are been identified and georeferenced with the GIS system approximately 12.5km of paths. This way the total inspected area during each detection session is approximately 317.6ha, equal to 10.6% of the entire protected area. After some simulation sessions, designed to detect and solve any potential problems due to the tracks (if necessary adapting them to the plant cover) and after the development of equipment for use, in late autumn/winter period (pre-reproductive period) were performed 5 official detection sessions, in each of which has been intercepted an average of 9.6 subjects (S.D.=±3.29), which have allowed to estimate an average density of 3.02 hares/100ha. Whereas the density of the hare in the areas of the ATCs is estimated between 1.5 and 1.8 units/100ha, the action of the species conservation put in place by the RNLLR proved itself substantially effective, although the density is still quite low, an issue that deserves to be studied.

### 3. Hunting management of the *Lepus europaeus* in the Rieti Province (Italy): species status after ten years of repopulation and hunting

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In the Rieti Province the *Lepus europaeus* is highly coveted hunting prey, but the data shows it's no longer able to meet the expectations of the hunters nor the absolute guarantee of preservation. In the Province the presence of the species is largely derived from the repopulation, made mostly using animal head imported or bred in captivity and released in the imminence of the killing. The Provincial Wildlife Hunting Planning (PFV) plans to limit the repopulation considering one-time actions to be carried out where considerable rarefactions occur. Regarding the Hare the PFV reiterates that the main management problem is the hunting wasn't proportioned to the real productivity of populations. Therefore repopulation should be occasional, given the ineffectiveness due to poor survival of the hares released. In practice, however, they have become recurring activities. Also, they should adopt hunting plans based on proper technical and scientific basis. For the progressive reduction of repopulation, in the Three-Year Management Plan (PTG) of the Territorial Hunting Areas (ATC-Rieti1, ATC-Rieti2) we wish for: the promotion and recovery of vital Hare units and the monitoring, the proper implementation of planned hunting, promotion of Restocking and Capture Zones(ZRC). In practice, however, those who manage the wildlife-hunting sector acquire and maintain consensus basically in relation to repopulating actions that, therefore, are not made on the basis of environmental suitability levels of the different regions, but based on the number of registered hunters. These dynamics make the restocking actions barely transparent and not all the data is of public domain. By analyzing those available, relating to repopulation made in 2005/2006 and 2008/2009 in 44 municipalities of the Province of Rieti (60.3% of the total) and 2 ZRCs, show that there has been a gradual reduction of releases: averagely 22.1hares/municipality (S.D.=±21.3) in 2005/2006; average 17.3hares/municipality (S.D.=±13.9) in 2006/2007; average 13.7hares/municipality (S.D.=±15.3) in 2007/2008; average 12.3hares/municipality (S.D.=±12.5) in 2008/2009. The trend is in line with the indications of the PFV, but to fully understand the management policies it should be checked if the decreasing number of released hares depends on increasing the price per head of the released hares or ATCs are allocating a gradually decreasing percentage budgets to the repopulation. In fact, despite all the taken actions, the average density of the Hare in pre-reproductive age in ATC territories is estimated between 1.5 and 1.8 units/100ha. In contrast, in the adjacent Regional Natural Reserve of Lungo and Ripasottile Lakes is estimated an average density of more than 3 heads/100ha; demonstrating the improved status of the species in an area not subject to either hunting or to repopulation. It can be concluded that in the face of ATC wildlife hunting planning, usually correct in technical terms, since they are subject to the supervision of the competent bodies (ISPRA, Provincial Administration, Regional Administration etc.) application stages that do not comply with the programming are followed. And regarding this discrepancy between planning and implementation, unfortunately, in Italy there are neither checks nor penalties against those responsible. All at the total expense of the conservation of species.



## 4. Hunting effort, preservation of hare and legislative dysfunctions: The case study of the Rieti Province (Italy)

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In Italy the Law 11.2.1992/157: "Regulations for the protection of warm-blooded wildlife and for hunting" in Article 1 states: "Hunting is permitted provided it does not conflict with the need of wildlife preservation...". Hunting is subject to preservation, a goal which should not be in jeopardy due to the number of killings. The number of animals left at the end of each hunting season must ensure the adequate increase in population of the species, allowing preservation and perpetuation of the hunting. The hunting calendar of the Lazio Region, a regulation from 1995 establishing an annual quality and quantity of the hunting, grants the killing of 5 hares/hunter. Not establishing the total theoretical regional hunting allowed, this regulation makes it a function of the number of hunters. Potentially the approximately 5,000 hunters who work permanently in the Province of Rieti can kill up to about 25,000 hares/year. The current management system, does not allow to monitor the ongoing overall sample, or to suspend the hunting on reaching the "stock of a sustainable hunting". The number of killings, in fact, becomes known only a few months after the end of the hunting season, when the hunting badges screening is run, where every hunter must write down the daily slaughtered preys. This study aims to test whether the system in place is an absolute guarantee of conservation of the species. To do this, the number of hares theoretically present in the whole Province of Rieti was estimated. To this end, in order to avoid errors of underestimation, in all the territory was given a good suitability to the presence of the species. Compared to hunting the provincial land management it is divided into two Hunting Areas (ATC-Rieti1, ATC-Rieti2). In both ATCs the Hare is monitored through Spot Light Census (SLC) in pre-reproductive age. The first has jurisdiction over approximately 113,000 ha, the average density is 1.48 units/100ha (S.D.=±0.68) and the estimated amount in about 1,670 hares. The second has jurisdiction over approximately 95,000ha, the average density (estimated at 5 sample areas) is 1.84 units/100ha (S.D.=±0.96) and the estimated amount in about 1,750 hares. For an estimated total of 3,420 hares. Giving to the SLC a tolerance of ±20%, the highest estimated presence of the species in ATCs in the pre-reproductive age is about 4,100 hares. Because in the Province of Rieti, Annual Profit Increase of the Hare is 100%, it is conceivable that at best the amount of the species at the start of hunting season is about 8,200 hares. Given that in the face of such amount is granted implicitly and theoretically a killing potential of about 25,000 hares, it is clear that, in fact, the adopted legislative system does not constitute an absolute guarantee of preservation of the species. To solve the issue it would be sufficient to impose on the ATCs the prior quantification of the annual sustainable killing (related to the updated status of the species and the number of hunters allowed) and to the hunters the daily recording of the shot animals on the ATCs site.

## 5. Diversity, extinction, and threat status in lagomorphs

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A quarter of all lagomorphs (pikas, rabbits, hares and jackrabbits) are threatened with extinction, including several monotypic genera. Genus species richness correlates with extinction risk in lagomorphs, but not in other mammals. Non-random extinction of small clades disproportionately threatens genetic diversity and phylogenetic history. Phylogenetic analyses suggest that lagomorph threat status was not related to body size, and there was no phylogenetic signal in extinction risk. Extinction risk was greater in areas of higher human population density and negatively correlated with anthropogenically modified habitat. Consistent with this, habitat generalists were less likely to be threatened. Our models did not predict threat status accurately for taxa that experience region-specific threats. Pressure from human populations is so severe and widespread that it overrides ecological, biological, and geographic variation in extant lagomorphs.

## 6. Effect of intra uterine position, vascularization and sex of the fetuses on their corporal composition in rabbits of local Algerian population

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The objective of this work was to examine the effect of intra uterine position, vascularization and sex of rabbit fetuses at 25 d of pregnancy on their corporal composition. Fifty seven unilaterally ovariectomized multiparous rabbit does were used in this study. The females were mated and sacrificed at 25 d of pregnancy. Immediately after, the uterine horn was examined in order to count the number of blood vessels reaching each implantation site, the position of each fetus within the uterine horn (oviduct, middle or cervix) and their sex by direct examination of gonads. Only the females with litter size near to the mean of the population were used (n = 14). For each female, three fetuses were chosen (one from the oviduct position, one from the cervix position and the last one from the middle position). The fetuses (n = 42) were frozen and stored at -20 °C until analysis. Representative samples of ground matter were freeze dried and analysis for dry matter, humidity, protein, ash, lipids and energy contents. The intra uterine position did not affect the ash content, dry matter and rate of humidity. Conversely, the protein, energy and lipid contents were significantly higher for the fetuses in oviduct position compared to the other positions (+15 %; P<0,05). The fetuses with implantation sites receiving equal or more than 6 blood vessels showed higher percentage of protein (+9%, P<0,05), lipids (+13%; P<0,05) and energy contents (+16%; P<0,05) than those receiving less. Finally, there were no differences for all the parameters measured between fetuses of sex male and female. In conclusion, at birth, the fetuses from the oviduct position or receiving higher number of blood vessels should have high probability of live related to higher fat and protein reserves.

## 7. Effect of anogenital distance before mating on behavior, litter size and its biological components in rabbits

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The aim of this work was to test in rabbit of local Algerian population, the effect of Ano-Genital Distance (AGD) before the mating on, sexual behavior, litter size and its components (ovulation rate and prenatal mortality) and sex ratio (ratio of male pups to females pups at birth). In total, 64 multiparous rabbit does were used in this experiment. At the moment of mating, the AGD was measured by three operators and the behavior of the females was noted. At 12 d *post coitum*, an endoscopy was realized on the pregnant females in order to measure the ovulation rate (number of non-hemorrhagic *corpora lutea*) and the number of implanted embryos (live and resorbed). At kindling, the number of pups (live and dead) and their sex were noted. The females with larger AGD were more aggressive (25,4% vs 8%;  $P < 0,01$ ) but presented similar receptivity rate when compared to the females with shorter AGD (82 vs 86%;  $P > 0,05$ ). At 12 d of pregnancy, the effect of the AGD was not significant on the ovulation rate (9,22 vs 9,35;  $P > 0,05$ ). However, the females with larger AGD presented higher early embryonic and fetal mortalities than those with shorter AGD (+45% and +57% respectively;  $P < 0,01$ ). The females with larger AGD gave birth to almost 62% male pups. Conversely, the females with shorter AGD gave birth to about 41% male pups. In conclusion, the AGD in rabbits has influenced the majority of the traits related to reproduction and more hormonal investigations are necessary in order to understand more the origin of results obtained.

## **8. Characterization of local Algerian population of rabbits: Study of the external morphometry and carcass traits**

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This work was undertaken in order to identify the rabbits of local Algerian population by the study of its external morphometry and carcass characteristics. In total 220 rabbits from both sex and at different ages (from 1 to 6 months) were used for the morphometry study. 12 morphological traits were measured and used to compare between young and adult in one hand and male and female in other hand. 60 male and female (30 per group) were sacrificed at the age of 6 months in order to study the traits of the carcass. The rabbit of local Algerian population is characterized by several colors of phenotypes. The weight of the rabbit at the adult age was  $2622 \pm 142$  g allowing the classification of this population in category of small breed. The majority of the morphometric parameters measured in this study were influenced significantly by the age of the animal. There were no differences between males and females for the majority of the traits except for, the distance between the eyes and the length of the head (+15% for the male;  $P < 0,05$ ). At the slaughtering, the females were slightly heavier than male (+8%;  $P < 0,05$ ) without any effect on the carcass yield. However, they presented more adiposity than males (+10 % for perirenal and inter scapular fat;  $P < 0,01$ ). This study is considered as preliminary research in order to characterize the rabbits of local Algerian population and more investigations by molecular methods seem necessary for its complete identification.

NOTE: Ali Berbar was unable to attend the conference. No poster was displayed.

## 9. Resurrection of the Tamaulipas white-sided jackrabbit: *Lepus altimirae*

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Abstract—American mammalogy as pioneered by C. H. Merriam was flawed in that his taxonomy was importantly determined by morphological measurements and pelage characteristics that were often highly variable. As an example, his and E. R. Hall's classification of different grizzly bear species and other mammals had the incongruity of inhabiting the same mountain range. Not only were some closely related taxa misclassified as separate species, other distinct species are in danger of being ignored. Such errors, formerly of only academic interest, now have legal complications due to the Endangered Species Act, which gives current systematics the power of law. Molecular genetics can be less ambiguous to resolve taxonomic units. A case in point is our resurrecting the Tamaulipan jackrabbit (*Lepus altamariae*) based on DNA evidence. This leporid, originally classified as *L. altimirae* by E. W. Nelson, and was later reclassified by E. R. Hall as a member of the *L. californicus* group based on morphological characters, where it remained until this genetic study. The *L. altimirae* classification is also more parsimonious with the biogeographical patterns of the region. We suspect that the systematic relationships of other lagomorphs are also in need of revision inclusive of DNA analyses (e.g., *L. townsendii*). We believe that the *L. californicus* group in particular should be tested in that several of the subspecies assigned to this species are not ecologically separated or isolated.

## 10. A new method for lagomorph density and abundance estimation using camera trap survey Random Encounter Models

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Camera traps are used to estimate densities or abundances using capture-recapture and, more recently, Random Encounter Models (REMs). We deploy REMs to describe an invasive-native species replacement process, and to demonstrate their wider application beyond abundance estimation. The Irish hare *Lepus timidus hibernicus* is a high priority endemic of conservation concern. It is threatened by an expanding population of non-native, European hares *L. europaeus*, an invasive species of global importance. Camera traps were deployed in thirteen 1 km squares, wherein the ratio of invader to native densities were corroborated by night-driven line transect distance sampling throughout the study area of 1652 km<sup>2</sup>. Spatial patterns of invasive and native densities between the invader's core and peripheral ranges, and native allopatry, were comparable between methods. Native densities in the peripheral range were comparable to those in native allopatry using REM, or marginally depressed using Distance Sampling. Numbers of the invader were substantially higher than the native in the core range, irrespective of method, with a 5:1 invader-to-native ratio indicating species replacement. We also describe a *post hoc* optimization protocol for REM which will inform subsequent (re-)surveys, allowing survey effort (camera hours) to be reduced by up to 57% without compromising the width of confidence intervals associated with density estimates. This approach will form the basis of a more cost-effective means of surveillance and monitoring for both the endemic and invasive species and is widely applicable to the density and abundance estimation of other lagomorphs.

## 11. Invasion ecology of non-native European brown hares and their impact on the endemic Irish hare

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The European brown hare (*Lepus europaeus*) is a highly successful invasive species worldwide. It was introduced to Ireland in the late 1800s for field sports with a substantial population discovered in Northern Ireland during 2005. Ecological Niche Modelling demonstrates that the endemic Irish hare (*Lepus timidus hibernicus*) is distinct from other mountain hare subspecies and is ecologically similar to the invading European hare. Both hare species have comparable niche breadths and almost complete niche overlap. There is the potential for strong interspecific interactions for space and resources. Projections of predicted climate change suggest the fundamental niche space available for the Irish hare is likely to contract whilst the that of the European hare is likely to expand in Ireland by 2070, increasing the likelihood of negative impacts of the invader on the native. Recent surveys suggest that the range of the invader expanded three-fold between 2005 and 2012-13. Camera trap Random Encounter Models captured spatial patterns in hare density and abundance which describe an invasive-native species replacement process, with invader outnumbering the native 5:1 in its core range. Expansion of its range suggests the European hare is dispersing at 0.73 km/year and backward extrapolation places the local origin of the population at ca. 1970 (considerably more recent than historical introductions). A social science questionnaire suggested that there is public support for Government intervention, specifically lethal culling. Support was strongest among respondents who were members of organisations that support countryside pursuits such as shooting. Invasive European hares represent an immediate threat to the endemic Irish hare. Rapid development and implementation of an invasive Species Action Plan (iSAP) is urgently required.



## **12. Long-term patterns in Iberian hare population dynamics in a protected area (Doñana National Park) in the SW Iberian Peninsula: effects of weather conditions, plant cover and predation**

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The Iberian hare (*Lepus granatensis* Rosenhauert, 1856) is a widely distributed endemic species in the Iberian Peninsula. Although knowledge of the species has increased considerably, there is still little information about the population dynamics and trends in the Iberian hare. The relative abundance and population trends of the Iberian hare were studied in the autumns of 1995–2012 in a protected area (Doñana National Park) by spotlighting. A total of 1,068 km of line transect sampling was carried out. The average relative abundance was 0.38 hare/km (sd = 0.63) in marshland and 3.6 hares/km (sd = 4.09) in ecotones. The Iberian hare population exhibited local inter-annual fluctuations and a negative population trend during the study period (1995-2012). The Iberian hare was low survival rates in marshland in a flooded year. We use linear model analysis to assess the importance of monthly rainfall, monthly surface flooding area and plant biomass/cover availability on the hare abundance in the marshland and ecotone areas. The flooding of the marshland in June, July and October favour hare abundance in the ecotone. The hare abundance in the marshland, was negatively affected by the herbaceous plant biomass and flooded area in November. It was observed a positive relationship between abundance indices and rainfall, and autumn flooding.

### 13. Identification of a new non-pathogenic lagovirus in brown hares (*Lepus europeaus*)

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European Brown Hare Syndrome (EBHS) is a viral disease mainly observed in European brown hare (EBH) (*Lepus europeaus*). Its causative agent (EBHSV) belongs to the *Lagovirus* genus and is highly related to Rabbit Hemorrhagic Disease Virus (RHDV). A third group of *Lagoviruses*, named rabbit caliciviruses (RCVs), detected in rabbits in Europe and Australia, cause silent infection of the intestinal tract without clinical signs and relevant lesions. The existence of a non-pathogenic EBHSV-like virus in hares was put forward for explaining some "unexpected" positive serological results obtained in EBH and other *Lepus* species from areas (Australia, South American and Central Africa) where the disease has never been detected.

We report the results of investigations performed to seek this putative virus eventually resulted in the first identification of a non-pathogenic Lagovirus in hares. On the basis of the present lagovirus nomenclature, we propose to preliminary name the new virus as "Hare Calicivirus" (HaCV).

Faecal and blood samples from 37 healthy hares, 1-2 months of age, born and reared in cages in a breeding hare farm of Brescia province (North Italy), were collected during summer 2014. The serological survey was repeated one year after (2015) testing 18 young hares from 7 separate cages to check the persistence of infection and virus circulation.

Total RNA was extracted from feces of hares and analyzed by RT-PCR using the universal primers for lagovirus able to amplify a conserved region of the vp60 gene. Three hares resulted RT-PCR positive for the presence of a lagovirus. The sequence analysis of the entire capsid protein vp60 gene showed an average of nucleotide identity of approximately 73% and 68% compared respectively to the EBHSV and RHDV sequences present in GenBank. To perform the phylogenetic analysis the entire vp60 gene sequences were aligned with those of lagoviruses available in GenBank. Thus, the three isolates were shown to be distinct from all previously described members of the genus *Lagovirus* and to form a new, separate genetic group in the clade of EBHSV.

Serological analysis was performed on blood samples using distinct MAbs-based ELISAs: i) a competition ELISA for Ab-EBHSV (EBHS specific); ii) an IgG ELISA able to detect EBHSV cross-reactive antibodies (lagovirus specific); iii) and iv) specific direct ELISAs for IgG and IgM anti-HaCV by using a specific recombinant antigen produced in baculovirus, adsorbed on the solid phase.

By using serological ELISAs contrasting results were obtained: in the case of competition ELISA most sera resulted negative with just few positive at very low titers, whereas most sera resulted positive in IgG ELISA with medium-high titers for cross-specific antibodies. Medium-high levels of IgM and/or IgG against the homologous HaCV were found in the sera of most hares tested both on 2014 and 2015, thus suggesting that HaCV was probably present since some time and was still circulating in the farm. This aspect and the lack of any sign of disease among the hares show that the viral infection has likely a subclinical course and thus the virus could be considered non-pathogenic at least since the final demonstration given by experimental infection.

## 14. Praying hares is the sole method used by carnivores for causing their death?

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Carnivores come into contact with many infectious agents due to their position at the top of the food chain. Thus, they could be potential carriers of infectious agents that occur in their prey species, even if they are not susceptible and not infected and do not develop clinical signs. This is the case of lagoviruses, which are specific agents of severe disease in rabbits (Rabbit Hemorrhagic Disease-RHD) and hares (European brown hare syndrome-EBHS). EBHS is a highly contagious disease of brown hares (*Lepus europaeus*) and, with lower frequency, of mountain hare (*Lepus timidus*) and Italian hare *Lepus corsicanus*). When introduced into a naive brown hare population, EBHSV achieves almost 100% morbidity. Mortality is about 50% in the adult age class but absent in young individuals <2-3 months of age, which become infected, do not exhibit clinical signs but seroconvert developing a long-lasting immunity. The virus is considered endemic in all European countries, forcing the adoption of surveillance programs for controlling the diffusion of the disease and the dynamics of hare populations.

During the annual serological surveillance of hares captured for restocking in a protected open area, managed as 'breeding-for-restocking' ground, one dead hare was found, collected and examined. Few days after, four red foxes were hunted in close proximity of the same area and then delivered to IZSLER for laboratory investigations, according to the Regional program for wildlife monitoring.

At necropsy the typical lesions suggestive of EBHS were found in the hare carcass i.e. petechial lung haemorrhages, friable, fatty and discolored liver, splenomegaly. The diagnosis of EBHSV was then confirmed in the liver and spleen homogenates by sandwich ELISA and RT-PCR. All the hare sera sampled during the capture operation tested positive in serological c-ELISA with medium-high titers, thus indicating an active circulation of EBHSV in that area. Thereafter, in order to elucidate the epidemiological role of predators and to confirm previous experimental data indicating the possibility that carnivores, after having predated diseased or dead lagomorphs, can excrete infectious lagoviruses with feces, we tested by RT-PCR for the presence of EBHSV the liver, spleen, mesenteric lymph nodes and contents of different part of the gut (duodenum, cecum and rectum) of the four red foxes. The intestinal content of one fox resulted virologically positive, whereas the other organs and all samples of the remaining three foxes resulted negative. Among the food debris present in the gastrointestinal contents of the positive fox we found some materials that were genetically identified as of hare origin. The 4 foxes were also serologically tested for EBHSV antibodies by c-ELISA with negative results. The identified EBHSV strains from hare and red fox were amplified, sequenced and compared. Partial sequence of VP60 gene amplified from the positive fox, showed a nucleotide identity of 96% compared to the Italian reference strain EBHSV\_BS89 (X98002).

These results proved, in natural condition, the possible epidemiological role of carnivores as passive vectors of EBHSV. In particular, red fox feeding on infected hares might contribute to spread infectious viral particles, thus promoting the persistence and occurrence of EBHS cases.

## 15. Comparison of four techniques for indexing rabbit abundance in arid scrub habitats

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Leporid abundance is indexed for a variety of reasons including to monitor population dynamics, assess prey availability for carnivores, and measure responses to environmental variation (e.g., drought, habitat impacts). We evaluated the efficacy of 4 techniques to survey for rabbits (black-tailed jackrabbits *Lepus californicus* and desert cottontails *Sylvilagus audubonii*) in arid shrublands and grasslands in the San Joaquin Valley of California. The 4 techniques were walking transects, aerial transects, spotlight transects, and scent stations. All 4 techniques were implemented in the Lokern Natural Area during April 2004. We evaluated each technique with respect to strengths, weaknesses, effort, and cost. Walking, spotlight, and helicopter transects all provided species-specific counts of individuals that can be used to derive an index of abundance (e.g., number/km) or to derive a density estimate by determining the effective transect width using distance measurements. Scent stations provided a visitation rate but the number of individuals and species could not always be determined. Walking and helicopter transects measured diurnal habitat use, spotlight transects measured nocturnal habitat use, and scent stations measured both. For walking and spotlight transects, leporid observability was reduced in shrublands compared to grasslands resulting in a habitat bias. Road availability can limit access for walking transects, spotlight transects, and scent stations but does not limit helicopter transects. Leporid indices in shrublands were 5.79/km on helicopter transects, 1.53/km on walking transects, 0.5/km on spotlight transects, and a 52% visitation rate on scent stations. Indices in grasslands were 2.94/km on spotlight transects, 0.27/km on walking transects, 0.04/km on helicopter transects, and a 40% visitation rate on scent stations. Scent station and walking transects were more labor-intensive than spotlight and helicopter transects. For two biologists working an average day, estimated achievement and costs (labor, materials, travel) were 25 scent stations (\$26.79/station), 80 km of helicopter transects (\$24.00/km), 30 km of walking transects (\$11.04/km), and 50 km of spotlight transects (\$6.80/km). Objectives, study site attributes, and budgets will determine which survey technique might yield the best results and be most cost-effective for a given project.

## 16. The rabbits of Okunoshima: Rewilding the domestic or domesticating the wild?

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Okunoshima Island, located in the Hiroshima Prefecture in southern Japan, has been host to a large population of feral rabbits since at least the 1970s, if not before. The rabbits of the island have access to limited vegetation and water, and thus rely on the tourists who feed them; the number of tourists has radically increased since 2006, and with that, the number of rabbits has increased as well, to a current estimated population of 1000. These rabbits display a fascinating combination of wild and domesticated traits, and the level of “wildness” or “tameness” differs based on where on the island the rabbits live. Our ethnographic research focuses on the rabbits of Okunoshima, and addresses their behavior and how they have been transformed by their liminal—and precarious—position and their relationships with the humans who, to some extent, shape their lives. Because they are both cared for, and threatened, by humans, the rabbits have developed a number of unique behaviors which would be of great interest to both domesticated and wild rabbit researchers.

## 17. Preliminary study on heavy metal concentrations of brown hares (*Lepus europaeus* Pallas, 1778) from central Turkey

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In this preliminary study, the amounts of heavy metals chromium (Cr), manganese (Mn), copper (Cu), zinc (Zn), selenium (Se), cadmium (Cd), mercury (Hg), and lead (Pb) in the kidney and liver tissues of 15 hares (*Lepus europaeus* Pallas, 1778) from Kırıkkale province located in central Turkey were investigated. Heavy metal concentrations in the samples prepared according to the standard methods were measured using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The wet weights of heavy metals found in tissues were, respectively, as follows: Cr<sub>liv</sub>; 1 mg kg<sup>-1</sup>, Cr<sub>kid</sub>; 1.02 mg kg<sup>-1</sup>, Mn<sub>liv</sub>; 4.80 mg kg<sup>-1</sup>, Mn<sub>kid</sub>; 6.00 mg kg<sup>-1</sup>, Cu<sub>liv</sub>; 2.34 mg kg<sup>-1</sup>, Cu<sub>kid</sub>; 1.91 mg kg<sup>-1</sup>, Zn<sub>liv</sub>; 40.51 mg kg<sup>-1</sup>, Zn<sub>kid</sub>; 32.65 mg kg<sup>-1</sup>, Se<sub>liv</sub>; 0.19 mg kg<sup>-1</sup>, Se<sub>kid</sub>; 1.15 mg kg<sup>-1</sup>, Cd<sub>liv</sub>; 0.83 mg kg<sup>-1</sup>, Cd<sub>kid</sub>; 4.49 mg kg<sup>-1</sup>, Pb<sub>liv</sub>; 2.19 mg kg<sup>-1</sup>, Pb<sub>kid</sub>; 1.23 mg kg<sup>-1</sup>. Hg, Cr, Pb and Cd concentrations found in the tissues were compared against the hygienic limits. Our results revealed that four samples of hare kidney exceeded the hygienic limit of Hg concentration. Cr concentrations came from nine samples of hare kidney and 11 samples of hare liver exceed the hygienic limits. Pb concentrations came from three samples of hare kidney and five samples of hare liver were higher than hygienic limits. The values of Cd concentrations exceeded the hygienic limits in both tissue of all hares. Consequently, our findings showed that the offal of hares were contaminated by the heavy metals. This might be due to the fact that Kırıkkale province has various industrial facilities and it is an important crossroad in terms of transportation. Moreover, there is usage of herbicides, pesticides and fertilizers containing potentially toxic trace elements in agricultural production in the study area. This study suggested that the Hg, Cr, Pb, and Cd metals, that were determined to be higher than the hygienic limits, could affect the vital functions of the hares in Kırıkkale province, and could cause a decreasing in population densities of the species by decreasing fertility in male and female.

## 18. Magnetic alignment in European hares? Preliminary results on form direction

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Mammals show a directional response to the geomagnetic field. This magnetic alignment represents a spontaneous, fixed directional response in which mammals align their bodies along or perpendicular to the magnetic field lines. We tested the potential effect of the geomagnetic field on the position of forms used by European hares (*Lepus europaeus*) in an arable landscape in Lower Austria. For 29 forms we determined their direction to the nearest 5° and classified them into the categories N, NE, E, SE, S, SW, W, NW. The statistical analysis with the help of both Fisher's Exact Test and Rayleigh Test revealed no significantly preferred alignment of the forms, although directions towards NE were most common (Fisher's Exact Test:  $p = 0.2441$ , Rayleigh-Test:  $p = 0.3365$ ). We suggest that the direction of forms should depend on field structure, crop types, main wind direction, etc. As the wind in our study site comes mainly from NW or SE, form direction was not related to this factor, too. To clarify whether magnetic alignment of hares is covered by numerous other factors related to habitat characteristics we are currently collecting data from more forms.

## 19. Long-term use of a rest area by brown hares in New Zealand

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Analysis of photographs taken whenever hares (*Lepus europaeus*) or rabbits (*Oryctolagus cuniculus*) were seen from our house revealed an extraordinary pattern. In 8 of the past 10 years, very occasionally each summer (November- February) a pair of hares spent 10-30 minutes sitting at exactly the same place near a fencepost. During this time one of the hares would lie flat on its side for 3-10 minutes while the other "stood guard" within about 1 m. As wild hares in New Zealand seldom live more than 4 years (Flux 1967) and some were individually recognisable, different pairs have certainly been involved. The same spot was used each year except in 2006-7, and the behaviour was not recorded elsewhere in the hares range. Once both hares lay down, but the roles of guard and rester were never reversed, and episodes ended when both ran off together. Rabbits occupy the same study area, and were photographed doing precisely the same thing at the same place; but rabbits often lay on their sides in other places too. An interspecific pair used the spot once, with a hare guarding a lying rabbit. Finally, and most remarkably, a pair of Australian magpies (*Gymnorhina tibicen*) copied this behaviour at the same spot, one lying on its side with its feet in the air while the other stood guard. The spot chosen is on a trail kept bare by horses running the fence-line, but other bare patches in the field are ignored, as are other parts of the trail. Having taken thousands of photographs of hares and rabbits in these fields over the years, I did not appreciate the significance of the spot by the post until 2014-15 when reviewing images for analysis, so observer bias is unlikely. Close examination revealed no pellets or unusual scent, and the aspect and shelter from wind seem no different from many other parts of the field. Has anyone seen such behaviour, or can offer an explanation?

Flux, J.E.C. 1967. Reproduction and body weights of the hare *Lepus europaeus* Pallas, in New Zealand. N. Z. J. Sci. 10: 357-401.

NOTE: John Flux was unable to attend the conference. No poster was displayed.



## 20. Genetic diversity of non-pathogenic rabbit lagoviruses in Europe

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*Rabbit hemorrhagic disease virus* (RHDV) is the aetiological agent of a highly pathogenic infectious disease of European rabbits *Oryctolagus cuniculus*. Its evolution shows relatively low genetic variability in the gene coding for the capsid protein VP60 but several genetic groups have been described, including the antigenic variant RHDVa. In 2010, a pathogenic lagovirus (RHDV2) affecting European rabbits that is related to but distant from RHDV was identified in France and spread throughout Western Europe. Since the 1990s, several non-pathogenic or mildly pathogenic lagoviruses have been characterised in domestic and/or wild rabbits. These viruses are genetically related to but distant from each other and from RHDV or RHDV2. In addition to the first non-pathogenic virus identified in Italy (RCV), two viruses for which the non-pathogenicity has been experimentally confirmed have been characterised: the French 06-11 strain discovered in domestic rabbits, and the Australian RCV-A1. The latter constitutes a new genotype and evolutionary analyses suggested that it has a European origin and has been introduced in Australia with introduced rabbits in the mid-19th century. However, RCV-A1 related viruses have not been collected and characterised in Europe yet. We have carried out a study aiming at characterizing non-pathogenic lagoviruses circulating in European wild rabbit populations.

Wild rabbit samples were collected in several distant locations in France between 2007 and 2015 (n = 380) and in Sweden in 2014 and 2015 (n = 97). Presence of lagovirus RNA was tested by RT-PCR and the strains were genotyped by sequence analyses. We successfully characterised two non-pathogenic lagovirus lineages. The first one is related to the 06-11 strain previously described in domestic rabbits in France, showing that it also circulates in wild populations. This lineage forms a unique genetic group with RHDV-RHDVa and RCV, which reveals a common ancestor. We propose the name RCV-E1 (Rabbit calicivirus Europe 1) for this lineage. The second one is closely related to RCV-A1 with which it forms a highly significant monophyletic group. We propose the name RCV-E2 for the European viruses belonging to this second lineage. Our data show that both RCV-E1 and RCV-E2 circulate throughout France and that they co-circulate at the population scale, while we have detected only RCV-E2 in Sweden. We obtained the entire VP60 sequence for four RCV-E1 and eight RCV-E2 strains including one strain isolated in Sweden. Phylogenetic analyses confirmed that RCV-E1 clustered with the Ashington strain collected in the UK. They also showed that RCV-E2 and RCV-A1 share a common ancestor, confirming the European origin of RCV-A1. Additionally, presence of one RCV-E2 strain grouped within RCV-A1 may suggest that several introductions of RCV-A1 ancestors have occurred in Australia. RCV-E2 distribution range seems larger than RCV-E1 range since RCV-E1 has not been detected in Swedish rabbits. In addition, RCV-E2 strains showed a higher genetic diversity than RCV-E1 suggesting that they have evolved in European rabbits over a longer time span. The molecular data acquired in this work contribute to our understanding of the evolutionary history of lagoviruses.

## 21. Geomorphic structure of pika habitats defined by airborne LiDAR

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Characterizing and mapping talus for pika research is important but difficult with field measures or common remote-sensing techniques. American pikas (*Ochotona princeps*) are most often found in taluses with rock diameters 0.2-1 m, but existing talus maps are limited to small areas, manually delineated from aerial imagery and local knowledge. Most aerial imagery cannot resolve talus characteristics important to pikas and have shadows and forest canopies that obscure the ground. Airborne LiDAR (light detection and ranging) can penetrate forests and describes microtopography in high detail that is likely sufficient to identify taluses suitable for pikas. We surveyed pikas within a recent LiDAR acquisition (9 pts/m<sup>2</sup>) in western Montana and measured rock sizes at 134 plots centered on either haypiles or areas unoccupied by pikas. We measured the strength of the relationship between rock sizes and LiDAR metrics for surface roughness to determine whether LiDAR can discriminate geomorphic features important to pikas. We then used logistic regression to discriminate areas around 206 haypiles from areas of talus unoccupied by pikas based on LiDAR metrics for microtopography. Mean rock size was positively correlated with the standard deviation of LiDAR return heights ( $R^2 = 0.40$ ,  $P < 0.001$ ). As expected, the probability of use by pikas had a significant quadratic relationship with the standard deviation of LiDAR return heights, such that highest use corresponded to mean rock size of 0.96 m. Results of this study indicate that airborne LiDAR is capable of describing taluses at resolutions meaningful to pikas, and this method should improve habitat mapping and hypothesis testing for this species. This study is our first step to examine new remote-sensing technologies for developing automated procedures to map talus and pika habitat at high resolutions across landscapes.

## 22. A practical guide to study brown hare leverets (*Lepus europaeus*) in the wild

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Since the brown hare (*Lepus europaeus*) is one of the best studied game species, it is surprising that little is known on the life stage of newly born offspring to independence. One reason for this may be that there was no practical method available to detect leverets. We used thermal imaging (introduced by Ulrich Voigt, Stiftung tierärztliche Hochschule Hannover), a specially trained sniffer dog and direct observations to locate leverets. In addition to a normal thermal imaging camera we used a thermal drone to search areas of high vegetation density. Knowing that brown hare does appear to suckle their young at very specific times we were able to track down some leverets by direct observations of nursing events. Using these methods we were able to detect 81 leverets in two different study sites in the Swiss lowlands. We found that it is essential to vary the choice of method depending on vegetation. The Hand-held thermal imaging device was suitable to search large areas with low vegetation. Even perfectly camouflaged and small animals down to the size of mice and small birds or their nests were easily detected from a distance of more than 200m. When vegetation density increases, the thermal drone was the more successful method. However where plants grew too dense the sniffing dog was the only method still working. Thermal imaging is a suitable method not only for detecting leverets but generally can be used to study cryptic endothermic animals such as for example ground-breeding birds. Furthermore it is convenient for behavioral observations in complete darkness. Recent technical progress made thermal imaging devices – including drones – becoming a gadget easy to use, with high performance and affordable. Hence we are confident that thermal imaging devices – hand-held and airborne – will become essential field equipment for many wildlife studies.

## 23. Back from the brink of extinction: Recovery of the riparian brush rabbit

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The riparian brush rabbit (*Sylvilagus bachmani riparius*; RBR) is considered to be one of the most endangered mammals in North America and is listed as an endangered species by both the State of California and the U.S. government. Until 1998, the only known population occurred within Caswell Memorial State Park (MSP), a small state park (250 acres; 101 ha) dominated by riparian oak forest on the Stanislaus River near Ripon, California. Because they were found only in Caswell MSP, it was believed that they required elements of climax riparian oak forest. Our confirmation in 1998 of a second population in the southern Sacramento/San Joaquin River Delta (South Delta), in non-forested and early successional riparian forest areas demonstrated that the former view of RBR habitat associations was inaccurate and that in fact they were more abundant in successional communities than in climax communities. They occupy riparian communities composed of willow thickets, blackberry, wild rose, and other successional shrubs and trees, as well as riparian forests dominated by oaks in the northern San Joaquin Valley of California. Such communities have been reduced to less than 1% of their historical extent, primarily by clearing natural vegetation, irrigated cultivation, impoundment of rivers, and stream channelization. However, thanks to a large and collaborative partnership, great strides have been made towards the recovery of riparian brush rabbits. In July 2002, with funding from federal and state partners, a Controlled Propagation and Reintroduction Program (CPRP) was initiated to place the riparian brush rabbit firmly on a path to recovery. Centered on the San Joaquin River National Wildlife Refuge (Refuge), the program included breeding rabbits in three large outdoor enclosures (each about 1 acre; 0.4 ha), releasing them into suitable habitat on the Refuge, and closely monitoring their movements and population dynamics. The rabbits were released into remnant riparian and newly-restored riparian habitat on the Refuge. By the time the CPRP was suspended (December 2013), a total of 1,496 riparian brush rabbits (762 females, 734 males) had been released on the Refuge, including private land under conservation easement. Despite the challenges of wildfires and severe flooding, the Refuge population has increased to where the hope is that the species will soon be eligible for down-listing or delisting. Conservation easements and land acquisitions along the San Joaquin and Stanislaus rivers have created more than 20 continuous river miles (32 km) of riparian habitat for the riparian brush rabbit and many other species, including the also endangered riparian woodrat (*Neotoma fuscipes riparia*), and more habitat restoration is ongoing. Further, riparian brush rabbits have been documented in remnant riparian habitat along the San Joaquin River downriver of both Refuge lands and Caswell MSP. Conservation efforts are also getting under way in the South Delta, and the overall recovery effort will be informed by population genetic studies that are also taking place. This is truly an endangered species success story that should be considered a model for other national and international efforts.

## 24. Evolutionary history of the cape hare (*Lepus capensis*) inferred from genetic variation

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The systematics of Lagomorph species is in numerous instances controversial and far from being resolved. Many of these species are closely related and share extensive morphological features and genetic variation, while others have vast geographic distributions and are possibly composed of many independent populations and evolutionary units. Hares (genus *Lepus*) from Africa are among the Lagomorphs with greater taxonomic uncertainties, and the cape hare (*Lepus capensis*) is one of the most puzzling species. This species occurs from Africa to China, and shows distinct phenotypes in different regions that led to the identification of 80 subspecies. The level of differentiation and divergence among the cape hare populations along its vast range of distribution is yet poorly known, since the existing studies on this complex were either local or did not take the evolutionary history and level of genetic isolation into account. In order to better understand the evolutionary history of the cape hare and produce information that may contribute to clarify its taxonomy, the population structure and phylogeographic patterns of the species were investigated. We sampled an important part of the range of the species and characterized the genetic variation of these populations using 18 newly developed microsatellites and sequences of one mitochondrial DNA and 10 nuclear DNA loci. Five geographically explicit evolutionary units were identified in the species. Interestingly, in the north-western part of Africa, mitochondrial DNA showed additional substructure, suggesting different levels of dispersal between sexes. We quantified levels of divergence among the evolutionary units using coalescent-based methods and found that the split between some of the lineages is as old as that found between other well-recognized hare species. These results suggest possible cryptic speciation within what is currently classified as *Lepus capensis*. However, the notion of species is multi-dimensional, and whether the taxonomy of the cape hare should be revised may depend on the collection of evidence of various sorts, including of non-genetic aspects of the biology of these hares.

## 25. Within-sample variation in snowshoe hare fecal glucocorticoid metabolite measurements

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Measures of stress hormones, specifically glucocorticoids and their metabolites, are being used increasingly to index the physiological stress burden experienced by diverse taxa. Although such measures can be obtained from a variety of biological matrices (e.g., blood, hair, urine), feces has proven to be particularly useful because it is often abundant, typically can be collected without disturbing the study animals, and numerous studies have shown that fecal glucocorticoid metabolite (FGM) concentrations reflect an integrated measure of circulating glucocorticoids, thereby providing biologically meaningful information about the physiological state of the animal that provided the sample. However, numerous abiotic and biotic factors can influence measures of FGM, particularly when samples cannot be collected immediately after defecation. In addition, FGM concentrations may not be evenly distributed throughout a fecal mass, thus variation in sampling protocols can lead to measures of FGM that do not accurately represent the physiological state of the animal that provided the sample. Using a captive collection of snowshoe hare (*Lepus americanus*), our goal was to inform sampling methodologies for both field and captive studies that seek to use measures of FGM concentrations to assess wildlife physiological stress. Our objectives were to: (a) quantify and compare within-sample variability of FGM concentrations using two different sampling techniques and (b) conduct a power analysis to determine the approximate number of samples needed for each sampling method to detect statistically significant differences in FGM concentrations. Preliminary analysis suggests that both sampling methodologies (i.e., Method 1: analyzing a subsample few pellets, Method 2: homogenizing the whole fecal mass and subsequently analyzing powdered subsamples) result in similar levels of within-sample FGM variation, although the average FGM concentrations were consistently higher among subsamples taken from the homogenized whole fecal mass (Method 2). Further our power analysis demonstrates that sufficient sample size is critical for being able to discern biological meaningful differences within and/or between study populations. As such, our work provides a useful sampling framework to inform future studies that will integrate measures of FGM to index the stress burden experienced by populations of conservation or management concern.

## 26. Patterns of genetic variation in populations of the pygmy rabbit (*Brachylagus idahoensis*) in California and Nevada, USA

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Pygmy rabbits (*Brachylagus idahoensis*) are the smallest leporids found in North America. They are extreme habitat and dietary specialists restricted to the big sagebrush (*Artemisia tridentata*) communities of the Great Basin Desert. While the species' geographic distribution includes most of the Great Basin, their specialized habitat requirements restrict and fragment their populations across their range. Little is known about the connectivity of these populations.

We examined the genetic variability of extant pygmy rabbit populations across the species' range in Nevada and California. Our aim was to begin to identify patterns of diversity and connectivity among these populations, and determine whether patterns of population structure correspond with potential biogeographic barriers. We extracted DNA from fresh fecal pellet samples collected from 19 sites across the study area. Using microsatellite and mitochondrial markers we identified the spatial distribution of genetic variation and inferred the regional connectivity across this portion of the range. Overall, local population subdivision across Nevada was low as evidenced by low  $F_{ST}$  values suggesting larger intermixing populations in the past. Populations in the Mono Basin of California showed relatively high differentiation from the remainder of the sampled range. The Mono Basin lies about 160 km from the nearest known contemporary pygmy rabbit populations in Nevada and are separated by inhospitable habitat in a relatively low elevation area. Pygmy rabbits face increasing pressures from habitat loss leading to greater fragmentation of populations. These data will add to ongoing efforts to ensure sustainable management of the pygmy rabbit throughout its range in California and Nevada.

## 27. Impact of an outbreak of RHDV2 on a semi-natural population of European rabbits in France

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Rabbit hemorrhagic disease (RHD) is one of the several possible causes of decline of wild rabbit (*Oryctolagus cuniculus*) populations in Europe. A new genotype of the RHD virus (RHDV), called RHDV2, has emerged in 2010 and is a new threat to previously weakened populations as this virus can infect individuals immunized against classical strains of RHDV. We assessed the demographic impact of a RHDV2 outbreak that occurred in a semi-natural wild rabbit population in a 0.2 ha enclosure. Rabbits were marked with RFID microchip and monitored by automatic detections of individuals. We assessed survival rates of individuals by multi-event capture-recapture modeling. Although rabbits were regularly vaccinated against RHDV, some cases of mortality due to RHDV2 were recorded in early 2011. An increased apparent mortality was observed for around 2 years, with two epidemic peaks in January-February 2011 and October 2012-March 2013 separated by a period of intermediate mortality. Monthly survival of non vaccinated individuals fell from 0.99 to 0.79 and 0.78 during the epidemic peaks. The analyses also revealed that individuals vaccinated against RHDV were impacted by RHDV2 too, but less than non-vaccinated ones. Indeed, monthly survival of vaccinated individuals fell only to 0.94 during the epidemic peaks. But this difference in survival between vaccinated and non-vaccinated individuals persisted even when there was no notable mortality, indicating the capacity of the virus to persist and cause mortalities a long time in the population. Overall, among the 70 adults known alive before the RHDV2 outbreak and irrespective of their vaccination status, their annual survival in absence of disease was around 0.68 and fell to 0.45 when exposed to the disease. Finally, around 15% of these adults were still alive at the end of the outbreak.



## 28. Interspecies hybridisation in pikas (*Ochotona*)

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Interspecies hybridisation is common in Leporidae. Therefore, mitochondrial trees in this group do not reflect species phylogeny. However, amongst pikas there is no actual information on hybridisation. We collected recent data on morphology and nuclear genes on one hand and mitochondrial genes on another, and interpret the incongruent results of these two datasets as a consequence of hybridisation. Near all cases of hybridisation with one exception, were found within *Ochotona* subgenus. Five species (or tentative species) of this subgenus turned out to be involved in hybridisation: *O. curzoniae*, *O. nubrica*, *O. cansus*, *O. thibetana*, "*O. (c.) morosa*". We list these cases below. The bigger part of the distribution range of *O. nubrica* contain specimens which bear mitochondrial DNA of *O. curzoniae*. However, we sampled *O. nubrica* from Mustang area, Nepal that probably possesses the only true representatives of *O. nubrica* with its own mtDNA. Probable hybridisation between *O. cansus* and "*O. (c.) morosa*" led to elimination of mtDNA of the latter in Qinling Mts. Analysis of nuclear genes shows that *O. cansus* and "*O. (c.) morosa*" are not sister species. Mitochondrial DNA of *O. cansus* was found also in specimens of *O. curzoniae* from Yushu, Qinghai. Another case of possible interspecies hybridisation took place in Wolong, Sichuan: local population of *O. thibetana* bears mtDNA of (most probably) *O. cansus*. Big genetic distance between modern *O. cansus* and this population of *O. thibetana* points to ancient character of hybridisation.

The only case of interspecies hybridisation in other subgenera concerns single occurrence of the hybrid between *O. turuchanensis* and *O. hyperborea* in Putorana Plateau, Russia.

Thus, outside *Ochotona* subgenus mitochondrial data could be successfully used for the species identification. Within this subgenus, mitochondrial data should be applied for taxonomic purpose with big care.

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NOTE: Although Andrey Lissovsky was unable to attend the conference, this poster was displayed.

## 29. Evolutionary history of Turuchan pika, *Ochotona turuchanensis*, after genomic analysis

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Turuchan pika was discovered in 2003 only (Lissovsky, 2003): this species was found inside Northern pika *O. hyperborea*, morphological difference between these two species is very shallow. Later studies found that Turuchan pika is a sister taxon for the third species — Alpine pika *O. alpina* (Lissovsky et al., 2007). Thus, morphological similarity between Turuchan pika and Northern pika does not reflect genetic proximity. Study of larger data on *O. alpina* revealed that this species is even paraphyletic relatively *O. turuchanensis* (Formozov et al., 2006). Turuchan pika and Alpine pika differ in size notably, besides they have different ecological preferences. Alpine pika inhabits higher elevations and prefers talus with bigger stones. Turuchan pika inhabits a large territory of Central Siberia without any talus, where it lives at the tops of hills near rock outcrops or just in crevices under the moss cover. Only population of Putorana Plateau inhabits tali at lower elevations. Thus, situation seems to be quite paradoxical: one population of *O. alpina* (if we consider Turuchan pika as a subspecies of Alpine pika treating paraphyly taxonomically) obtains its own ecological niche and is a sibling-"species" to Northern pika.

Multilocus data, using "species tree" approach, showed very close proximity of these two taxa again (Melo-Ferreira et al., 2015). Analysis of single loci, however, revealed specific alleles in two nuclear introns (of 13 studied) that were very different from alleles of *O. alpina*, but occupied sister position to the latter species. Thus we can hypothesize that close proximity of Turuchan pika and Alpine pika on phylogenetic trees could be a result of recent hybridisation with elimination of some part of ancestral alleles in *O. turuchanensis*. In order to test this hypothesis, we carried out Genotyping by Sequencing (GBS) analysis of whole genome of *O. hyperborea*, *O. alpina* and *O. turuchanensis* specimens.

We studied pooled samples consisting of five specimens from each of the three species. In total we got 14383 polymorphic SNP loci, well defined in all three pools. Analysis of allele frequencies of the loci by statistical modelling for inferring the patterns of population splits and mixtures, as implemented in treemix software, rejects the hypothesis of *O. hyperborea* participation in hybridisation. Hybrid origin of *O. turuchanensis* however is not rejected: both ancestors are suggested to be from alpina clade, with one parental genome close to *alpina-turuchanensis* common ancestor. Thus, we should compare two alternative hypotheses: either *O. turuchanensis* is a recent branch within *O. alpina* evolution with dramatic morphological shift, or *O. turuchanensis* recently suffered hybridisation with another, unknown yet pika from alpina (s.l.) clade. These hypotheses will be further evaluated by Bayesian factor estimation approach, using population history simulation.

NOTE: Andrey Lissovsky was unable to attend the conference. Poster #29 was not displayed.

### 30. Conservation status review of all the lagomorphs in Mexico

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Mexico is considered the country with the third ranking highest diversity of species of lagomorphs worldwide, with 15 species: 10 rabbits (9 *Sylvilagus* species and *Romerolagus diazi*), and 5 hares species (*Lepus*). Mexico has approximately 55% of the species that live in the American continent and the largest number of endemic lagomorph species (7) on the same continent, of which 6 are located on islands or small areas less than 300 km<sup>2</sup>. In spite of the high species diversity and numerous endemic species: 1) The knowledge of different aspects of the basic biology and natural history is scarce in almost all species, even in species with wide distribution and considered as generalists (*L. californicus*, *L. alleni*, *S. audubonii*). In particular, *S. insonus* is the rarest and least understood of all lagomorphs in Mexico and the world; it is considered to be possibly extinct; 2) Some the wild populations of lagomorphs have been declining at an accelerated rate, due to habitat alteration, deforestation or clearing of natural habitats for agriculture, overgrazing by cattle and sheep, increased urbanization, development (increasing their degree of isolation), and global climate change. All these factors particularly affect endangered and endemic species (*R. diazi*), or species with restricted ranges (*S. robustus*; *S. insonus*), but also populations of species considered historically widely distributed, such as *S. bachmani* in the Baja California Peninsula, *S. floridanus* in the Yucatan Peninsula, *S. gabbi* in southeastern Mexico, *S. cunicularius* in central and western Mexico, and *L. callotis* in northwestern, central, and southern Mexico; and 3) Excessive hunting is another anthropogenic factor that has caused some drastic decreases in population density, such as with *L. flavigularis*, a lagomorph species with one of the highest risks of extinction in Mexico. In insular populations, the introduction of exotic species (rodents, and domestic cats and goats), along with wild predators, can cause a serious risk of extinction in species such as *S. mansuetus*, *S. graysoni*, and *L. insularis*. Although recent efforts have generated knowledge about the state of conservation and threat factors of some lagomorph species, it is still urgently necessary to get funds to carry out detailed and long-term studies, and to propose management plans and conservation of their habitats in collaboration with society and academic and institutional sectors.

## 31. The role of natural selection in the evolution of lagomorphs

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Recent advances in high throughput sequencing have revolutionized our ability to collect and analyze massive amounts of genetic information from traditionally non-model species. RNA-sequencing is one of the most popular techniques, which allows collecting information about the transcriptome of species, the part of the genome that is transcribed into mRNA and later translated into proteins. This gives us the opportunity to understand the major patterns of molecular evolution of genes at several evolutionary depths, from species to families or orders. In this work we analyze the transcriptome of three species of hares together with available data on protein coding genes from other Lagomorphs. We align the orthologs and use codon-based methods to infer whether positive selection has affected the evolution of genes at particular sites in some of the major braches of the phylogeny. This will provide valuable insights on the occurrence and nature of adaptive evolution that lies at the very foundation of several of the Lagomorph groups, such as leporids and hares.

## 32. Dispersal patterns in European rabbit

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Dispersal is a behavioral process, fundamental in ecology and evolution as it affects individual fitness and population demography. In social species like European rabbit, natal dispersal can be defined as the movement of juveniles from their natal social group to their first breeding group. Adult dispersal is the departure of adult individuals from their current social group to join another social group, permanently or temporary.

Radiotelemetry or global positioning systems provide means to measure such movement patterns. However, for small mammals their use has limitations because of the small size and weight of juvenile individuals that cannot carry such material. We therefore used experimental conditions to investigate dispersal patterns and characteristics of dispersers in European rabbit.

Rabbits live in polygynous groups that use and defend warrens for resting during the day. Our study was carried out in a rearing enclosure, divided into four separate warrens with a common grazing area. Here, dispersal cannot be driven by food availability as rabbits are artificially fed throughout the year. The rabbits, individually marked with a microchip, could access each warren enclosure from the grazing area through small tubes equipped with a detection device to record their movements between warrens. Rabbits can visit other warrens but are considered disperser when they settle out of their original warren (natal for juvenile and current for adults). We thus obtained sequence of warren use for each individual and developed a method to identify and characterize dispersal movements between the four social groups. For both juveniles and adults, we looked at the proportion of dispersing individuals in each sex and the timing of dispersal. For natal dispersal, we also looked at the influence of birth date on the probability of dispersing.

Our results showed that natal dispersal is male biased with 18% of juvenile males moving to another social group vs only 5% of juvenile females. Natal dispersal occurs at the age of 136 days on average and mainly between June and September, and to a lower extent in December. There was no effect of month of birth on dispersal probability. Adult dispersal follow roughly the same pattern with 22% of adult males moving to another social group but only 8% of adult females dispersing. Adult dispersal did not occur randomly during the year but was aggregated in two periods, similar to natal dispersal: one main period in summer (April – August) and a second period in winter (October – February). The first dispersal peak in spring–summer suggests that the composition of the social groups changes during the breeding season, possibly when early born juveniles become competing adults. The dispersal peak in winter could be driven by the need to avoid competition for breeding with relatives and the need to settle in a group before the start of the mating season.

Our study provides new insights on characteristics of dispersal movements in rabbit such as timing of dispersal or age at dispersal.

### 33. Evaluating performance of two GPS technologies to quantify space use by pygmy rabbits

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Global positioning system (GPS) technology has enhanced the temporal and spatial resolution at which animal location data can be collected, and recent advances in GPS receivers permit their deployment on mammals weighing <500 g. One such technological advance, rapid-fixing technology, allows for improved battery life by reducing the time to first fix by postponing the recovery of ephemeris data and procession of satellites to estimate locations until the device has been recovered from the animal. Previous work using this technology, however, is limited. Our goal was to evaluate transmitter performance of 2 types of GPS telemetry (traditional and rapid-fix) on a small, semi-fossorial lagomorph, the pygmy rabbit (*Brachylagus idahoensis*), to examine how errors might influence conclusions about fine-scale patterns of space use and resource selection. We performed 30 stationary tests in a range of shrub canopy covers for each type of device and compared fix success rates and location errors. We also fitted adults with collars that carried rapid-fixing GPS telemetry units programmed to collect locations at 15-min intervals in east-central Idaho, USA, during winter and summer 2015; adults were fitted with traditional GPS collars programmed at 60-min intervals during winter 2016 and summer trials are on-going. We predicted both types of devices would show similar fix success rates (FSR), but the extended time to first fix for traditional GPS would lead to increased accuracy. We also predicted that cover provided by shrubs and burrows would reduce performance of the GPS collars. When deployed on animals, FSR for the rapid-fix collars averaged 72% during winter and 86% during summer, whereas FSR for traditional technology averaged 75% during winter. Preliminary analysis of location errors at stationary trial locations suggest reduced errors with traditional devices. As expected, fix success of GPS collars in burrows was significantly reduced relative to above ground. Effects of shrub cover on GPS performance also were variable, but less pronounced than burrow cover. Furthermore, functioning of rapid-fixing devices was highly variable and unpredictable, whereas the traditional devices consistently performed well. These trials will be used to quantify GPS errors to inform the scale at which interpretations of resource selection and space use patterns are appropriate, and to provide information for application of GPS technology on other small lagomorphs.

### 34. Things that go bump in the night: Nighttime antipredator behavior of pygmy rabbits

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Cryptic prey species must balance the need to forage, find mates, and care for young with the risk of being depredated. One strategy for meeting maintenance needs and avoiding predators is flexibility in the timing of activity to avoid peak periods of predation risk. The success rate of nocturnal predators is known to be higher under a full moon, and many prey species will shift activity from nocturnal time periods under a new moon to crepuscular time periods under a full moon. Pygmy rabbits (*Brachylagus idahoensis*) are an important prey species for many predators in the sagebrush steppe of the Great Basin and Intermountain West of the United States, and predation is the primary cause of mortality in the species. Our objective was to evaluate the activity patterns of free-ranging, adult pygmy rabbits to test for an effect of moon phase on the timing of activity. We hypothesized that pygmy rabbits could be active at any time of the day, but under a full moon nighttime activity would be significantly lower than activity during crepuscular periods when compared with new moon phases. We fit 24 adult rabbits with accelerometers set to sum and record motion at 1-minute intervals for  $\geq 14$  days in the summer and winter of 2015. We used a repeated measures ANOVA and Tukey's HSD to compare mean activity during the dawn, dusk, night, and day time periods in both seasons. Pygmy rabbits demonstrated a crepuscular activity pattern under a full moon in both summer and winter, but there was no significant difference between dawn, dusk, and nighttime activity under new moons in either season. Crepuscular activity under a full moon was especially pronounced during the winter when perception of predation risk may be amplified by increased moonlight illumination and reduced vegetative cover. Undoubtedly, there are many factors that influence activity patterns of prey species, however, our data suggest that predation risk, or the perception thereof, is a key factor in the timing of activity of pygmy rabbits.

## 35. Characterizing population differentiation and genetic variation in candidate genes in mountain hare populations with distinct coat color phenotypes

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Coat color is one of the strongest phenotypic adaptations to local environments. More remarkable is the ability that some boreal animals have to change seasonally their coat color to mimic the surrounding environments, periodically covered with snow. Several species of hares possess this important phenological trait and intraspecific variation in the phenotype can sometimes be found. The mountain hare, *Lepus timidus*, is one of such species. For example, while the norm of the species is to change the coat color from a brown summer coat to a white winter one (such as in Scandinavia or the Alps), Irish populations retain a brown coat year-round and those from the Faroe Islands show a grey winter coat. In addition, specimens with black coat were collected in Sweden and included in the collections of the Swedish Natural History Museum. In this work, we use microsatellites to characterize differentiation among these mountain hare populations, which are either geographically isolated or carry distinct seasonal coat color phenotypes, or both. In addition, we sequenced genes that are commonly known to be involved in determining coat color phenotypes, in order to understand if variation at these genes may be responsible for the variation of the trait in the mountain hare.



## 36. Community level effects of the new variant of the rabbit hemorrhagic disease virus (RHDV) in Iberian ecosystems

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The European rabbit (*Oryctolagus cuniculus*) is a keystone species in Mediterranean ecosystems, with ecosystem engineering effects all across its distribution range, and where it is the staple prey for over 40 predator species. Recently, a new variant of the rabbit hemorrhagic disease virus (RHDV2 or RHDVb) emerged in France, and quickly spread throughout the Europe affecting natural populations of both rabbit subspecies (*Oryctolagus cuniculus cuniculus* and *O. c. algirus*). This disease is shaping the demographic patterns of rabbit populations, with subsequent strong impacts in the conservation of endangered predators. However, the dynamics, epidemiology and population-level impacts of this disease are largely unknown. Using yearly population monitoring data for the period of 2003-2015, we quantitatively assess the impact of RHDV2 on natural rabbit populations and in two endangered endemic apex predator populations: the Iberian lynx (*Lynx pardinus*) and the Spanish Imperial eagle (*Aquila adalberti*). Additionally, specific target populations were subjected to detailed monthly demographic monitoring, and both dead animals found in the field and hunted were screened for RHDV using molecular techniques for the period of 2012-2015. We observed 50-60% declines in the natural populations of European rabbits following the arrival of RHDV2, supporting the high mortality rates associated with this etiological agent. These decreases were followed by immediate decreases of 65.7% in Iberian lynx and 45.5% in Spanish Imperial eagle fecundities, and a year-lagged reduction in these apex predators breeding populations. We found that 83.5% of rabbit carcasses found in the field were RHDV2-positive, and there are currently three different virus forms simultaneously circulating in natural rabbit populations. Most RHDV2-positive carcasses were found during the breeding season, and a high proportion of affected animals were juveniles. These results suggest a higher susceptibility of young rabbits, and support a scenario where RHDV2 imposes a demographic constraint in the natural demographic dynamics of European rabbit populations by impairing the adequate recruitment of new individuals. We advocate that RHDV2 as strong a community-level effect, by controlling the population dynamics of a keystone species, hence acting as a keystone species itself. The current demographic trend of natural rabbit populations can have severe implications for the conservation of rabbit-reliant predators in the Iberian Peninsula. This model system stresses the importance of including diseases as functional players in the dynamics of terrestrial trophic webs.

NOTE: Pedro Monterroso was unable to attend the conference. No poster was displayed.

### **37. Effect of parasitosis and virosis on the physiologic condition and population dynamics of wild European rabbit (*Oryctolagus cuniculus*)**

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The wild European rabbit constitutes a key species in the Mediterranean ecosystems, mainly because it represents the bulk of the diet of a wide variety of Iberian predators. These include the Iberian lynx (*Lynx pardinus*) and the Spanish imperial eagle (*Aquila adalberti*), both seriously threatened. Despite being historically numerous and widespread on the Iberian Peninsula, the European rabbit populations have undergone a dramatic decline in the last century due to the emergence of two viral diseases, habitat loss and an excessive hunting pressure, within others. Certainly emerging and re-emerging infectious diseases play an important role in the population dynamics of wild species. Undoubtedly, they are one of the main threats in wildlife conservation. Despite of many years of research, there are still many knowledge gaps regarding the dynamics of infectious agents in their individual hosts, and what determines their impact on life-history traits. The present PhD project is aimed at studying eco-epidemiological parameters from a wild population by using as a model species the European wild rabbit (*Oryctolagus cuniculus*) and the two main viral diseases that affect them (myxomatosis, and rabbit hemorrhagic disease). We will use the data collected in an ongoing project entitled: 'Scientific monitoring and enhancement of *Oryctolagus cuniculus* populations in the Hornachuelos Natural Park. This project provides an outstanding opportunity to study and monitor the emergence and development of diseases on wild rabbit populations. Furthermore interactions between pathogens with abiotic and biotic factors could be identified, as well as the ultimate effects on the rabbit population dynamics. The main aim is to improve strategies against diseases and minimizing their impact on natural populations. Ultimately the results from this PhD would improve management plans for the conservation of keystone species of the Mediterranean ecosystems and therefore the endangered predators that prey upon them.

### **38. Non-invasive genetic monitoring of the mountain hare (*Lepus timidus*): Results from a pilot study in the Swiss Alps**

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Climate change, human disturbance and potential hybridisation with the European hare (*Lepus europaeus*) are considered to be serious threats for the mountain hare in the Alps. Therefore, reliable and accurate monitoring methods are needed to survey future population trends. We used non-invasive genetic sampling (NIGS) data to obtain size estimates of a mountain hare population in the Swiss Alps. We evaluated and compared the effectiveness of systematic, opportunistic and combined NIGS approaches. Extraction success rate of faecal samples and their age-dependent variation as well as the quality of genetic parameters were used as measures of effectiveness. Based on a spatially explicit capture-recapture (SECR) sampling, we estimated the minimum population size and sex ratio. We found that extraction success decreased with the time since faecal excretion. The systematic sampling was more effective for finding unique mountain hare individuals than the opportunistic sampling. Depending on the sampling design, the population size estimate was highest for the systematic sampling and lowest for the combined sampling but differed only by 0.4 hares per 100 ha. Our study illustrates that NIGS is a powerful and effective tool for monitoring elusive species such as the mountain hare in mountainous terrain.

### **39. Risk of predation influences the habitat use of the Alpine mountain hare (*Lepus timidus*) during the reproductive period**

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Habitat use of mountain hares (*Lepus timidus*) is subjected to strong trade-offs between foraging needs and predator avoidance. Identifying habitat use of mountain hares may help to determine crucial and limited environmental resources and has strong implications for habitat management. We investigated the relative importance of habitat characteristics providing foraging or shelter for mountain hares in the Swiss Alps at the small scale during the reproductive period. Habitat use was assessed non-invasively by considering the spatio-temporal distribution of faecal pellets that were sampled along systematically distributed transects. The highest densities of pellet locations were found in the timberline zone. Heterogeneous habitats with high diversity of vegetation types and/or a high abundance of saplings were strongly used. The availability of shelter was more important than the availability of food. In the reproductive period, when food is overabundant, hares strongly used dense habitats that offered protection from predators. Therefore, a structurally heterogeneous ecotone at the upper timber line with a mosaic of different vegetation types and hiding structures should be maintained or created in the management of the species. Complementing habitat management with visitor steering concepts is important to minimize potential negative effects of leisure activities during the reproductive period of the mountain hare.

## 40. Optimization and accuracy of faecal pellet count estimates of population size: The case of European rabbits in extensive breeding nuclei

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Cost-effective indices to estimate relative abundances of species are crucial for their management and conservation. As an example, population indices are needed to monitor extensive breeding nuclei used for translocating wild rabbit populations. Based on counts of faecal pellets from four high density rabbit nuclei in southwest Spain, (i) we assess the accuracy of this population index in high-density populations, and (ii) present a simulation approach to evaluate how the reduction of the counting effort affects accuracy of the population estimates. Our findings suggest that this method provides a valid estimate in high-density rabbit populations, and, notably, estimates would have not substantially changed after a reduction of 45-65% in the number of counted plots depending on variation on rabbit density between nuclei. We provide a framework that managers and other scientists could use to improve data collection of pellet counts in order to optimize their chances of detecting relative abundance estimates of rabbits. In addition, we present a new R function to implement our approach that can easily be applied in a variety of monitoring programs for other species based on count data. This will likely help to reduce field-effort in different studies without compromising population indices estimates.

## 41. Does fencing extensive breeding plots affect European wild rabbit perceived risk of predation?

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During the second half of the 20th century, European rabbit populations (*Oryctolagus cuniculus*) declined sharply across the species' native range, the Iberian Peninsula, mainly as a result of viral diseases and the loss of suitable habitat. This decline has been exacerbated with the recent outbreak of a new variant of the rabbit haemorrhagic disease virus. Following the rabbit decline, several management programmes, mainly through rabbit translocations, were established in Iberia to provide populations for hunting (as it is one of the most important small game species) and for endangered predators that rely on this prey. Since the success of these earlier wild rabbit translocations was generally low, several research projects were carried out in France and Spain with the aim of investigating potential solutions to improve rabbit translocation programs. This situation led to a new concept in wild rabbit translocation programs, such as the use of in situ extensive rabbit captive breeding fenced plots, which are now widely used for both conservation and hunting management purposes. The plot serves two main roles, as 1) soft release receptor site, and, with time, as 2) a potential donor population. We expect that fencing a plot may favour rabbit survival and rabbit body condition due to reduction of stress caused by risk of predation, which may also alter rabbit behaviour (e.g. group size, distance to cover, and rabbit main activity). In order to assess how fences may affect the perceived risk of predation of rabbits inhabiting breeding plots (subspecies *O.c. algirus*), we carried out 1) direct observations of single individuals and rabbits in groups, 2) estimate body condition, and 3) calculate rabbit survival in two breeding plots in SW Spain, one of them being fenced to completely exclude terrestrial carnivore predators. The plots consisted of a grassland field approximately 4 ha in size with 18 artificial warrens built-in. Overall, rabbits in fenced plot spent most of the time eating and tended to group in larger numbers, but they did not move further from cover. In the unfenced plot, rabbits spent more time in alert, and were the larger groups of rabbits ( $\geq 4$ ) who moved far from the cover. No differences were found in rabbit body condition between plots. However, fences did favour rabbit survival by reducing mortality by terrestrial carnivores, although mortality by birds of prey was higher in the fenced plot, what could explain the higher aggregation of rabbits in this plot. Our results provide evidences that reducing predation risk by limiting carnivore presence not only increases rabbit survival but also modifies rabbit behaviour in breeding plots.

## 42. Home range use by the European hare (*Lepus europaeus*) analysed at a fine temporal scale

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An animal's home-range use is influenced by the landscape type. European hares (*Lepus europaeus*) have up to now only been studied in agricultural areas with medium to large field sizes. In agricultural areas with small field sizes, European hares' locomotor behaviour is expected to be more localised. GPS-tracking provides the opportunity to investigate an animal's home-range use at fine temporal scales. In this study, we tracked nine European hares by means of GPS and VHF collars during the vegetation period in an agricultural area with small field sizes in Lower Austria. In particular, we were interested in the hares' space use at a fine temporal scale such as during active and resting periods within single 24-hour periods. Furthermore, we compared calculations (day-day distances and day-night distances travelled) based on GPS and VHF data. European hares' home-range size was smaller and the distance between places of activity and resting was shorter, in this agricultural area with small fields than has ever been measured in other agricultural areas with larger fields. European hares expressed a preference for areas near field edges while both being active and resting during the vegetation period. Our findings suggest that with GPS data it is possible to distinguish properly between questions regarding the movement path and the frequented area by an animal, whereas with VHF data these two parameters may be difficult to separate. In conclusion, our results show that in areas where accessibility to resources are high, such as agricultural areas with small field sizes, the European hare is able to reduce its home-range size to almost half of the minimum size that has been recorded so far in other habitats. As small home ranges involve less energy expenditure for movement, our results suggest that animals living in agro-ecosystems may benefit from small fields.

### 43. Pikas reject the low snowfall/high mortality hypothesis

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In 1978 one of us (ATS) formulated a hypothesis which has gained considerable traction in the pika literature, particularly with regard to climate change extrapolations and conditions that may lead to extinction of pikas throughout part of their geographic range. In *Ecology* [78:133-139] it was claimed that there could be high density-independent mortality in years of low or early snowmelt, thus providing more vacant territories on pika talus habitat. Further, it was demonstrated using data on water content of snow on 1 April (California Cooperative Snow Survey) that the snowpack in the Sierra Nevada is highly variable. The potential mechanism that could lead to increased mortality in years of low snowpack made intuitive sense. Not having an insulative layer of snow to protect them from cold may force pikas to excessively increase their metabolism overwinter to thermoregulate, and they may run out of energy reserves before the onset of spring and emergence of fresh vegetation. Many researchers have picked up on this hypothesis – low snowpack leads to higher pika mortality across the intermontane West – and many have even stated it as fact.

In winter 2014-2015 the Sierra experienced its lowest snowfall in recorded history. So, what better year to clarify and test this hypothesis? Water content of snow on 1 April 2015 was at an historic low – only 5% of average. The previous low for the date was 25% of average in 2014 and 1977. Most snow-course data in the central Sierra were this low or worse. Mammoth Pass with an average water content of snow of 42.2 inches had only 0.6 inches (1.3% of average) in 2015. In Yosemite National Park, Dana Meadows was 7.6% of average, while Tuolumne Meadows was only 0.7% of average. No snow on 1 April was recorded at Big Pine Creek, Rock Creek Lakes, or South Lake. There was essentially no snow.

We are seasoned pika field biologists familiar with the Sierra Nevada and its American pikas (*Ochotona princeps*). In summer 2015 there were healthy populations of pikas at every locality where pikas had been seen in the previous summer. Some haypile sites that had been abandoned in summer 2014 were occupied in summer 2015. Huge haypiles were observed, and most populations of pikas appeared to be saturated (all territories occupied). There was no evidence that the nearly total lack of snow caused unusual overwinter mortality in Sierra pikas. The pikas rejected the low snowfall/high mortality hypothesis.



## 44 Physiological stress response of snowshoe hares (*Lepus americanus*) to environmental enrichment

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Captive wildlife often serve as models for a variety of research endeavors, although chronic stress associated with experimental treatments or living conditions sometimes can drive animals into a pathological state. For example, research animals may experience unnatural conditions (e.g., restrictive housing, disruptive social environments) that can cause an endocrine response resulting in elevated glucocorticoid (i.e., stress hormone) levels. However, the use of environmental enrichment, an animal husbandry principle that seeks to improve the quality of captive animal care by identifying and providing stimuli that encourage normal activities, has been shown to reduce stress in captive animals. Using a captive colony of snowshoe hares (*Lepus americanus*, n = 12) housed at an environmental facility at the NCSU College of Veterinary Medicine (CVM) and fecal cortisol metabolite (FCM) concentrations as a measure of physiological stress responses, we examined the effectiveness of environmental enrichment for reducing stress in captive animals. Specifically, we quantified the extent to which different enrichment treatments (e.g., edible, non-edible) influenced FCM concentrations relative to a control treatment (i.e., no enrichment) over 3 trials. Although at the treatment level, edible enrichment had no significant influence on FCM concentrations compared to the control treatment ( $P= 0.696$ ), we found that access to non-edible enrichment resulted in a slight decrease in FCM concentrations ( $P= 0.062$ ). In addition, we found that within the edible enrichment treatment, access to fresh loblolly pine (*Pinus taeda*) resulted in significantly lower FCM concentrations ( $P = 0.032$ ), which suggests that this edible item was effective at reducing snowshoe hare stress. Understanding how environmental enrichment can be used to mitigate stress experienced by captive wildlife is critical for enhancing the long-term care of research animals and for obtaining reliable results from studies that use measures of stress to draw conclusions about the physiological responses of study animals to experimental treatments. Our research provide a framework that other researches and captive wildlife managers can use to measure the effectiveness of environmental enrichment for enhancing the well-being of wild animals housed in captivity.

## 45. Strong reactive movement response of the medium-sized European hare to elevated predation risk in short vegetation

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Reactive movement responses of prey are affected by habitat characteristics, such as cover, which determine predation risk. Open habitats with low cover facilitate predator detection, movement and escape, while closed habitats reduce the ability to detect predators and hinder movement. We performed a field experiment using non-lethal predators to study the reactive movement responses of medium-sized prey in patches with different vegetation characteristics related to elevated predation risk. Ten GPS collared, free-ranging European hare (*Lepus europaeus*) were repeatedly subjected to a leashed dog and two humans in an experimental cross-over design. Linear Mixed Models were used to assess the effect of the treatment and its interaction with vegetation parameters on the movement behaviour of European hare. The reactive movement response was best explained by the model that included the interaction between elevated predation risk and vegetation structure. A strong immediate response was found in short vegetation up to one hour after the treatment ended. The effect extended beyond the duration of the treatment and was synchronised with the resting and foraging period over the next 24 hrs. The distance covered between resting and foraging grounds was negatively affected, while use of less risky, low-quality vegetation during resting and foraging was favoured. Medium-sized prey species exhibit strong behavioural responses to the perceived predation risk, which we demonstrate here for the European hare. An elevated predation risk, for example by dogs, can trigger costly behavioural responses in these medium-sized prey species.

## 46. Feral cat issue and negative impact on the Amami rabbit on Amami-Ohshima Island and Tokunoshima Island, Japan

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Feral cats *Felis silvestris catus* have caused extinctions and population declines of many endemic species on numerous islands around the world. Island endemic species are vulnerable to invasive predators because of their lack of anti-predator behavior and life history responses. Amami-Ohshima Island (712 km<sup>2</sup>) and Tokunoshima Island (248km<sup>2</sup>) in most southern Japan are also one of those islands and have many endemic species, such as the Amami rabbit *Pentalagus furnessi*, Ryukyu long-tailed giant rat *Diplothrix legata*, and Amami-Ohshima Island has the Amami spiny rats *Tokudaia osimensis* and Tokunoshima Island has the Tokunoshima spiny rat *T. tokunoshimensis*. So, there has been concern that feral cats have negative impacts on the endangered endemic mammals on the islands. However no diet study and actual habitat survey of feral cat has been conducted to support the necessity of an urgent feasible feral cat management for the island. Therefore, we analyzed feral cat diet using scats analysis and estimated population size by sensor camera technique in the islands. The number of prey, percentage of prey, frequency of occurrence (the percentage of scats in a sample containing a particular prey item), percentage of biomass (biomass of the same prey item divided by the total consumed biomass × 100) and daily consumed biomass (DCB) were estimated especially in Amami-ohshima Island. Three endangered endemic mammals were the main prey species of the feral cat diet (68.5 % of total DCB) on Amami-ohshima Island (n=102, 2009-2011). The percent contributions of these species on DCB were; long-tailed giant rat (34.7%), Amami spiny rat (21.9%), and Amami rabbit (12.0%) on Amami-ohshima Island and long-tailed giant rat (33%), Tokunoshima spiny rat (33.3%), Amami rabbit (33.3%) and birds (50.0%) on Tokunoshima Island (n=6, 2014). Mammals species, especially endangered endemic mammals were main prey species of feral cat diet on the islands. As many as 600-1,300 feral cats on Amami-ohshima Island and 150-200 feral cats on Tokunoshima Island inhabiting in forests of endangered species habitat in 2015 were estimated. Therefore, feral cats are likely to be having a significant impact on endangered endemic mammals on the islands. To protect of these endemic species and ecosystem on the islands, urgent elimination of feral cats from endangered species habitat and appropriate management of supply source of house cat are necessary.

## 47. Histological analyses of European brown hare (*Lepus europaeus* Pall.) testes and ovaries from various regions, seasons and ages from Bulgaria

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In order to be analyzed the histological structure of testes and ovaries of various regions from Bulgaria, seasons of the year and ages (determined by dry eye lens weights), 20 testes and 10 ovaries of European brown hares were investigated from 11 hunting grounds (2 from Northern Bulgaria, and 9 from Southern Bulgaria). The samples were manipulated, according to the routine histological technology, colored with hematoxylin-eosyn and prepared as microscope durable preparations. After the histological analyses of the testes, we determinate, that the testes (10, 3 ± 0, 20, diameter ± SD, mm) from young males (up to 1 year,  $n = 5$ ) had single spermatozoa, but the active and ripe ones were missing. We observed incomplete spermatogenesis up to level spermatogoniums. At the adult males (above 1 year,  $n = 5$ ) with macroscopic small testes (11, 7 ± 0, 19, diameter ± SD, mm), taken in inactive reproductive period (October – December), a part of the spermatozoa were ripe, but they were not active. The small channels were with suppressed spermatogenesis up to level primary spermatocytes. At the adult males ( $n = 5$ ) with macroscopic medium size of testes (17, 7 ± 0, 04, diameter ± SD, mm), taken in inactive reproductive period, almost all spermatozoa were ripe, but they also were inactive. In the most of the small channels, there were missing elongated spermatids. At the adult males ( $n = 5$ ) with macroscopic big testes (20, 6 ± 0, 19, diameter ± SD, mm), taken in active reproductive period (January – September), all spermatozoa were ripe and active. We observed small channels with ripe elongated spermatids and small channels in a late stage of the spermatogenesis. All investigated testes were with normal anatomic structure, according to the season, which supposed good reproductive possibilities. After detailed microscope analysis, there were not recorded presence of anomalies for the spermatozoa and abnormal structural changes of the testes, which confirms that the anomalies of European brown hare spermatozoa were comparatively rare (Kozdrowski et al. 2006). For the adult males (above 1 year, with different testes size), we observed a difference in the structure of the testes depending on the beginning of the active reproductive period in various regions of the country, which is probably due to their geographical origin. From the results we determinate, that the reproductive organs in female European brown hares were with a normal anatomic structure, at various ages, which supposes good reproductive possibilities. At the young females (up to 1 year,  $n = 5$ ), still had not formed corpus luteum. At the adult females in reproductive age (above 1 year,  $n = 5$ ), we observed tertiary follicles, which were located on a small part of the ovary and there were clearly seen the presence of degenerated corpus luteum.

## 48. European brown hare (*Lepus europaeus* Pall.) testis weights from different seasons and ages from Bulgaria

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Testis weights on 315 European brown hare from different ages were measured during the whole year around in Bulgaria and testicular activity was studied. From the results, we determinate, that the active reproductive season finish at end of August and beginning of September, whereupon follow period of calm when the testis weights were lowest. In September, October, and the beginning of November, the weights of the testes were low. The first indications for starting of the new reproductive season were shown in November, gradually with growth of the testis weights. In January the testis weights reached high levels, as the highest weights were measured in May-June and with a bit lower values in July and August. After this follow low weights, when the calm period started in September and the testis growth is reactive in December. All adult males (above 1 year old, determined by dry eye lens weights,  $n = 160$ ), had high significant values ( $p < 0, 0001$ ) of testis weights during the whole year, compare with all young ones (under 1 year old, determined by dry eye lens weights,  $n = 155$ ). The results showed that normal reproductive cycle with periods of activity and calm in male European brown hares from Bulgaria. The distribution of testes weights, compared to the various ages, follows the same sequence, as at the sperm density (we assigned sperm quantity for each individual in the epididymides to five classes: 1 – no sperm, 2 – very little, 3 – moderately present, 4 – high amount, 5 – massive amount). View of the fact that the density of sperm is defined by the tail of the epididymis, and the weights were measured from the testes, the connection between them confirms the proofs about the existence of a normal reproductive cycle with stages of activity and standstill. The heaviest testes had the biggest quantity of sperm in the epididymis, independently from the season. We defined, that the quantity of sperm and the weights of the testes were in direct dependency ( $p < 0, 0001$ ), as exactly following the line of activity and standstill in the seasons.