Università degli Studi di Torino
Department of Live Science and System Biology

Phenotypic adaptations to high altitude: effects of environmental variables on rodents’ morphology, behaviour, and physiology in the Alps

Ilaria Melcore

PhD Programme Coordinator: **Prof. Silvia Perotto**

Academic Years: **2019 - 2022**

Tutor: **Prof. Sandro Bertolino**
Co-Tutor: **Prof. Zbyszek Boratyński**
Academic Discipline (SSD): **Ecology**

Università degli Studi di Torino

Tesi di Dottorato di Ricerca in Scienze Biologiche
e Biotecnologie Applicate

PhD Thesis in Biological Sciences

and Applied Biotechnologies

Phenotypic adaptations to high altitude: effects of environmental variables on rodents' morphology, behaviour, and physiology in the Alps

Ilaria Melcore

Tutor: Prof. Sandro Bertolino

Co-tutor: Prof. Zbyszek Boratyński

XXXV Cycle:

2019 – 2022

Table of contents

LIST OF CHAPTERS

This doctoral thesis is structured in a general introduction, a collection of one published paper, three unpublished manuscripts and a final discussion. The chapters with the results are based on these manuscripts:

CHAPTER II: Melcore I., Boratyński Z., Bertolino S. Rodents in the Alps follow Allen's rule but contradict Bergmann's rule (*Manuscript*)

CHAPTER III: Melcore I., Demitri A., Bertolino S., Boratynski Z., Nokelainen O. Camouflage accuracy: a study of Alpine rodents across altitudinal levels (*Manuscript*)

CHAPTER IV: Melcore I., Gargano N., Boratyński Z., Cerri J., Bertolino S. Comparing behavioural traits in four species of rodents along an altitudinal gradient in the Alps (*Manuscript*)

CHAPTER V: Melcore I., Bertolino S., Boratyński Z. 2022. Variation of rodents' body temperature across elevation in Alps. *Physiological and Biochemical Zoology 95(6)*

Front cover by I. Melcore, Parco Nazionale Gran Paradiso, 2021.

ABSTRACT

Mountain ecosystems are hotspots for biodiversity and endemism. At the same time, they are threatened by increasing warming due to climate change, which is more than double than in lowlands. This makes mountains one of the world's most vulnerable ecosystems and therefore of great conservation concern. To protect mountain species from extinction a basic understanding of their ecology and physiology is required, which, however, is largely lacking due to the practical and logistical difficulties of conducting research in these landscapes. Despite this, mountains offer an excellent study system by providing an elevation gradient and great variability of habitats, facilitating the study of species' adaptations to changing environmental conditions. This thesis focuses on temperate mountains, which are highly seasonal, resulting in high resource variability across the year, driven by temperature and snow cover. The first part focuses on larger-scale patterns of morphological adaptation and species distribution along elevation gradients. The second part investigates four ground-dwelling forest rodents' physiological and behavioural adaptations: *Apodemus flavicollis, A. sylvaticus, A. alpicola* and *Clethrionomys glareolus.*

Previous literature on mountain ecology describes important patterns in species' biology and ecology. When looking at morphological traits, Bergmann's and Allen's rules have been supported by some studies that found larger bodies and shorter extremities in animals living at high elevations. However, many exceptions to these rules have been noted, showing a preponderance of smaller individuals at higher elevations. In Chapter 2 we analysed the direction of phenotypic variation along elevation gradients in four sympatric rodents' species occurring along a wide elevational range. We found that two species with the wider altitudinal distribution both support (*C. glareolus*) and contradict (*A. flavicollis*) the Allen's and Bergmann's rules.

Animal colouration has been related to fitness in many species. The animal to background matching may result in a lower predation risk for prey species or, conversely, greater hunting success for predators. In Chapter 2, it is shown that this animal to background match is a species-

dependent factor, and it is linked with elevation. This factor divides species with narrow habitat niches from more generalist species that exploit a broader range of environmental conditions. This is particularly evident in our results, where we show how two different groups of rodents adopt two opposite colouration strategies.

In Chapter 3, species' behavioural traits in relation to elevation are considered. The shy-bold continuum is a trait linked to an individual’s personality. Individuals may display varying levels of boldness or shyness that can affect the outcome of daily ecological challenges, such as competing for food, habitat selection, and foraging under the pressure of predation. However, as boldness is controlled by physiological components and can be heritable, it can be subject to evolution, following natural selection in subsequent generations. Here, we compared the inter-specific variation of behavioural traits in sympatric wild rodent species and analysed this variation along a gradient of elevation. In our results, we observed an opposite behaviour between *Apodemus* spp. and *C. glareolus*, with the first more explorative and bold than the second one, and we hypothesised a different genetic and physiological bases of these strategies both probably advantageous for the survival of these species in Alpine environment.

The cost for thermoregulation in many taxa, including rodents, increases with elevation, with great differences in physiological adjustment between high and low elevation. A negative trend of body temperature along elevation has been supported by some studies that focused on the physiological response of highland rodents during winter. In Chapter 4, it is shown that this negative trend in body temperature is not confirmed during the reproductive season when species are exposed to higher air temperature. This result was similar for males and females, suggesting that the high costs of reproduction of females might not be the main reason for the observed pattern. It could be hypothesised that such a mechanism is related to mild tissue hypoxia and greater ventilation of individuals from higher elevations. If true, it would suggest that even weak but chronic hypoxia can have a fitness effect in small endotherms, perhaps influencing on their aerobic capacity performance.

For adequate species protection in mountain areas, it is necessary to better understand species' adaptations. Here, we have contributed to the knowledge of what drives rodent species distributions across elevation gradients by investigating a broad spectrum of these adaptations. We analysed the physiological, behavioural, and morphological mechanisms of alpine rodents, testing the complex interplay between elevation and habitat on these characteristics. Thanks to our results, it is possible to predict the profound impact that phenomena such as deforestation, habitat degradation, landscape changes and even more the ongoing climatic changes can have on species adapted to the Alpine environment. On this basis, further studies could elaborate the determinants of these ecological mechanisms in Alpine rodent populations by linking them with fitness components, such as reproductive success and survival rate, under different environmental conditions, investigating environmental variables to ultimately develop management strategies for the conservation of diverse Alpine communities.

CHAPTER I − Introduction

The importance of mountains to global biodiversity

Mountain regions, here defined to include not only mountain slopes and peaks but also valleys and adjacent foothills (Rahbek et al. 2019), are home to a remarkable proportion of Earth's terrestrial vertebrate species (Körner et al. 2017; Noroozi et al. 2018; Myers et al. 2000; Martin et al. 2021). Weathering and erosion in mountainous ecosystems lead to highly intricate environmental heterogeneity, ultimately providing high biodiversity conditions (Antonelli et al. 2018). In particular, mountain can support hotspots of species richness, including endemisms (Noroozi et al. 2018), due to the compression of a wide range of ecosystems into a relatively short distance, and often provide islands of suitable habitat, isolated from unfavourable surrounding lowlands (Keppel et al. 2016). This thesis focuses on the Alpine landscape, which is characterized by strong natural gradients and considerable spatial and temporal heterogeneity, which give rise to a great diversity of habitats and species (Theurillat et al 2003). Furthermore, human land use has shaped biodiversity, especially below the treeline, where most grasslands are artificial. The Italian Alps, in particular, host a wide range of species. Some of them are alpine endemism, such as the alpine field mouse (*Apodemus alpicola*), others are widespread species both at high and low altitudes, as bank voles (*Clethrionomys glareolus*) and yellow-necked mouse (*A. flavicollis*). Studying biodiversity in mountains is, therefore, of global importance for conservation.

Mountain habitats are far less impacted by anthropogenic disturbance and show lower rates of land-use change (Martin 2001) than lowland habitats. Apart from their role as refugia during past (non-anthropogenic) climate-driven species extinctions (Rahbek et al. 2019), they provide a current refuge not only for cold-adapted species, but also for lowland generalist species that have suffered recent population declines due to land-use change, including agricultural intensification and urbanisation (Scridel et al. 2018).

At the same time, mountains show a more than doubled rate of warming compared to the global average (Brunetti et al. 2009; Oyler et al. 2015; Wang et al. 2016). Global average surface temperatures increased by 0.85 °C between 1880 and 2012 and are likely to rise by a further 1.0–3.7 °C by the end of this century (IPCC 2014), causing many species to shift their geographic ranges. In mountain environments temperatures increased by 1 °C between 1880 and 2012. Even though these changes are not well studied, and general patterns are not yet understood, this level of warming is expected to cause a phenological shift toward earlier springs across trophic levels and potentially prolonged summers (Asam et al. 2018; Jabis et al. 2020; Migliavacca et al. 2008; Vitasse et al. 2021. Moreover, an increasing frequency of extreme weather events, such as late snowfalls in spring, is observed in mountains (Brundl et al 2020), potentially disturbing important phenological events, such as reproduction and migration in birds. Finally, habitat shifts resulting from climatic changes lead to overlap between species that did not interact or compete over some limited resources in the past, affecting the composition of animal and plant communities (Inouye et al., 2000; Réale et al., 2003).

Adaptations to high elevations

Due to their significant environmental variation, mountains provide a wide array of habitats along an elevational gradient at a reduced spatial scale. Animals face this ecological variability with specific adaptations. As altitude increases, the duration of the vegetative period and biomass production decreases, and plant phenology is delayed (Hille & Cooper 2015). As a result, trophic resources are reduced and more scattered across space, predatory pressure decreases, as well as the structure and diversity of vegetation (Laiolo et al., 2004; Nagy & Grabherr, 2009; Hürlimann et al., 2019). At high elevations, animals need to cope with higher precipitation, windier climatic conditions, high solar radiation, lower ambient temperature and lower oxygen partial pressure, which result in a range of physiological and behavioural adaptations. Additionally, the high daily and seasonal variability in all environmental conditions, such as environmental temperature, forces animals to react flexibly to these changes. Species, need to cope with extremely high and low temperatures, or dry and wet conditions, often within a short timeframe (Levy et al. 2011; Careau et al. 2012). Contrary to short-term acclimatization - and immediate physiological response to environmental change - high altitude adaptation also means irreversible physiological responses associated with inheritable changes, evolved for high altitude environments. Environmental conditions can be challenging for many animals, especially small endothermic mammals and birds. As body size decreases, the surface area over which heat is lost increases relative to the volume of tissue available for heat production. Small mammals also have a limited capacity for insulation with either fat accumulation or fur composition compared to larger animals (Scholander 1955). For many species, the temperatures at high altitude are often below their thermoneutral zone - the narrow range of ambient temperatures where body temperature can be maintained solely using basal metabolic rates - especially during winter (Levy et al., 2011). Therefore, small endotherms at high altitudes are challenged with high demands for thermogenesis, likely contributing to their very high daily energy expenditures, and possibly restricting the aerobic activity (Hayes 1989). The ability of high-altitude animals to maintain adequate aerobic performance could result from a combination of genetic specializations, environmentally induced plasticity (i.e., acclimatization, developmental plasticity, etc.), and the interaction between these two processes (Storz 2010).

Morphological adaptations

Along elevation gradients, numerous factors shape morphological adaptations. Bergmann's rule explains morphological differences across climatic gradients, predominantly from warmer to colder environments (Meiri and Dayan 2003; Gohli & Voje, 2016). Expanding on Bergmann’s rule (Bergmann, 1847, Mayr, 1956), body sizes should exhibit a positive correlation with elevation. This can result from the selection of larger animals with a lower surface-volume ratio than smaller animals, thus radiating less body heat per unit of mass. However, exceptions to this rule have been reported (Eastman et al., 2012, Grieco & Rizk, 2010, Tomassini et al., 2014, Yom-Tov et al., 2006, Yom-Tov et al., 2003, Yom-Tov et al., 2012, Yom-Tov et al., 2013), showing a preponderance of smaller individuals at higher elevations (Lundblad and Conway 2019). This could be explained as smaller bodies require lower total energy intake, which might be a limiting factor in high elevation habitats with seasonally variable resource availability. Thus, it has been suggested that food availability may be a driver of body size in mammals contradicting Bergmann's rule (Yom-Tov et al., 2006).

Physiological adaptations

In endotherms living in cold and hypoxic conditions at high altitudes, there are ecologically important measures of whole-organism performance, such as capacities for sustained exercise and thermogenesis, which are directly related to aerobic metabolism. An animal's rate of aerobic metabolism can be measured as the oxygen (O_2) consumption rate because oxygen is required for ATP synthesis via oxidative phosphorylation in the mitochondria (Storx, 2021). The most obvious high altitude challenges for endothermic vertebrates are the reduced partial pressure of oxygen and the generally cooler temperatures. This reduction of atmospheric oxygen can result in serious depletion of O_2 in the tissues, impairing metabolism. This not only may affect the maintenance of regular activity but also may impair the capacity to maintain a constant internal temperature in the face of cooler ambient temperatures (Storz et al. 2010; Cheviron and Brumfield, 2012; Lövy et al., 2022). Another challenge is maintaining adequate hydration as several responses to high altitude hypoxia (low O_2) also lead to increased water loss. One of the first physiologic responses to hypoxia is an increase in ventilation (breathing) to increase the oxygen partial pressure in the lungs, helping to compensate for the drop in oxygen partial pressure in the atmosphere. However, increased ventilation results in increased respiratory water loss. Down-regulation of the kidney system results in increased urinary output (Yanagisawa et al. 2012). These responses necessitate an increase in water intake to avoid dehydration. Another strategy adopted by high-altitude animals is the increase in haemoglobin (Hb) affinity with O_2, which improves the amount of oxygen transported to tissues. The high Hb-O_2 affinity in alpine populations is associated with a greater maximal rate of O_2 consumption at high altitudes. This allows for greater aerobic activity and thermogenic capacity in the active, non-hibernating rodents (Storz, 2007; Cheviron and Brumfield, 2012; Natarajan et al. 2013).

Behavioural adaptations

The activity rate of a species is shaped not only by internal signals but also by external factors, such as distribution and abundance of resources, competition, predation risk and climatic factors (i.e. rainfall or temperature; Halle & Stenseth 2000; Körtner & Geiser 2000; Fragaszy et al., 2004). The optimal amount, duration and distribution of activities are influenced by the availability of energy which is strongly related to the distribution of food resources and ambient temperature (Daan & Aschoff 1982; Angilletta et al. 2010). In particular, the energy balance of small endotherms can be challenged by variations in the ambient temperature due to a relatively high surface-to-volume ratio and, therefore, to a low heat capacity which increases the energy demand for thermoregulation (McNab 2002). Maintaining body temperature within certain limits is essential for homeostasis; animals could, therefore, modify their activity rhythms to cope with ambient temperature changes, in order to avoid episodes of hyper or hypothermia (Sassi 2015). Due to their significant environmental variation, mountains represent a valuable context where environmental conditions influence animal behaviour, both within and between populations. Among behavioural traits, the so-called "bold-shy" continuum, where individuals show consistent differences in their degree of risk-prone behaviour (Ioannou et al. 2008; Réale et al. 2007; Zidar et al. 2017), was found to have an adaptive value when environmental conditions change rapidly (e.g., rodents, Dammhan et al., 2020). Environmental conditions associated with elevation gradients, can equally select bold and shy behavioural traits. Bold animals could be favoured by their greater exploratory activity and might dominate sparsely distributed resources (Huntingford et al., 2012). On the other hand, when resources are scarce and scattered, selection could favour shy behaviours, characterised by less active and slower animals, minimizing energy consumption in such harsh conditions (Mettke-Hofmann et al., 2002).

Adaptation of colourations

Animal colouration is shaped by multiple selective pressures, including camouflage, communication, and thermoregulation (Zimova et al., 2018), and has been related to fitness in many species (Kappers et al., 2020; Morosinotto et al., 2020; Roff & Fairbairn, 2013). An advantage of camouflage may result in a lower predation risk for prey species or, conversely, greater hunting success for predators (Galeotti et al., 2003; Zimova et al., 2018). In addition to the selective advantage of camouflage, a potential role of thermoregulation as an ecological driver of fur colouration has been proposed and has received empirical support (Caro, 2005; Clusella Trullas et al., 2007; Stuart-Fox et al., 2017). Since the adaptive value of camouflage is mainly based on the similarity of individuals to visual characteristics of the environmental background, specific phenotypic characteristics should only be effective in a limited set of environments (Hughes, Liggins, & Stevens, 2019; Ruxton et al., 2004; Stevens & Merilaita, 2009; Thayer, 1896). Animals have many ways to optimize camouflage. For example, they may evolve a generalist tactic ('imperfect camouflage'), which confers a reasonable level of concealment across a range of habitat types but fails to be optimal in any (Houston et al. 2007). Alternatively, some species may adopt more specialized camouflage, which provides better protection in specific environments but constrains the use of other habitats at the cost of increased vulnerability (Michalis et at., 2017). Thus, it can be predicted that animals inhabiting a heterogeneous range of environments should adopt a more generalist and compromise camouflage tactic. In contrast, sedentary and less exploratory species should rely on more specialized camouflage (Fennell et al., 2018; Hughes et al., 2019; Merilaita et al. 2017). Alpine environments are characterized by extreme and changing climatic conditions, which make the species inhabiting them particularly diversified in terms of adaptive strategies. Along the altitudinal gradient, we assist to a variation of structure and complexity of a habitat which may affect the habitats and animal colour and lighting patterns (Cuthill, 2019).

Effect of Global Warming

In seasonal environments, the optimal exploitation of food resources by species at higher trophic levels requires synchronization with productivity at lower trophic levels, namely plant phenology and insect emergence for rodents (Kristensen et al. 2015; Kubelka et al. 2022). The need for synchronization is likely to be particularly marked at high latitudes and elevations, given the relatively short vegetation and breeding season. Recently, phenological shifts across taxa under climate change have been observed in alpine habitats (Chen et al., 2011; Vitasse et al. 2021). Phenological mismatches can drastically affect animals' fitness if a minimum of resources is not available to raise offspring (Corkery et al. 2019). In addition to potential phenological mismatches in seasonal environments, extreme weather events are likely to become more frequent with global warming, with cold snaps, late snowfall and high precipitation affecting reproductive success (Kluen et al. 2011; Moreno et al. 2015).

Aims of the thesis

This thesis aims to determine the mechanisms underlying phenotypic variation of mountain rodent species, in order to predict the impacts of environmental change on this particular ecosystem. In the first part, a broader perspective is taken by considering four rodents' species, yellow-necked mouse *Apodemus flavicollis*, wood mouse *A. sylvaticus*, alpine field mouse *A. alpicola* and bank vole *Clethrionomys glareolus*, investigating interspecific variation in morphological traits across an elevational gradient, and the potential habitat factors affecting this variability. The variation in animal colouration to background match according to habitat structure and sex is described in Chapter 2 using digital images of the animals and their respective backgrounds. Behavioural traits described at the species level are investigated in Chapter 3, comparing two behaviours associated to the shy-bold trait, to observe their variation between species and the effect of elevation and habitat complexity.. These studies shall help to understand how rodents might react and adapt to the future landscape and climate change, i.e. phenological shifts in spring progression and exceptional (more extreme) weather conditions. Chapter 4 presents the results on the thermoregulatory adaptations of these four species, considering body temperature and the effect of elevation, habitat structure, sex, and body condition. This is the first study on the body temperature of these wild rodent species across altitudes during their reproductive season. In Chapter 5, we discuss the overall implications of the results of the thesis in a broader context of environmental change and propose the next crucial research steps needed for a better understanding of species adaptations to alpine habitats.

Reference

Angilletta, M. J. Jr, Cooper, B. S., Schuler, M. S. & Boyles, J. G. 2010: The evolution of thermal physiology in endotherms. Front Biosci. 2, 861—881.

Antonelli, A., Kissling, W.D., Flantua, S.G.A., Bermúdez, M.A., Mulch, A., Muellner-Riehl, A.N., Kreft, H., Linder, H.P., Badgley, C., Fjeldså, J., et al. (2018). Geological and climatic influences on mountain biodiversity. Nat. Geosci. *11*, 718–725.

Asam, S., Callegari, M., Matiu, M., Fiore, G., De Gregorio, L., Jacob, A., Menzel, A., Zebisch, M., and Notarnicola, C. (2018). Relationship between Spatiotemporal Variations of Climate, Snow Cover and Plant Phenology over the Alps - An Earth Observation-Based Analysis. Remote Sens. *10*, 1757. DOI:10.3390/rs10111757

Bergmann, C. (1847). Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Göttinger Stud.*1*, 595–708.

Brundl A.C., Sallé L., Lejeune L.A., Sorato E., Thiney A., Chaine A.S., Russell A.F. 2020.) Elevational Gradients as a Model for Understanding Associations Among Temperature, Breeding Phenology and Success. Front. Ecol. Evol. 8:563377. doi: 10.3389/fevo.2020.563377.

Brunetti, M., Lentini, G., Maugeri, M., Nanni, T., Auer, I., Böhm, R., and Schöner, W. (2009). Climate variability and change in the Greater Alpine Region over the last two centuries based on multi-variable analysis. Int. J. Climatol. *29*, 2197–2225.

Careau V. and T.Jr. Garland. 2012. Performance, personality, and energetics: correlation, causation, and mechanism. Physiol Biochem Zool 85:543–571.

Caro, T. (2005). The adaptive significance of coloration in mammals. *BioScience*, *55*(2), 125–136. https://doi.org/10.1046/j.1095-8312. 2003. 00190.x

Chen IC Hill JK Ohlemüller R Roy DB Thomas CD . 2011. Rapid range shifts of species associated with high levels of climate warming. Science. 333:1024–1026.

Cheviron, Z.A., and R.T. Brumfield. 2012. Genomic insights into adaptation to high-altitude environments. Heredity 108:354–361.

Clusella Trullas, S., van Wyk, J. H., & Spotila, J. R. (2007). Thermal melanism in ectotherms. *Journal of Thermal Biology*, *32*(5), 235–245. https://doi.org/10.1016/j.jther bio.2007.01.013

Corkery, C.A., Nol, E., and Mckinnon, L. (2019). No effects of asynchrony between hatching and peak food availability on chick growth in Semipalmated Plovers (*Charadrius semipalmatus*) near Churchill, Manitoba. Polar Biol. *42*, 593–601.

Cuthill, I. C., Stevens, M., Sheppard, J., Maddocks, T., Párraga, C. A., & Troscianko, T. S. (2005). Disruptive coloration and background pattern matching. *Nature*, *434*, 72–74. https://doi.org/10.1038/nature03312

Daan, S. & Aschoff, J. 1982: Circadian contributions to survival. In: Vertebrate Circadian Systems. (Aschoff, J., Dann, S., Groos, G. A., eds). Springer, Berlin Heidelberg, pp. 305—321.

Eastman LM, Morelli TL, Rowe KC, Conroy CJ, Moritz C. (2012). Size increase in high elevation ground squirrels over the last century. Global Change Biology, 18,1499--1508.

Fennell, J. G., Talas, L., Baddeley, R. J., Cuthill, I. C., Life, B., Building, S., & Acquisition, F. (2018). Optimising colour for camouflage and visibility: The effects of the environment and the observer’s visual system. *Journal of the Royal Society Interface*. https://doi.org/10.1098/rsif.2019.0183

Fragaszy, D. M., Visalberghi, E. & Fedigan, L. M. 2004: The Complete Capuchin: The Biology of the Genus Cebus. Cambridge University Press, Cambridge, UK.

Galeotti, P., Rubolini, D., Dunn, P. O., & Fasola, M. (2003). Colour polymorphism in birds: Causes and functions. *Journal of Evolutionary Biology*, *16*(4), 635–646. https://doi.org/10.1046/j.1420-9101.2003.00569.x

Gohli J, Voje KL. An interspecific assessment of Bergmann’s rule in 22 mammalian families. BMC evolutionary biology. 2016 Dec;16(1):1-2.

Grieco T, Rizk O. (2010). Cranial shape varies along an elevation gradient in Gambel's white-footed mouse (*Peromyscus maniculatus gambelii*) in the Grinnell Resurvey Yosemite transect. Journal of Morphology, 271, 897-909

Halle, N. & Stenseth, S. C. 2000: Activity Patterns in Small Mammals, an Ecological Approach. Springer-Verlag, Berlin.

Hayes JP. 1989. Altitudinal and seasonal effects on aerobic metabolism of deer mice. J. Comp. Physiol. B 159:453–59

Houston, A. I., Stevens, M., & Cuthill, I. C. (2007). Animal camouflage: Compromise or specialize in a 2 patch-type environment? *Behavioral Ecology, 18*, 769–775. https://doi.org/10.1093/beheco/arm039

Hughes, A., Liggins, E., & Stevens, M. (2019). Imperfect camouflage: How to hide in a variable world? *Proceedings of the Royal Society B: Biological Sciences, 286*, 20190646. https://doi.org/10.1098/rspb.0646

Huntingford, F.A., Tamilselvan, P., Jenjan, H., 2012. Why do some fish fight more than others? Physiol. Biochem. Zool. 85, 585–593.

Inouye, D.W., Barr, B., Armitage, K.B. & Inouye, B.D. (2000). Climate change is affecting altitudinal migrants and hibernating species. Proc. Natl. Acad. Sci. USA 97, 1630–1633.

Ioannou, C.C., Payne, M. & Krause, J. 2008. Ecological consequences of the bold–shy continuum: the effect of predator boldness on prey risk. Oecologia 157, 177. https://doi.org/10.1007/s00442-008-1058-2

IPCC, 2014: Climate Change 2014: In: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. (Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Farahani, E., Kadner, S., Seyboth, K., Adler, A., Baum, I., Brunner, S., Eickemeier, P., Kriemann, B., Savolainen, J., Schlömer, S., von Stechow, C., Zwickel, T. & Minx, J. C. eds). Cambridge University Press, United Kingdom and New York, NY, USA.

Jabis, M.D., Winkler, D.E., and Kueppers, L.M. (2020). Warming acts through earlier snowmelt to advance but not extend alpine community flowering. Ecology *101*, e03108.

Kappers, E. F., de Vries, C., Alberda, A., Kuhn, S., Valcu, M., Kempenaers, B., & Both, C. (2020). Morph-dependent fitness and directional change of morph frequencies over time in a Dutch population of Common buzzards *Buteo buteo*. *Journal of Evolutionary Biology*, *33*(9), 1306–1315. https://doi.org/10.1111/jeb.13675

Keppel G., Todd P. Robinson, Grant W. Wardell-Johnson, Colin J. Yates, Kimberly P. Van Niel, Margaret Byrne, Antonius G. T. Schut, A low-altitude mountain range as an important refugium for two narrow endemics in the Southwest Australian Floristic Region biodiversity hotspot, *Annals of Botany*, Volume 119, Issue 2, January 2017, Pages 289–300, https://doi.org/10.1093/aob/mcw182

Kluen, E., de Heij, M.E., andBrommer, J.E. (2011). Adjusting the timing of hatching to changing environmental conditions has fitness costs in blue tits. Behav. Ecol. Sociobiol. *65*, 2091–2103.

Körner, C., Jetz, W., Paulsen, J., Payne, D., Rudmann-Maurer, K., and M. Spehn, E. (2017). A global inventory of mountains for bio-geographical applications. Alp. Bot. *127*, 1–15.

Körtner, G. & Geiser, F. 2000: Torpor and activity patterns in free-ranging sugar gliders Petaurus breviceps (Marsupialia). Oecologia 123, 350—357

Kristensen, N.P., Johansson, J., Ripa, J., and Jonzén, N. (2015). Phenology of two interdependent traits in migratory birds in response to climate change. Proc. R. Soc. B Biol. Sci. *282*, 20150288.

Kubelka, V., Sandercock, B.K., Székely, T., and Freckleton, R.P. (2022). Animal migration to northern latitudes: environmental changes and increasing threats. Trends in Ecology & Evolution *37*, 30–41.

Levy O., T. Dayan, and N. Kronfeld-Schor. 2011. Adaptive thermoregulation in golden spiny mice: the influence of season and food availability on body temperature. Physiol Biochem Zool 84:175-184.

Lövy M., Boratyński Z., Okrouhlík J., Svačinová L., Meheretu Y., Sumbera R. (2022). Alternative highland adaptations: organ masses and fur insulation in Afroalpine rodents. Journal of Zoology, in press.

Lundblad, C.G., and Conway, C.J. (2019). Variation in Selective Regimes Drives Intraspecific Variation in Life History Traits and Migratory Behavior along an Elevational Gradient. J. Anim. Ecol. *00*, 1–15. https://doi.org/10.1111/1365-2656.13134

Mayr E. Geographical character gradients and climatic adaptation. *Evolution*. 1956;10:105-108. https://www.proquest.com/scholarly-journals/geographical-character-gradients-climatic/docview/10193289/se-2.

Martin, K. (2001). Wildlife in alpine and sub-alpine habitats. In Wildlife-Habitat Relationsships in Oregon and Washington., (Johnson, D.H. and T.A. O'Neil. Oregon State University Press), pp. 239–260

Martin, K., Altamirano, T.A., de Zwaan, D.R., Hick, K.G., Vanderpas, A., and Wilson, S. (2021). Avian ecology and community structure across elevation gradients: The importance of high latitude temperate mountain habitats for conserving biodiversity in the Americas. Glob. Ecol. Conserv. *30*, e01799.

Mettke-Hofmann, C., Winkler, H., Leisler, B., 2002. The significance of ecological factors for exploration and neophobia in parrots. Ethology 108, 249–272

Merilaita, S., Scott-Samuel, N. E., & Cuthill, I. C. (2017). How camouflage works. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*, 20160341. https://doi.org/10.1098/

rstb.2016.0341

McNab, B. K. 2002: The Physiological Ecology of Vertebrates: A View from Energetics. Cornell University Press, New York.

Meiri, S., and Dayan, T. (2003). On the validity of Bergmann's rule: On the validity of Bergmann's rule. J. Biogeogr. *30*, 331–351.

Michalis, C., Scott-samuel, N. E., Gibson, D. P., Cuthill, I. C., & Michalis, C. (2017). Optimal background matching camouflage. *Proceedings of the Royal Society B*, *284*, 20170709. https://doi.org/10.1098/rspb.2017.0709

Migliavacca, M., Cremonese, E., Colombo, R., Busetto, L., Galvagno, M., Ganis, L., Meroni, M., Pari, E., Rossini, M., Siniscalco, C., et al. (2008). European larch phenology in the Alps: can we grasp the role of ecological factors by combining field observations and inverse modelling? Int. J. Biometeorol. *52*, 587–605.

Moreno, J., González-Braojos, S., and Ruiz-de-Castañeda, R. (2015). A spring cold snap is followed by an extreme reproductive failure event in a mountain population of Pied Flycatchers *Ficedula hypoleuca*. Bird Study *62*, 466–473.

Morosinotto, C., Brommer, J. E., Lindqvist, A., Ahola, K., Aaltonen, E., Karstinen, T., & Karell, P. (2020). Fledging mass is color morph specific and affects local recruitment in a wild bird. *The American Naturalist*, *196*(5), 609–619. https://doi.org/10.1086/710708

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature *403*, 853–858.

Natarajan, C., N. Inoguchi, R.E. Weber, A.Fago, H. Moriyama, and J.F. Storz. 2013.Epistasis among adaptive mutationsin deer mouse hemoglobin. Science340:1324–1327.

Noroozi, J., Talebi, A., Doostmohammadi, M., Rumpf, S.B., Linder, H.P., and Schneeweiss, G.M. (2018). Hotspots within a global biodiversity hotspot - areas of endemism are associated with high mountain ranges. Sci. Rep. *8*, 10345.

Oyler, J.W., Dobrowski, S.Z., Ballantyne, A.P., Klene, A.E., and Running, S.W. (2015). Artificial amplification of warming trends across the mountains of the western United States. Geophys. Res. Lett. *42*, 153–161.

Rahbek C., M. K. Borregaard, B. Hermansen, D. Nogués-Bravo, J. Fjeldså. (2019). Mountain Regions of the World.

Réale, D., McAdam, A.G., Boutin, S. & Berteaux, D. (2003). Genetic and plastic responses of a northern mammal to climate change. Proc. Roy. Soc. Lond. B 270, 591–596

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev 82(2):291–318

Roff, D. A., & Fairbairn, D. J. (2013). The costs of being dark: The genetic basis of melanism and its association with fitness-related traits in the sand cricket. *Journal of Evolutionary Biology*, *26*(7), 1406–1416. https://doi.org/10.1111/jeb.12150

Ruxton, G. D., Sherratt, T. N., & Speed, M. P. (2004). *Avoiding attack: The evolutionary ecology of crypsis, warning signals and mimicry*. New York, NY: Oxford University Press.

Sassi P.L., Taraborelli P., Albanese S., Gutierrez A. 2015. Effect of Temperature on Activity Patterns in a Small Andean Rodent: Behavioral Plasticity and Intraspecific Variation. Ethology121(2015) 840–849

Scridel, D., Brambilla, M., Martin, K., Lehikoinen, A., Iemma, A., Matteo, A., Jähnig, S., Caprio, E., Bogliani, G., Pedrini, P., et al. (2018). A review and meta-analysis of the effects of climate change on Holarctic Mountain and upland bird populations. Ibis *160*, 489–515.

Scholander PF. 1955. Evolution of climatic adaptation in homeotherms. Evolution 9:15–26

Stevens, M., & Merilaita, S. (2009). Animal camouflage: Current issues and new perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*, 423–427. https://doi.org/10.1098/rstb.

Storz, J.F. 2007. Hemoglobin function andphysiological adaptation to hypoxia inhigh-altitude mammals. Journal of Mam-malogy 88(1): 24–31

Storz, J.F., G.R. Scott, and Z.A. Cheviron. 2010. Phenotypic plasticity and genetic adaptation to high altitude hypoxia in vertebrates. The Journal of Experimental Biology 213:4125–4136.

Storz JF. High-Altitude Adaptation: Mechanistic Insights from Integrated Genomics and Physiology. Mol Biol Evol. 2021 Jun 25;38(7):2677-2691. doi: 10.1093/molbev/msab064. PMID: 33751123; PMCID: PMC8233491.

Stuart-Fox, D., Newton, E., & Clusella-Trullas, S. (2017). Thermal consequences of colour and near-infrared reflectance. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1724). https://doi.org/10.1098/rstb.2016.0345

Thayer, A. H. (1896). The law which underlies protective coloration. *The Auk*, *13*, 477–482. https://doi.org/10.2307/4068693

Theurillat J.P., A. Schlüssel, P. Geissler, A. Guisan, C. Velluti, and L. Wiget . 2003. Vascular plant and bryophyte diversity along elevational gradients in the Alps. In L. Nagy, G. Grabherr, C. Körner, and D. B. A. Thompson editors. Alpine Biodiversity in Europe. Berlin, Germany Springer. pp. 185–193.

Tomassini A, Colangelo P, Agnelli P, Jones G, Russo D. (2014). Cranial size has increased over 133 years in a common bat, *Pipistrellus kuhlii*: a response to changing climate or urbanization? Journal of Biogeography, 41, 944--953.

Vitasse, Y., Ursenbacher, S., Klein, G., Bohnenstengel, T., Chittaro, Y., Delestrade, A., Monnerat, C., Rebetez, M., Rixen, C., Strebel, N., et al. (2021). Phenological and elevational shifts of plants, animals and fungi under climate change in the European Alps. Biol. Rev. *96*, 1816–1835.

Wang, Q., Fan, X., and Wang, M. (2016). Evidence of high-elevation amplification versus Arctic amplification. Sci. Rep. *6*, 19219.

Yanagisawa, K., O. Ito, S. Nagai, and S. Onishi. 2012. Electrolyte-carbohydrate beverage prevents water loss in the early stage of high-altitude training. The Journal of Medical Investigation 59:102–110. https://www.jstage.jst.go.jp/article/jmi/59/1%2C2/59_1%2C2_102/_pdf.

Yom-Tov Y, Heggberget T, Wiig Ø, Yom-Tov S (2006). Body size changes among otters, *Lutra lutra*, in Norway: the possible effects of food availability and global warming. Oecologia, 150, 155-160.

Yom-Tov Y, Yom-Tov S, Angerbjorn A (2010) Body size of the weasel *Mustela nivalis* and the stoat *M. erminea* in Sweden. Mammalian Biology. Zeitschrift für Säugetierkunde, 75, 420-426.

Yom-Tov Y, Yom Tov S, Baagøe H (2003) Increase of skull size in the red fox (*Vulpes vulpes*) and Eurasian badger (*Meles meles*) in Denmark during the twentieth century: an effect of improved diet? Evolutionary Ecology Research, 5, 1037-1048.

Yom-Tov Y, Yom-Tov S, Jensen T, Baagoe H (2012) No recent temporal changes in body size of three Danish rodents. Acta Theriologica, 57, 59-63.

Yom-Tov Y, Yom Tov S, Macdonald D, Yom Tov E. (2007) Population cycles and changes in body size of the lynx in Alaska. Oecologia, 152, 239-244.

Yom-Tov Y, Yom Tov S, Zachos FE (2013) Temporal and geographical variation in skull size of the red fox (*Vulpes vulpes*) and the Eurasian badger (*Meles meles*) in Austria. Biological Journal of the Linnean Society, 108, 579-585.

Zidar J., Balogh A., Favati A., Jensen P., Leimar O., Løvlie H. 2017. A comparison of animal personality and coping styles in the red Junglefowl. Animal Behaviour 130 (2017) 209e220

Zimova, M., Hackländer, K., Good, J. M., Melo-Ferreira,J., Alves, P. C., & Mills, L. S. (2018). Function and underlying mechanisms of seasonal colour moulting in mammals and birds: What keeps them changing in a warming world? *Biological Reviews*, *93*(3), 1478–1498.

CHAPTER II

Rodents in the Alps follow Allen's rule but contradict Bergmann's rule

Ilaria Melcore[1], Zbyszek Boratyński[2], Sandro Bertolino[1]

[1] Department of Life Sciences and Systems Biology, University of Turin, Via Accademia Albertina 13, 10123 Torino, Italy.

[2] Association BIOPOLIS, CIBIO-InBIO Associate Laboratory, Research Center in Biodiversity and Genetic Resources, University of Porto, Porto, Portugal

Manuscript

Abstract

The study of phenotypic variations along gradients can provide information on the mechanisms that drive the distribution of species and therefore can be a useful indicator of environmental change. In mountain, numerous factors can shape morphological adaptations, including ultraviolet radiation, temperature, air pressure and oxygen levels, and food availability. We analysed three years data to measure the direction of phenotypic variation along elevation gradients in four sympatric rodent species occurring along a wide elevation range. We modelled inter-specific change in foot length and body mass with elevation and found an opposite pattern between the two groups: in *Apodemus* foot length increased along elevation while body mass decreased; in *C. glareolus* foot length was shorter than in *Apodemus* species, and not affected by elevation, while body mass increased along this gradient. Another interesting result was the relationship between body temperature and body mass. In particular, in *C. glareolus* smaller bodies had higher body temperature, but this was not the case in *Apodemus* species. The results of our exploratory analysis show the potential of studying species living along broad elevation gradients, as they are exposed to diverse climatological and ecological conditions and this environmental variation is thus expected to drive morphological variation.

Keywords: Alps, body mass, foot length, morphological traits, rodents.

Running head: Morphological variation in Alpine rodents

INTRODUCTION

Morphology can provide information on the ability of species to respond to a changing environment (Hoffmann & Parsons, 1991; Yom-Tov & Yom-Tov, 2005). Both spatial and temporal differences in habitat variability significantly influence the variation in morphology within species (Wauters et al., 2007; Hopkins, 2008; Jetz *et al*., 2009; Ozgul *et al*., 2010; Tranquillo et al., 2022). In particular, many studies have focused on the change in body size in response to warmer environments (Porter & Kearney, 2009; Gardner et al., 2011). Although change in body size or a similar phenotypic response is one of the most evident adaptations of species to environmental change (Barnosky et al., 2003), genetic adaptation of other traits may be a further response to such events (Stockwell et al., 2003; Davis et al., 2005; Leech & Crick, 2007).

In mountain environments, numerous factors can shape morphological adaptations. Variables related to elevation include ultraviolet radiation, temperature, air pressure and oxygen levels. Bergmann's Rule, which says that population and species of larger size are found in colder environments, is often used to explain morphological differences across climate gradients (Bergmann, 1847). This common trend in body size can result from the selection of larger animals which have lower surface-to-volume ratio than smaller animals, and so they radiate less body heat per unit of mass. Allen's rule predicts that animals living in cold climates should evolve shorter limbs and extremities to minimize the surface area, thus dissipating less heat (Allen, 1877). However, many exceptions to these rules have been reported (e.g. Eastman et al., 2012, Grieco & Rizk, 2010, Tomassini et al., 2014, Yom-Tov et al., 2013), particularly among birds, which show a preponderance of smaller individuals at higher elevations (Lundblad & Conway 2019). A hypothesis to explain these opposite patterns could be that smaller bodies require less energy intake, which could be advantageous in high altitude habitats, where resource availability varies seasonally. These contradictory trends suggest that there may be other factors contributing to the change in body size patterns (Wolverton *et al*., 2009; McNab, 2010), one of which it could be the nutrition and food availability (Langvatn & Albon, 1986; Geist, 1987; Wauters et al., 2007).

Recent global warming and its consequences, such as rising temperatures at higher altitudes, early snowmelt and greening, and an extended growing season, lead to a high heterogeneity in the magnitude and direction of responses in size of animals (Gardner et al., 2011). Some research has reported an increase in body size as some species respond positively to nutritional changes or increased food availability because of early greening and more extended growing season (Yom-Tov & Yom-Tov, 2005; Yom-Tov *et al*., 2006; McNab, 2010; Ozgul *et al*., 2010; Eastman et al., 2012). Other studies, however, have shown that due to the increase in spring and summer temperatures (Luterbacher et al., 2004; Brunetti et al. 2009; Oyler et al. 2015; Wang et al. 2016), individuals reduce foraging time to avoid heat stress (Aublet et al., 2009) with consequent reduction in body size (Gardner et al. 2011; Mason et al., 2014). Thus, studying trait variation within and across species along environmental gradients can be used to understand potential reactions to future global changes. Rodents living in mountain habitats are interesting model species to study such variation since they rich a good compromise in the accuracy of matching across different habitat types (Nokelainen et al. 2020) and thanks to their genetic and adaptive plasticity (Hürlimann et al. 2019), they show a more rapid population response to habitat variations (Vessey et al. 2007). Nevertheless, comparative studies in mountain habitats used by small mammals' species are few (Armstrong, 1977) and, consequently, we still have a limited understanding and information on the mechanisms and the habitat use strategies behind those species adapted to live at high altitudes.

In this study, we explored the existence of intra and interspecific variation between four rodent species in two phenotypic traits: body size and body mass, and the presence of sexual dimorphism in these traits. In particular, we wanted to test whether, increasing in elevation, rodents follow Bergman' and Allen's rules. We predicted 1) differences in body size and mass along the elevation, with smaller animals of lower body mass in the higher elevation, as they may be advantaged by having lower daily energy-requirements than larger and heavier individuals. We therefore evaluated whether 2) smaller bodies have a lower body temperature, due to a lower absolute energy requirement. Finally, we also tested for 3) difference in these traits associated to variation between trapping sites, characterized by variation in type and overall availability of the major food resources. We also investigated sexual dimorphism in these traits (Bednarz & Zwolak, 2022; see also in squirrel, Wauters et al. 2007).

METHODS

Study area

In this analysis, we used data collected during two sampling periods: the first one during 2017 and the second during 2020 and 2021, within the Gran Paradiso National Park, in the Graian Alps (northwestern Italy). Trapping was conducted during the breeding season of the target species, in spring and summer. In 2017, seven altitudinal levels separated 300 m of altitude were selected, between 800 and 2,600 m a.s.l. At each altitudinal level, two trapping sites were identified (except for the highest altitudinal level with only one trapping site), for a total of thirteen trapping locations. Each trapping area was composed of a line with 40 sampling points distanced 10 m from each other, where 120-140 live traps (Sherman traps, Ugglan and Longworth) were placed in groups of 3-4 traps. Each transect was surveyed in two sessions in May-July and July-September with traps left active for three days. During the years 2020 and 2021, from April to September, we sampled three wide altitudinal ranges (from 400 to 800 m, from 900 to 1,300 and from 1,500 to 1,822 m a.s.l). Four trapping sites were identified at each altitudinal level for a total of twelve trapping locations. Days of trapping at high- and low-altitude sites were randomly alternated, and the order of trapping was recorded. Each trapping area consisted of a line of 30 sampling points with 60 live traps (Sherman traps, Ugglan and Longworth) placed in groups of 2, located every 10 m (Figure 1) and left active for one week. Traps were baited with apple, sunflower seeds and peanut butter and provided cotton as bedding. The animal’s sex was recorded, along with body mass (Pesola spring balance; ±1 g), foot length and other body measurements (see Melcore et al., 2022). Each captured individual was assigned to an age class (young and adults, based on fur characteristics and body dimensions, e.g. Gurnell and Flowerdew 2019), and tissue ear samples were collected and used in genetic barcoding of the *Apodemus* species. In 2020 and 2021, body temperature was also recorded using a rectal thermocouple (RET-3; accuracy: ± 0.1 C°) connected to a thermometer (model PST095) on adult individuals acclimatised to laboratory conditions during the active phase of the species' daily cycle, around 10:00 p.m (see Melcore et. al, 2022).

Statistical analysis

For the analysis, we considered only adult individuals of four species: yellow-necked mouse *Apodemus flavicollis*, wood mouse *A. sylvaticus*, alpine field mouse *A. alpicola* (with a minimum body mass of 15 g for *Apodemus* spp.) and bank vole *Clethrionomys* g*lareolus* (with a minimum body mass of 13 g). All continuous variables (foot length, body mass, elevation, and body temperature) were log10 transformed before analyses to improve normality in the distributions of residuals.

We conducted two separate analyses. For the first analysis, we used the whole database to evaluate whether the four species differed in foot length and body mass. Since data were non normalized, we used Kruskal-Wallis ANOVA and then pairwise.wilcox.test with corrections for multiple testing (Benjamini–Hochberg correction; Beyer, 2002) for pairwise comparisons. We explore the effect of elevation (from 390 to 2286 m a.s.l.) on foot length, and body mass (dependent variables) within each species. For the second analysis, we used the database of the years 2020-2021 to assess for each species the effect of trapping site, sex, and body temperature (continuous variables) on foot length and body mass. For these analyses we applied generalized linear mixed models (glmmADMB package) accounting for variation among years (random factor; Bolker et al. 2012; R Core Team 2019). The goodness of models fit was compared using the Likelihood Ratio Test (hereafter LRT; package "lmtest" in R), to compare the full model, where all the variables were included, with the reduced models, excluding one variable at the time. (Self et al., 1992).

RESULTS

In total, 1,197 individuals have been analysed for the period 2017-2021, out of which 396 individuals belonged to *A. flavicollis,* 168 to *A. sylvaticus,* 98 to *A. alpicola*, and 535 to *C. glareolus.* For the second analysis 680 individuals were analysed, out of which 154 individuals belonged to *A. flavicollis,* 112 to *A. sylvaticus,* 66 to *A. alpicola*, and 348 to *C. glareolus.*

Plant diversity was correlated with trapping sites (Kruskal-Wallis chi-squared = 98.29, df = 11; $p < 0.001$). Foot length correlate with body mass only in *C. glareolus* ($r = 0.12$, $p = 0.002$), but not in the *Apodemus* species ($p \geq 0.11$). The four species differed in the foot length (Kruskal-Wallis chi-squared = 801.5, df = 3, $p < 0.001$) and body mass (Kruskal-Wallis chi-squared = 14.471, df = 3, $p = 0.002$). In particular, the average foot length was significantly smaller in *C. glareolus* (mean ± SD = 18.2 ± 1.29) than in *Apodemus* spp. (*A. flavicollis* = 22.8 ± 1.39; *A. sylvaticus* = 22.7 ± 1.82, *A. alpicola* = 22.7 ± 1.51). The average body mass was smaller in *C. glareolus* (26.5 ± 6.23) compared to *A. flavicollis* (28.2 ± 7.09), followed by *A. sylvaticus* (27.9 ± 6.24) and *A. alpicola,* (27.2 ± 6.84; Fig. 2; Table S1).

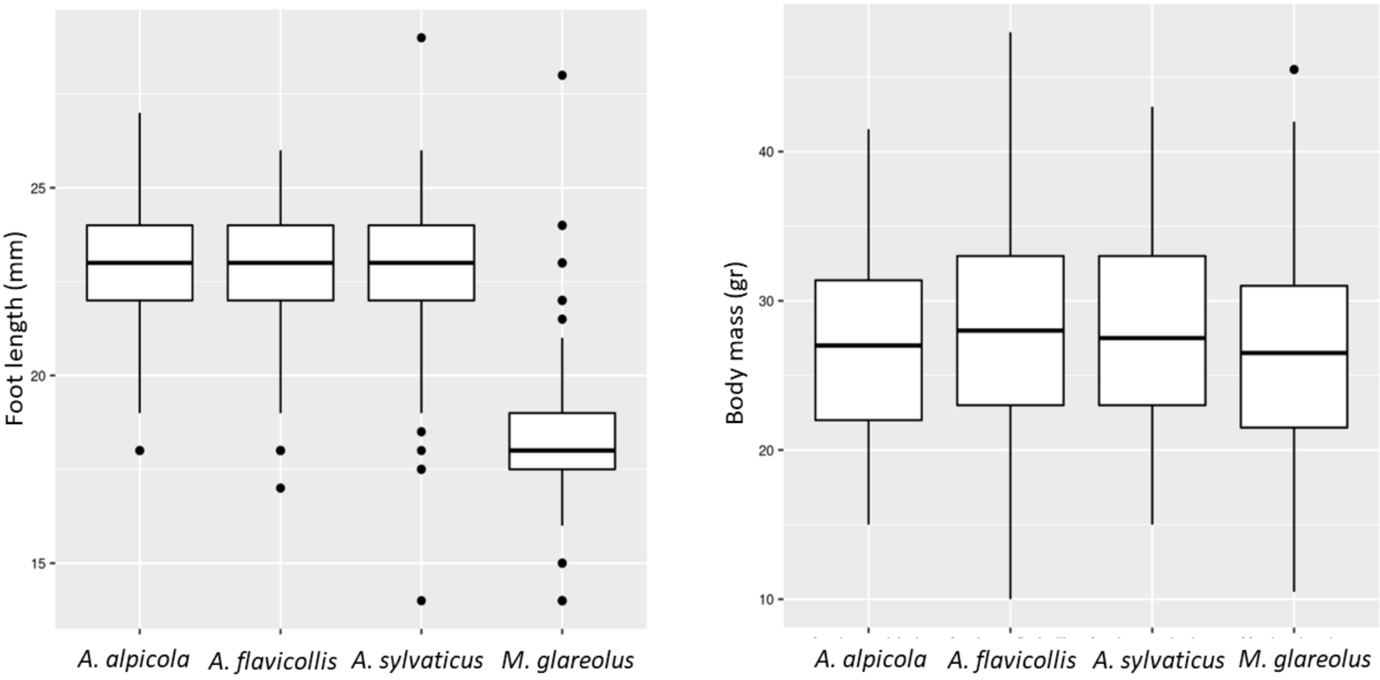


Figure 2. Boxplots comparing foot length and body mass for each species.

From the first analysis with the whole database, we observed that in *A. flavicollis* foot length increase ($\beta \pm SE = 0.086 \pm 0.041$; $z = 2.090$; $p = 0.03$) and body mass decrease ($\beta \pm SE = -0.092 \pm 0.031$, $z = -2.93$, $p = 0.003$) along elevation, while in *C. glareolus* body mass increase ($\beta \pm SE = 0.058 \pm 0.027$, $z = 2.13$, $p = 0.03$) along the altitudinal gradient.

From the second analysis, the outputs of the Likelihood Ratio Test showed that for the foot length, trapping site was the main variable improving the fit of the model in *A. flavicollis* ($\chi^2 = 26.47$, $p = 0.005$), together with sex ($\chi^2 = 4.38$, $p = 0.03$; Fig. 3; Table S2.1), where female showed smaller foot (mean ± SD = 21.8 ± 1.35) than males (22.3 ± 1.55), and in *A. sylvaticus* ($\chi^2 = 27.86$, $p = 0.003$; Table S2.2). In *A. alpicola* only sex seems to influence the fit of the model ($\chi^2 = 4.41$, $p = 0.03$; Fig. 3; Table S2.3). Similar results were obtained for the body mass, where trapping site was the main variable improving the fit of the model in all the four species (*A. flavicollis*: $\chi^2 = 47.15$, $p < 0.001$; *A. sylvaticus*: $\chi^2 = 22.15$, $p = 0.02$; *A. alpicola*: $\chi^2 = 38.63$, $p < 0.001$; Table S2). However, in

A. sylvaticus the best model was the one also including sex ($\chi^2 = 4.21$, $p = 0.02$), and in *A. alpicola* the one including foot length ($\chi^2 = 7.55$, $p = 0.004$).

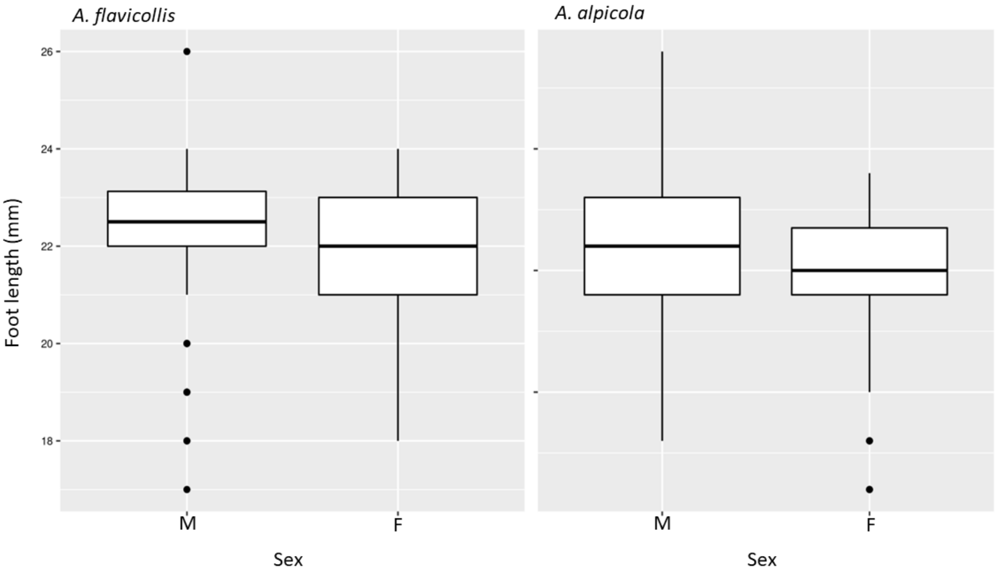


Figure 3. Boxplot showing the difference in foot length between male and female in *A. flavicollis* and *A. alpicola*.

In *C. glareolus* (Table S2.4), none of the tested variables affected the foot length. However, for the body mass trapping site ($\chi^2 = 47.67$, $p < 0.001$), sex ($\chi^2 = 4.54$, $p = 0.03$), with female heavier (28.2 ± 5.88) than males (27.2 ± 6.29) and body temperature ($\chi^2 = 6.91$, $p = 0.008$; Fig. 4) were found to improve the model fit. Indeed, we found that in *C. glareolus,* individuals with low body mass had higher body temperature but this relation was not observed in the other species.

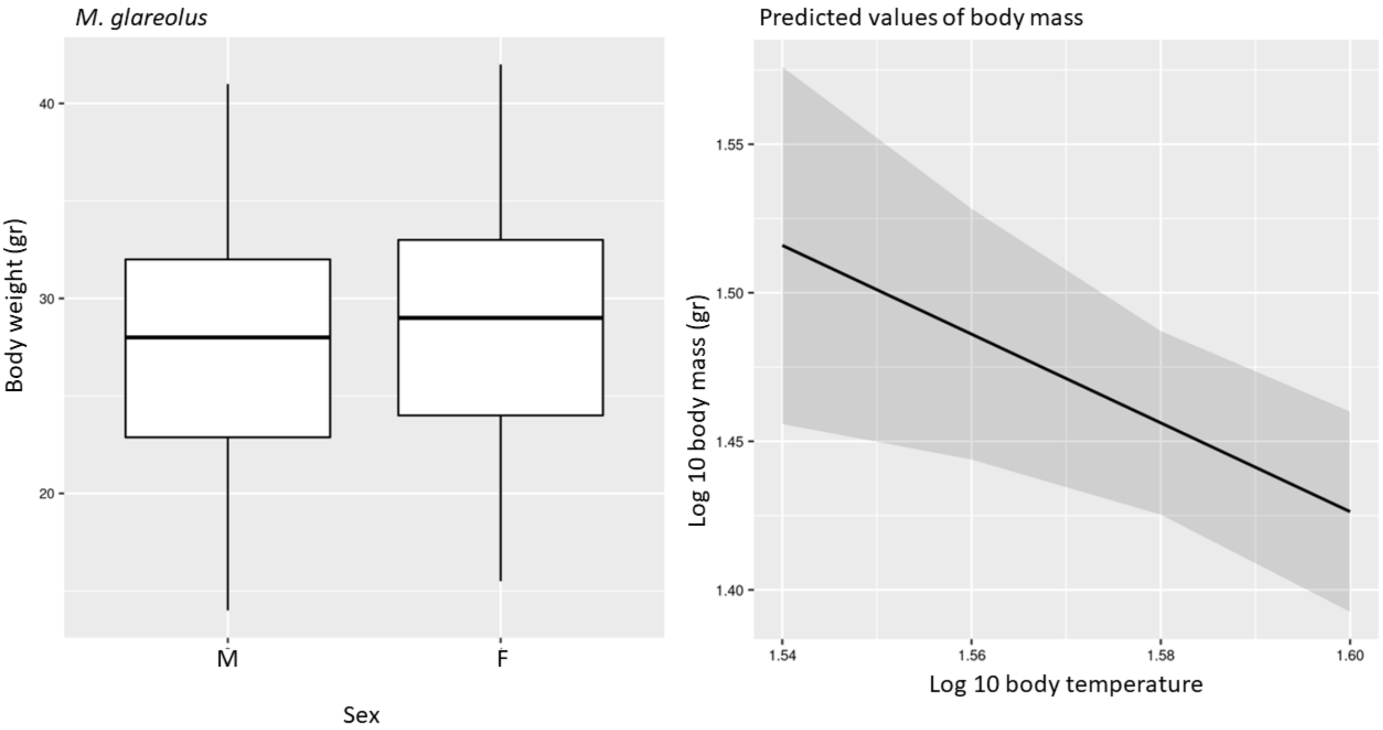


Figure 4. Left: Boxplot showing the difference in foot length between male and female in *C. glareolus*. Right: Marginal effect showing the predicted values of body mass in function of body temperature variation in *C. glareolus*.

DISCUSSION

In this study, we presented how body morphological characteristics differ between species, trapping sites and sexes. We found an opposite pattern between the two groups, *Apodemus* spp. and *C. glareolus*, with body mass decreasing in *A. flavicollis* and increasing in *C. glareolus* at high altitudes.

Interestingly, the two species with the broader altitudinal distribution support (*C. glareolus*) and contradict (*A. flavicollis*) the Allen' and Bergmann' rules. The mechanisms for both rules lay behind optimization of thermoregulatory costs, reducing heat dissipation in animals with smaller extremities, and increasing thermal inertia in larger individuals. In our results, *C. glareolus* had small extremities, and its body mass increased along the elevation, following both rules. There is some evidence in birds (*Pyrrhocorax pyrrhocorax, P. graculus*), and in amphibians and turtles

(Alho et al., 2011) of the prevalence of larger individuals (i.e. higher body mass) with relatively shorter extremities (e.g. legs) at high altitudes (Laiolo and Rolando 2008, Meiri and Dayan 2003, Poblete et al. 2018). This can result from the selection of larger animals with short extremities, which have a lower surface-to-volume ratio than smaller animals and thus a lower body heat dispersion per unit of mass. However, in the same area, *A. flavicollis* contradicts both rules, as it exhibited longer feet and lower body mass along the elevation. Previous studies have observed that lower body mass at high altitudes occurs commonly and becomes particularly pronounced with increasing altitudes (Kayser, 1994; also in red squirrels, see Wauters et al., 2007 and Tranquillo et al., 2022). The reduction in body size could be partially connected to a reduced energy intake at high altitudes where resources are limited (Westerterp & Kayser 2006). We can suppose that the two rodent species examined here have been affected differently by high altitude selection: *C. glareolus* developed morphological adjustment to avoid heat dissipation, while *A. flavicollis* instead found an advantage of having a smaller body in order to reduce the amount of energy needed (Bears et al. 2008). Other hypotheses to explain such trend could be that a variation in size (and/or processing time) of major food types, could have affected these differences. For example, studies on red squirrels showed that individual are bigger where they feed on larger seeds (Wauters et al., 2007). It is also possible that *Apodemus,* which are more explorative rodents compared to *C. glareolus* (Dammhahn et al. 2020), at higher altitudes have longer feet and slimmer body to optimize mobility when food resources are more limited.

An interesting result was observed in *C. glareolus,* where we found a negative relationship between body temperature and mass, with animals with smaller bodies having higher body temperatures. A previous study showed a similar correlation in birds (Prinzinger et al., 1990), and we confirmed the same in this rodent species. It could be that in rodents, smaller bodies need more energy for searching for resources and shelter, especially in unstable environments, such as mountains. This could suggest that several physiological mechanisms are involved to generate heat and cope with cold environmental conditions, such as the active organs involved in energetically demanding aerobic activity (Abumrad, 2017; Jastroch et al., 2021; RodriguezSerrano & Bozinovic, 2009). This hypothesis, however, needs to be corroborated by other studies considering the effects of physiological measures of the animals, such as the organ masses and the brown adipose tissue.

The ability of species to adapt, is of vital importance to their survival in a changing environment. Species must be able to adjust either through evolutionary changes or phenotypic plasticity, or face risk of extinction under ongoing rapid habitat and climatic changes. Species living along broad elevational gradients provide an excellent opportunity to study morphological variation, as they are exposed to diverse climatic and ecological conditions, which could drive morphological variation. This study indeed showed that some species such as *A. flavicollis,* which have a typical lowland distribution and probably only recently colonised high-altitude areas, and thus not yet well adapted to such environment, is under stronger selection by the instable food resources availability. Others such as *C. glareolus,* which was found at high density population in high altitude areas, adopt more thermoregulatory strategies to preserve heat dissipation. These results suggest a species-specific morphological adaptations imposed by the mountain environment.

Reference

Aublet JF, Festa-Bianchet M, Bergero D, Bassano B. (2009) Temperature constraints on foraging behaviour of male Alpine ibex (*Capra ibex*) in summer. Oecologia. 159: 237-247. 10.1007/s00442-008-1198-4.

Abumrad, N. A. (2017). The liver as a hub in thermogenesis. Cell Metabolism, 26(3), 454–455.

Alho, J. S., Herczeg, G., Laugen, A. T., Räsänen, K., Laurila, A., and Merilä, J. (2011). Allen's rule revisited: quantitative genetics of extremity length in the common frog along a latitudinal gradient J. Evol. Biol. 24, 59–70. doi: 10.1111/j.1420-9101.2010.02141.x

Allen, J.A. (1877). The influence of Physical conditions in the genesis of species. Radical Review. 1: 108–140.

Barnosky AD, Hadly EA, Bell CJ (2003) Mammalian response to global warming on varied temporal scales. Journal of Mammalogy, 84, 354–368

Bednarz PA, Zwolak R. (2022). Body mass and sex, but not breeding condition and season, influence open-field exploration in the yellow-necked mouse. Ecol Evol. 27;12(3):e8771

Bears, H., Drever, C., and Martin, K. (2008). Comparative morphology of dark-eyed juncos Junco hyemalis breeding at two elevations: a common aviary experiment. J. AVIAN Biol. 39, 152–162.

Bergmann, C. (1847). Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Göttinger Stud.1, 595–708.

Beyer, W. H. CRC Standard Mathematical Tables, 31st ed. Boca Raton, FL: CRC Press, pp. 536 and 571, 2002.

Bolker B., H. Skaug, A. Magnusson, and A. Nielsen. 2012. Getting started with the glmmADMB package.

Brunetti, M., Lentini, G., Maugeri, M., Nanni, T., Auer, I., Böhm, R., and Schöner, W. (2009). Climate variability and change in the Greater Alpine Region over the last two centuries based on multi-variable analysis. Int. J. Climatol. *29*, 2197–2225.

Dammhahn, M., Mazza, V., Schirmer, A. *et al.* Of city and village mice: behavioural adjustments of striped field mice to urban environments. *Sci Rep* **10,** 13056 (2020).

Davis MB, Shaw RG, Etterson JR (2005) Evolutionary responses to changing climate. Ecology, 86, 1704–1714.

Eastman, L. M., Morelli, T. L., Rowe, K. C., Conroy, C. J., & Moritz, C. (2012). Size increase in high elevation ground squirrels over the last century. *Global Change Biology*, *18*(5), 1499-1508.

Gardner JL, Peters A, Kearney MR, Joseph L, Heinsohn R (2011). Declining body size: a third universal response to warming? Trends in Ecology and Evolution, 26, 285– 291.

Geist V (1987) Bergmann's rule is invalid. Canadian Journal of Zoology, 65, 1035–1038.

Grieco T, Rizk O. (2010) Cranial shape varies along an elevation gradient in Gambel's white-footed mouse (*Peromyscus maniculatus gambelii*) in the Grinnell Resurvey Yosemite transect. Journal of Morphology, 271, 897-909.

Gurnell and Flowerdew. 2019. The Live trapping Small Mammals: A Practical Guide. Mammal Society

Hoffmann AA, Parsons PA (1991) Evolutionary Genetics and Environmental Stress. Oxford University Press, Oxford

Hopkins SSB (2008) Reassessing the mass of exceptionally large rodents using toothrow length and area as proxies for body mass. Journal of Mammalogy, 89, 232–243.

Jastroch, M., Polymeropoulos, E. T., & Gaudry, M. J. (2021). Pros and cons for the evidence of adaptive non-shivering thermogenesis in marsupials. Journal of Comparative Physiology B, 191(6), 1085–1095

Jetz W, Ashton KG, La Sorte FA (2009) Phenotypic population divergence in terrestrial vertebrates at macro scales. Ecology Letters, 12, 1137–1146.

Kayser, B. (1994). Nutrition and energetics of exercise at altitude. Theory and possible practical implications. Sports Med. 17:309–323

Laiolo, P., and Rolando, A. (2008). Ecogeographic correlates of morphometric variation in the Red-billed Chough Pyrrhocorax pyrrhocorax and the Alpine Chough Pyrrhocorax graculus. Ibis 143, 602–616.

Langvatn R, Albon SD (1986) Geographical clines in body weight of Norwegian red deer: a novel explanation of Bergmann's rule? Holarctic Ecology, 9, 285–293.

Leech DI, Crick HQP (2007) Influence of climate change on the abundance, distribution and phenology of woodland bird species in temperate regions.Ibis, 149, 128–145.

Lundblad, C.G., and Conway, C.J. (2020). Variation in Selective Regimes Drives Intraspecific Variation in Life History Traits and Migratory Behavior along an Elevational Gradient. J Anim Ecol *89*, 397–411.

Luterbacher J, Dietrich D, Xoplaki E, Grosjean M, Wanner H: European seasonal and annual temperature variability, trends, and extremes since 1500. Science. 2004, 303: 1499-1503. 10.1126/science.1093877

Mason, T.H., Apollonio, M., Chirichella, R. *et al.* Environmental change and long-term body mass declines in an alpine mammal. *Front Zool* **11**, 69 (2014). https://doi.org/10.1186/s12983-014-0069-6

McNab BK (2010) Geographic and temporal correlations of mammalian size reconsidered: a resource rule. Oecologia, 164, 13–23

Meiri, S., & Dayan, T. (2003). On the validity of Bergmann's rule: On the validity of Bergmann's rule. J. 295 Biogeogr. 30, 331–351.

Melcore I., Zbyszek B., Bertolino S. (2022). Variation of rodents' body temperature across elevation in Alps. PBZ. Vol. 96 (6).

Ozgul A, Childs DZ, Oli MK et al. (2010) Coupled dynamics of body mass and population growth in response to environmental change. Nature, 466, 482–485.

Oyler, J.W., Dobrowski, S.Z., Ballantyne, A.P., Klene, A.E., and Running, S.W. (2015). Artificial amplification of warming trends across the mountains of the western United States. Geophys. Res. Lett. *42*, 153–161.

Porter WP, Kearney M (2009) Size, shape, and the thermal niche of endotherms. Proceedings of the National Academy of Sciences of the United States of America, 106, 19666–19672.

Prinzinger R., Prebmar A. and Schleucher E. (1990). Body temperature in birds. Camp. Biochem. Physiol. Vol. 99A, No. 4, pp. 499-506.

Poblete, Y., Gutiérrez, V., Cid, V., Newsome, S.D., Sabat, P., and Vasquez, R.A. (2018). Intraspecific variation in exploratory behavior and elevational affinity in a widely distributed songbird. Oecologia186, 931–938.

Rodr´ıguez-Serrano, E., & Bozinovic, F. (2009). Interplay between global patterns of environmental temperature and variation in nonshivering thermogenesis of rodent species across large spatial scales. Global Change Biology, 15: 2116–2122.

Self, S. G., Mauritsen, R. H., & Ohara, J. (1992). Power calculations for likelihood ratio tests in generalized linear models. *Biometrics*, 31-39.

Stockwell CA, Hendry AP, Kinnison MT (2003) Contemporary evolution meets conservation biology. Trends in Ecology & Evolution, 18, 94–101.

Tomassini A, Colangelo P, Agnelli P, Jones G, Russo D. (2014). Cranial size has increased over 133 years in a common bat, *Pipistrellus kuhlii*: a response to changing climate or urbanization? Journal of Biogeography, 41: 944--953.

Tranquillo C, Wauters LA, Santicchia F, Preatoni D, Martinoli A. (2022). Living On the edge: morphological and behavioural adaptations to a marginal high-elevation habitat in an arboreal mammal. Integrative Zoology. Doi: 10.1111/1749-4877.12679

Wang, Q., Fan, X., and Wang, M. (2016). Evidence of high-elevation amplification versus Arctic amplification. Sci. Rep. *6*, 19219.

Wauters LA, Vermeulen M, Van Dongen S, Bertolino S, Molinari A, Tosi G, Matthysen E (2007). Effects of spatio-temporal variation in food supply on red squirrel *Sciurus vulgaris* body size and body mass and its consequences for some fitness components. Ecography 30: 51-65.

Westerterp, K.R.; Kayser, B. Body mass regulation at altitude. Eur. J. Gastroenterol. Hepatol. 2006, 18, 1–3.

Wolverton S, Huston MA, Kennedy JH, Cagle K, Cornelius JD (2009) Conformation to Bergmann’s rule in white-tailed deer can be explained by food availability. American Midland Naturalist, 162, 403–417.

Yom-Tov Y and Yom-Tov J (2005) Global warming, Bergmann’s rule and body size in the masked shrew Sorex cinereus Kerr in Alaska. Journal of Animal Ecology, 74, 803–808.

Yom-Tov Y, Yom-Tov S, Wright J, Thorne CJR, Du Feu R (2006) Recent changes in body weight and wing length among some British passerine birds. Oikos, 112, 91– 101.

Yom-Tov Y, Yom-Tov S, Zachos FE. (2013). Temporal and geographical variation in skull size of the red fox (*Vulpes vulpes*) and the Eurasian badger (*Meles meles*) in Austria. Biological Journal of the Linnean Society, 108:579-585.

Appendix

Table S1. Pairwise Wilcox test for foot length and body mass. Table show the p-values of the pairwise comparisons between the four species with corrections for multiple testing. Values in bold are statistically significant.

Foot length				Body mass			
Pairwise Wilcox test: Kruskal-Wallis chi-squared = 801.5, df = 3, $p < 0.001$				Pairwise Wilcox test: Kruskal-Wallis chi-squared = 14.47, df = 3, $p = 0.002$			
	A. alpicola	*A. flavicollis*	*A. sylvaticus*		*A. alpicola*	*A. flavicollis*	*A. sylvaticus*
A. flavicollis	0.84	-	-	*A. flavicollis*	0.33	-	-
A. sylvaticus	0.84	0.68	-	*A. sylvaticus*	0.55	0.60	-
C. glareolus	**< 0.001**	**< 0.001**	**< 0.001**	*C. glareolus*	0.58	**0.001**	0.06

Table S2. Single species analysis for foot length and body mass. Likelihood ratio test of the generalised linear model testing the effect of trapping site, sex and body temperature on foot length and body mass of the animals. Df = model's degree of freedom; df = degree of freedom of the test statistic; logLik = loglikelihood; χ^2 = chi square statistic test; p = p value. In bold are shown the significant p-values of the models where the excluded tested variable was significant on the model fit.

2.1) *A. flavicollis*

foot length	Df	LogLik	Df	χ^2	P-value
Full model: ~ trapping site + sex + body temperature	15	335.52			
- body temperature	14	335.31	-1	0.41	0.51
- sex	14	333.37	-1	4.29	**0.038**
- trapping site	4	322.28	-11	26.47	**0.005**
~ 1	2	320.63	-13	29.78	**0.005**

body mass	Df	LogLik	Df	χ^2	P-value
Full model: ~ trapping site + sex + body temperature + foot length	16	159.64			
- foot length	15	158.98	-1	1.32	0.24
- body temperature	15	159.21	-1	0.87	0.34
- sex	15	159.54	-1	0.42	0.51
- trapping site	5	136.07	-11	47.15	< **0.001**
~ 1	2	133.65	-15	51.98	< **0.001**

2.2) *A. sylvaticus*

foot length	Df	LogLik	Df	χ^2	P-value
Full model: ~ trapping site + sex + body temperature	15	221.33			
- body temperature	14	221.27	-1	0.11	0.73
- sex	14	221.33	-1	0.0003	0.98

- trapping site	4	207.40	-11	27.86	**0.003**
~ 1	2	206.98	-13	28.7	**0.007**

body mass	Df	LogLik	Df	χ^2	P-value
Full model: ~ trapping site + sex + body temperature + foot length	16	109			
- foot length	15	109	-1	0.002	0.96
- body temperature	15	108.98	-1	0.02	0.86
- sex	15	106.89	-1	4.21	**0.04**
- trapping site	6	97.91	-11	22.15	**0.02**
~ 1	2	96.28	-14	25.43	**0.03**

2.3) *A. alpicola*

foot length	Df	LogLik	Df	χ^2	P-value
Full model: ~ trapping site + sex + body temperature	15	139.34			
- body temperature	14	139.07	-1	0.53	0.46
- sex	14	137.13	-1	4.41	**0.03**
- trapping site	4	135.96	-11	6.76	0.81
~ 1	2	134.77	-14	9.13	0.76

body mass	Df	LogLik	Df	χ^2	P-value
Full model: ~ trapping site + sex + body temperature + foot length	16	75.16			
- foot length	15	71.38	-1	7.55	**0.005**
- body temperature	15	75.05	-1	0.20	0.64
- sex	15	75.15	-1	0.01	0.88
- trapping site	5	55.84	-11	38.63	< **0.001**
~ 1	2	52.27	-14	45.76	< **0.001**

2.4) *C. glareolus*

foot length	Df	LogLik	Df	χ^2	P-value
Full model: ~ trapping site + sex + body temperature	15	721.43			
- body temperature	14	721.42	-1	0.02	0.89
- sex	14	721.39	-1	0.07	0.78
- trapping site	5	712.66	-1	17.54	0.09
~ 1	2	712.52	-13	17.82	0.16

body mass	Df	LogLik	Df	χ^2	P-value
Full model: ~ trapping site + sex + body temperature + foot length	16	320.77			
- body temperature	15	317.31	-1	6.91	**0.008**
- sex	15	318.50	-1	4.54	**0.03**
- trapping site	5	296.93	-11	47.67	**< 0.001**
~ 1	2	292.97	-14	55.60	**< 0.001**

CHAPTER III

Camouflage accuracy in Alpine rodents across altitudinal levels

Ilaria Melcore[1], Annalisa Demitri[1], Sandro Bertolino[1],

Zbyszek Boratyński[2]

[1] Department of Life Sciences and Systems Biology, University of Turin, Via Accademia Albertina 13, 10123 Turin, Italy

[2] Association BIOPOLIS, CIBIO-InBIO Associate Laboratory, Research Center in Biodiversity and Genetic Resources, University of Porto, Porto, Portugal

Abstract

Mountains provide a vast and diverse range of habitats which vary along an elevational gradient, influencing animal adaptations. Natural selection act on phenotypic variation which includes differences in morphological traits such as animal colouration. Camouflage is one of the most efficient anti-predator strategies as it makes it difficult for the predator to detect or recognize the prey. We investigated camouflage in four rodent species inhabiting elevation gradient in Alps. Specifically, we estimated the accuracy of camouflage to distinguish between generalist and specialist strategies and investigated the effect of elevation and some microhabitat element on chromatic component (hue), brightness and mismatches of animal dorsal fur. We observed that all species shared a similar high accuracy of camouflage in the hue of the dorsal fur, perfectly matching their habitat colouration. However, for the brightness component of the colour, we did not observe the same optimal camouflage as before, and in none of the studied species the brightness of the animal and of substrate were correlated. At the species level, we found different camouflage strategies which varied between species and along the elevational gradient. In *Apodemus alpicola* and *Clethrionomys glareolus* we registered higher value in brightness, probably due to the near-white radiance spectrum of higher altitude. *A. sylvaticus* exhibits darker coloration compared to the other species and to its habitat, and this colouration became lighter along the elevation. This may suggest that in the habitats this species lives, may not be under strong selection for background matching in its coloration, but for some other reason of coloration (e.g., thermoregulation). Regarding the dominant wavelength, we observed an opposite trend between *C. glareolus* and *A. flavicollis,* with the first showing lower wavelengths (redder colouration) compared to the other *Apodemus* spp., and the latter becoming more redder (lower hue) along the elevation, suggesting an evolutionary and adaptive differential effect to altitude. Thus, we can suppose that fur colouration can influence the activity patterns and other ecological factors of the species, such as behaviour or thermoregulation.

Keywords: Rodents, dominant wavelength, mismatch, elevation, species comparison

INTRODUCTION

Mountains are characterized by strong natural gradients and large spatial and temporal heterogeneity (Theurillat et al 2003). Due to their significant environmental variation, mountains provide a vast and diverse range of habitats which vary along an elevational gradient, influencing animals' adaptations. The adaptations of rodents to mountain environments involves not only physiological performance to deal with the harsh high-altitude condition, but also include specific behavioural, morphological, and phenotypical adaptations, which are usually not unique to mountain animals, but they may be especially prominent in extreme habitats (Sears et al. 2009).

Natural selection acts on phenotypic variation influencing animal adaptation which can be defined as heritable phenotypic change (Draghi, 2019). Among the most classical examples of adaptive evolution is the emergence of colour polymorphism, particularly with the evolution of camouflage colouration in animals (Endler et al., 1988; Mappes et al., 2014). Camouflage is one of the most efficient anti-predator strategies as it makes it difficult for the predator to detect or recognize the prey, increasing its similarity to the surrounding natural environment or to objects contained therein (Bond & Kamil, 2002; Duarte, Stevens, & Flores, 2018; Cuthill, 2019; Nokelainen et al., 2019). There are different types of camouflage strategies (e.g., background matching, disruptive colouration, self-shadow concealment and many others; Merilaita, 2011). Here, we considered the background-matching, which is the most common camouflage type. The background-matching can be defined as the adaptation of an animal's body colouration to the habitat colouration, to reduce its visibility to predators (Endler, 1978; Merilaita et al., 2017). Animals have many ways to optimize camouflage, in relation to their distribution range and the complexity of their habitats (Nokelainen et al, 2020). In diverse habitats, composed by different background elements animals can both evolve a generalist tactic, in which it matches well with the different visual backgrounds but its camouflage is not optimal in any of them (Houston et al., 2007; Hughes et al., 2019) or can adopt a specialist tactic to match one of the backgrounds well, which, however, results in greater visibility and vulnerability in other habitats (Cuthill, 2019). In homogeneous habitat, instead, animals may adopt more specialised camouflage, which provides better protection in specific environments (Kjernsmo & Merilaita, 2012; Michalis et al., 2017; Fig. 1).

Figure 1. Different camouflage accuracy in two specie: *Apodemus flavicollis* (top) and *Myodes glareolus* (bottom)

Although the phenotype-environment match is well known and studied across different taxa (Barbosa et al., 2008; Kang et al., 2012; Wilson-Aggarwal et al., 2016), it is very challenging to quantify camouflage directly while checking different types of natural backgrounds (Macedo et al., 2022). Furthermore, until now the tools available to study such mechanisms were not very adequate and for these reasons there are still very few direct studies on camouflage in nature (Nokelainen et al., 2020) and in particular on species inhabiting mountain habitats.

In this work, we investigated camouflage in rodent species inhabiting elevation gradients in the Alps. We estimated the accuracy of camouflage of these species in relation to their background to distinguish between generalist and specialist camouflage strategies and investigated the effect of elevation and the habitat structure on fur colour properties and mismatch between the animal and its background. We expected 1) low animal-background mismatch (more specialist strategies) to be found at high elevations since species have to match in more homogeneous habitat. On the contrary, species at lower elevations should have evolved higher animal-background mismatch (generalist strategy), averaging the backgrounds that they experience. We predicted that 2) individual from higher elevation are darker (lower brightness values), because the substrate is also darker, and vegetation is more scarce compared to lower elevation (Rios & Álvarez-Castaneda, 2012; Reguera et al. 2014). Finally, we expected that 3) sympatric species will not differ significantly in the dominant wavelength (hue), but we expected within the species, individuals at higher altitude to be redder (lower values of hue), because red pigmentations are more prevalent in habitats with exposure to high solar radiation (high UV exposure; Cooper, 2010).

METHODS

Study area

The study area was located in the Piedmont region (North-western Italy), within the Gran Paradiso National Park territory, in the Graian Alps. A peculiarity of the territory is the presence of diffuse rocky areas, located mainly above the altitudinal limit of the trees, sometimes even at lower altitudes. Deciduous forests at low elevations (400-1000 m), were composed of European beech (*Fagus sylvatica*), followed by sycamore maple (*Acer pseudoplatanus*), large-leaved linden (*Tilia platyphyllos*), European ash (*Fraxinus excelsior*), poplar (*Populus* spp.), birch (*Betula* spp.) and chestnut (*Castanea sativa*) species. Coniferous forests, at high elevations (1800-2000 m) were dominated by larch (*Larix decidua*), often mixed with Norway spruce (*Picea abies*). At mid-elevation (1000-1800 m) the mixed forests were composed by several deciduous and coniferous species.

Trapping and handling

The sampling season started on April 15 and ended on September 15, 2020, during the breeding season of the target species and the study was repeated in the same period during 2021. The days of capture were randomly alternated at high and low altitude sites to avoid the effect of seasonality in rodent moulting. We identified 12 trapping sites distanced at least 5 km from each other, distributed along an elevational gradient, from 400 to 1822 m a.s.l (Figure A1 and Table A1). Each trapping site was composed of a line of 30 live traps (Sherman traps, 229 mm x 89 mm x 76 mm in size), located every 10 m. Traps were set up in the field for one week and checked at dawn and dusk. Traps were baited with apple, sunflower seeds, and peanut butter, and provided with cotton as bedding. Trapped species were yellow-necked mouse *Apodemus flavicollis* (hereinafter *AF*), wood mouse *A. sylvaticus* (*AS*), alpine field mouse *A. alpicola* (*AA*) and bank vole *Clethrionomys glareolus* (*CG*; also known as *Myodes glareolus*; Kryštufek et al., 2020). Two of these species, *AF* and *CG* had a wide altitudinal range, the other two *AS* and *AA* are mainly distributed in the low and

the high altitudinal gradient, respectively. Tissue samples were collected from the ear of captured animals and used to identify the different *Apodemus* species through barcoding (Aljanabi et al. 1997). Only adult individuals (based on fur characteristics and body dimensions, e.g. Gurnell and Flowerdew 2019; see also Melcore et al. 2022) were selected for the camouflage analysis to have well-formed fur coat. Within each plot we registered the elevation (GPS Garmin Etrex 32x).
We collected vegetation data in seven plots located within the trapping line: the first plot was centered around the first trap, and the following plots were placed every five traps. Each plot was constituted by a 5 x 5-m square with a live trap located in the center. The tree/bush species present in the plot were identified, and percentage of cover of each shrub species, and of the herbaceous and rocky cover at the ground level were visually estimated. The percentage of shrub coverage was calculated to account for variation in plant diversity (Shannon Index) and vegetation coverage.

Image analysis

To quantify colour phenotypes and their match to the background, both the animals and their habitat backgrounds were digital photographed directly in the field. Three photos were taken for each animal in three different points around the trap (within a range of maximum 3 m) in order to include in the analyses, the different elements of the habitat in which the animal was captured. The photographic scene focused on the entire dorsal view of the animal (excluding the tail) and the habitat background, with a grey colour reference scale (Fig 1). Photographs were taken during the morning traps check (around 9 am) and from similar height from the ground (about 1 m) under natural light conditions. The photographs were all taken using a Nikon D3100 camera with basic lens (50 mm). Images were analysed with ImageJ (version 1.5k) an open-source Java-based image processing program developed to display, edit, analyse, and process different image file formats. Before starting with photo analyses, we calibrated the camera, using a standard colour chart (X-Rite color Checker passport) to create a cone-catch model in ImageJ. Then, the images were standardised (equalised) with white and black standards using an ImageJ plugin, micaToolbox

(Troscianko & Stevens, 2015). The process of elaboration of the photographs involved various procedural steps (available on the ImageJ guide). The images were scaled to reflectance values in red, green and blue (RGB) layers. For each image, we measured two regions of interest (ROIs), one of the entire dorsum of the animal and one of the background, and we calculated the RGB values (0-255) for both these ROIs. Since for each animal were taken three photos in the different habitat background around the trap where it was captured, we associated the average value of the three background RGBs to each individual.

To analyse the mismatch between animal colouration and its background, we transformed RGB values to HSV colour space (hue, saturation, and value or brightness) (using “rgb2hsv” R package). The HSV value gives information about the dominant wavelength (hue), the purity of the colour (saturation), and the brightness of the target (value). HSV are a colour space closer to how colour, and consequently prey camouflage, is perceived (Ajmal et al., 2018). Since the species studied were both crepuscular and nocturnal and considering that quite large changes in the spectrum of light can also occur within forests due to the diversity in vegetation structure and habitat type (Warrant & Johnsen, 2013), we decided to consider only hue and brightness values in the analyses. Saturation could be not as important as the other camouflage parameters, as some predators vision models could interpret saturation poorly (Kelber & Osorio, 2010). Then, for each individual we calculated mismatches as the difference in the value of the animal dorsum against the background in which it was found.

Statistical analysis

Continuous variables (elevation, vegetation cover, herbaceous and rocky coverage, plant diversity, hue, brightness and mismatches values) were $\log_{10}$ transformed before analyses to improve normality of distributions of their residuals. We used one-way ANOVA test and then pairwise t tests comparisons with corrections for multiple testing (Benjamini–Hochberg correction; Beyer, 2002), to analyse the differences in camouflage adaptations between the different species.

First, we tested how elevation, vegetation cover, herbaceous and rocky coverage, and plant diversity (independent variables) affected camouflage accuracy using a generalised linear model. We conducted four separate analyses for animal hue, brightness and the respective mismatches as dependent variables, assuming Gaussian distribution. We included species as a factor, and in analysis for hue and brightness only, we included habitat hue and brightness as continuous predictors in the model. We tested two-way factorial interaction between species and elevation to test for the differences among species in camouflage. Lack of multicollinearity among predictors was confirmed with variance inflation factors (< 2.5). The goodness of models fit was compared using the Likelihood Ratio Test (package "lmtest" in R), which determine whether adding complexity to the model makes the model significantly more accurate (Self et al., 1992).

Second, to determine species-specific camouflage responses to habitat characteristics, we constructed similar to above analyses but separately for the four species ensuring we had enough records for conclusive statistical investigation (N>30).

RESULTS

We captured a total of 461 individuals: 118 *AF*, 95 *AS*, 46 *AA*, 202 *CG*. When testing correlations between animal and habitat colouration, we found a significant correlation for hue (Pearson's correlation: $r = 0.66$, $t = 19.02$, $p < 0.001$) but not for brightness ($r = -0.02$, $df = 459$, $t = -0.42$, $p = 0.67$).

Hue and brightness values

Hue of the habitat was correlated with plant diversity ($r = 0.12$, $t = 2.78$, $p = 0.005$), but not with the other predictors (elevation, herbaceous, rocky and vegetation coverage; $p \geq 0.77$). Brightness of the habitat was not correlated with any of the predictors ($p > 0.33$).

The four species differed in the hue ($F = 3.27$, $df = 3$, $p = 0.021$) and brightness ($F = 3.75$, $df = 3$, $p = 0.010$) values. In particular, the average hue value was significantly smaller in *CG* (mean ± SD =

91.050 ± 28.8) than in *Apodemus* spp. (*AF* = 100.876 ± 33.96, *AS* = 101.185 ± 32.97, *AA* = 103.613 ± 28.23). The average brightness was significantly smaller in *AS* (0.037 ± 0.02) compared to *CG* and *AA* which had a similar brightness value (0.041 ± 0.01), followed by *AF* (0.038 ± 0.02; Fig. 2; Table S2).

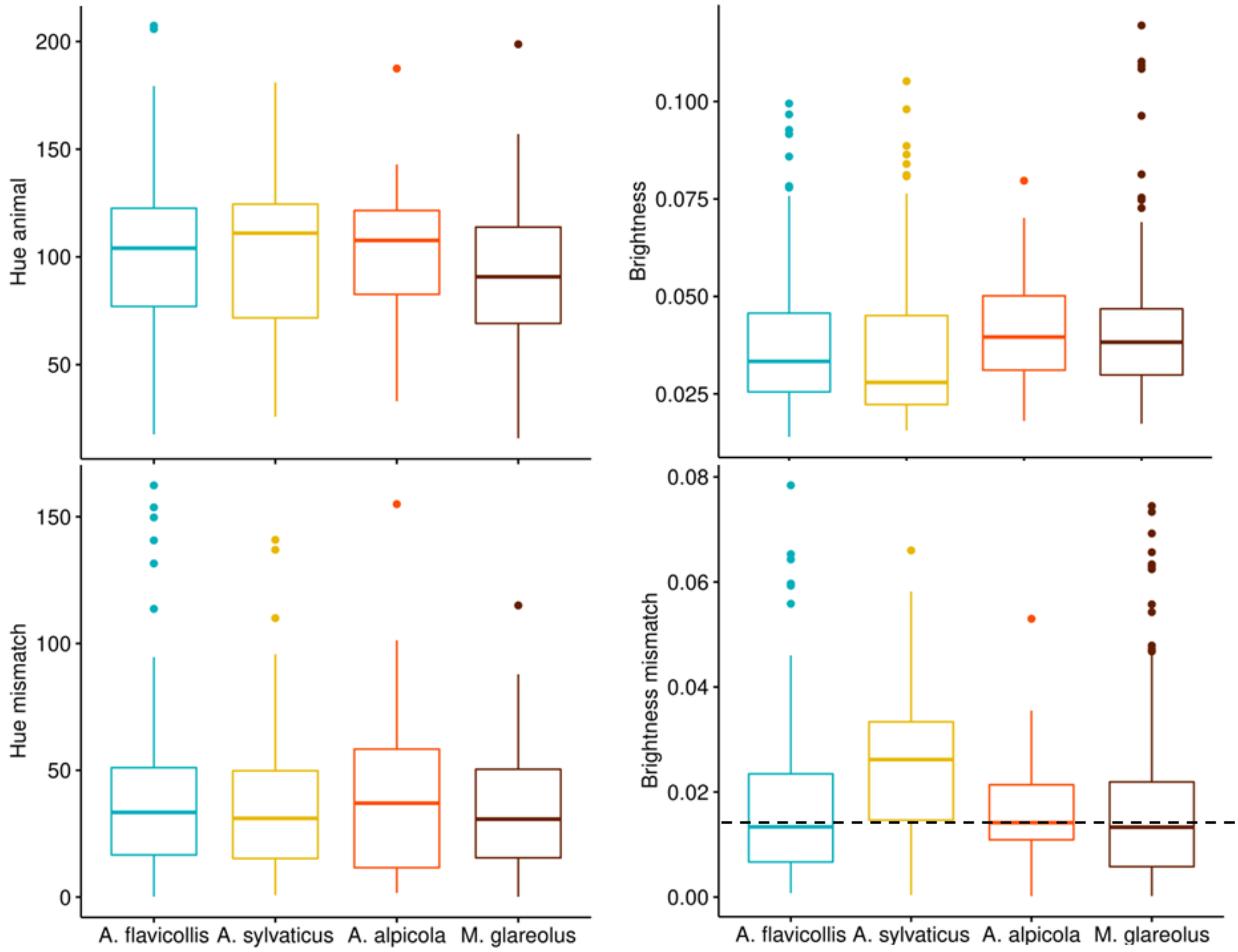


Figure 2. Boxplot of hue, brightness and the respective mismatches for the four species.

Results from the LRT analyses for hue and brightness, including species as factor, showed that the interaction between elevation and species improved the fit of the model both for hue (χ^2 = 33.59, p

< 0.001) and for brightness (χ^2 = 35.36, p < 0.001). For animal hue, the best model included the habitat hue (χ^2 = 276.39, p < 0.001), while for brightness the vegetation cover (χ^2 = 4.92, p = 0.02) and plant diversity (χ^2 = 4.92, p = 0.017). The other covariates considered here had no clear effect on model fit either for the hue (p ≥ 0.13) and for the brightness (p ≥ 0.12; Table 1).

Table 1. Likelihood ratio test of the generalised linear model testing the effect of elevation, species and their interaction, herbaceous, rocky and vegetation coverage, plant diversity and hue of the habitat on animals' hue on animals' hue (a) and brightness of the habitat on animals' brightness (b). Df = model's degree of freedom; df = degree of freedom of the test statistic; logLik = loglikelihood; X^2 = chi square statistic test; p = p value. In bold are shown the significant p-values of the models where the excluded tested variable was significant on the model fit.

(a)			Hue_animal		
	Df	df	logLik	X^2	p
Full model: ~ species* elevation + hue_habitat + herbaceous + rocky + vegetation cover + plant diversity	14		349.690		
- interaction species * elevation	11	-3	332.860	33.59	**< 0.001**
- plant diversity	13	-1	348.680	2.01	0.15
- vegetation cover	13	-1	348.710	1.95	0.16
- rocky	13	-1	348.550	2.27	0.13
- herbaceous	13	-1	348.790	1.79	0.18
- hue_habitat	13	-1	211.490	276.39	**< 0.001**
- elevation	10	-4	332.810	33.74	**< 0.001**
- species	8	-6	324.680	50.01	**< 0.001**
~ 1	2	-12	184.960	329.45	**< 0.001**

(b)			Brightness_animal		
	Df	df	logLik	X^2	p
Full model: ~ species* elevation + brightness_habitat + herbaceous + rocky + vegetation cover + plant diversity	14		167.220		
- interaction species * elevation	11	-3	149.530	35.36	**< 0.001**
- plant diversity	13	-1	164.750	4.92	**0.017**
- vegetation cover	13	-1	164.750	4.92	**0.021**
- rocky	13	-1	167.130	0.17	0.67
- herbaceous	13	-1	166.030	2.36	0.12
- brightness_habitat	13	-1	167.200	0.02	0.88
- elevation	10	-4	143.300	47.82	**< 0.001**
- species	8	-6	148.200	38.02	**< 0.001**
~ 1	2	-12	133.170	68.09	**< 0.001**

At the species level, the results for hue values from LRT analysis, showed that hue of the habitat had a positive effect in all the four species (*AF:* $\chi^2 = 81.42$, $p < 0.001$; *AS:* $\chi^2 = 88.67$, $p < 0.001$; *AA*: $\chi^2 = 15.17$, $p < 0.001$; *CG*: $\chi^2 = 76.76$, $p < 0.001$; Fig 3). Elevation had a negative effect on the hue in *AF* ($\chi^2 = 11.06$, $p < 0.001$) and a positive effect in *CG* ($\chi^2 = 9.92$, $p = 0.001$); for *AA* we found a negative effect of herbaceous cover ($\chi^2 = 10.77$, $p = 0.001$) and a positive effect of vegetation cover ($\chi^2 = 10.25$, $p = 0.001$; Fig. 2; Table S4). The results for brightness showed no effect of the tested variables on the brightness of animals in *AF* and *AA* ($p \geq 0.06$), while in *AS* we observed a negative effect of the brightness of the habitat ($\chi^2 = 11.58$, $p < 0.001$) and a positive effect of elevation ($\chi^2 = 33.74$, $p < 0.001$). In *CG* we observed a positive effect of the herbaceous ($\chi^2 = 8.40$, $p = 0.003$) and plant diversity ($\chi^2 = 9.83$, $p = 0.001$; Fig.3; Table S4).

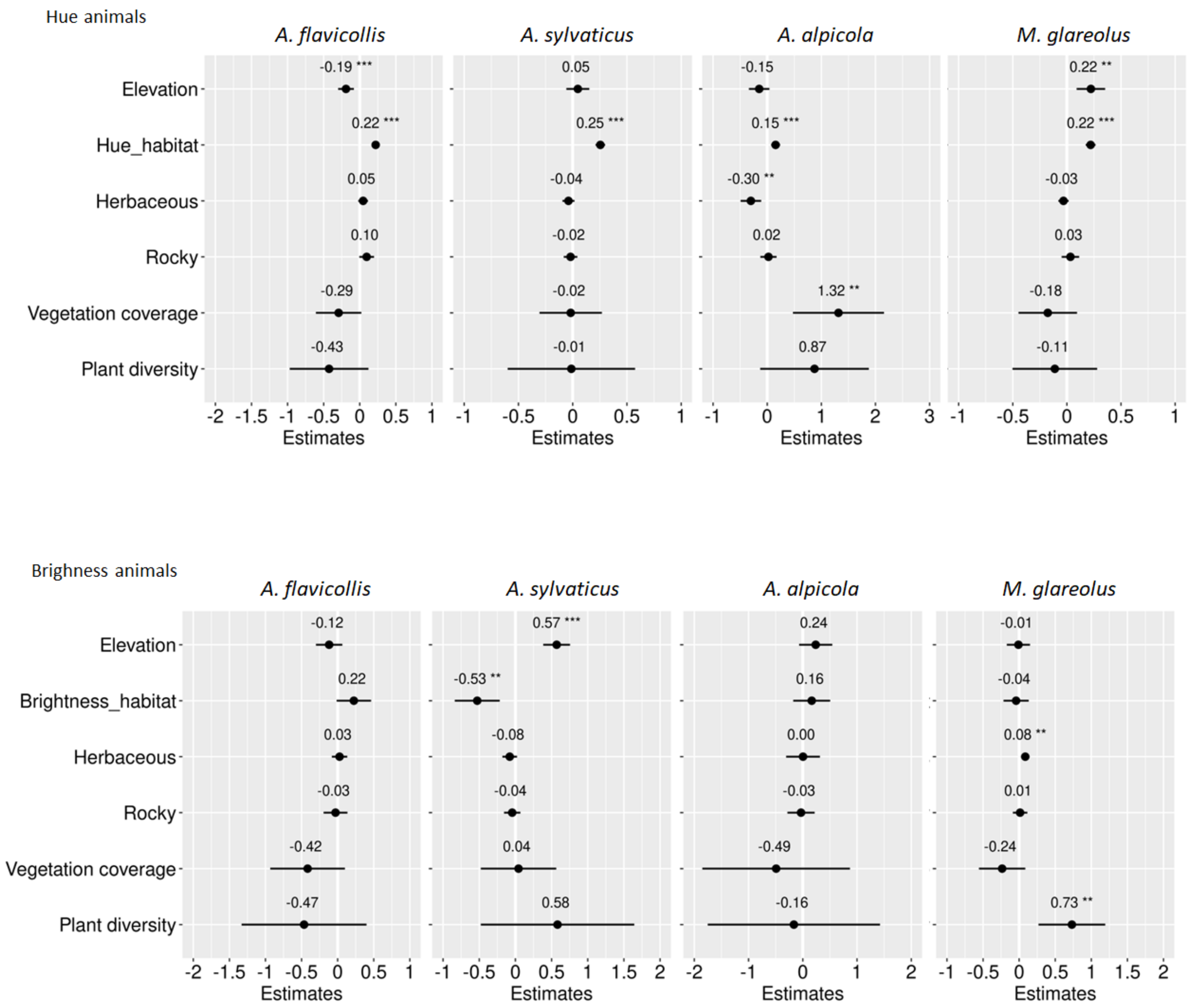


Figure 3: Plot from the summary of the regression model of species hue values (top) and brightness values (bottom). The stars indicate the significance level of the tested covariates.

Mismatches

The four species differed in the brightness mismatch (F = 7.77, df = 3, p < 0.001) but not for the hue mismatch (F = 0.36, df = 3, p = 0.78). In particular, brightness mismatch was significantly higher in *AS* (0.0261 ± 0.0146), followed by *CG* and *AF*, where the values were similar (mean ± SD = 0.0172 ± 0.0155), and *AA* (0.0162 ± 0.0104; Fig. 4; Table S3).

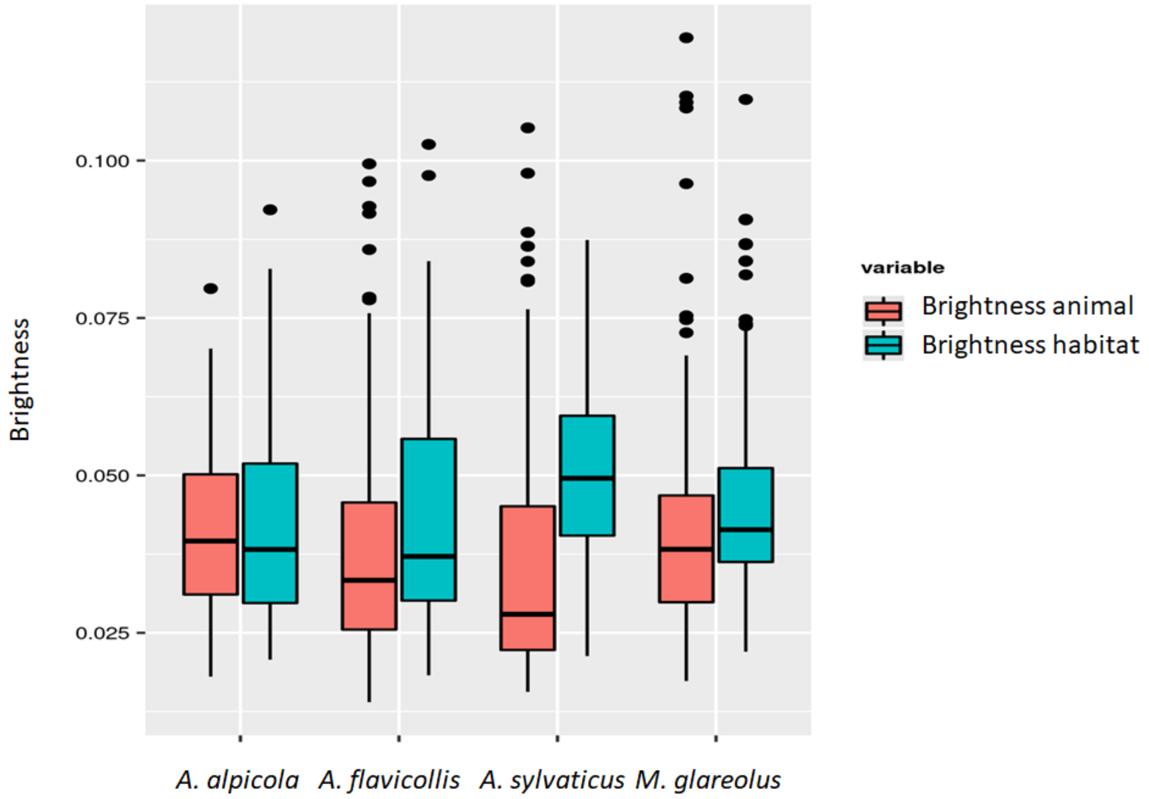


Figure 4. Boxplot of brightness value of the animal (red) and the habitat (blue) for the four species

Analyses for mismatches showed that the species-elevation interaction had an effect in improving the model fit for hue mismatch ($\chi^2 = 16.13$, $p < 0.001$), while for brightness mismatch only species seemed to influence the model fit ($\chi^2 = 20.65$, $p = 0.002$). No other relation with any of the tested covariates was found in the mismatches ($p \geq 0.15$; Table 2).

Table 2. Likelihood ratio test of the generalised linear model testing the effect of elevation, species and their interaction, herbaceous, rocky and vegetation coverage, and plant diversity on hue and brightness mismatches. Df = model's degree of freedom; df = degree of freedom of the test statistic; logLik = loglikelihood; χ^2 = chi square statistic test; p = p value. In bold are shown the significant p-values of the models where the excluded tested variable was significant on the model fit

			Hue mismatch			Brightness mismatch		
	Df	df	logLik	χ^2	p	logLik	χ^2	p

Full model: ~ species* elevation + herbaceous + rocky + vegetation cover + plant diversity	13		-2092.1			1385.5		
- interaction species * elevation	10	-3	-2100.1	16.14	**< 0.001**	1383.4	4.32	0.22
- plant diversity	12	-1	-2092.3	0.52	0.46	1385.5	0.02	0.87
- vegetation cover	12	-1	-2092.7	1.31	0.25	1385.4	0.28	0.59
- rocky	12	-1	-2093.1	2.06	0.15	1385.0	1.14	0.28
- herbaceous	12	-1	-2092.1	0.00	0.95	1385.2	0.64	0.42
- elevation	9	-4	-2103.0	21.91	**< 0.001**	1383.4	4.32	0.36
- species	7	-6	-2104.0	23.83	**< 0.001**	1375.2	20.65	**0.002**
~ 1	2	-11	-2107.1	30.12	**0.001**	1372.8	25.34	**0.008**

At the species level, the results for hue mismatch from LRT analysis, showed that elevation had a positive effect in *AF* (χ^2 =5.14, p = 0.023) and *AS* (χ^2 =5.08, p = 0.024). We also observed a negative effect of the vegetation coverage in *AF* (χ^2 = 5.67, p = 0.017), and a positive effect of plant diversity in *CG* (χ^2 =5.28, p = 0.021). None of the other tested variable improved the fit of the single-species models for hue mismatch (p ≥ 0.07; Fig. 3; Table S5). For the brightness mismatch we observed in *AF* a low effect of the rocky cover (χ^2 = 3.95, p = 0.046), while in *AA* there was a moderate positive effect of the vegetation coverage (χ^2 = 5.18, p = 0.022) and a strong negative effect of the herbaceous coverage (χ^2 = 9.34, p = 0.002). In *MG* and *AS* none of the tested variables had a clear effect on the model fit (p ≥ 0.07; Fig. 5; Table S5).

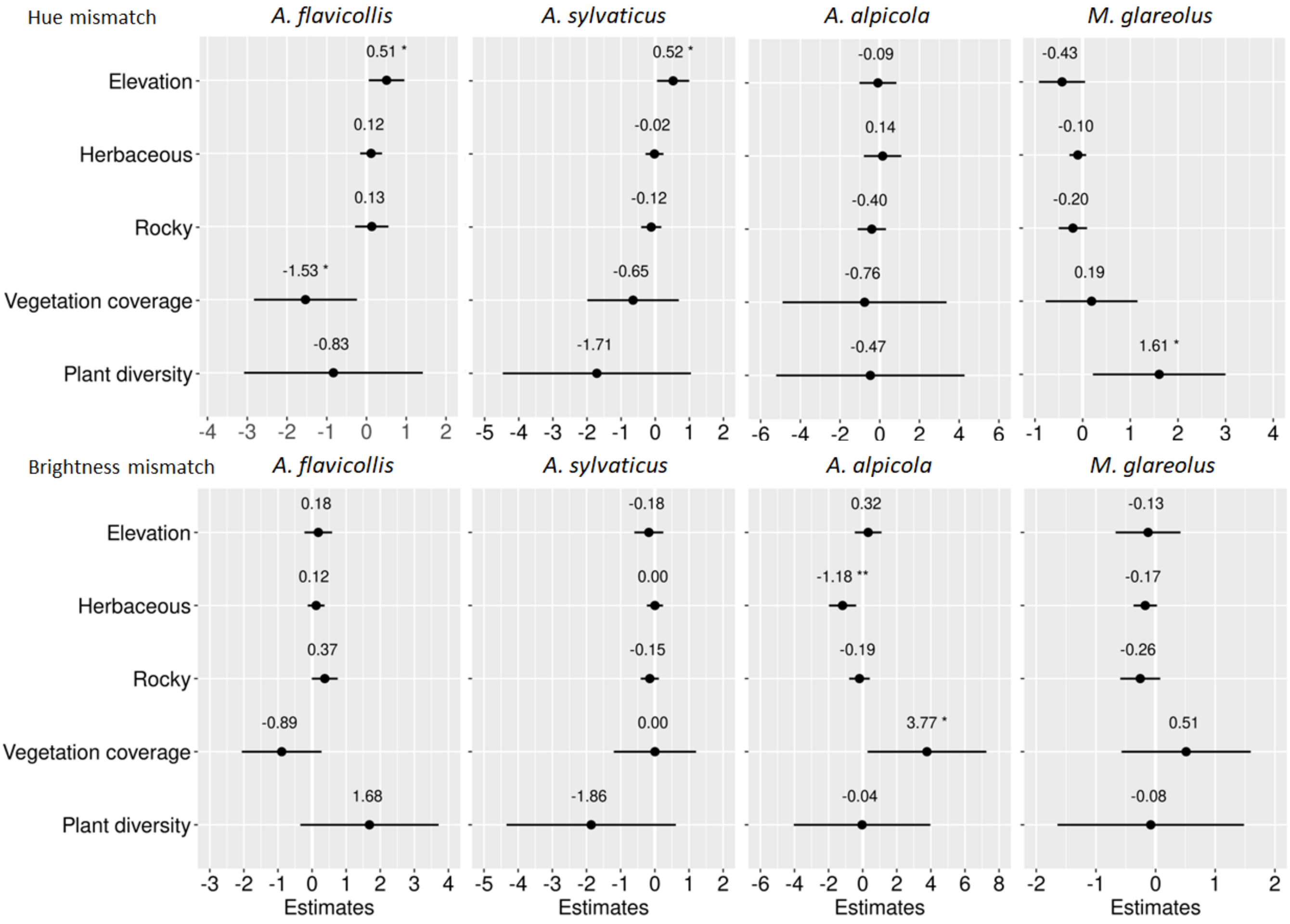


Figure 5: Plot from the summary of the regression model of species hue mismatch (top) and brightness mismatch (bottom). The stars indicate the significance level of the tested covariates.

DISCUSSION

Rodents' community living in the Alps provides a fruitful model system to study how animals adopt different strategies to survive in their habitat (Kay & Hoekstra, 2008). In this study, we presented results from analyses on camouflage in four sympatric rodents' species and showed that species differed in their camouflage adaptations. We also showed how fur colouration characteristics (hue and brightness) have a different role among the two groups of rodents (i.e., mice vs bank voles), perhaps reflecting differential species activity patterns and microhabitat use.

Hue, saturation and brightness (HSV, also known as HSB) are alternative representations of the RGB color model to align with the way human vision perceives color-making attributes more closely. In HSV a color with maximum value/brightness is analogous to shining a white light on a coloured object (e.g. shining a bright white light on a red object causes the object to still appear red, just brighter and more intense, while shining a dim light on a red object causes the object to appear darker and less bright; Ihaka et al. 2022; Ottosson & Björn, 2022). Thus, HSV is a valuable analysis tool, because it extensively characterises colour variation across the visible spectrum of 400-700 nm (which is also the human visible spectrum), making interpretation somewhat receiver independent. For these analyses we decided to consider only hue and brightness values because saturation in the predators' vision models could be interpreted poorly (Kelber & Osorio, 2010).

Following the hypothesis that predator is the major selective pressure driving background matching in rodents' colouration, animal colour characteristics should correlate with the corresponding values of backgrounds (Boratyński et al., 2014, 2017). In general, our results are consistent with this hypothesis and showed that all species shared a similar high accuracy of camouflage in the chromatic component of the colour (hue) of the dorsal fur, perfectly matching their habitat colouration. However, for the brightness component of the colour, we did not observe the same optimal camouflage since in none of the studied species the brightness of the animal correlated with the substrate, except in *AS* which exhibits darker coloration compared to its habitat. This may suggest that this species may not be under strong selection for background matching in its coloration, but for some other reason of coloration (i.e., thermoregulation, immune defence, genetic components etc.; Dreiss et al., 2016).

At the species level we observed a different pattern in the colour components here analysed, and the differential effect of some habitat characteristics, such as elevation and microhabitat variables, suggesting that in addition to the optimised camouflage achieved by each species, there are differential effects of the habitat at the local scale that must be taken into consideration (Macedo et al. 2022).

Regarding the brightness component of the colour, our second prediction that darker colourations are predominant at higher elevation is not confirmed by our results. In fact, *AA* showed lighter colouration compared to the lowland species *AS*. This could be due to the habitat used by the highland species, which is restricted to high altitudes grassland dominated by rocky scree habitats and sparce larch woodland with a rich herbaceous layer (Storch & Lütt, 1989;). Open habitats are dominated by a near-white radiance spectrum (Dreiss et al., 2016), which probably lead this species to evolve brightness camouflage strategy in such brighter habitats. Lighter colouration was observed also in *CG,* and it was positive correlated with plant diversity and herbaceous coverage. This result may reflect the microhabitat used by this species, mostly found in grass covered habitat and in coniferous forests (Gurnell, 1985; Mazurkiewicz 1994; Canova, 1993).

Regarding the dominant wavelength (hue), our results showed a differential strategy between the two groups, *Apodemus* spp. vs bank vole, consequently rejecting our third hypothesis that sympatric rodents may share similar colouration component. In fact, *CG* showed lower wavelength, and thus reddish colourations, compared to *Apodemus* species. However, a reversal trend is observed along the elevation: *AF* become more reddish (lower hue, confirming our fourth hypothesis) while *CG* become less reddish (higher hue). This result could be due to two factors. On the one hand *AF* has a typical low-land distribution well adapted to deciduous forests and colonised high-altitude environment only recently (Reutter et al., 2003). In our study areas it was found at high elevation only at low population density. These ecological characteristics may have influenced its wavelength, probably for thermoregulation mechanisms and for protection from solar radiation (Nor, 2001). This result was also supported by the increasing in hue mismatch with elevation, supporting the hypothesis of recent adaptation to the high altitude (Rios & Álvarez-Castañeda, 2011). On the contrary, in *CG,* and also in *AA*, hue was higher at high elevation, where the species were captured mainly in rocky environments, with herbaceous cover and coniferous forests with low canopy, suggesting an anti-predatory strategies in hue component for these species. A hypothesis could be that lighter colouration and higher wavelength at high altitude might be expected to be less noticeable in habitats that are often snow-covered or lacking dark vegetation, to the detriment of heat absorption in these cold environments. There is also experimental evidence showing that both aerial (Dice, 1947; Kaufman, 1974) and terrestrial (Brown, 1965) predators select against conspicuously coloured mice. Thus, we can suppose that fur colouration can influence the activity patterns and other ecological factors of the species, such as behaviour or thermoregulation. The colouration matching patterns may be an essential parameter to be considered in the analysis for camouflage, as natural environments are variable in visual texture (e.g. river banks, gravel of various sizes, and rocks; Michalis et al., 2017). This sets different strategies for species that live in such habitats (Dimitrova & Merilaita, 2014; Kjernsmo & Merilaita, 2012). Habitat characteristics such as elevation, the vegetative, herbaceous and rocky cover at the ground level, and the plant diversity have to be considered as they determine the level of natural selection pressure (Rios & Álvarez-Castaneda, 2012). For example, in our study plant diversity, vegetation and herbaceous coverage were the main factors affecting both the hue and the brightness of the species in a differential way (Körner, 2007; Macedo et al. 2022). The data showed that selection acts differentially on fur dominant wavelength and brightness and could be used to explain the mechanisms underlying the choice of one camouflage strategy over the other in sympatric species. However, it is important to point out that hue should be used with caution to interpret background matching, as the vision system of relevant predators is not known. HSV is a valuable analysis tool, because it extensively characterises colour variation across the visible spectrum of 400-700 nm (which is also the human visible spectrum), making interpretation somewhat receiver independent. However, the camouflage strategy in the dominant wavelength using HSV-hue could be an indirect signal, but not direct evidence, because it is not known whether predators are sensitive at those wavelengths that the hue of HSV indicates. In future studies, a model of animal vision could be conducted, to provide evidence of the animal vision system, and whether it is able to detect the wavelengths registered here. An analysis of other variables, i.e. spatial and temporal scales, are needed to have a better understand of fur colour evolutionary adaptation to habitat characteristics.

This study sets the stage for a more detailed analysis of the differences in fitness in relation to animal-background similarity, which are needed to explore the relative importance of camouflage and thermoregulation as selective drivers of fur colouration in alpine rodents. So far, no empirical study has addressed the fitness of the camouflage strategies under natural conditions, and the selection forces driving the observed variation in their spatial distribution remain unknown.

Acknowledgments

We thank Gran Paradiso National Park for logistical support, Noemi Gargano for help with fieldwork, and Alfredo Santovito for providing material and support for the laboratory analysis. Financial support and material were provided by the University of Turin and the CBIO.

Reference

Ajmal, A., Hollitt, C., Frean, M., & Al-Sahaf, H. (2018, 19-21 Nov. 2018). A Comparison of RGB and HSV Colour Spaces for Visual Attention Models. Paper presented at the 2018 International Conference on Image and Vision Computing New Zealand (IVCNZ).

Ashby KR (1972) Patterns of daily activity in mammals. Mammal Rev 1:171–185

Bolker B., H. Skaug, A. Magnusson, and A. Nielsen. 2012. Getting started with the glmmADMB package.

Bond, A. B., & Kamil, A. C. (2002). Visual predators select for crypticity and polymorphism in virtual prey. *Nature*, *415*, 609‒613. https://doi.org/10.1038/415609a

Brown LN 1965. Selection in a population of housemice containing mutant individuals. Journal of Mammalogy 46: 461–465.

Canova L. 1993. Resource partitioning between the bank vole *Clethrionomys glareolus* and the wood mouse *Apodemus sylvaticus* in woodland habitats. Boll Zool. 60:193–198.

Caro, T. (2005). The adaptive significance of colouration in mammals. *BioScience*, *55*(2), 125‒136. https://doi.org/10.1046/j.1095-8312. 2003. 00190.x

Cone, R. D. (2006). Studies on the physiological functions of the melanocortin system. *Endocrine Reviews*, *27*(7), 736‒749.

Cooper, I.A. 2010. Ecology of sexual dimorphism and clinal variation in colouration in a damselfly. Am. Nat. 176: 566–572

Cuthill, I. C. (2019). Camouflage. Journal of Zoology, 308(2), 75-92. doi:10.1111/jzo.12682

Galeotti, P., Rubolini, D., Dunn, P. O., & Fasola, M. (2003). Colour polymorphism in birds: Causes and functions. *Journal of Evolutionary Biology*, *16*(4), 635‒646. https://doi.org/10.1046/j.1420-9101.2003.00569. X

Gurnell J. 1985. Woodland rodent communities. [In: The ecology of woodland rodents, bank voles and wood mice. J. R. Flowerdew, J. Gurnell and J. H. W. Gipps, eds]. Symposia of the Zoological Society of London 55: 377–402.

Delhey K., Dale J., Valcu M., Kempenaers B. (2020) Why climate change should generally lead to lighter coloured animals. Current Biology. 30: R1406-R1407

Dice LR 1947. Effectiveness of selection by owls of deermice (Peromyscus maniculatus) which contrast in color with their background. Contributions from the Laboratory of Vertebrate Biology, University of Michigan 34: 1–20.

Draghi J. (2019). Phenotypic variability can promote the evolution of adaptive plasticity by reducing the stringency of natural selection. J Evol Biol. 32:1274–1289

Dreiss, A., Henry, I., Ruppli, C., Almasi, B., & Roulin, A. (2010). Darker eumelanic barn owls better withstand food depletion through resistance to food deprivation and lower appetite. *Oecologia*, *164*(1), 65−71.

Dreiss, A. N., Séchaud, R., Béziers, P., Villain, N., Genoud, M., Almasi, B., Jenni, L., & Roulin, A. (2016). Social huddling and physiological thermoregulation are related to melanism in the nocturnal barn owl. *Oecologia*, *180*(2), 371−381. https://doi.org/10.1007/s0044 2-015-3491-3

Duarte, R. C., Stevens, M., & Flores, A. A. V. (2018). The adaptive value of camouflage and colour change in a polymorphic prawn. *Scientific Reports*, *8*, 1−10. https://doi.org/10.1038/s4159 8-018-34470 -z

Ducrest, A. L., Keller, L., & Roulin, A. (2008). Pleiotropy in the melanocortin system, colouration and behavioural syndromes. *Trends in Ecology & Evolution*, *23*(9), 502−510. https://doi.org/10.1016/j.tree.2008.06.001

Endler, J. A. (1978). A Predator's View of Animal Colour Patterns. In M. K. Hecht, W. C. Steere, & B. Wallace (Eds.), Evolutionary Biology (pp. 319-364). Boston, MA: Springer US.

Endler, J. A. & Greenwood, J. J. D. Frequency-dependent predation, crypsis and aposematic coloration. Philos. Trans. R. Soc. B Biol. Sci 319, 505–523

Fennell, J. G., Talas, L., Baddeley, R. J., Cuthill, I. C., Life, B., Building, S., & Acquisition, F. (2018). Optimising colour for camouflage and visibility: The effects of the environment and the observer's visual system. *Journal of the Royal Society Interface*. https://doi.org/10.1098/rsif.2019.0183

Goldenberg, J., Bisschop, K., D'Alba, L., & Shawkey, M. D. (2022). The link between body size, colouration and thermoregulation and their integration into ecogeographical rules: a critical appraisal in light of climate change. *Oikos*, *2022*(6), e09152.

Körner, C. 2007.The use of "altitude" in ecological research. Trends Ecol. Evol, 22, 569–574.

Kryštufek, B., Tesakov, A. S., Lebedev, V. S., Bannikova, A. A., Abramson, N. I., and Shenbrot, G. (2020). Back to the future: the proper name for red-backed voles is *Clethrionomys* Tilesius and not *Myodes* Pallas. *Mammalia* 84, 214–217. doi: 10.1515/mammalia-2019-0067

Halle, S. (2000). Voles—small graminivores with polyphasic patterns. *Activity patterns in small mammals: an ecological approach*, 191-215.

Hanlon R.T & J.B Messenger. 1987. Adaptive Colouration in Young Cuttlefish (*Sepia Officinalis* L.): The Morphology and Development of Body Patterns and Their Relation to Behaviour. Philosophical Transactions of The Royal Society B Biological Sciences

Harrison X.A., L. Donaldson, M.E. Correa-Cano, J. Evans, D.N. Fisher, C.E.D. Goodwin, B.S. Robinson, D.J. Hodgson, and R. Inger. 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. PeerJ 6:e4794.

Hille, S. M., & Mortelliti, A. (2011). Microhabitat partitioning of Apodemus flavicollis and Myodes glareolus in the sub-montane Alps: a preliminary assessment. *Hystrix, the Italian Journal of Mammalogy*, *21*(2).

Houston, A. I., Stevens, M., & Cuthill, I. C. (2007). Animal camouflage: Compromise or specialize in a 2 patch-type environment? *Behavioral Ecology*, *18*, 769‒775. https://doi.org/10.1093/beheco/arm039

Hughes, A., Liggins, E., & Stevens, M. (2019). Imperfect camouflage: How to hide in a variable world? *Proceedings of the Royal Society B: Biological Sciences*, *286*, 20190646. https://doi.org/10.1098/rspb.2019.0646

Ihaka R, Murrell P, Hornik K, Fisher JC, Stauffer R, Wilke CO, McWhite CD, Zeileis A. Color Spaces: S4 Classes and Utilities. R Project: colorspace package (Article). Retrieved 27 January 2022.

ImageJ software. Webpage tutorial: http://www.empiricalimaging.com/about/brief-introduction/

Kappers, E. F., de Vries, C., Alberda, A., Kuhn, S., Valcu, M., Kempenaers, B., & Both, C. 2020. Morph-dependent fitness and directional change of morph frequencies over time in a Dutch population of Common buzzards *Buteo buteo*. *Journal of Evolutionary Biology*, *33*(9), 1306‒1315. https://doi.org/10.1111/jeb.13675

Kaufman DW 1974. Adaptive coloration in Peromyscus polionotus: experimental selection by owls. Journal of Mammalogy 55: 271–283.

Kay E.H., Hoekstra H.E. 2008. Rodents. *Current Biology* 18:406-410.

Kelber A. 2006: Nocturnal colour vision – not as rare as we might think. *J. Exp. Biol.* 209*:* 781–788.

Kelber A. & Lind O. 2010: Limits of colour vision in dim light. *Ophthal. Physl. Opt.* 30*:* 454–459.

Kelber, A., & Osorio, D. (2010). From spectral information to animal colour vision: experiments and concepts. Proceedings of the Royal Society B: Biological Sciences, 277(1688), 1617-1625. doi:doi:10.1098/rspb.2009.2118

Kjernsmo, K., & Merilaita, S. (2012). Background choice as an anti-predator strategy: The roles of background matching and visual complexity in the habitat choice of the least killifish.

Proceedings of the Royal Society B: Biological Sciences, *279*, 4192–4198. https://doi.org/10.1098/rspb.2012.1547

Knitlová, M. and Horáček I. 2017. Late Pleistocene-Holocene paleobiogeography of the genus *Apodemus* in Central Europe. PLoS ONE 12 (3): e0173668

Lai, Y. C., Shiroishi, T., Moriwaki, K., Motokawa, M., & Yu, H. T. (2008). Variation of coat color in house mice throughout Asia. *Journal of Zoology*, *274*(3), 270-276.

Macedo, T., Campos, J., Nokelainen, O., Scott-Samuel, N. E., & Boratyński, Z. (2022). The effect of spatial and temporal scale on camouflage in North African rodents. Biological Journal of the Linnean Society.

Mappes, J., Kokko, H., Ojala, K. & Lindström, L. Seasonal changes in predator community switch the direction of selection for prey defences. Nat. Commun. 5, 5016

Mazurkiewicz M. 1994. Factors influencing the distribution of the bank vole in forest habitats. Acta Theriologica 39: 113–126.

Merilaita, S., & Stevens, M. (2011). Crypsis through background matching. Animal camouflage: mechanisms and function, 17-33.

Merilaita, S., Scott-Samuel, N. E., & Cuthill, I. C. (2017). How camouflage works. Philosophical Transactions of the Royal Society B-Biological Sciences, 372(1724). doi:10.1098/rstb.2016.0341

Michalis, C., Scott-samuel, N. E., Gibson, D. P., Cuthill, I. C., & Michalis, C. (2017). Optimal background matching camouflage. *Proceedings of the Royal Society B*, *284*, 20170709. https://doi.org/10.1098/rspb.2017.0709

Morosinotto, C., Brommer, J. E., Lindqvist, A., Ahola, K., Aaltonen, E., Karstinen, T., & Karell, P. (2020). Fledging mass is colour morph specific and affects local recruitment in a wild bird. *The American Naturalist*, *196*(5), 609–619. https://doi.org/10.1086/710708

Nokelainen, O., Maynes, R., Mynott, S., Price, N., & Stevens, M. (2019). Improved camouflage through ontogenetic colour change confers reduced detection risk in shore crabs. *Functional Ecology*, *33*(4), 654.

Nokelainen, O., Brito J.C., Scott-Samuel, N.E., Valkonen, J.K., Boratyński Z. (2020). Camouflage accuracy in Sahara–Sahel desert rodents. *J Anim Ecol,* 89: 1658−1669.

Nor S.M.D. 2001. Elevational diversity patterns of small mammals on Mount Kinabalu, Sabah, Malaysia. Global Ecol Biogeogr 10:41-62.

Ottosson, Björn (8 September 2021). "Okhsv and Okhsl" (Blog post). GitHub. Retrieved 27 January 2022.

Poblete, Y., Gutiérrez, V., Cid, V., Newsome, S.D., Sabat, P., and Vasquez, R.A. (2018). Intraspecific variation in exploratory behavior and elevational affinity in a widely distributed songbird. Oecologia *186*, 931–938.

Pyron, R. A., Costa, G. C., Patten, M. A. & Burbrink, F. T. Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. Biol. Rev. 90, 1248–1262

Reguera, S., Zamora-Camacho, F. J., & Moreno-Rueda, G. (2014). The lizard Psammodromus algirus (Squamata: Lacertidae) is darker at high altitudes. *Biological Journal of the Linnean Society*, *112*(1), 132-141.

Reutter, B. A., Helfer, V., Hirzel, A. H., & Vogel, P. (2003). Modelling habitat-suitability using museum collections: an example with three sympatric Apodemus species from the Alps. *Journal of Biogeography*, *30*(4), 581-590.

Rezende, E. L. & Diniz-Filho, J. A. F. Phylogenetic analyses: comparing species to infer adaptations and physiological mechanisms. Compr. Physiol 2, 639–674

Rios, E. & Álvarez-Castañeda S.T. 2011. Pelage color variation in pocket gophers (Rodentia: Geomyidae) in relation to sex, age and differences in habitat. Mammalian Biology.77: 160-165.

Roff, D. A., & Fairbairn, D. J. (2013). The costs of being dark: The genetic basis of melanism and its association with fitness-related traits in the sand cricket. *Journal of Evolutionary Biology*, *26*(7), 1406−1416

Roulin, A., Bize, P., Tzaud, N., Bianchi, M., Ravussin, P. A., & Christe, P. (2005). Oxygen consumption in offspring tawny owls *Strix aluco* is associated with colour morph of foster mother. *Journal of Ornithology*, *146*(4), 390−394.

Roulin, A., & Ducrest, A. L. (2011). Association between melanism, physiology and behaviour: A role for the melanocortin system. *European Journal of Pharmacology*, *660*(1), 226−233. https://doi.org/10.1016/j. ejphar.2011.01.036

San-Jose, L. M., & Roulin, A. (2017). Genomics of colouration in natural animal populations. *Philosophical Transactions of the Royal Society*

B: Biological Sciences, *372*(1724). https://doi.org/10.1098/rstb.2016.

0337

Sears M.W., J.P. Hayes, M.R. Benta, and D. McCormick. 2009. Out in the cold: physiological capacity influences behaviour in deer mice. Funct Ecol 23:774-783

Spitzenberger F, Englisch H. 1996. Die Alpen wald maus (*Apodemus alpicola* Heinrich, 1952) in Österreich. Mammalia Austriaca 21. Bonn Zool Beitr. 46: 249–260.

Spitzenberger F. 2001. Die Säugetierfauna Österreichs. Bundesministerium für Land- und Forstwirtschaft. Wien: Umwelt und Wasserwirtschaft.

Storch G, Lütt O. 1989. Artstatus der Alpenwaldmaus, *Apodemus alpicola* Heinrich, 1952. Z Saugetierkd. 54: 337–346.

Troscianko, J., & Stevens, M. (2015). Image calibration and analysis toolbox – a free software suite for objectively measuring reflectance, colour and pattern. Methods in Ecology and Evolution, 6(11), 1320-1331

Vogel P. 1995. *Apodemus sylvaticu*s (L., 1758), *Apodemus flavicollis* (Melchior, 1834), *Apodemus alpicola* Heinrich, 1952. In: Hausser J, editor. Säugetiere der Schweiz—Verbreitung, Biologie und Ökologie, Basel: Birkhäuser Verlag; pp. 178–282.

Walsberg, G. E. (1983). Coat colour and solar heat gain in animals. *BioScience*, *33*(2), 88−91. https://doi.org/10.2307/1309169

Walsberg, G. E. (1991). Thermal effects of seasonal coat change in three subarctic mammals. *Journal of Thermal Biology*, *16*(5), 291−296.

Walsberg, G. E., & Schmidt, C. A. (1989). Seasonal adjustment of solar heat gain in a desert mammal by altering coat properties independently of surface colouration. *Journal of Experimental Biology*, *142*(1), 387−400.

Warrant, E. J., & Johnsen, S. (2013). Vision and the light environment. *Current Biology*, *23*(22), R990-R994.

Wooldridge, T. B., Kautt, A. F., Lassance, J. M., McFadden, S., Domingues, V. S., Mallarino, R., & Hoekstra, H. E. (2022). An enhancer of Agouti contributes to parallel evolution of cryptically colored beach mice. *Proceedings of the National Academy of Sciences*, *119*(27), e2202862119.

Zimova, M., Hackländer, K., Good, J. M., Melo-Ferreira, J., Alves, P. C., & Mills, L. S. (2018). Function and underlying mechanisms of seasonal colour moulting in mammals and birds: What keeps them changing in a warming world? *Biological Reviews*, *93*(3), 1478−1498.

Appendix

Figure A1. Land use map of the PNGP (Park Plan 2019)

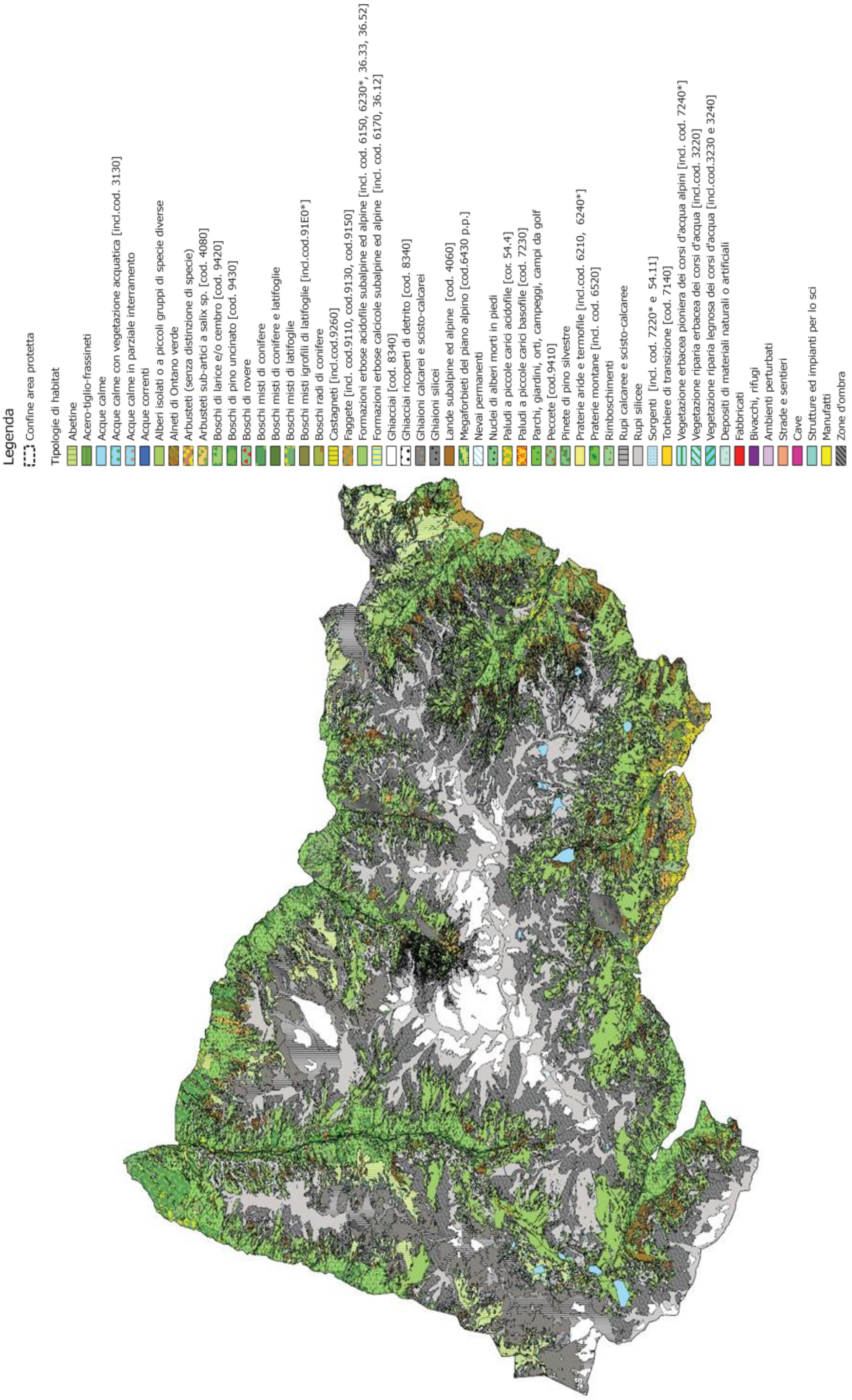


Table S1. Coordinates (longitude; latitude) of the twelve trapping location, the weather stations and the laboratories:

	Trapping locations		Weather stations		Laboratories	
1	7.62805	45.40565	7.57330	45.55680	7.52220	45.49770
2	7.61568	45.40899	7.48720	45.51350	7.31445	45.45137
3	7.59606	45.42822	7.61110	45.42000		
4	7.43559	45.42309	7.54100	45.40890		
5	7.54968	45.50748	7.43820	45.42670		
6	7.31445	45.45137	7.41150	45.43840		
7	7.39580	45.45295	7.31140	45.45280		
8	7.48709	45.51901	7.20910	45.44110		
9	7.47173	45.52610				
10	7.51484	45.55321				
11	7.21039	45.43669				
12	7.56948	45.55651				

Table S2. Pairwise t test for animals' hue and brightness values. Table show the p-values of the pairwise comparisons between the four species with corrections for multiple testing. Values in bold are statistically significant.

Hue animals				Brightness animals			
One-way ANOVA test: F = 3.27, df = 3, p = 0.021				One-way ANOVA test: F = 3.75, df = 3, p = 0.010			
	A. alpicola	*A. flavicollis*	*A. sylvaticus*		*A. alpicola*	*A. flavicollis*	*A. sylvaticus*
A. flavicollis	0.60	-	-	*A. flavicollis*	0.23	-	-
A. sylvaticus	0.60	0.60	-	*A. sylvaticus*	**0.05**	0.23	-
M. glareolus	**0.05**	**0.05**	**0.05**	*M. glareolus*	0.85	0.16	**0.013**

Table S3. Pairwise t test for animals' hue and brightness mismatches. Table show the p-values of the pairwise comparisons between the four species with corrections for multiple testing. Values in bold are statistically significant.

Hue mismatch				Brightness mismatch			
One-way ANOVA test: F = 0.36, df = 3, p = 0.78				One-way ANOVA test: F = 7.77, df = 3, p < 0.001			
	A. alpicola	*A. flavicollis*	*A. sylvaticus*		*A. alpicola*	*A. flavicollis*	*A. sylvaticus*
A. flavicollis	0.91	-	-	*A. flavicollis*	0.87	-	-
A. sylvaticus	0.91	0.91	0.91	*A. sylvaticus*	**0.015**	**< 0.001**	-
M. glareolus	0.91	0.91	0.91	*M. glareolus*	0.61	0.61	**< 0.001**

Table S4. Single species analysis for hue and brightness. Likelihood ratio test of the generalised linear model testing the effect of elevation, herbaceous, rocky and vegetation coverage, plant diversity and hue oh the habitat on animals' hue and brightness of the habitat on animals' brightness. Df = model's degree of freedom; df = degree of freedom of the test statistic; logLik = loglikelihood; χ^2 = chi square statistic test; p = p value. In bold are shown the significant p-values of the models where the excluded tested variable was significant on the model fit.

			Hue_animal			Brightness_animal		
A. flavicollis	Df	df	logLik	χ^2	p	logLik	χ^2	p
Full model: ~ elevation + hue_habitat + herbaceous + rocky + vegetation cover + plant diversity	8		79.300			33.990		
- plant diversity	7	-1	78.245	2.11	0.14	33.389	1.21	0.27
- vegetation cover	7	-1	77.732	3.13	0.07	32.651	2.67	0.10
- rocky	7	-1	77.746	0.48	0.07	33.923	0.13	0.71
- herbaceous	7	-1	78.430	1.74	0.18	33.863	0.51	0.47
- hue_habitat /- brightness_habitat	7	-1	38.580	81.42	**< 0.001**	32.173	3.63	0.06
- elevation	7	-1	73.770	11.06	**< 0.001**	33.123	1.73	0.18
~ 1	2	-6	33.345	91.91	**< 0.001**	28.854	10.27	0.11
A. sylvaticus	Df	df	logLik	χ^2	p	logLik	χ^2	p
Full model: ~ elevation + hue_habitat + herbaceous + rocky + vegetation cover + plant diversity	8		87.964			33.181		
- plant diversity	7	-1	87.948	0.03	0.86	32.542	1.27	0.25
- vegetation cover	7	-1	87.954	0.01	0.88	33.166	0.02	0.86
- rocky	7	-1	87.730	0.46	0.49	32.855	0.65	0.41
- herbaceous	7	-1	87.027	1.87	0.17	31.912	2.53	0.11
- hue_habitat /- brightness_habitat	7	-1	43.628	88.67	**< 0.001**	27.389	11.58	**< 0.001**
- elevation	7	-1	87.782	0.36	0.54	16.310	33.74	**< 0.001**
~ 1	2	-6	34.651	106.62	**< 0.001**	11.340	43.67	**< 0.001**
A. alpicola	Df	df	logLik	χ^2	p	logLik	χ^2	p
Full model: ~ elevation + hue_habitat + herbaceous + rocky + vegetation cover + plant diversity	8		43.089			22.504		
- plant diversity	7	-1	41.512	3.15	0.07	22.478	0.05	0.82
- vegetation cover	7	-1	37.962	10.25	**0.001**	22.191	0.62	0.42

- rocky	7	-1	43.026	0.12	0.72	22.464	0.08	0.77
- herbaceous	7	-1	37.700	10.77	**0.001**	22.503	0.00	0.97
- hue_habitat /- brightness_habitat	7	-1	35.506	15.17	**< 0.001**	21.943	1.12	0.28
- elevation	7	-1	41.840	2.49	0.11	21.084	2.84	0.09
~ 1	2	-6	26.537	33.10	**< 0.001**	20.105	4.79	0.57
C. glareolus	Df	df	logLik	χ^2	p	logLik	χ^2	p
Full model: ~ elevation + hue_habitat + herbaceous + rocky + vegetation cover + plant diversity	8		133.270			100.087		
- plant diversity	7	-1	133.130	0.28	0.59	95.169	9.83	**0.001**
- vegetation cover	7	-1	132.520	1.49	0.22	99.006	2.16	0.14
- rocky	7	-1	132.920	0.70	0.40	100.050	0.07	0.79
- herbaceous	7	-1	132.550	1.45	0.22	95.882	8.40	**0.003**
- hue_habitat /- brightness_habitat	7	-1	94.895	76.76	**< 0.001**	99.969	0.23	0.62
- elevation	7	-1	128.310	9.92	**0.001**	100.080	0.01	0.90
~ 1	2	-6	80.444	105.66	**< 0.001**	86.160	27.85	**< 0.001**

Table S5. Single species analysis for hue and brightness mismatches. Likelihood ratio test of the generalised linear model testing the effect of elevation, herbaceous, rocky and vegetation coverage, and plant diversity on hue and brightness mismatches of the animals. Df = model's degree of freedom; df = degree of freedom of the test statistic; logLik = loglikelihood; χ^2 = chi square statistic test; p = p value. In bold are shown the significant p-values of the models where the excluded tested variable was significant on the model fit.

			Hue mismatch			Brightness mismatch		
A. flavicollis	Df	df	logLik	χ^2	p	logLik	χ^2	p
Full model: ~ elevation + herbaceous + rocky + vegetation cover + shannon	7		-79.471			-67.313		
- plant diversity	6	-1	-79.753	0.56	0.45	-68.721	2.81	0.09
- vegetation cover	6	-1	-82.306	5.67	**0.017**	-68.503	2.37	0.12
- rocky	6	-1	-79.679	0.41	0.51	-69.290	3.95	**0.046**
- herbaceous	6	-1	-79.834	0.72	0.39	-67.788	0.94	0.32
- elevation	6	-1	-82.041	5.14	**0.023**	-67.734	0.84	0.35
~ 1	2	-5	-84.542	10.143	0.07	-71.800	8.97	0.11
A. sylvaticus	Df	df	logLik	χ^2	p	logLik	χ^2	p
Full model: ~ elevation + herbaceous + rocky + vegetation cover + shannon	7		-58.235			-48.018		
- plant diversity	6	-1	-59.040	1.61	0.20	-49.197	2.35	0.12
- vegetation cover	6	-1	-58.728	10.98	0.32	-48.018	0.008	0.99
- rocky	6	-1	-58.563	0.65	0.41	-48.673	1.30	0.25
- herbaceous	6	-1	-58.250	0.03	0.86	-48.018	0.001	0.98
- elevation	6	-1	-60.778	5.08	**0.024**	-48.379	0.72	0.39
~ 1	2	-5	-62.611	8.75	0.11	-49.956	3.87	0.56
A. alpicola	Df	df	logLik	χ^2	p	logLik	χ^2	P
Full model: ~ elevation + herbaceous + rocky + vegetation cover + shannon	7		-29.386			-21.599		
- plant diversity	6	-1	-29.409	0.04	0.82	-21.599	0.002	0.98
- vegetation cover	6	-1	-29.466	0.16	0.68	-24.193	5.18	**0.022**
- rocky	6	-1	-30.122	1.47	0.25	-21.830	0.46	0.49
- herbaceous	6	-1	-29.441	0.10	0.74	-26.273	9.34	**0.002**
- elevation	6	-1	-29.409	0.04	0.82	-21.988	0.77	0.37

~ 1	2	-5	-30.738	2.70	0.74	-27.025	10.85	**0.05**
C. glareolus	Df	df	logLik	χ^2	p	logLik	χ^2	p
Full model: ~ elevation + herbaceous + rocky + vegetation cover + shannon	7		-122.90			-146.53		
- plant diversity	6	-1	-125.54	5.28	**0.021**	-146.54	0.01	0.91
- vegetation cover	6	-1	-122.94	0.15	0.69	-146.98	0.89	0.34
- rocky	6	-1	-123.84	1.87	0.17	-147.71	2.35	0.12
- herbaceous	6	-1	-123.56	1.32	0.24	-148.08	3.10	0.07
- elevation	6	-1	-124.49	3.17	0.07	-146.64	0.21	0.64
~ 1	2	-5	-132.62	19.44	**0.002**	-149.47	5.88	0.31

CHAPTER IV

Analysis of boldness in sympatric rodents along an altitudinal gradient in the Western Italian Alps

Ilaria Melcore[1], Noemi Gargano[1], Zbyszek Boratyński[3], Jacopo Cerri[2],
Sandro Bertolino[1]

Affiliations:

1. Department of Life Sciences and Systems Biology, University of Turin, Via Accademia Albertina 13, 10123 Torino, Italy.
2. Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Via Vienna 2, 07100, Sassari, Italy.
3. BIOPOLIS, CIBIO-InBIO Associate Laboratory, Research Center in Biodiversity and Genetic Resources, University of Porto, Porto, Portugal

Manuscript

Abstract

Behavioural responses are particularly important to quantify species-specific habitat influences on natural selection. Mountains, with their wide range of habitats, can be regarded as a valuable context where the environmental conditions influence animal behaviour. We studied the inter-specific variation in the escape tendency and the mobility behaviours in four alpine rodents' species, a bank vole (*Clethrionomys glareolus*) and three *Apodemus* mouse, to evaluate the differential boldness degree and its variation with elevation. A zero-altered Gamma GLMs revealed that the two groups of rodents (*Clethrionomys* vs. *Apodemus*) differed in both these traits. The latency time to first jump, an index of escape behaviour, was approximately 42% lower in *Apodemus* mice than in *C. glareolus*, in which 46.2% of individuals did not jump during the entire experiment. Also, the mobility, measured as the distance travelled, was 55% lower in *C. glareolus* compared to *Apodemus* mice. However, the leave-one-out cross validation did not detect any effect of elevation, neither on the distance travelled, nor on the latency time to the first jump. The apparent absence of any environmental influence on the behaviours here considered, could also indicates the presence of a genetic basis that regulates their expression in such species. These results can be interpreted in the context of differential habitat use and microhabitat segregation due to interspecific competition. In fact, the differentiation of niches (spatial, trophic and temporal) between the two taxa may have led to differences in behavioural traits.

Introduction

Mammals show behavioural responses to various limiting factors, such as anthropogenic activities (Wong & Candolin, 2015), or food resources (Bayliss & Choquenot, 2002), which can be particularly important to understand species adaptation, in particular in the context of environmental changes (Hager, 2010).

Apart from traits acquired through learning and cultural transmission (e.g., primates, Lefebvre 1995, e.g., cetaceans, Simões-Lopes et al. 2016), a substantial portion of behavioural variation is heritable (Titterington et al., 2022; Bubac et al., 2020). Therefore, environmental pressures can drive the extent to which animal species could express behavioural adaptation (Wong & Candolin, 2015). Among the behavioural traits, the so-called “bold-shy” continuum (or degree of boldness), where individuals within and between populations show consistent differences in their degree of risk-prone behaviour (Ioannou et al., 2008; Réale et al., 2007; Zidar et al., 2017), was found to have an adaptive value in face of rapidly changing environmental conditions (e.g., rodents, Dammhan et al., 2020). Bold individuals have often a high level of general mobility and are more explorative, take more risks, learn more quickly (Sneddon, 2003), exhibit greater aggression, and readily form persistent routines (Sih et al., 2004). By contrast, shy individuals show neophobia, with comparatively lower levels of activity and general mobility, taking less risks (Wilson et al., 1993; Koolhaas et al., 1999; Carere et al., 2005), and paying attention to external stimuli, adjusting cautiously to changes in the environment. The degree of boldness displayed by an individual is consistently expressed in a range of behavioural tests (Frost et al., 2007) and it can carry a substantial portion of additive genetic variation, therefore responding to natural selection (van Oers et al., 2004). Whereas boldness seems to increase fitness under stable environmental conditions, shy individuals seem to respond better to changing environments (Le Coeur et al., 2015; Santicchia et al., 2018).

Mountains are characterized by pronounced habitat variation along an elevational gradient, often across small ranges of latitude and longitude. Part of the natural selection in these habitats is exerted through reduced productivity: as altitude increases, environmental temperature, the duration of vegetative period and biomass decrease, and plant phenology is delayed (Hille & Cooper, 2015). Energetic resources are reduced and more spatially scattered, and the structure and diversity of vegetation decreases at higher altitudes (Laiolo et al., 2004; Nagy & Grabherr, 2009; Hürlimann et al., 2019). Thus, the habitat heterogeneity, here considered as plant diversity (Delarue et al., 2015), is another important factor that can strongly affect behavioural traits (Abramsky et al., 1992), as its elements represent a protection from predators during animals foraging and dispersion (Delarue et al., 2015). Heterogeneous habitat presents a high and complex structural variation, a considerable vegetational density, greater wealth, and plant richness, due to the increase in microhabitats availability (Kovalenko et al., 2012; Delarue et al., 2015). On the other hand, homogeneous habitat exhibits environmental and ecological characteristics that tend to be relatively stable over time (Delarue et al., 2015; Williams et al., 2002).

Mountains therefore represent a valuable context where environmental conditions may influence animal behaviour. Rodents are particularly suitable to study how the environment can influence individual behaviours, being among the mammals with the lowest age of first reproduction (Wootton, 1987), the highest reproductive rates (Butet & Leroux, 1993) and a very "fast" life history (Dobson & Oli, 2008). These characteristics make rodents particularly responsive to selective pressures, being capable of selecting fitness-related behavioural traits (Dammhahn et al., 2020), at fine spatial scales (Smolka et al., 2012) or across short temporal scales (Harris & Munshi-Shout, 2017).

To evaluate if behavioural characteristics of the species determine their altitudinal distribution, here we analysed escape and mobility behaviours, in four sympatric rodents, which co-occur in the Western Italian Alps and are partially segregated on the elevation gradient (400 to 1,800 m a.s.l.): the bank vole *Clethrionomys glareolus* (also known as *Myodes glareolus*; Kryštufek et al., 2020) and three *Apodemus* mice (the yellow-necked mouse *A. flavicollis*, the wood mouse *A. sylvaticus* and the alpine field mouse *A. alpicola;* Fig.1).

We predicted that elevation had a non-neutral effect over boldness-related traits (H_1), either because food resources may often become rarer and patchier, favouring bolder individuals with a greater exploration potential (Hurlimann et. al., 2019), or on the other hand favouring less active and slower animals which maximize energy-saving (Sears et al., 2009; Sears et al., 2006). We also predicted that species distributed across a wide altitudinal range, such as *C. glareolus* and *A. flavicollis*, which have therefore adapted to different types of habitats and variable environmental conditions (Stenseth, 1985; Haapakoski & Ylönen 2010; Hille & Mortelliti, 2010), would have had a greater inter-individual variability in level of boldness as they may respond faster to variable and changing environments (H_2, Ruperto et al., 2023). In line with field evidence indicating that species of the genus *Apodemus* are more reactive to manipulation than voles (Galsworthy, 2005), we also hypothesised species-specific differences in boldness-related traits between the four species (H_3). Finally, we predicted a sex-dependent effect affecting boldness, as found for *A. flavicollis* where males are more exploratory than females given their greater reproductive potential (H_4) (Bednarz & Zwolak, 2022).

MATERIAL AND METHODS

Study area and animal trapping

The study area was located in the Piedmont region (North-western Italy), within the territory of the Gran Paradiso National Park and nearby areas. Twelve trapping sites were identified and distributed along an elevation gradient, from 400 to 1822 m a.s.l (Table. A1). At each trapping site, a line of 30 live traps (Sherman traps, 229 x 89 x 76 mm in size), located every 10 m, was placed. Traps were set up in the field for one week and checked at dawn and dusk, being baited with apple, sunflower seeds, and peanut butter, and provided with cotton as bedding. We registered the elevation (using a GPS Garmin Etrex 32x) and geographic exposure of each trapping site (values from 0° north and deviation from that exposure moving clockwise and anti-clockwise to 180° as maximum value as south). The percentage covered by shrub and tree species in each plot was measured to evaluate plant diversity, represented by the Shannon index (see Appendix A1 for description of vegetation plot).

Animals were captured between the 15th of April and the 15th of September 2020, during the breeding season of the target species. Capture days were randomly alternated at high and low altitude sites to avoid the effect of delay in seasonality along the altitudinal gradient. Trapped animals were marked with fur-clipping, sexed and weighted. Only adult individuals (excluding evidently pregnant or lactating females) of the trapped species (based on fur characteristics and body dimensions, e.g. Gurnell & Flowerdew, 2019) with a minimum body weight of 15 g for *Apodemus* spp. and 13 g for *C. glareolus*, were selected for the behavioural experiments to reduce potential effects of body growth and ontogeny on the expression of behavioural traits (Balčiauskiene et al., 2009a; Balčiauskiene et. al., 2009b). Captured animals were transported to the closest of the three available laboratories (located at 497 m, 972 m and 1065 m a.s.l.; Table. A1). Animals were kept in captivity in individual cages with food and water at libitum for no more than 24 h and then released at their capture location. All experimental procedures were approved by the national bioethical committee (Ministry for health, protocol n. 618/2020-PR), ISPRA - Institute for environmental protection and research, (protocol n. 13486 del 23/3/2020) and trapping and handling of animals were authorized by the Gran Paradiso National Park (protocol n. 0003037/2020 del 18/08/2020) and the Province of Turin (Authorization n. 618/2020-PR).

Behavioural trials

To estimate the level of boldness we measured escape behaviour and the mobility of animals in open field test (Šíchová et al., 2014; Koski, 2014). The novel environment presented by the open field test provides a contrast for comparing similar behaviour in the field (Réale & Dingemanse, 2012). Experiments were conducted during the active phase of the species daily cycle (around 10:30 pm). First, the animal was transferred from its cage into a cylinder in the centre of the arena (see Appendix A2 for description of arena structure), to accommodate animal to arena for 1 min. After removing the cylinder, individual behaviour was recorded for 600 seconds using a camera (Panasonic Lumix DMC-FT30EG-A) fixed above the arena. Afterwards the individual was recovered and placed into its cage. After each trial, the arena was cleaned with 75% alcohol before the next trial, to eliminate urine, fecal pellets and scents that could affect the behaviour of the next animal (Mazzamuto et al., 2018).

Available software facilitates quantification of various aspects of animal behaviour, using commercial automatic tracking systems. Mobility and escape behaviour were assessed using EthoVision XT 14.0 (Noldus et al., 2001) by measuring respectively the total distance (in centimeter) covered by each animal (Henry et al., 2010), and the latency to the first jump (in second), as an attempt by the animal to leave the arena by jumping toward the edge of the walls (Marics et al., 2014). We considered the latency and distance travelled as a metric for boldness degree: as distance travelled increases and latency time decreases, the individual boldness degree increases (Eccard & Herde, 2013). To standardise the length of the videos before the analysis, we manually checked each video, cutting the seconds before the exact moment the cylinder was removed from the arena and considered a total of 570 s thereafter.

Statistical analyses: effect of elevation and habitat variables on the escape behaviour and mobility

First, we tested the correlation between elevation, and habitat heterogeneity, measured through the Shannon's index, in the various species (Spearman's rank correlation: *A. alpicola* = 0.76; *A. flavicollis* = - 0.02; *A. sylvaticus* = -0.57; *C. glareolus* = 0.70). We used Bayesian Generalized Linear Models (GLM) to evaluate the effect of elevation on the escape behaviour and mobility of *A. alpicola* (n = 33), *A. flavicollis* (n = 60), *A. sylvaticus* (n = 43) and *C. glareolus* (n = 106). The first hypothesis (H_1) was tested by including a linear predictor for the effect of elevation, which was found to be correlated, although with different strengths or directions, to the Shannon's diversity index. As the four species have different elevational distribution, we standardised and centered elevation values for each species, then we added the species as a covariate and used an interaction term to disentangle species-specific effects of elevation, as well as to test for species-specific differences (H_3) in boldness related-traits. Finally, we controlled for potential differences in spatial exploration due to sex (Bednarz & Zwolak, 2022).

We fit two separate models, one for the latency time and another one for travelled distances. Based on a preliminary exploration of the data, we used a Gamma distribution with a log link to model the distance travelled by the different individuals. However, due to zero-inflation, as some animals had not moved during the experiment, we used a Hurdle Gamma distribution to model the latency time. Zero-altered models, also known as Hurdle models, differently from zero-inflated models quantify separately the probability that the response is zero, or some other non-zero value (Zuur et al., 2017). Model selection was based on a stepwise backward approach, based on leave-one-out cross validation (Vehtari, 2017). To improve model regularisation, we adopted moderately informative prior distributions for regression slopes, setting them according to a normal distribution with mean equal to 0 and variance equal to 2, as suggested by Lemoine (2019). Parameter estimation was based on Markov Chains Monte Carlo methods with 4 chains, 5,000 iterations and a burn-in of 2,000 iterations. We used the Gelman-Rubin statistics, as well as the graphical inspection of posterior distributions of model parameters, to check for convergence. Finally, we also explored model residuals to detect the non-linearity of covariates and used posterior predictive checks to test for the goodness of fit of our data to the model.

To test H_2 and measure the inter-individual variability of the latency time and the distance travelled we calculated the Coefficients of Variation for each species using the Modified signed-likelihood ratio test (MSLRT) for the equality of CVs' (Krishnamoorthy & Lee, 2014) in R (package "cvequality"; Marwick et al., 2022). Statistical analyses were carried out in R (R Core Team 2022) and STAN (Carpenter et al., 2017), with the package 'brms' (Bürkner et al., 2017).

RESULTS

Behavioural experiments were conducted on 242 individuals (124 females and 118 males): 136 *Apodemus* spp. and 106 to *C. glareolus* (57 females and 49 males). After genotyping *Apodemus* spp.

were determined as: 60 *A. flavicollis* (36 females and 24 males), 43 *A. sylvaticus* (10 females and 33 males) and 33 *A. alpicola* (21 females and 12 males). We found a negative correlation between latency time (before zero-transformation) and mobility (r = - 0.53).

Leave-one-out cross validation did not detect any effect of elevation, neither on the distance travelled, nor on the latency time to the first jump. Moreover, it did not detect any difference in these two quantities, between the two sexes (Table 1).

As for species-specific differences, *C. glareolus* travelled smaller distances (mean ± SE= 2854.6 ± 267.7 cm) than species from the genus *Apodemus: A. flavicollis* = 6258.3 ± 390.9; *A. sylvaticus* = 6321 ± 411.7; *A. alpicola* = 6441.9 ± 423.6 cm (Fig. 2). Moreover, although individuals of *C. glareolus* did not have longer latency times than individuals from the genus *Apodemus,* model selection indicated that the zero-inflation parameter varied between species (Table 1). This was in line with the fact that 46.2% of *C. glareolus* did not jump during the experiment, while only about 11% of *Apodemus* did so (Fig. 3).

Finally, we found a significant difference between the species in the variance of latency to the first jump (MSLRT = 39.50, $p < 0.001$), which was high in *C. glareolus* (CV = 96.56) followed by *A. flavicollis* (CV = 45.4), *A. alpicola* (CV = 37.7) and *A. sylvaticus* (CV = 31.7; Fig. 4). For the distance travelled we did not find any significant difference between the species in the variance of the data (p = 0.35).

DISCUSSION

We compared the inter-specific variation of behavioural traits in sympatric wild rodent species and analysed this variation along a gradient of elevation. “Boldness” (or the corollary “shyness”) is defined as “an individual’s reaction to any risky situation” (Réale et al., 2007). Therefore, in line with other studies conducted on rodents (Stratton et al., 2021; McMahon et al., 2023; Krebs et al., 2019), we expected the behavioural trait “boldness” to affect the escape behaviour, as well as the distance travelled, and the area covered in the novel environment represented by the arena. Distance travelled is a behaviour that can be linked to the shy-bold continuum with individuals traversing longer distances usually considered bolder (Peignier et al., 2022; Huntingford et al., 2012). In this study, the latency to the first jump and the distance travelled were negatively correlated. This allowed us to use the distance travelled to corroborate results from the latency time.

We found evidence that different species can exhibit a specific behavioural type, not rejecting H_3. In this study, *C. glareolus* was predominantly a shy species, as it was more likely that animals did not jump when placed in a novel arena. In support of this finding, a study by Galsworthy (2004) highlighted the freezing behaviour of *C. glareolus* during manipulation and arena tests. The distance travelled by this species was much shorter than in *Apodemus* spp., confirming the less mobility attitude and, therefore, perhaps shyness trait of *C. glareolus*. On the contrary, high level of boldness were previously described in *A. sylvaticus* (Dammhahn et al., 2020). Here we confirmed that high level of boldness can be present in other *Apodemus* mice, such as *A. flavicollis* and *A. alpicola.* Contrary to hypothesis H_4, no significant differences in the level of boldness were found between males and females, in accordance with other studies on differences in exploratory behaviour between the sexes (Hürlimann et al., 2019; Lodewijckx, 1984). The high variance of the escape behaviour (measured as latency to the first jump) in *C. glareolus,* confirmed our hypotheses (H_2) that generalists species adapted to different microhabitat and to use different type of food resources, have a greater plasticity to adjust their behaviours, compared to other species, such as *Apodemus,* which are more specialists on diet (granivorous) and habitat (forests) that tend to perform well in a particular habitat type (Boon et al., 2008; Gray et al., 2000; Sneddon, 2003). In fact, our studied species are typical forest dwellers, but a certain degree of flexibility is possible both at habitat and microhabitat level, especially in mountain areas. Some, like *C. glareolus,* can be found in a wide range of habitats, such as deciduous, mixed and coniferous forests, usually associated to forest edges (Niethammer & Krapp, 1982), but are often also found inside the forest, at places with high canopy cover of old trees and with good cover of high shrubs. This species is also found to be very flexible in adapting its habitat use when resources become scarce (Hille & Mortelliti, 2010). Other species, like the *Apodemus* spp., outperform in more restricted habitat types. Although occasionally found in coniferous forests, *A. flavicollis* is a species related mostly to mature broad-leaved and mixed forests. It is mainly associated with forest edges (Hille & Mortellini, 2010), where it lives in sympathy with *A. sylvaticus* (Spagnesi & De Marinis, 2002), sharing the same ecological niches. At high altitudes, *A. flavicollis* lives in partial syntropy with *A. alpicola* in *Alnus viridis* shrubs and in open habitats characterised by herbaceous patches, rocky debris and low woody vegetation (Debernardi et al., 2003). In mixed subalpine forests *A. flavicollis* is found in sympatry with *C. glareolus*, leading to a differential use of the microhabitats by the two rodents (Hille & Mortelliti, 2010). This segregation is predicted to reduce interspecific competition, especially in favour of *C. glareolus* to avoid the more aggressive and bigger *A. flavicollis*, which can attack and expel it from resources (Andrzejewski & Olszewski, 1963). This differentiation of niches (spatial, trophic and temporal) between the two taxa may have led to differences in behavioural traits (Probst & Probst, 2023).

However, contrary to our prediction formulated in $H_{1,}$ elevation did not affect the latency time, nor the distance travelled in the four species. This finding could be due to the moderate altitudinal gradient considered here, which therefore does not fully represent the harsh environmental conditions of high altitudes. Species-specific behaviours are almost always created and influenced by social interactions and environmental influence. The apparent absence of any environmental influence on the behaviours here considered, could also indicates the presence of a genetic basis that regulates their expression in such species (De Meester, 1996; Haraway et al., 1998).

We are aware that considering only one behavioural trait (degree of boldness) in the analyses is a limitation of the study. Integrate the analysis of behaviours associated with the boldness continuum, such as latency to emerge from the trap (Brehm et al., 2019) and response to manipulation (Brehm & Mortelliti, 2022), together with other behavioural traits, such as individuals’ activity in a non-risky and a non-novel environment (Réale et al., 2007), could give greater support to the hypotheses presented here. A comprehension of the behavioural adaptation could become even more critical considering climate change scenarios where the increasing temperatures, especially at high altitudes, are expected to cause species movement and new species interactions. Integrative studies on the genetic and physiological mechanisms underlying species behaviour, their selective benefits and microhabitat use will help to clarify how rodents have adapted to their environments. This will shed light on the adaptation of mountain species to this peculiar and extreme habitat. In this study we have shown the two opposite strategies adopted by sympatric species of rodents, proposing a possible extension of the behavioural concept, from the individual level to the species level.

Acknowledgments

We thank Gran Paradiso National Park for logistical support, Annalisa Demitri for helping with fieldwork, Xabi Salgado Irazabal for providing technical support for EthoVision Software, and Alfredo Santovito for providing material and support for the laboratory analysis. Financial support and material were provided by the University of Turin and the CBIO.

Figures

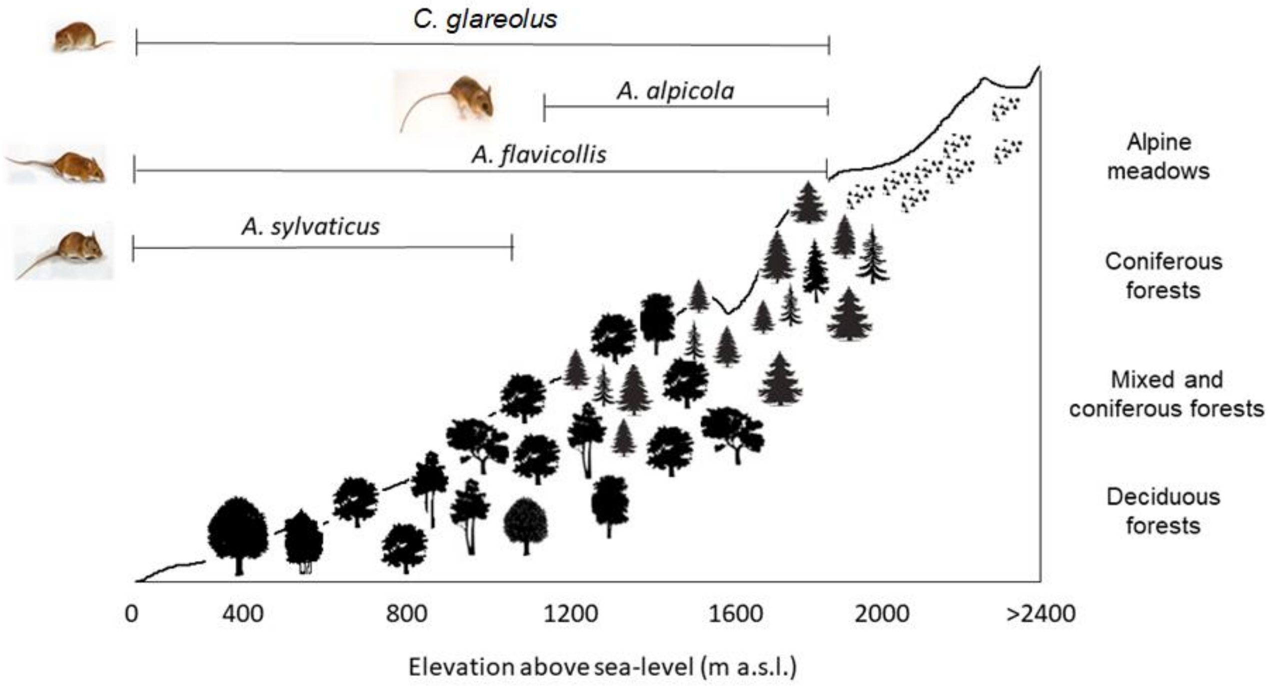


Fig. 1. Distribution of deciduous, mixed and coniferous forests and study species (*Apodemus flavicollis*, *A. sylvaticus*, *A. alpicola* and *Clethrionomys glareolus*) across elevational gradient (400 to 1,822 m. a.s.l.) in Alps. Bars represent the altitudinal distribution where species were captured.

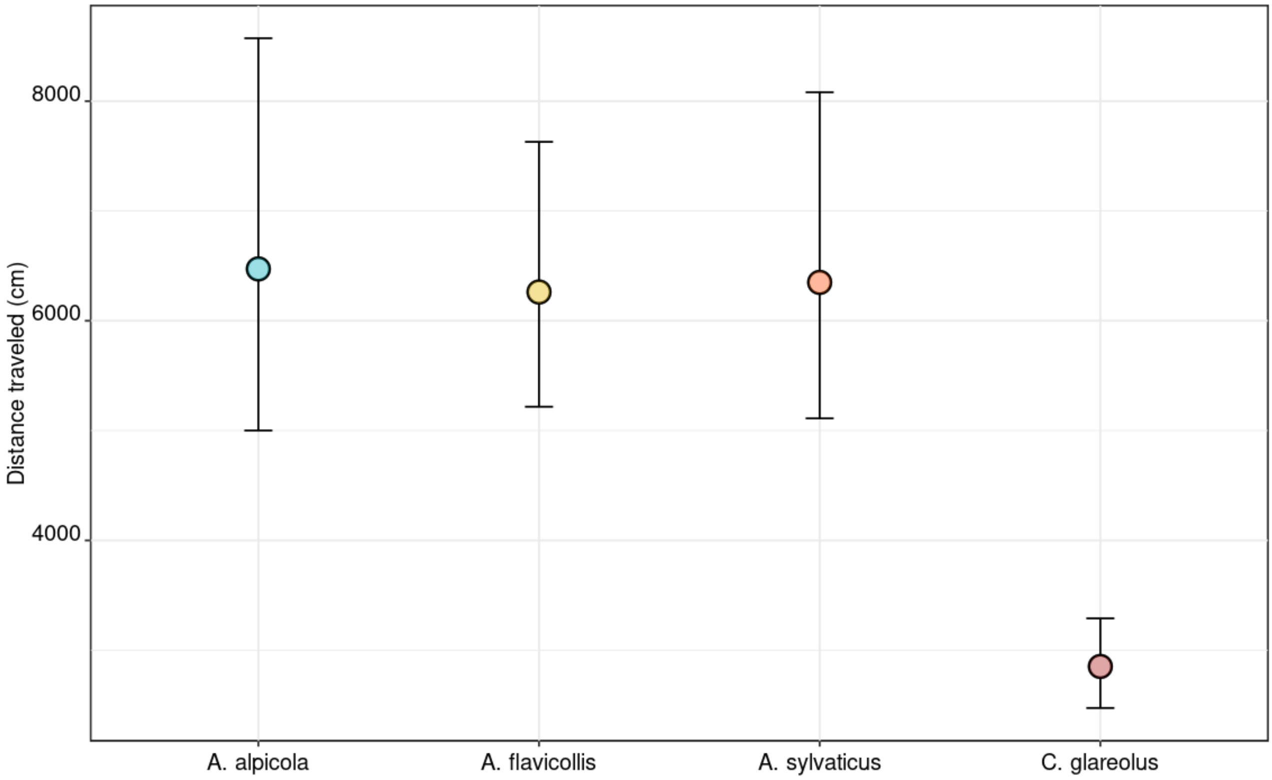


Fig. 2. Conditional effect, showing interspecific differences in the distance travelled by individuals during the arena test.

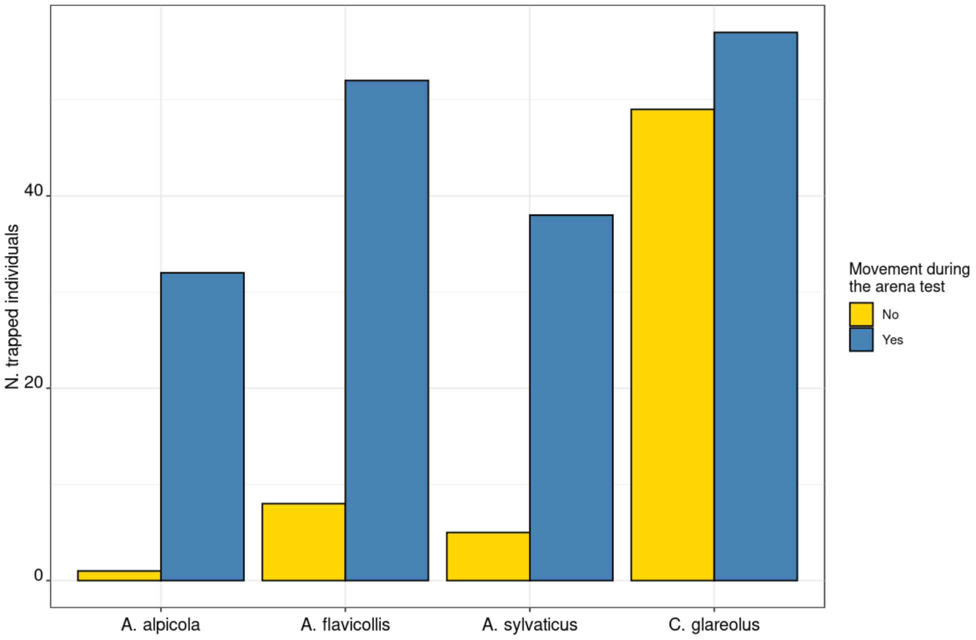


Fig. 3. Number of trapped individuals, that did not move at all, during the arena test, between the four species.

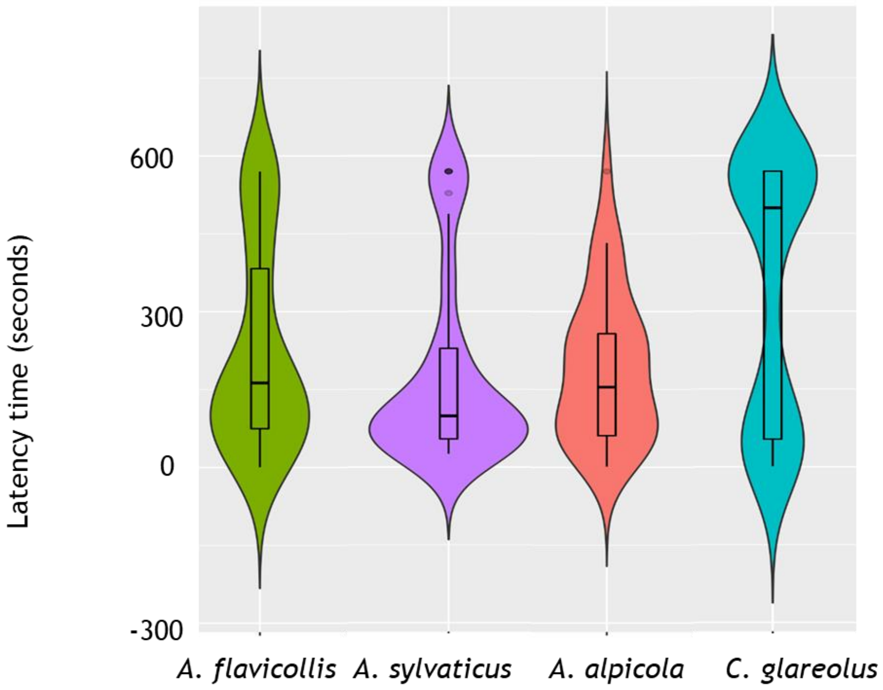


Fig. 4. Boxplot of the variance coefficient of the latency to the first jump in the four species examined.

Table 1. Overview of model selection, for the distance travelled and latency times, through Bayesian leave-one-out cross validation. The table represents model formula and the expected log-pointwise predictive density (ELPD, the closer to zero the better). For latency times, zero values corresponds to odds ratio of the Hurdle gamma, representing the probability that values were zero. As the four species were treated as a factor variable with sum-to-zero contrasts, differences in the hu parameter are not directly interpretable from a table. But the model where zero-inflation varied by species was retained by cross validation. For a graphical interpretation, we encourage you to read Fig. 3, where the number of animals that did not jump was reported for each species. A null model is added for comparison for both measures.

Distance travelled	
Model formula	ELPD ± S.E.
Distance ~ elevation + species + sex + (elevation X species)	2265.0 ± 13.6
Distance ~ elevation + species + (elevation X species)	-2264.3 ± 13.7
Distance ~ elevation + species	-2263.5 ± 12.9
Distance ~ species	-2262.6 ± 12.6
Distance ~ 1	-2287.7 ± 9.7
Latency times	
Model formula	ELPD ± S.E.
Non-zero values ~ elevation + species + sex + (elevation X species) Zero values ~ elevation + species	-1209.7 ± 38.6
Non-zero values ~ elevation + species + sex + (elevation X species) Zero values ~ species	-1209.0 ± 38.7
Non-zero values ~ elevation + species + sex + (elevation X species) Zero values ~ 1	-1226.8 ± 37.0
Non-zero values ~ elevation + species + (elevation X species) Zero values ~ species	-1207.8 ± 38.6
Non-zero values ~ elevation + species Zero values ~ species	-1206.6 ± 38.5
Non-zero values ~ species Zero values ~ species	-1205.9 ± 38.5
Non-zero values ~ species Zero values ~ species	-1204.6 ± 38.3

Reference

Balčiauskiene, L., L. Balčiauskas, and A. Čepukiene. (2009a). Growth of the bank vole *Myodes glareolus* in the non-vegetative period in ne lithuania. Est. J. Ecol. 58:86–93.

Balčiauskienė, L., L. Balčiauskas, and A. Čepukienė. (2009b). Demographic and morphometric parameters of the yellow-necked mouse (*Apodemus flavicollis*) in late autumn-early spring in Lithuania. Acta Biol. Univ. Daugavp 9:25–34.

Bayliss P. & D. Choquenot. 2002. The numerical response: rate of increase and food limitation in herbivores and predators. Phil. Trans. R. Soc. Lond.

Bednarz, P. A., & Zwolak, R. (2022). Body mass and sex, but not breeding condition and season, influence open-field exploration in the yellow-necked mouse. Ecology and evolution, 12(3), e8771. https://doi.org/10.1002/ece3.8771

Benus, R. F., Bohus, B., Koolhaas, J. M., & Van Oortmerssen, G. A. (1991). Heritable variation for aggression as a reflection of individual coping strategies. Cellular and Molecular Life Sciences, 47(10), 1008–1019.

Boon, A.K., Réale, D., Boutin, S. (2008). Personality, habitat use, and their consequences for survival in North American red squirrels *Tamiasciurus hudsonicus.* Oikos, 117: 1321-1328

Brehm, A. M., Mortelliti, A., Maynard, G. A., & Zydlewski, J. (2019). Land-use change and the ecological consequences of personality in small mammals. Ecology Letters, 22, 1387-1395.

Brehm, A. M. & Mortelliti, A. (2022). Small mammal personalities generate context dependence in the seed dispersal mutualism. PNAS, 119: e2113870119

Bubac, C. M., Miller, J. M., & Coltman, D. W. (2020). The genetic basis of animal behavioural diversity in natural populations. *Molecular Ecology*, *29*(11), 1957-1971.

Bürkner, P. C. (2017). brms: An R package for Bayesian multilevel models using Stan. Journal of statistical software, 80, 1-28.

Butet A. & Leroux A.B., 1993: Effect of prey on predator's breeding success. A 7- year study on common vole (Microtus arvalis) and Montagu's harrier (Circus pygargus) in a West France marsh. Acta Ecologica, 14 6): 857-865.

Carere, C., Drent, P. J., Privitera, L., Koolhaas, J. M., & Groothuis, T. G. (2005). Personalities in great tits, Parus major: stability and consistency. Animal Behaviour, 70(4), 795-805.

Carpenter, B., Gelman, A., Hoffman, M. D., Lee, D., Goodrich, B., Betancourt, M., ... & Riddell, A. (2017). Stan: A probabilistic programming language. Journal of statistical software, 76(1).

Dammhahn, M., Mazza, V., Schirmer, A. *et al.* Of city and village mice: behavioural adjustments of striped field mice to urban environments. *Sci Rep* **10,** 13056 (2020).

De Meester, L. 1996. Evolutionary potential and local genetic differentiation in a phenotypically plastic trait of a cyclical parthenogen, *Daphnia magna*. Evolution 50: 1293 – 1298.

Dobson, F. S., & Oli, M. K. (2008). The life histories of orders of mammals: fast and slow breeding. *Current Science*, 862-865.

Eccard, J.A., Herde, A. Seasonal variation in the behaviour of a short-lived rodent. BMC Ecol 13, 43 (2013). https://doi.org/10.1186/1472-6785-13-43

Frost AJ, Winrow-Giffen A, Ashley PJ, Sneddon LU. Plasticity in animal personality traits: does prior experience alter the degree of boldness? Proc Biol Sci. 2007 Feb 7;274(1608):333-9. doi: 10.1098/rspb.2006.3751. PMID: 17164196; PMCID: PMC1702388.

Gould T.D., Dao D.T., Kovacsics C.E. (2009). Mood and anxiety related phenotypes in mice: characterization using behavioral tests. Humana Press

Gray, S.J., Jensen, S.P., Hurst J.L. (2000). Structural complexity of territories: preference, use of space and defence in commensal house mice *Mus domesticus.* Anim. Behav., 60: 765-772

Gurnell and Flowerdew. 2019. The Live trapping Small Mammals: A Practical Guide. Mammal Society

Hager, S. B. (2010) The Diversity of Behavior. Nature Education Knowledge 4(2):66

Hankin R.K.S. (2022). cmvnorm: The Complex Multivariate Gaussian Distribution. R package version 1.0-7. https://CRAN.R-project.org/package=cmvnorm

Haapakoski, M., and H. Ylönen. 2010. Effects of fragmented breeding habitat and resource distribution on behavior and survival of the bank vole (Myodes glareolus). Population ecology 52:427-435

Haraway, Maury M.; Maples, Ernest G. (1998). In Greenberg, Gary; Haraway, Maury M. (eds.). *Comparative Psychology: A Handbook*. Taylor & Francis. pp. 191–7.

Harris, S. E., & Munshi-South, J. (2017). Signatures of positive selection and local adaptation to urbanization in white-footed mice (*Peromyscus leucopus*). Molecular Ecology, *26*(22), 6336-6350.

Henry BL, Minassian A, Young JW, Paulus MP, Geyer MA, Perry W. (2010). Cross-species assessments of motor and exploratory behavior related to bipolar disorder. Neurosci Biobehav Rev.;34:1296-1306. doi:10.1016/j.neubiorev.2010.04.002

Hille, S.M. & Cooper, C.B. (2015) Elevational trends in life histories: revising the pace-of-life framework. Biological Reviews, 90, 204–213.

Hille, S.M. & Mortelliti A. (2010). Microhabitat partitioning of *Apodemus flavicollis* and *Myodes glareolus* in the sub-montane alps: a preliminary assessment. Hystrix It. J. Mamm. 21: 157-163

Huntingford, F.A., Tamilselvan, P., Jenjan, H., 2012. Why do some fish fight more than others? Physiol. Biochem. Zool. 85, 585–593.

Hürlimann ML, Martin JGA & Bize P. 2019. Evidence of phenotypic correlation between exploration activity and resting metabolic rate among populations across an elevation gradient in a small rodent species. Behavioral Ecology and Sociobiology, 73:131

Ioannou, C.C., Payne, M. & Krause, J. 2008. Ecological consequences of the bold–shy continuum: the effect of predator boldness on prey risk. Oecologia 157, 177.

Koolhaas JM, Korte SM, de Boer SF, van der Vegt BJ, van Reenen CG et al., (1999). Coping styles in animals: Current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23: 925–935.

Krebs, R., Linnenbrink, M., Guenther, A. (2019). Validating standardised personality tests under semi-natural conditions in wild house mice (*Mus musculus domesticus*). Ethology. 125: 761–773. https://doi.org/10.1111/eth.12930

Krishnamoorthy, K., Lee, M. (2014). Improved tests for the equality of normal coefficients of variation. Comput Stat 29, 215–232.

Kruschke, J. K., & Liddell, T. M. (2018). The Bayesian New Statistics: Hypothesis testing, estimation, meta-analysis, and power analysis from a Bayesian perspective. Psychonomic bulletin & review, 25(1), 178-206.

Laiolo P, Caprio E, Rolando A (2004) Can forest management have season-dependent effects on bird diversity? Biodivers Conserv 13:1925–1941

Le Cœur, Thibault, M., Pisanu, B., Thibault, S., Chapuis, J-L., Baudry, E. (2015). Temporally fluctuating selection on a personality trait in a wild rodent population, *Behavioral Ecology*, 26: 1285–1291,

Lefebvre, L. Culturally transmitted feeding behaviour in primates: Evidence for accelerating learning rates. Primates 36, 227–239 (1995). https://doi.org/10.1007/BF02381348

Lemoine, N. P. (2019). Moving beyond noninformative priors: why and how to choose weakly informative priors in Bayesian analyses. Oikos, 128(7), 912-928.

Lodewijckx, E. (1984). The influence of sex, sexual condition and age on the exploratory behaviour of wild wood mice (*Apodemus sylvaticus* L.). *Behavioural Processes, 9*(4), 431–444. https://doi.org/10.1016/0376-6357(84)90028-7

Loggins, A. A., Shrader, A. M., Monadjem, A., & McCleery, R. A. (2019). Shrub cover homogenizes small mammals' activity and perceived predation risk. *Scientific reports*, *9*(1), 1-11.

Marics, I., Malapert, P., Reynders, A., Gaillard, S., & Moqrich, A. (2014). Acute heat-evoked temperature sensation is impaired but not abolished in mice lacking TRPV1 and TRPV3 channels. PLoS One, 9(6), e99828.

Mazzamuto M.V., Cremonesi G., Santicchia F., Preatoni D., Martinoli A., Wauters L.A. (2018). Rodents in the arena: a critical evaluation of methods measuring personality traits. Ethology Ecology & Evolution. doi: 10.1080/03949370.2018.1488768

McMahon, E. K., Farhan, S., Cavigelli, S. A. (2023). How do we characterize temperament? Broad testing of temperament across time and contexts in low-variable conditions. Animal Behaviour, 195, 29-42. https://doi.org/10.1016/j.anbehav.2022.10.007

Melcore I., Zbyszek B., Bertolino S. (2022). Variation of rodents' body temperature across elevation in Alps. PBZ. Vol. 96 (6).

Nagy, L., Grabherr, G. (2009). The Biology of Alpine Habitats. Oxford University Press, 390. https://doi.org/https://doi.org/10.1657/1938-4246-42.1.130a.

Noldus L.P., Spink A.J., Tegelenbosch R.A. (2001). EthoVision: a versatile video tracking system for automation of behavioral experiments. Behavior Research Methods Instruments, & Computers. 33 (3):398 – 414

Peignier, M., Araya-Ajoy, Y.G., Bégué, L. *et al.* (2022). Exploring links between personality traits and their social and non-social environments in wild poison frogs. *Behav Ecol Sociobiol* 76, 93. https://doi.org/10.1007/s00265-022-03202-9

Probst R & Probst R. (2023). High frequency of *Apodemus* mice boosts inverse activity pattern of bank voles, *Clethrionomys glareolus,* through non-aggressive intraguild competition. Animals 13: 981. https://doi.org/10.3390/ani13060981

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev 82(2):291–318

Réale, D., & Dingemanse, N. J. (2012). Animal personality. Els.

Ruperto, E. F., Menéndez, J., Taraborelli, P. A., Scattolón, F. O., & Sassi, P. L. (2023). Behavioral responses of two small-sized rodents, Phyllotis vaccarum and Abrothrix andina, to energy challenges of high-altitude habitats in the Andes Mountains. *Ethology*, *129*(3), 117-132.

Santicchia, F., Gagnaison, C., Bisi, F., Martinoli, A., Matthysen, E., Bertolino, S., & Wauters, L. A. (2018). Habitat-dependent effects of personality on survival and reproduction in red squirrels. *Behavioral Ecology and Sociobiology*, *72*(8), 1-13.

Šíchová K, Koskela E, Mappes T, Lantová P, Boratyński Z. (2014). On personality, energy metabolism, and mtDNA introgression in bank voles, *Myodes glareolus*. Anim Beh, 92:229-237

Sih, A., Bell, A., Johnson, J.C. (2004). Behavioral syndromes: an ecological and evolutionary overview, Trends in Ecology & Evolution. 19: 372-378,

Simões-Lopes, Paulo C., Daura-Jorge, Fábio G. and Cantor, Maurício. 2016. Clues of cultural transmission in cooperative foraging between artisanal fishermen and bottlenose dolphins, Tursiops truncatus (Cetacea: Delphinidae). Zoologia (Curitiba). v. 33, n. 06

Smolka, J., Baird, E., Byrne, M. J., El Jundi, B., Warrant, E. J., & Dacke, M. (2012). Dung beetles use their dung ball as a mobile thermal refuge. Current Biology, 22(20), R863–R864

Sneddon, L. U. (2003). The bold and the shy: individual differences in rainbow trout. Journal of Fish Biology, 62(4), 971-975.

Stenseth, N. C. 1985. Geographic distribution of Clethrionomys species. Annales Zoologici Fennici 22:215-219.

Stratton, J.A., Nolte, M.J. & Payseur, B.A. (2021). Evolution of boldness and exploratory behavior in giant mice from Gough Island. Behav Ecol Sociobiol 75, 65. https://doi.org/10.1007/s00265-021-03003-6

Terrien, J., Perret, M., & Aujard, F. (2011). Behavioral thermoregulation in mammals: a review. *Frontiers in Bioscience-Landmark*, *16*(4), 1428-1444.

Titterington, F. M., Knox, R., Morrison, S. J., & Shirali, M. (2022). Behavioural Traits in Bos taurus Cattle, Their Heritability, Potential Genetic Markers, and Associations with Production Traits. *Animals*, *12*(19), 2602.

van Oers, K., Drent, P., de Jong, G. *et al.* Additive and nonadditive genetic variation in avian personality traits. *Heredity* **93**, 496–503 (2004). https://doi.org/10.1038/sj.hdy.6800530

Vehtari, A., Gelman, A., & Gabry, J. (2017). Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. Statistics and computing, 27(5), 1413-1432.

Verdolin, J. L. (2006). Meta-analysis of foraging and predation risk trade-offs in terrestrial systems. *Behav Ecol Sociobiol* **60**, 457–464

Wilson, D. S., Coleman, K., Clark, A. B., & Biederman, L. (1993). Shy-bold continuum in pumpkinseed sunfish (Lepomis gibbosus): An ecological study of a psychological trait. Journal of comparative psychology, 107(3), 250.

Wong B.B.M. & U. Candolin. 2014. Behavioural responses to changing environments. Behavioral Ecology (2015), 26(3), 665–673. doi:10.1093/beheco/aru183

Wootton, J. T. (1987). The effects of body mass, phylogeny, habitat, and trophic level on mammalian age at first reproduction. *Evolution*, *41*(4), 732-749.

Zidar J., Balogh A., Favati A., Jensen P., Leimar O., Løvlie H. 2017. A comparison of animal personality and coping styles in the red Junglefowl. Animal Behaviour 130 (2017) 209e220

Zuur, A. F., Ieno, E. N., & Saveliev, A. A. (2017). Spatial, temporal and spatial-temporal ecological data analysis with R-INLA. Volume II. GAM and Zero-Inflated Models. Highland Statistics Lt.

APPENDIX

Table A1. Coordinates (longitude; latitude) of the twelve trapping location, the weather stations and the laboratories:

	Trapping locations		Weather stations		Laboratories	
1	7.62805	45.40565	7.57330	45.55680	7.52220	45.49770
2	7.61568	45.40899	7.48720	45.51350	7.31445	45.45137
3	7.59606	45.42822	7.61110	45.42000		
4	7.43559	45.42309	7.54100	45.40890		
5	7.54968	45.50748	7.43820	45.42670		
6	7.31445	45.45137	7.41150	45.43840		
7	7.39580	45.45295	7.31140	45.45280		
8	7.48709	45.51901	7.20910	45.44110		
9	7.47173	45.52610				
10	7.51484	45.55321				
11	7.21039	45.43669				
12	7.56948	45.55651				

APPENDIX A1. Vegetation plot

We collected vegetation data in seven plots located within the trapping line: first plot was placed with the first trap in the centre, the following plots placed every 5 traps. Each plot was represented by a 5x5 m squares with a live-trap located in the centre. All the trees/bush species presented within the plot have been detected and cover of each species was estimated visually. We assigned to each species one class of coverage out of five (<5%; 5-25%, 25.1-50%, 50.1-75%, 75.1-100%). We calculated the midpoint of each assigned coverage class (Wikum & Shanholtzer, 1978), and we sum them to obtain the vegetation coverage of each trapping location. We calculated plant diversity using the Shannon diversity index (Rad et al., 2009). In order to calculate the proportional abundance needed for this index, we assigned a score to each coverage class (1: <5%; 2: 5-25%; 3: 25.1-50%; 4: 50.1-75%; 5: 75.1-100%) and divided each score by the sum of the scores of all the species at each site.

APPENDIX A2. Arena structure

For the behavioural experiments, the individuals were transported to a laboratory and placed individually in special cages for rodents. The laboratory was protected from any source of light and from any other type of external disturbance. To carry out the behaviour experiments, each individual was placed inside an arena. The arena was composed by a wood box (100×100×100 cm), wholly covered with a thick white plastic sheet (Gould et al., 2009). Open field trials were recorded using a video camera (Panasonic Lumix DMC-FT30EG-A) fixed on a tripod, and a set of 4 red light lamps to illuminate the arena without affecting the animal's behaviour.

CHAPTER V

Variation of rodents' body temperature across elevation in Alps

Ilaria Melcore[1], Sandro Bertolino[1], Zbyszek Boratyński[2]

[1] Department of Life Sciences and Systems Biology, University of Turin, Via Accademia Albertina 13, 10123 Turin, Italy

[2] Association BIOPOLIS, CIBIO-InBIO Associate Laboratory, Research Center in Biodiversity and Genetic Resources, University of Porto, Porto, Portugal

Running title: Altitude thermal adaptation in mammals

Published in: Physiological and Biochemical Zoology, Volume 95, Issue 6

ABSTRACT

Understanding the physiological adaptations of animals living in mountain landscapes is a key to predicting the responses of individuals to environmental changes, especially those imposed by climate. In this regard, the mechanism of body temperature regulation is particularly important in determining the physiological limits for activity under hypoxic and climatic stressful conditions. Numerous studies have shown that body temperature of rodents and other animals decreases with elevation and with extreme winter temperatures. But surprisingly, it is still not known how body temperature of wild animals varies along elevation during their reproductive seasons. We studied variation in core body temperature among individuals (N = 294) of four rodent species (*Apodemus flavicollis, Apodemus sylvaticus, Apodemus alpicola, Myodes glareolus*) and its association with elevation (from 400 to 1,822 m asl), accounting for variation in environment temperature, microhabitat structure, body condition, and sex. Results showed a strong positive effect of elevation and a more limited effect of environmental temperature on rodents' body temperature during spring and summer. The effect of elevation on body temperature was influenced neither by interactions with environmental temperature or sex nor by the microhabitat structure. We suggest that in normothermic condition at high elevations, small mammals experiencing hypoxic exposure might show physiological adjustments for increasing efficiency of thermoregulation in low oxygen conditions.

Keywords: adaptation, altitude, hypoxia, heterotermy, normothermia, rodents

INTRODUCTION

Regulation of body temperature (T_b) is a key aspect of endothermic physiology (Hankenson et al. 2018), where animals maintain high and relatively stable core temperature optimizing cellular physiological processes (Chappell et al. 2007). Individual temperature varies between and within species, and this variation can reflect adaptive and plastic responses to environmental heterogeneity (Heinrich 1977; Geiser 2004; Angilletta et al. 2010). Over the past years, evidence has accumulated describing variable physiological mechanisms across elevational gradients (Careau 2012; Cheviron et al. 2012, 2013; Dzal 2018). High altitudes are characterized by decreased ambient temperatures and oxygen partial pressure (Storz et al. 2010), challenging animals' T_b regulation (Tattersall and Milsom 2009). Animals of relatively small bodies (< 100 g), such as many mammals and birds, are under increased pressure because of their high surface-to-volume ratio, resulting in high relative energetic demands to support temperature regulation (Robertson et al. 2019).

In mountains, air temperature decreases by approximately 0.55 C°–0.65 C° every 100 m of altitude increase (Anslow and Shawn 2002). Animals adapted to high altitudes and exposed to cold environments may apply physiological adjustments to increase heat production, or thermogenesis (e.g., by increased vascularization of brown adipose tissue and upregulation of mitochondrial thermogenic respiration; Shabalina et al. 2013). Maintaining high T_b by endothermic mechanisms under low air temperatures is physiologically costly and depends on the degree and duration of cold exposure and habitat productivity (Bastos et al. 2021). Hypoxic conditions can further limit T_b regulation, as the exposure to decreased partial pressure of oxygen can limit aerobic physiology (Chappell and Snyder 1984; Cadena and Tattersall 2014). T_b also has a clear sex-dependent pattern in response to environmental constraints. For instance, post pubertal female mice can have 0.2 C° – 0.5 C° higher temperatures than male mice (Marques et al. 2017). However, it is not clear how sexual differences in T_b can interact with plastic and adaptive responses to other factors, such as elevation (Marques et al. 2017).

Endothermic thermoregulation physiology demands a high amount of energy to maintain a stable and high T_b (Wooden and Walsberg 2002). Vegetation provides important food resources and habitat for rodents, and T_b regulation may be affected by fluctuation in availability of food resources (Bastos et al. 2021). Fluctuating and harsh conditions at high altitudes result in lower plant diversity and coverage (Nor 2001) and reduced seed production (Hodkinson 2008). Decreased food diversity, abundance, and predictability and shelter availability expose rodents to nutrients restriction and variable air temperatures (Nagy and Grabherr 2009; Camacho et al. 2019). In response to these environmental challenges, animals may adopt an effective energy-saving strategy, such as daily torpor, which can reduce energy expenditures (Ruf and Geiser 2015). Despite the many factors that may affect T_b in animals living across altitudes, surprisingly few studies have collected data at the individual level and tested how environmental and individual predictors affect variation in T_b in the wild (but see, e.g., Boratyński et al. 2021).

Here, we studied variation in T_b in four rodent species across a moderate altitudinal gradient in the Alps during their reproductive season. In high altitudes, individuals are exposed to decreasing air temperature and oxygen partial pressure. If air temperature influences rodents' T_b, it can be predicted that individuals inhabiting higher altitudes would have higher T_b (i.e., as a result of increased heat production). On the other hand, when air temperature becomes too low, animals from high altitudes could evolve low T_b to decrease the body-environment temperature gradient and costs of homeothermic thermoregulation. We also predicted that a sex-dependent pattern of T_b and habitat variation, such as slope exposure and plant diversity, richness, and coverage, could further modulate the elevation effects.

MATERIAL AND METHODS

Study Area

The study area was located in Piedmont region (north-western Italy), within the territory of the Gran Paradiso National Park, in the Graian Alps (fig. 1, top). We identified 12 trapping locations distanced at least 5 km from each other, spanned across a moderate elevational gradient, from 400 to 1,822 m asl (fig. 1, bottom). Deciduous forests, at low elevations (400–1,000m), were composed of European beech (*Fagus sylvatica*), followed by sycamore maple (*Acer pseudoplatanus*), large-leaved linden (*Tilia platyphyllos*), European ash (*Fraxinus excelsior*), *Populus* spp., *Betula* spp., and chestnut (*Castanea sativa*). Mixed forests, at mid elevations (1,000–1,800 m), were composed of species belonging to deciduous and coniferous trees. Coniferous forests, at high elevations (1,800–2,000 m), were dominated by larch (*Larix decidua*), often mixed with European spruce (*Picea abies*)

Trapping and Handling of Animals

Trapping was conducted during the breeding season of the target species, from April 15 to September 15, 2020. Days of trapping at high- and low-altitude sites were randomly alternated, and the order of trapping was recorded. Each trapping location consisted of a line of 30 live traps (Sherman traps, 229 mm x 89 mm x 76 mm in size) located every 10 m. Traps were set at sunset and inspected at sunrise. Each trapping session lasted 1 wk. Traps were baited with apple, sunflower seeds, and peanut butter, and cotton was provided as bedding. Trapped animals were marked with fur clipping and weighed. Adult individuals of the trapped species (based on fur characteristics and body dimensions; e.g., Gurnell and Flowerdew 2019 — yellow-necked mouse (*Apodemus flavicollis*), wood mouse (*Apodemus sylvaticus*), alpine field mouse (*Apodemus alpicola*), and bank vole (*Myodes glareolus*) — with a minimum body weight of 15 g for *Apodemus* spp. and 13 g for *M. glareolus* were transported to the laboratory (fig. 1, top), kept in individual cages, and provided with water.

Rectal T_b was measured on postabsorptive animals acclimatized to laboratory conditions for approximately 14 h. T_b was measured during the active phase of the species' daily cycle, around 10:00 p.m. on the same day of capture. A rectal thermocouple (RET-3; accuracy: ± 0.1 C°) connected to the thermometer (model PST095) was inserted 2 cm into the animal's rectum for

10 s. Animals with $T_b < 35$ °C (eight individuals probably in hypothermic condition) or $T_b > 41$ °C (one individual who may have suffered from fever) were excluded from analyses. Animal's sex was recorded, along with body weight (Pesola spring balance; ± 1 g) and head width and head, ear, foot, body, and tail lengths measured with calipers (Mitutoyo 530–119 calipers; ± 0.02 mm). Tissue ear samples were collected and used in genetic bar coding of the *Apodemus* species. Animals were kept for no more than 24 h and then released to the location of capture. All experimental procedures were approved by the national bioethical committee (Ministry for Health, 618/2020-PR; Institute for Environmental Protection and Research, protocol 13486 del 23/3/2020), and trapping and handling of animals were authorized by the Gran Paradiso National Park (0003037/2020 of 18/08/2020) and the Province of Turin (618/2020-PR).

Environmental Data

Daily minimum air temperature was selected as the probable driving variable for T_b adaptation in nocturnal animals to estimate thermal stress. We obtained these data from a meteorological database available online (ARPA Piedmont; http://www.arpa.piemonte.it). Eight of 12 weather stations were located within 2 km of and at the same altitude as the trapping sites. The remaining four weather stations were located within 5 km of and at the same altitude as the trapping sites (fig. 1, top). Mean values of daily minimum air temperature (TEmean) for 30 d before trappings were used in the analysis.

The number of shrub and tree species was included in the analyses to account for variation in plant richness. The percentage of shrub and tree species coverage was calculated to account for variation in plant diversity and vegetation coverage. We collected vegetation data in seven plots located within the trapping line: the first plot was centered around the first trap, and the following plots were placed every five traps. Each plot was constituted by a 5 x 5-m square with a live trap located in the center. The tree/bush species present in the plot were identified, and cover of each species was estimated visually.

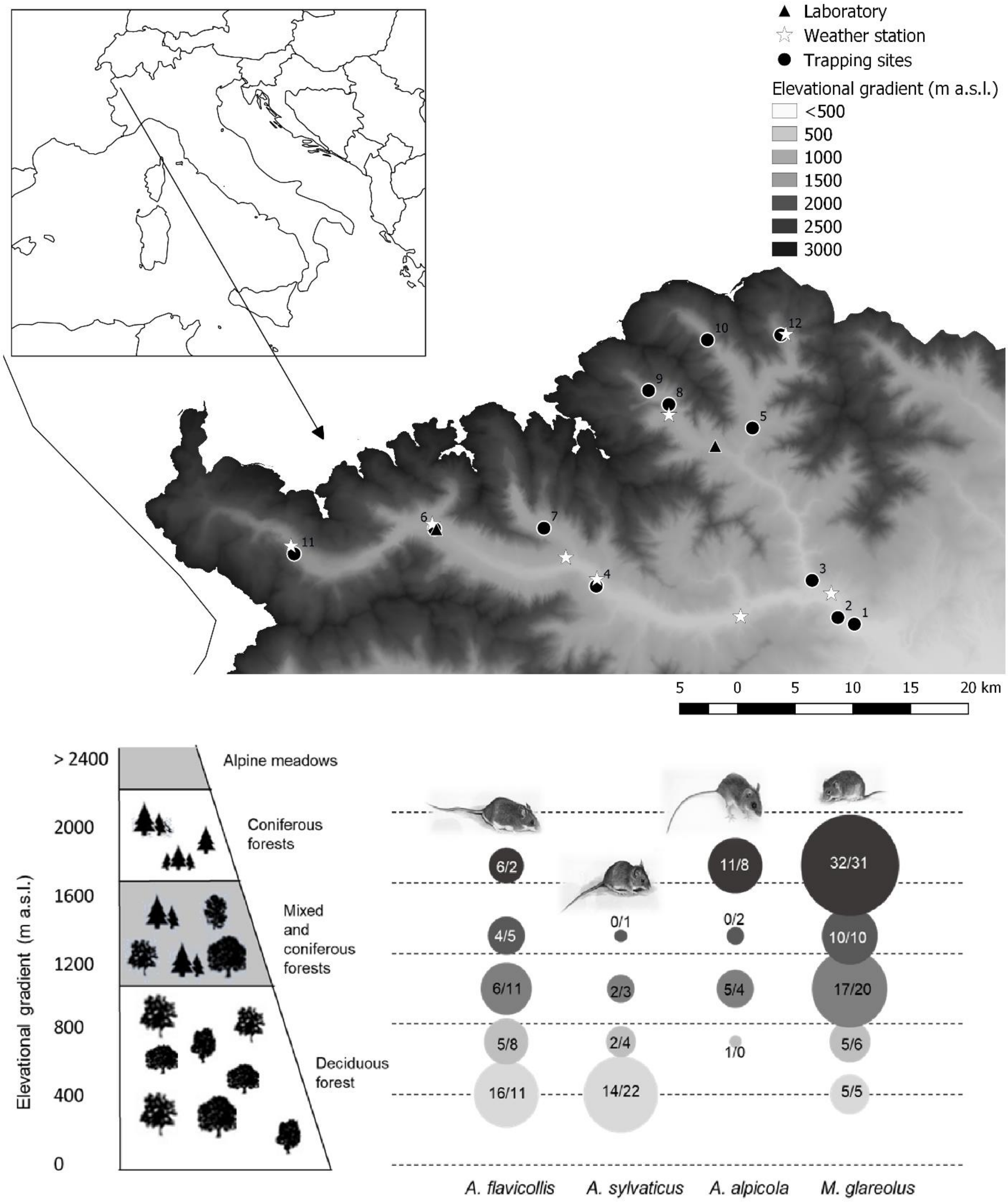


Figure 1. Top, location of the Gran Paradiso National Park (45° 31′ 7.02″ N, 7° 16′ 2.22″ E) and the 12 trapping sites, weather stations, and laboratories (table A1). Bottom, habitats (deciduous, mixed, and coniferous forests) and species (*Apodemus flavicollis, Apodemus sylvaticus, Apodemus alpicola*, and *Myodes glareolus*) studied across an elevational gradient (400–1,822 m asl) and numbers of captured female/male rodents.

We assigned to each species one class of coverage out of five (<5%, 5%–25%, 25.1%–50%, 50.1%–75%, 75.1%–100%). We then calculated the midpoint of each assigned coverage class (Wikum and Shanholtzer 1978) and summed them to obtain the vegetation coverage of each trapping location. We calculated plant diversity using the Shannon diversity index (Rad et al. 2009). To calculate the proportional abundance needed for this index, we assigned a score to each coverage class (1 for <5%, 2 for 5%–25%, 3 for 25.1%–50%, 4 for 50.1%–75%, 5 for 75.1%–100%) and divided each score by the sum of the scores of all of the species at each site. Within each plot, we registered elevation (GPS Garmin eTrex 32x) and geographic exposure of the trapping site (values from 0° as north and deviation from that exposure moving clockwise and counter clockwise to 180° as maximum value as south).

Statistical Analyses

All continuous variables (T_b, elevation, TEmean, plant richness, plant diversity, vegetation coverage, and exposure) were log10 transformed before analyses to improve normality of distributions of their residuals. To assess how T_b (response variable) varied as a function of elevation, minimum environment temperature, plant richness and diversity, vegetation coverage, exposure, and sex (predictors), we applied generalized linear mixed models accounting for variation among trapping locations (random factor), body size (head width), and condition (continuous predictors), using the package glmmADMB (Bolker et al. 2012; R Core Team 2019).

Body condition was calculated as residual variation from body mass regressed against head width (e.g., Boratyński et al. 2010). We evaluated T_b variation across all rodent species and tested differences among species (fixed factor). We account for two seasons (spring and summer) included in this study as fixed factors. To evaluate differential responses to elevation among females and males, a two-way interaction between elevation and sex was tested. To assess whether air temperature influences response to elevation, a two-way interaction between elevation and minimum air temperature was tested. Nonsignificant interactions were excluded from the analysis by applying a stepwise backward reduction approach. Lack of multicollinearity among predictors was confirmed with variance inflation factors (< 2.5). The best model was selected according to the lowest Akaike information criterion (Harrison et al. 2018). To test whether movement of the animals to the laboratory could have affected our results, we ran an additional analysis including the absolute difference in elevation between the laboratory and the trapping site. Such effect was insignificant (absolute elevation difference: β (SE) = -0.0129 (± 0.0115), z = -1.13, p = 0.26); therefore, it was excluded from the final analysis. To investigate consistency of detected results, separate analyses for each species were also performed.

RESULTS

T_b was measured in 294 individuals (141 females and 153 males), out of which 153 belong to the genus *Apodemus* and 141 are *Myodes glareolus* (69 females and 72 males). After genotyping, *Apodemus* spp. were determined to be 74 *A. flavicollis* (37 females and 37 males), 48 *A. sylvaticus* (18 females and 30 males), and 31 *A. alpicola* (17 females and 14 males; fig. 1; see also fig. A1 and table A2 [fig. A1 and tables A1–A4 are available online]). Across a gradient of elevation (mean: 1,084 m asl; first quartile: 723 m asl; third quartile: 1,564 m asl), we recorded TEmean of 10.4 °C, with a difference of 4.6 °C between mean TEmean at the highest (7.7 °C) and lowest (12.3 °C) elevations. Elevation was negatively correlated with TEmean (Pearson product moment correlation: r = -0.16, p = 0.003) and positively correlated with vegetation coverage (r = 0.44, p < 0.001) and plant diversity (r = 0.19, p < 0.001), while there was no correlation with plant richness (r = 0.05, p = 0.29).

We found that rodents' T_b increased with increasing elevation (β ± SE = 0.023 ± 0.007, z = 3.53, p < 0.001; table 1; fig. 2). We also found higher T_b in females than in males (β = 0.004 ± 0.001, z = 3.85, p < 0.001; fig. 2). The interactions between elevation and TEmean (β = -0.064 ± 0.037, z = -1.75, p = 0.08) and between elevation and sex (β = -0.004 ± 0.005, z = -0.77, p = 0.44) did not affect animals' T_b. We found a positive effect of minimum environmental temperature on T_b (β =

0.027 ± 0.006, z = 5.93, p < 0.001) but only in the analysis including 10 rodents captured in April, when TEmean's were very low (tables 1, A3).

The positive covariation between T_b and elevation was detected in separate analyses for *A. flavicollis* (β = 0.020 ± 0.006, z = 3.08, p = 0.003) and *M. glareolus* (β = 0.024 ± 0.008, z = 2.75, p = 0.006), but not for *A. alpicola* (β = - 0.035 ± 0.107, z = - 0.33, p = 0.74), and the effect was negative for *A. sylvaticus* (β = - 0.071 ± 0.025, z = - 2.85, p = 0.007; fig. 3; table A4).

Table 1. Generalized mixed analysis to quantify effect of elevation (and other predictors) on rodents' body temperature.

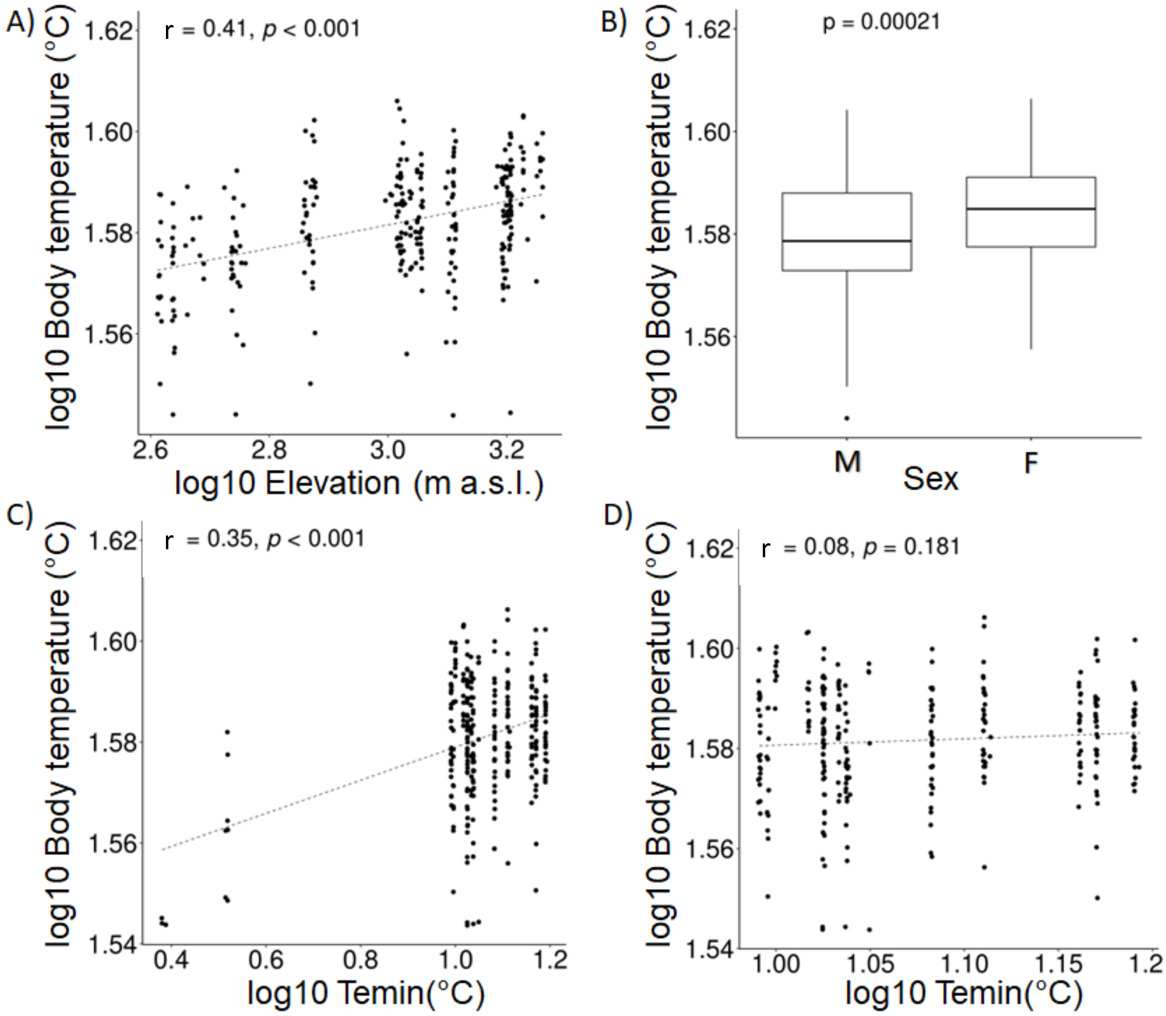
	β (SE)	*z*	*p*
A. flavicollis	0.003 (0.002)	1.23	0.22
M. glareolus	0.001 (0.002)	0.38	0.70
A. sylvaticus	0.0003 (0.002)	0.11	0.91
Seasonality (1, 2)	0.001 (0.003)	0.22	0.82
Elevation	0.023 (0.007)	3.53	< **0.001**
T_{Emean}	0.009 (0.015)	0.59	0.55
Sex (0,1)	0.004 (0.001)	3.85	< **0.001**
Richness	0.003 (0.011)	0.27	0.78
Vegetation cover	-0.002 (0.011)	-0.24	0.80
Plant diversity	-0.001 (0.003)	-0.48	0.63
Exposure	-0.001 (0.003)	-0.27	0.78
BC	-0.007 (0.006)	-1.10	0.27
Head width	0.002 (0.019)	0.13	0.90

NOTE. The model included log10-transformed variables (elevation; mean daily minimum air temperature, TEmean; plant richness; plant diversity; vegetation coverage; exposure; head width) and body condition (BC) as continuous variables, and seasonality (1 for spring and 2 for summer), sex (1 for female, 0 for male) and species (A. flavicollis, M.

glareolus, A. sylvaticus and A. alpicola) as categorical factors. Area was set as random effect: Variance = 2.4e-06, SD = 0.001549. R^2 = 0.2278; Adjusted R^2 = 0.2035.

Figure 2. Variation in rodent body temperature as predicted by (A) elevation, (B) sex and daily minimum air

co



W

0.0

± (

T_b

wi

z =

Nc

A4

DI

To

mc

W

inhabiting the entire elevation gradient. As expected, T_b in females was higher than in males, but this sex difference did not influence the elevation gradient. Air temperature influenced T_b only in one species, and habitat structure, as defined by plant richness and diversity and vegetation coverage, did not substantially affect T_b variation.

Rodents' T_b increased in our study with elevation, on average by 0.2 °C in mice and 0.3°C in voles every 200 m. In mountain habitats, especially during cold seasons, animals inhabiting high elevation are exposed to harsh environmental conditions. Low ambient temperatures and hypoxia (Storz et al. 2010) together can affect T_b regulation (Tattersall and Milsom 2009). For example, captive populations of deer mice (*Peromyscus maniculatus*) originating from highlands expressed higher oxygen consumption and T_b compared with captive populations from lowlands (Ivy and Scott 2017).

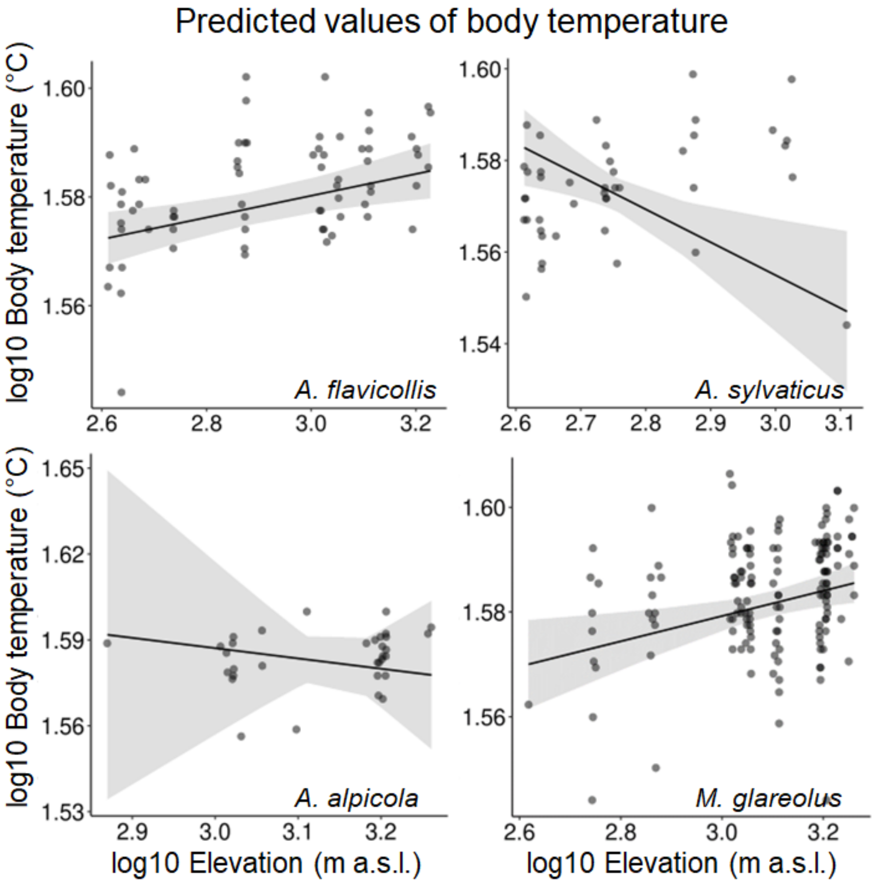


Figure 3. Predicted values of body temperature from regression models plotted against elevation, for *A. flavicollis, A. sylvaticus, A. alpicola* and *M. glareolus.*

In general, our results are consistent with these findings and also showed that wild rodents captured at higher elevations in the Alps maintained higher T_b during warm seasons compared with animals captured at lower elevations (figs. 1, 2). These results were consistent when considering species distributed across the complete elevation gradient studied here (*Apodemus flavicollis* and *Myodes glareolus*; fig. 3). The results were not significant in high-altitude species (*Apodemus alpicola*). In contrast, in species with lowland distribution (*Apodemus sylvaticus*), with very low densities at high altitudes (fig. 1), T_b was positively influenced by air temperature, and it was lower at higher elevations (fig. 3; table A4). The mechanisms behind these detected patterns are not clear. It may be hypothesised that plastic and adaptive responses in ventilatory performance (Ivy and Scott 2017) and a-globin affinity for oxygen (Chappell and Snyder 1984; Sears et al. 2009), imperative for aerobic performance, are involved in differential responses among species. The cost for thermoregulation in homeothermic endotherms is high and increases with decreasing ambient temperature (Levy et al. 2011). Consequently, in species adapted to warm conditions, it would be energy efficient to reduce the body-environment temperature gradient (Gordon 2017). In our study, highland species did not reflect the T_b gradient according to air temperature, suggesting that they may be well adapted to low temperatures. However, we must be careful with this interpretation, as it could be influenced by the relatively weak environmental gradient during our study seasons, particularly for the highland species. We found that, on average, lowlands (12.3 °C at 400 m) were only 4.6 °C warmer than highlands (7.7 °C at 1,800 m). This difference in temperature along elevationis lower than expected (7.7 °C–9.1 °C for 1,400m of altitudinal range; Anslow and Shawn 2002) and may have made it difficult to detect the thermal environmental effects experienced by those species. Despite the weak air temperature gradient, the effect of air temperature on the T_b of the lowland species was evident, suggesting distinct physiological adaptation in *A. sylvaticus* compared with other *Apodemus* mice. Assessing T_b fluctuation in wild performing animals will provide detailed information about daily regulatory mechanisms and confirm patterns detected here on animals transferred to the common laboratory condition for measurements.

Our results showed that rodents from higher elevations expressed higher T_b than those from lower elevations. For most of the studied species, the effect was independent from variation in air temperature, habitat productivity, and body size or condition, suggesting that there are other mechanisms behind the detected pattern. It could be hypothesised that such a mechanism is related to mild tissue hypoxia and greater ventilation of individuals from higher elevations (Chappell and Snyder 1984). If true, it would suggest that even weak but chronic hypoxia can have a fitness effect in small endotherms, perhaps influencing their aerobic capacity performance. A comprehension of the thermoregulation mechanisms of studied species could become even more critical considering climate change scenarios where the warmer temperatures in high altitudes are expected to become more frequent (Sears et al. 2009). Integrative studies on thermoregulation (i.e., behavioural adaptations, physiological mechanisms, and their selective benefits) and microhabitat use will help clarify how rodents adapted to extreme highland environments and shed light on the possibility for them to adapt to increasing air temperatures.

Acknowledgments

We thank Gran Paradiso National Park for logistical support, Annalisa Demitri and Noemi Gargano for help with fieldwork, and Alfredo Santovito for providing material and support for the laboratory analysis. Financial support and material were provided by the University of Turin and the CBIO. We thank the anonymous reviewers whose comments/suggestions helped improve the manuscript.

Reference

ARPA Piedmont, Regional Agency for the Protection of the Environment of Piedmont. 2021. Daily meteo-hydrographic data [Data file]. Retrieved from: www.arpa.piemonte.it.

Angilletta M.J.Jr., B.S. Cooper, M.S. Schuler, and J.G. Boyles. 2010. The evolution of thermal physiology in endotherms. Front Biosci 2:861-81.

Anslow F.S. and M.J. Shawn. 2002. An investigation of local alpine terrestrial lapse rates in the Canadian Rockies. pp. 1. Proceedings of the 32nd Annual Arctic Workshop, INSTAAR, University of Colorado, Boulder, 1.

Bastos B., N. Pradhan, P. Tarroso, J.C. Brito, and Z. Boratyński. 2021. Environmental determinants of minimum body temperature in mammals. J Vertebr Biol 70:1-12.

Bolker B., H. Skaug, A. Magnusson, and A. Nielsen. 2012. Getting started with the glmmADMB package.

Boratyński Z., T.A. Mousseau, and A.P. Møller. 2021*a*. Individual quality and phenology mediate the effect of radioactive contamination on body temperature in Chernobyl barn swallows. Ecol Evol 11:9039–9048.

Boratyński Z., E. Koskela, T. Mappes, and A. Oksanen. 2010*b*. Sex-specific selection on energy metabolism – selection coefficients for winter survival. J Evol Biol 23:1969-1978.

Cadena V. and G.J. Tattersall. 2014. Body temperature regulation during acclimation to cold and hypoxia in rats. J Therm Biol 46:56-64.

Camacho L.F., P. Chávez, and D.G. Tirira. 2019. Elevation and wind exposure shape the habitat preferences of the Andean cottontail *Sylvilagus andinus* (Lagomorpha: Leporidae). Mamm Biol 94:1-3.

Careau V. and T.Jr. Garland. 2012. Performance, personality, and energetics: correlation, causation, and mechanism. Physiol Biochem Zool 85:543–571.

Chappell M.A., K.A. Hammond, R.A. Cardullo, G.A. Russell, E.L. Rezende and C. Miller. 2007*a*. Deer mouse aerobic performance across altitudes: effects of developmental history and temperature acclimation. Physiol Biochem Zool 80:652–662.

Chappell M.A., and L.R.G. Snyder. 1984*b*. Biochemical and physiological correlates of deer mouse alpha-chain hemoglobin polymorphisms. Proc Natl Acad Sci USA 81:5484–5488.

Cheviron Z.A., G.C. Bachman, and J.F. Storz. 2013*a*. Contributions of phenotypic plasticity to differences in thermogenic performance between highland and lowland deer mice. J Exp Biol 216:1160-1166.

Cheviron Z.A., G.C. Bachman, A.D. Connaty, G.B. McClelland, and J.F. Storz. 2012*b*. Regulatory changes contribute to the adaptive enhancement of thermogenic capacity in high-altitude deer mice. Proc Natl Acad Sci USA 109:8635-8640.

Dzal Y.A. and W.K. Milsom. 2018. Hypoxia alters the thermogenic response to cold in adult homeothermic and heterothermic rodents. J Physiol 597:4809-4829.

Geiser F. 2004. Metabolic rate and body temperature reduction during hibernation and daily torpor. Annu Rev Physiol 66:239-274.

Gordon C.J. 2017. The mouse thermoregulatory system: Its impact on translating biomedical data to humans. Physiology and behavior 179:55-66.

Gurnell and Flowerdew. 2019. The Live trapping Small Mammals: A Practical Guide. Mammal Society

Hankenson F.C., J.O. Marx, C.J. Gordon, and J.M. David. 2018. Effects of rodent thermoregulation on animal models in the research environment. Comp Med 68:425-438.

Harrison X.A., L. Donaldson, M.E. Correa-Cano, J. Evans, D.N. Fisher, C.E.D. Goodwin, B.S. Robinson, D.J. Hodgson, and R. Inger. 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. PeerJ 6:e4794.

Heinrich B. 1977. Why have some animals evolved to regulate a high body temperature? Am Nat 111:623-640.

Hodkinson I.D. 2008. Terrestrial insects along elevation gradients: species and community responses to altitude. Biol Rev 80:489-513.

Ivy C.M. and G.R. Scott. 2017. Control of breathing and ventilatory acclimatization to hypoxia in deer mice native to high altitudes. Acta Physiol 221:266-282.

Levy O., T. Dayan, and N. Kronfeld-Schor. 2011. Adaptive thermoregulation in golden spiny mice: the influence of season and food availability on body temperature. Physiol Biochem Zool 84:175-184.

Marques D.A., D. de Carvalho, G.S.F. da Silva, R.E. Szawka, J.A. Anselmo-Franci, K.C. Bícego, and L.H. Gargaglioni. 2017. Influence of estrous cycle hormonal fluctuations and gonadal hormones on the ventilatory response to hypoxia in female rats. European J Physiology 469:1277-1286.

Nagy L. and G. Grabherr. 2009. The biology of alpine habitats. Oxford University Press 390.

Nor S.M.D. 2001. Elevational diversity patterns of small mammals on Mount Kinabalu, Sabah, Malaysia. Global Ecol Biogeogr 10:41-62.

Rad J.E., M. Manthey, and A. Mataji. 2009. Comparison of plant species diversity with different plant communities in deciduous forests. Int J Environ Sci Te 6:389-394.

Robertson C.E., G.J. Tattersall, and G.B. McClelland. 2019. Development of homeothermic endothermy is delayed in high altitude native deer mice (*Peromyscus maniculatus*). P R Soc B 286:20190841-20190841.

Ruf T. and F. Geiser. 2015. Daily torpor and hibernation in birds and mammals. Biol Rev 90:891-926.

Sears M.W., J.P. Hayes, M.R. Benta, and D. McCormick. 2009. Out in the cold: physiological capacity influences behaviour in deer mice. Funct Ecol 23:774-783.

Shabalina I.G., N. Petrovic, J.M. de Jong, A.V. Kalinovich, B. Cannon, and J. Nedergaard. 2013. UCP1 in brite/beige adipose tissue mitochondria is functionally thermogenic. Cell Rep 5:1196–1203.

Storz J.F., G.R. Scott, and Z.A. Cheviron. 2010. Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. J Exp Biol 213:4125-4136.

Tattersall G.J. and W.K. Milsom. 2009. Hypoxia reduces the hypothalamic thermogenic threshold and thermo sensitivity. J Physiology 587:5259-5274.

Wikum D.A. and G.F. Shanholtzer. 1978. Application of the Braun-Blanquet cover-abundance scale for vegetation analysis in land development studies. Environ Manage 2:323-329.

Wooden K.M. and G.E. Walsberg. 2002. Effect of environmental temperature on body temperature and metabolic heat production in a heterothermic rodent, *Spermophilus tereticaudus*. J Exp Biol 205:2099-2105.

Online Material

Table A1. Coordinates (longitude; latitude) of the twelve trapping location, the weather stations and the laboratories:

	Trapping locations		Weather stations		Laboratories	
1	7.62805	45.40565	7.57330	45.55680	7.52220	45.49770
2	7.61568	45.40899	7.48720	45.51350	7.31445	45.45137
3	7.59606	45.42822	7.61110	45.42000		
4	7.43559	45.42309	7.54100	45.40890		
5	7.54968	45.50748	7.43820	45.42670		
6	7.31445	45.45137	7.41150	45.43840		
7	7.39580	45.45295	7.31140	45.45280		
8	7.48709	45.51901	7.20910	45.44110		
9	7.47173	45.52610				
10	7.51484	45.55321				
11	7.21039	45.43669				
12	7.56948	45.55651				

Table A2. Descriptive statistics of phenotypic traits measured for *Apodemus flavicollis, A. sylvaticus, A. alpicola* and *Myodes glareolus* in the Gran Paradiso National Park divided into three altitudinal levels. Abbreviation: wt (body weight); hl (head length); hw (head width); bl (body length); BT (body temperature); SD (standard deviation).

A. flavicollis	< 1000 m a.s.l.		1000-1500 m a.s.l.		>1500 m a.s.l.		Female	Male
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	
wt (g)	30.0 (5.18)	21-43	30.5 (6.67)	19-43	26.1 (10.22)	17-40	30.5 (5.81)	31.6 (7.41)
hl (mm)	27.9 (2.81)	21.8-34	28.04 (2.70)	20.1-32	28.8 (2.88)	24-32.1	27.9 (2.86)	28.4 (2.60)
hw (mm)	15.5 (1.36)	10.2-17.9	15.6 (1.11)	13-17.9	15.3 (1.22)	13.5-17	15.3 (1.31)	15.5 (1.19)
bl (mm)	79.0 (7.69)	62-96	80.5 (6.45)	68-90	75.1 (10.92)	60-92	78.7 (6.8)	81 (8.2)
BT (°C)	37.6 (1.15)	35-40	38.3 (0.67)	37-40	38.7 (0.65)	37.5-39.5	38.3 (0.85)	37.96 (0.84)

A. sylvaticus

wt (g)	29.8 (4.71)	22-43	28.5 (8.49)	18-38	-	-	27.7 (4.59)	30.8 (5.42)
hl (mm)	27.0 (2.38)	22.9-32	29 (3.29)	23-32	-	-	26.9 (2.11)	27.6 (2.63)
hw (mm)	15.4 (1.08)	13.2-17.9	15.2 (0.74)	14-16	-	-	15.4 (0.98)	15.3 (1.01)
bl (mm)	75.8 (7.35)	57-93	80.8 (9.78)	65-92	-	-	76.6 (6.04)	77.4 (8.78)
BT (°C)	37.3 (0.98)	35-39.7	37.9 (1.56)	35-39.6	-	-	37.8 (0.90)	37.3 (1.04)

A. alpicola

wt (g)	-	-	28.8 (6.83)	17-40	29 (6.24)	18-40	28.5 (5.73)	29.7 (7.16)
hl (mm)	-	-	27.9 (2.04)	25-32	28.8 (1.44)	26-32	28.2 (1.82)	28.9 (1.65)
hw (mm)	-	-	15.8 (1.06)	14-17	15.5 (1.46)	13-18.9	15.4 (1.28)	15.8 (1.28)
bl (mm)	-	-	80.5 (8.94)	68-95	79.2 (8.12)	65-94	79 (7.45)	81 (9.42)

BT (°C)	-	-	38.3 (1.09)	36-39.8	38.5 (0.69)	37.1-39.8	38.5 (0.53)	38.2 (1.20)
M. glareolus								
wt (g)	25 (5.20)	14-36	26.8 (5.83)	14.5-35.5	30 (6.89)	13.5-38	27.9 (5.98)	27.1 (6.32)
hl (mm)	26.9 (4.09)	19.6-34	26.95 (2.33)	22-31.2	27.8 (2.99)	11.5-33	27.9 (3.13)	27.8 (2.52)
hw (mm)	14.5 (0.89)	12-16.2	14.92 (0.81)	13.8-16	15.1 (0.90)	13-17	14.9 (0.79)	14.8 (0.84)
bl (mm)	79.8 (7.52)	68-94	81.5 (9.29)	60-98	85.7 (10.12)	60-99	82.8 (8.27)	82.9 (9.84)
BT (°C)	37.7 (1.17)	35-39.8	38.3 (0.93)	36.2-40.4	38.7 (0.83)	36.9-40.1	38.5 (0.86)	38.1 (1.0)

NOTE. Abbreviations: wt (body weight); hl (head length); hw (head width); bl (body length); BT (body temperature); SD (standard deviation).

Table A3. Significance of the effect between body temperature and the tested variables in mixed-effects models.

	β (SE)	*z*	*p*
A. flavicollis	0.003 (0.002)	1.17	0.24
A. sylvaticus	0.001 (0.003)	0.33	0.74
M. glareolus	0.001 (0.002)	0.36	0.72
Season (1, 2)	-0.001 (0.002)	-0.42	0.67
Elevation	0.027 (0.005)	5.05	< **0.001**
T_{Emean}	0.023 (0.006)	3.93	< **0.001**
Sex (0,1)	0.004 (0.001)	3.94	< **0.001**
Plant richness	0.007 (0.009)	0.71	0.48
Vegetation coverage	-0.005 (0.009)	-0.53	0.59
Plant diversity	-0.002 (0.003)	-0.58	0.56
Exposure	-0.002 (0.003)	-0.57	0.57
BC	-0.007 (0.006)	-1.02	0.31
Head width	0.002 (0.020)	0.09	0.93

NOTE. The models included $\log_{10}$-transformed variables (elevation; daily minimum air temperature, T_{Emean}; plant richness; plant diversity; vegetation coverage; exposure; head width) seasonality (1 for spring and 2 for summer) and body condition (BC) as continuous variables, sex (1 for female, 0 for male) and species (*A. flavicollis*, *M. glareolus*, *A. sylvaticus* and *A. alpicola*) as categorical factors. Bolded text highlights statistically-significant results.

Table A4. Single species models in which ten records with very low T_{Emean} (< 9.8 °C) were excluded. Species were analysed separately: (a) *A. flavicollis (*N = 69), (b) *A. sylvaticus (*N = 43), (c) *A. alpicola (*N = 33), (d) *M. glareolus (*N = 151). The effect of seasonality and the interaction effect between elevation and sex and elevation and T_{Emean} were excluded from these analyses due to the small sample size.

(a)

Apodemus flavicollis	β (SE)	*z*	*p*
Elevation	0.020 (0.006)	3.08	**0.003**
T_{Emean}	0.016 (0.026)	0.63	0.52
Sex (0,1)	0.006 (0.002)	2.53	**0.013**
Richness	0.0002 (0.018)	0.01	0.99
Plant diversity	0.001 (0.006)	0.22	0.82
Vegetation cover	0.007 (0.016)	0.46	0.64
Exposure	- 0.004 (0.004)	-0.91	0.36
BC	- 0.010 (0.013)	- 0.75	0.45
Head width	0.022 (0.030)	0.74	0.46

(b)

Apodemus sylvaticus	β (SE)	*z*	*p*
Elevation	- 0.071 (0.025)	- 2.85	**0.007**
T_{Emean}	0.233 (0.062)	3.75	< **0.001**
Sex (0,1)	0.003 (0.003)	1.05	0.29
Richness	-0.006 (0.033)	-0.19	0.84
Plant diversity	- 0.019 (0.009)	- 2.13	0.040
Vegetation cover	- 0.044 (0.021)	- 2.08	0.044
Exposure	0.011 (0.005)	2.09	0.044
BC	-0.027 (0.026)	- 1.02	0.31
Head width	- 0.061 (0.059)	- 1.03	0.30

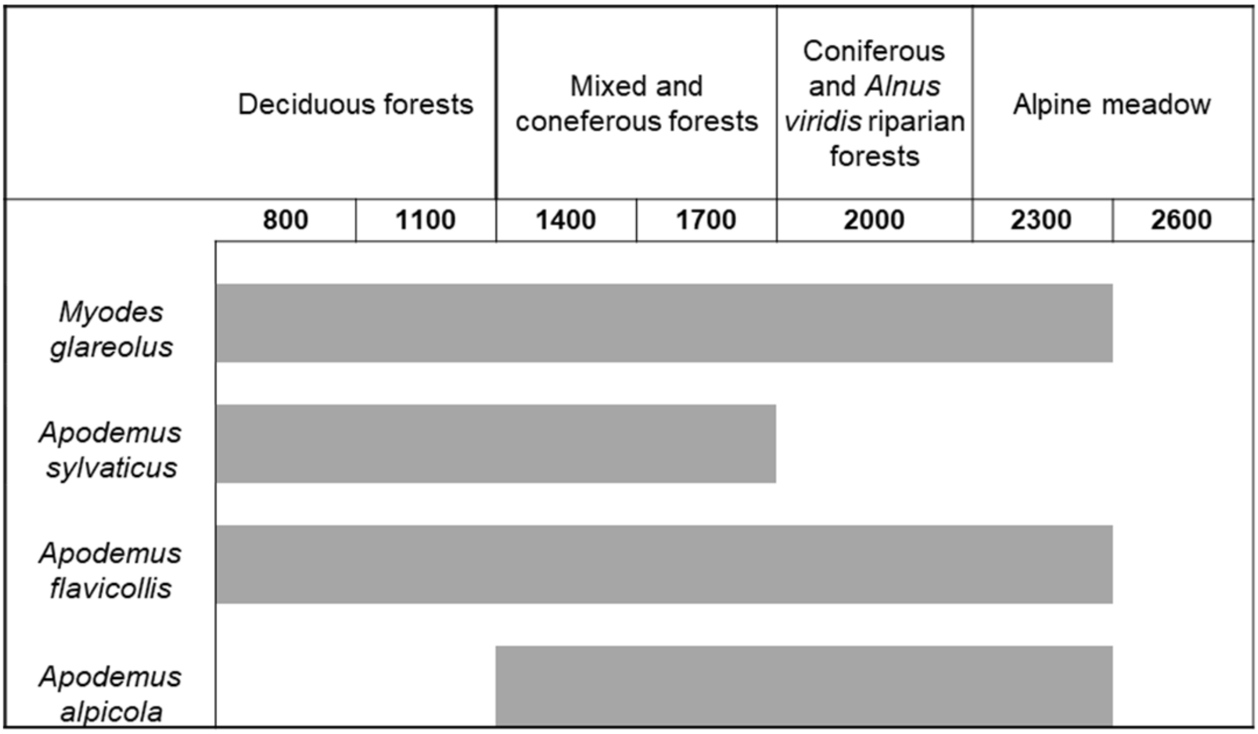
(c)

Apodemus alpicola	β (SE)	z	p
Elevation	-0.035 (0.107)	-0.33	0.74
T_{Emean}	0.031 (0.129)	0.24	0.81
Sex (0,1)	0.002 (0.004)	0.60	0.54
Richness	0.047 (0.051)	0.92	0.36
Plant diversity	0.021 (0.019)	1.11	0.27
Vegetation cover	- 0.054 (0.054)	- 1.01	0.32
Exposure	0.010 (0.022)	0.48	0.63
BC	- 0.046 (0.027)	- 1.70	0.10
Head width	- 0.026 (0.056)	- 0.46	0.64

(d)

Myodes glareolus	β (SE)	*z*	*p*
Elevation	0.024 (0.008)	2.75	**0.006**
T_{Emean}	0.003 (0.017)	1.18	0.85
Sex (0,1)	0.003 (0.001)	2.37	**0.018**
Richness	0.004 (0.012)	0.37	0.70
Plant diversity	- 0.007 (0.004)	- 1.70	0.091
Vegetation cover	0.007 (0.016)	0.48	0.63
Exposure	0.002 (0.004)	0.60	0.54
BC	- 0.011 (0.009)	- 1.26	0.21
Head width	- 0.010 (0.036)	-0.29	0.76

Figure A1. Altitudinal range distribution of the study species within the Gran Paradiso National Park.



CHAPTER VI − General Discussion

Discussion

The rodent community from the alpine environment provides a fruitful model to study how animals adapt to their habitat. This PhD project aimed to increase the knowledge of mountain rodents' ecology and their adaptations to alpine habitats, focusing on their morphological, physiological, and behavioural strategies. Using a multi-species approach by selecting four species widely distributed in different habitat types, *Apodemus flavicollis, A. sylvaticus, A. alpicola* and *Clethrionomys glareolus*, we were able to analyse and compare their adaptive strategies along an altitudinal gradient and see the effect of habitat variables on these species.

Phenotypic plasticity is assumed to be a good predictor of resilience to environmental change, as high plasticity facilitates range shifts or adaptation to changing conditions. In Chapter 2 we investigated whether four rodent species across a wide elevational range (400 to 1,800 m a.s.l.) show intra-specific variations in morphological traits. By analysing biometrical data collected during 2017 and from 2020 to 2021, we found two opposite patterns between the two groups of rodents: *Apodemus* spp. and *C. glareolus*. We found that at high altitude body weight increases in *C. glareolus,* which had also shorter foot length than *Apodemus* species, supporting Bergmann' (larger individuals in colder environments) and Allen' (shorter limbs in colder climates). In *Apodemus* species instead, we observed a general increase in foot length along the elevation, while body weight decreases, contradicting both these rules. These results suggest that variables other than temperature might explain the variation in these morphological traits. Higher elevations require a range of physiological adaptations due to factors besides the low ambient temperature, such as hypoxia and reduced food resources. Different hypotheses have been proposed. The impact of a change in body weight during exposure to high-altitude hypoxia is not well known. Weight loss could result from caloric restriction during prolonged exposure to hypoxia (Karwi et al., 2019). It is hypothesised that lower body weight in hypoxia exposure would promote reduced ventricular hypertrophy through physiologic upregulation mechanisms. Exposure to hypoxic environments induces cardiopulmonary alterations that allow the maintenance of circulatory demands and tissue homeostasis in conditions of limited oxygen availability (Ostadal et al., 1998). Thus, a caloric restriction is a potent dietary intervention to produce beneficial cardiac effects (Kobara et al., 2015; Melo et al., 2016). Another hypothesis on the role of body weight in the adaptations at high altitudes concerns the effect of the resource availability hypothesis, which predicts a positive association between body size and the amount of food resources in a habitat (Rosenzweig, 1968; Blackburn et al., 1999; Blackburn & Hawkins, 2004; Virgós et al., 2011). In mountain environment, plant productivity is one of the leading forces acting on rodents' survival: as altitude increases, environmental temperature, the duration of the vegetative period and biomass decrease, and plant phenology is delayed (Hille & Cooper, 2015). Trophic resources are reduced and more scattered across space, and the structure and diversity of vegetation decrease (Laiolo et al., 2004; Nagy & Grabherr, 2009; Hürlimann et al., 2019). In these conditions, lower body masses may be beneficial when resources are scarce and highly seasonal, and energy intake is thus limited. Thus, we can suppose that the two groups of rodents here examined have been affected in a different way from high altitude selection: *C. glareolus* developed morphological adjustment to avoid heat dissipation, while *A. flavicollis* instead found advantage of having a smaller body in order to reduce the amount of energy needed (Bears et al. 2008). It is also possible that *Apodemus* at higher altitudes have longer feet and slimmer body to optimize mobility when food resources are more limited. Studying the trait variation within and across species along environmental gradients can be used to understand potential reactions to future global changes, and thus highlights the importance of increasing the data collection on rodents and other taxa. Here, we have shown that rodents inhabiting similar habitats over a large elevation gradient have high phenotypic variation.

Most of the ecology studies done so far in mountain systems are mainly conducted on single species, and the comparison of ecological strategies and their driving factors in the Alpine mountains is rather scarce. In chapters 3-5, we moved from studying single-species phenotypical, behavioural and physiological adaptations to species comparison across an elevational gradient, trying to identify a common pattern of these mechanisms.

In Chapter 3, we have considered the phenotypical variation in fur colouration across an elevational gradient. We have shown that species differed in their camouflage adaptations and that the colour characteristics have variable importance among the species. Animal adaptation is an outcome of natural selection, which shapes inheritable phenotypic change (whether on a molecular or morphological level), resulting in increased fitness of individuals in a particular environment (Boomsma, 2016). Classic examples of adaptive evolution are related to the emergence of colour polymorphism, particularly with the evolution of camouflage colouration in animals (Endler & Greenwood, 1988; Mappes et al., 2014). Camouflage is one of the most efficient anti-predatory strategies, and it is a functional trait characterised by the correspondence between the colour/pattern of the organisms and their background to avoid being discovered by predators (Roff & Fairbairn, 2013; Kappers et al., 2020; Morosinotto et al., 2020). In mammals, colouration depends primarily on the deposition of two forms of melanin, red to pink pheomelanin and black to brown eumelanin, during hair growth. The regulation of the expression of two antagonist genes (melanocortin-1 receptor and Agouti signalling protein) determines the proportion of expression of the darker or lighter form (Hubbard et al., 2010). We observed that fur colouration in our target species was strictly linked with the background colouration, indicating a high match with their respective habitats. However, under stressful conditions, the production of one of the two forms of melanin (darker or lighter) can have different expression levels (Galván et al., 2012). Indeed, in our study, we observed a different trend between the two rodents' groups: *Apodemus* spp. and *C. glareolus*. This result could be due to two factors. On the one hand *A. flavicollis* has a typical low-land distribution well adapted to deciduous forests and colonised high-altitude environment only recently (Reutter et al., 2003). In our study areas it was found at high elevation only at low population density. These ecological characteristics may have influenced its wavelength, probably for thermoregulation mechanisms and for protection from solar radiation (Nor, 2001). This result was also supported by the increasing in hue mismatch with elevation, supporting the hypothesis of recent adaptation to the high altitude (Rios & Álvarez-Castañeda, 2011). On the contrary, in *C. glareolus* and also in *A. alpicola,* hue was higher at high elevation, where the species were captured mainly in rocky environments, with herbaceous cover and coniferous forests with low canopy, suggesting an anti-predatory strategies in hue component for these species. A hypothesis could be that lighter colouration and higher wavelength at high altitude might be expected to be less noticeable in habitats that are often snow-covered or lacking dark vegetation, to the detriment of heat absorption in these cold environments. There is also experimental evidence showing that both aerial (Dice, 1947; Kaufman, 1974) and terrestrial (Brown, 1965) predators select against conspicuously coloured mice. Thus, we can suppose that fur colouration can influence the activity patterns and other ecological factors of the species, such as behaviour or thermoregulation. The different patterns of colouration here presented, suggest that selection may be stronger in the mountain habitat, acting on a physiological and genetic level. Thus, we can suppose that fur colouration can influence the activity patterns and other ecological factors of the species, such as behaviour or thermoregulation. This result requires future insight with research focusing on the strength of selection during ontogenetic development and the effectiveness of this selection on animal fitness.

Behavioural strategies have been considered in Chapter 4, where we compared the inter-specific variation of behavioural traits in sympatric wild rodent species and analysed this variation along a gradient of elevation. Predatory pressures mainly determine and select animal behaviour (Lima, 1998). However, preys must trade off their antipredator responses with other functions, such as foraging, mating or thermoregulate (e.g., Gilliam & Fraser, 1987). An animal's propensity to take a risk is often described along an axis of boldness and shyness. A high probability of taking risks is defined as boldness, and a low probability is defined as shyness. Individuals may display varying levels of boldness or shyness that can affect the outcome of daily ecological challenges, such as competing for food (Dingemanse et al., 2004), habitat selection (Pay et al., 2022), and foraging under the pressure of predation (Lima, 1998; Stamps, 2007). Consequently, boldness and shyness can affect reproduction and survival, and thus, ultimately affect fitness. However, as foraging is a trait typically related to boldness (Dochtermann, et al., 2015; Mousseau & Roff, 1987; Sih et al., 2004), this behaviour can have underlying physiological components and can be heritable (Chuang & Riechert, 2022; Brown, 2007), so it can be subject to evolution following natural selection in subsequent generations (Réale, 2003). In our results, we observed two opposite behaviours that emerged between the two groups of rodents examined. *Apodemus*, which showed consistency in bold and exploratory behaviours in the three species (*A. flavicollis, A. sylvaticus* and *A. alpicola*), and *C. glareolus* which instead showed high variability in the escape behaviour, but with a predominance of shy individuals, who mainly froze during the arena experiment. The absence of any effect of environmental variables on these traits suggests the existence of genetic and physiological factors underlying the behavioural traits of the species. Voles and mice diverged by approximately 20-40 million years ago (Horner et al., 2007; Honeycutt, 2009), and it could be hypothesised that this degree of divergence led to the evolution at the genetic and physiological levels of distinct behavioural strategies, both advantageous for the different species. More studies are needed to analyse the existence of a physiological trend at the species level, and considering survival success and reproductive strategies as measures of the consequences that specific behavioural strategies can have on animals' fitness.

Homeothermy is a defining trait for endothermic mammals, and the capacity for sustained metabolic heat production (or thermogenesis) is considered one of the hallmarks of mammalian evolution (Crompton, 1978). Changes in thermogenic performance can be achieved *via* physiological adjustments that occur within the lifetime of individuals (phenotypic plasticity) and genetically based changes that occur across generations (genetic adaptation) (Garland & Carter, 1994; Hammond et al., 2002; Storz et al., 2010; Swanson, 2010). Prolonged cold stress can strongly affect the capacity for metabolic heat production, particularly in small homeothermic endotherms (Conley & Porter, 1986; Hayes & O'Connor, 1999). Indeed, due to their high surface-to-volume ratio rodents have relatively high energy demands to support temperature regulation (Robertson et al., 2019). These conditions become even more energetically critical in mountain habitats where cold, wind, hypoxia and scarce food resources represent an important selective pressure. In the last Chapter 5, we considered the fundamental aspect of the adaptations of rodents to the mountain environment, analysing the effect that the altitude and habitat variables have on the thermoregulation of the species. We have shown how body temperature varies along an altitudinal gradient in wild populations and during the breeding season of species (spring and summer). Most of the previous works on this topic (Cheviron et al., 2012, 2013; Careau, 2012; Dzal, 2018) have addressed the physiological responses of animals when exposed to the extreme winter cold at high elevations. When rodents are exposed to cold and hypoxic conditions, they may apply physiological adjustments to increase heat production through vascularization of brown adipose tissue, up-regulating mitochondrial thermogenic respiration (Shabalina et al., 2013), through increasing ventilatory performance (Ivy & Scott, 2017), and the α-globin affinity for oxygen (Chappell & Snyder, 1984; Sears et al., 2009). On the other hand, maintaining high body temperature by endothermic mechanisms under low air temperatures is physiologically costly and depends on the degree and duration of cold exposure and habitat productivity (Bastos et al., 2021). When air temperature becomes too low, animals from high altitudes enter a torpor state to decrease the body-to-environment temperature gradient and costs of homeothermic thermoregulation. However, our study showed that during their reproductive period, rodents from high elevations have higher body temperatures compared to the conspecific from lower elevations. This result was similar for males and females, suggesting that the high costs of reproduction of females might not be the main reason for the observed pattern. Body temperature increased by 0.2 °C in *Apodemus* species and 0.3 °C in *C. glareolus.* For most of the studied species, the effect was independent from variation in air temperature, habitat productivity and body size or condition, suggesting other mechanisms behind the detected pattern. A hypothesis is that this physiological mechanism is related to mild tissue hypoxia and greater ventilation of individuals from higher elevations (Chappell & Snyder, 1984), which could be advantageous for small endotherms, perhaps influencing their aerobic capacity performance.

A cross-analysis between the four adaptive mechanisms presented in this thesis, could provide more information on rodents' resilience and resistance capacities to challenging mountain environments. This becomes even more important given climate change, particularly evident in the mountains. In recent years we are witnessing extreme and highly variable climatic conditions (Hille & Cooper, 2015), greater drought also at high altitudes, with a consequent reduction in trophic availability, a melting of glaciers and an early melting of snow, and a progressive shift of vegetation to higher elevations.

The overall data showed that selection acts differentially on the species and could be used to explain the mechanisms underlying the choice of one strategy over the other in sympatric species.

Future perspectives

Within the years of this PhD project, we have collected far more data than we could present here. We plan to continue the work on our model species combining yet many other challenging aspects of their biology. In particular we want to analyse core body temperature data collected during 2021, where the animals were exposed to food deprivation, to detect the differential thermoregulation mechanism of the species from high and low altitudes, when food resources are scarce. Furthermore, we want to combine body temperature and camouflage data, to test the hypothesis advanced for *A. flavicollis* and *A. alpicola,* that the darker colouration found at high elevation have a thermoregulatory purpose. Another important aspect that we want to investigate are the anti-predator strategies, both for the camouflage and the behavioural aspects, taking into account the predator perspective, in particular of its visual capacity, and to analyse how this influences the effectiveness of prey camouflage.

This study sets up several avenues of potential research. It is impossible to say whether the species difference found in this project is the result of phenotypic plasticity or evolutionary changes.

Furthermore, since the data were collected in a moderate geographic area, it may be the result of a specific environmental adaptation. For these reasons, I propose first, a long-term continuous data collection from the same area, to identify the direction of species changes. In this way, we can examine seasonality variation as well as long-term patterns in morphological, physiological, and behavioural changes. Furthermore, recording environmental conditions data, we can attribute particular phenotypic changes to environmental parameters. Secondly, it would be useful to study the same parameters here considered in other mountain areas to provide more information on how these species are responding to environmental change in different areas. Combining multiple species and geographic areas will allow for a broad overview of what drives overall alpine rodent populations.

Reference

Allen, J. A. (1877). The influence of physical conditions in the genesis of species. Radical Review, 1, 108–140.

Bergmann C. (1847). Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse *Göttinger Studien* 3: 595–708.

Blackburn TM, Gaston KJ, Loder N, 1999. Geographic gradients in body size: a clarification of Bergmann's rule. Divers Distrib 5:165–174.

Blackburn TM, Hawkins BA, 2004. Bergmann's rule and the mammal fauna of northern North America. Ecography 27:715–724.

Brown C, Burgess F, Braithwaite VA (2007) Heritable and experiential effects on boldness in a tropical poeciliid. Behavioral Ecology and Sociobiology 62: 237–243

Caro T. Contrasting colouration in terrestrial mammals. Philos Trans R Soc Lond B Biol Sci. 2009 Feb 27;364(1516):537-48.

Careau V. and T.Jr. Garland. 2012. Performance, personality, and energetics: correlation, causation, and mechanism. Physiol Biochem Zool 85:543–571.

Chappell M.A., and L.R.G. Snyder. 1984. Biochemical and physiological correlates of deer mouse alpha-chain hemoglobin polymorphisms. Proc Natl Acad Sci USA 81:5484–5488

Cheviron Z.A., G.C. Bachman, A.D. Connaty, G.B. McClelland, and J.F. Storz. 2012. Regulatory changes contribute to the adaptive enhancement of thermogenic capacity in high-altitude deer mice. Proc Natl Acad Sci USA 109:8635-8640.

Cheviron Z.A., G.C. Bachman, and J.F. Storz. 2013. Contributions of phenotypic plasticity to differences in thermogenic performance between highland and lowland deer mice. J Exp Biol 216:1160-1166.

Chuang, A., & Riechert, S. E. (2022). Does spatial sorting explain leading edge personality types in a spider's non-native range?. *Ethology*, *128*(4), 317-330.

Conley, K. E. and Porter, W. P. (1986). Heat loss from deer mice (*Peromyscus*): evaluation of seasonal limits to thermoregulation. *J. Exp. Biol.* 126, 249-269.

Crompton AW, Taylor CR, Jagger JA. (1978) Evolution of homeothermy in mammals. Nature 272, 333–336. (doi:10.1038/272333a0)

Dingemanse NJ, Both C, Drent PJ, Tinbergen JM (2004) Fitness consequences of avian personalities in a fluctuating environment. P R Soc B 271: 847–852.

Dzal Y.A. and W.K. Milsom. 2018. Hypoxia alters the thermogenic response to cold in adult homeothermic and heterothermic rodents. J Physiol 597:4809-4829.

Endler JA, Greenwood JJD. (1988). Frequency-dependent predation, crypsis and aposematic colouration. *Philos. Trans. R. Soc. B Biol. Sci.* 319:505–523.

Galván I., Erritzøe J., Wakamatsu K. & Møller A. P. (2012). High prevalence of cataracts in birds with pheomelanin-based colouration. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 162, 259–64

Garland, T., Jr and Carter, P. A. (1994). Evolutionary physiology. Annu. Rev. Physiol. 56, 579-621.

Gilliam JF, Fraser DF (1987) Habitat selection when foraging un-der predator hazard: a model and a test with stream-dwellingminnows. Ecology 68:1856–1862

Gohli J, Voje KL, 2016. An interspecific assessment of Bergmann's rule in 22 mammalian families. BMC Evol Biol 16:222.

Hayes, J. P. and O'Connor, C. S. (1999). Natural selection on thermogenic capacity of high-altitude deer mice. *Evolution* 53, 1280-1287.

Hammond, K. A., Chappell, M. A. and Kristan, D. M. (2002). Developmental plasticity in aerobic performance in deer mice (*Peromyscus maniculatus*). *Comp. Biochem. Physiol.* **133A**, 213-224.

Hubbard J. K., Uy J. A. C., Hauber M. E., Hoekstra H. E. & Safran R. J. (2010).Vertebrate pigmentation: from underlying genes to adaptive function. Trends Genet. 26, 231–9

Karwi, Q. G., Zhang, L., Altamimi, T. R., Wagg, C. S., Patel, V., Uddin, G. M., et al. (2019). Weight loss enhances cardiac energy metabolism and function in heart failure associated with obesity. *Diabetes Obes. Metab.* 21, 1944–1955. doi: 10.1111/dom.13762

Kobara, M., Furumori-Yukiya, A., Kitamura, M., Matsumura, M., Ohigashi, M., Toba, H., et al. (2015). Short-term caloric restriction suppresses cardiac oxidative stress and hypertrophy caused by chronic pressure overload. *J. Card. Fail* 21, 656–666. doi: 10.1016/j.cardfail.2015.04.016

Koolhaas, J.M., S.F. de Boer, B. Buwalda, B.J. van der Vegt, C. Carere, A.G.G. Groothuis

Honeycutt R. Rodents (Rodentia). In The Timetree of Life (eds Shedlock, A. & Edwards, S.) 490–494 (Oxford University Press, 2009).

Horner, D. S. et al. (2007). Phylogenetic analyses of complete mitochondrial genome sequences suggest a basal divergence of the enigmaticrodent Anomalurus. BMC Evol. Biol. 7: 16-46. How and why coping systems vary among individuals. D.M. Broom (Ed.), Coping with challenge: Welfare in animals including humans, Dahlem University Press, Dahlem (2001), pp. 197-209

Ivy C.M. and G.R. Scott. 2017. Control of breathing and ventilatory acclimatization to hypoxia in deer mice native to high altitudes. Acta Physiol 221:266-282

Lima SL, 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. Adv Study Behav27:215–290.

Maestri R, Luza AL, Barros LD, Hartz SM, Ferrari A et al. , 2016. Geographical variation of body size in sigmodontine rodents depends on both environment and phylogenetic composition of communities. J Biogeogr 43:1192–1202.

Mappes J, Kokko H, Ojala K, Lindström L. (2014). Seasonal changes in predator community switch the direction of selection for prey defences. Nat. Commun. 5:5016.

Mayr E, 1956. Geographical character gradients and climatic adaptation. Evolution 10:105–108.

Malenka RC, Nestler EJ, Hyman SE (2009). "Chapter 10: Neural and Neuroendocrine Control of the Internal Milieu". In Sydor A, Brown RY (ed.). *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience* (2nd ed.). New York: McGraw-Hill Medical. pp. 246, 248–259

Melo, D. S., Costa-Pereira, L. V., Santos, C. S., Mendes, B. F., Costa, K. B., Santos, C. F., et al. (2016). Severe calorie restriction reduces cardiometabolic risk factors and protects rat hearts from ischemia/reperfusion injury. *Front. Physiol.* 7:106. doi: 10.3389/fphys.2016.00106

Noppe, G., Dufeys, C., Buchlin, P., Marquet, N., Castanares-Zapatero, D., Balteau, M., et al. (2014). Reduced scar maturation and contractility lead to exaggerated left ventricular dilation after myocardial infarction in mice lacking AMPKalpha1. *J. Mol. Cell Cardiol.* 74, 32–43. doi: 10.1016/j.yjmcc.2014.04.018

Ostadal, B., Ostadalova, I., Kolar, F., Pelouch, V., and Dhalla, N. (1998). “Cardiac adaptation to chronic hypoxia,” in *Myocardial Preservation and Cellular Adaptation*, eds E. E. Bittar and D. K. Das (London: Press INC), 43–60. doi: 10.1016/s1569-2590(08)60007-5

Pay, J.M., Patterson, T.A., Proft, K.M., Cameron, E.C., Hawkins, C.E., Koch, A.J., Wiersma, J.M., Katzner, T.E. (2022). Considering behavioral state when predicting habitat use: Behavior-specific spatial models for the endangered Tasmanian wedge-tailed eagle. Biological Conservation. 274, 109743.

Réale D, Festa-Bianchet M (2003) Predator-induced natural selection on temperament in bighorn ewes. *Animal Behaviour* 65: 463–470

Robertson C.E., G.J. Tattersall, and G.B. McClelland. 2019. Development of homeothermic endothermy is delayed in high altitude native deer mice (*Peromyscus maniculatus*). P R Soc B 286:20190841-20190841.

Rosenzweig ML, 1968. The strategy of body size in mammalian carnivores. Am Midl Nat 80:299–315.

Sears M.W., J.P. Hayes, M.R. Benta, and D. McCormick. 2009. Out in the cold: physiological capacity influences behaviour in deer mice. Funct Ecol 23:774-783.

Shabalina I.G., N. Petrovic, J.M. de Jong, A.V. Kalinovich, B. Cannon, and J. Nedergaard. 2013. UCP1 in brite/beige adipose tissue mitochondria is functionally thermogenic. Cell Rep 5:1196–1203.

Stamps, JA (2007) Growth-mortality tradeoffs and “personality traits” in animals. Ecol Lett 10: 355–363

Storz, J. F., Scott, G. R. and Cheviron, Z. A. (2010). Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. *J. Exp. Biol.* 213, 4125- 4136.

Swanson, D. L. (2010). Seasonal metabolic variation in birds: functional and mechanistic correlates. *Curr. Ornithol.* 17, 75-129.

Veenema, A.H., O.C. Meijer, R.E. de Kloet, J.M. Koolhaas, B. Bohus (2003). Differences in basal and stress-induced HPA regulation of wild house mice selected for high and low aggression. Horm. Behav., 43 (2003), pp. 197-204

Vickery W, and Bider J (1981) The influence of weather on rodent activity. J Mammal:140–145

Virgós E, Kowalczyk R, Trua A, de Marinis A, Mangas JG et al. , 2011. Body size clines in the European badger and the abundant centre hypothesis. J Biogeogr 38:1546–1556.

Walsberg, G.E. (1983). Coat colour and solar heat gain in animals. BioScience. 33:88–91.

Wróbel, A., and Bogdziewicz, M. (2015). It is raining mice and voles: which weather conditions influence the activity of *Apodemus flavicollis* and *Myodes glareolus*?. *Eur J Wildl Res* **61**, 475–478.

ACKNOWLEDGMENTS

Many people have been involved in this project through moral, academic and financial support. First of all, I want to thank Giancarlo, as this achievement would not have been possible without his daily and constant encouragement, his trust and his deep love. Prof. Sandro Bertolino, my academic supervisor and mentor during this process, was always ready and patient to guide me through all stages, trying to get the best out of me. I appreciate all the work done with the guidance of him to achieve this. While not on my academic committee, I would like to express my appreciation to Dr. Zbyszek Boratynsky (University of Porto), whose comments have substantially enhanced the scope of this thesis. I also thank Prof. Alfredo Santovito (University of Turin) who carried out all the genetic analyses for my thesis and provided valuable feedback to substantially improve the work done. I want to thank also Dr. Luc Vauters (ULaval, Quebec), and Zbigniew Borowski (University of Poland) whose participation as external examiners was important to better illustrate the contributions provided on this thesis. I also thank my two graduate students (Noemi Gargano and Annalisa Demitri) with whom I spent many days in the field to collect data for this study, and my family who also encouraged me at every stage of my life. I also thank the Gran Paradiso National Park, which provided all the authorizations to complete my data collection, providing me with the guesthouse free of charge. The University of Turin provided additional funding to complete the fieldwork.