High frequency of melanistic bank voles *Clethrionomys glareolus* in a mountain area of Central Italy (Latium, Lepini Mountains)

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Abstract:
Melanism is a phenotypic trait often driven by selective pressures such as thermoregulation or crypsis. This study reports on a population of bank voles (*Clethrionomys glareolus*) in the Lepini Mountains (Central Italy) characterized by a remarkably high prevalence of melanistic individuals. While this phenotype was ubiquitous within the study area—exceeding the frequency of the wild-type reddish coloration—it was absent in surrounding regions. Genetic analyses revealed no significant differentiation from neighbouring populations, suggesting that the observed melanism represents a local adaptation rather than a lineage divergence.

Keywords: small mammals, melanism, local adaptation, mimicry, Cricetidae.

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Melanistic bank vole in a mountain area of Central Italy

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HIGH FREQUENCY OF MELANISTIC BANK VOLES *CLETHRIONOMYS GLAREOLUS* IN A MOUNTAIN AREA OF CENTRAL ITALY (LATIUM, LEPINI MOUNTAINS)

ABSTRACT

Melanism is a phenotypic trait often driven by selective pressures such as thermoregulation or crypsis. This study reports on a population of bank voles (*Clethrionomys glareolus*) in the Lepini Mountains (Central Italy) characterized by a remarkably high prevalence of melanistic individuals. While this phenotype was ubiquitous within the study area—exceeding the frequency of the wild-type reddish coloration—it was absent in surrounding regions. Genetic analyses revealed no significant differentiation from neighbouring populations, suggesting that the observed melanism represents a local adaptation rather than a lineage divergence.

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INTRODUCTION

The adaptations exhibited by species and individuals formed the basis for the development of Darwinian and Wallacean theories of natural selection (Darwin, 1859; Wallace, 1864). Research into the evolutionary advantages that can derive from these adaptations has been conducted since the nineteenth century, and among these, coat or plumage colouration is considered one of the most important (e.g. Mullen & Hoekstra, 2008; Olsen et al., 2010). Variation in coat or plumage colouration has been included in classic studies on evolution and adaptation (e.g., Cott, 1940) as well as covered by recent comprehensive revisions (e.g. Eizirik & Trindade, 2021), with emphasis often placed on ecological and behavioral implications (e.g., Caro, 2005 regarding mammals). Variability in these traits served as important genetic markers for model animals before molecular techniques (e.g. by Mendelian genetics), aiding early gene mapping and genomic studies (Hoekstra, 2006).

Melanism has evolved convergently across various taxa in response to environmental pressures such as thermoregulation and mimicry (Trullas et al., 2007). In ectotherms, melanism is predominantly driven by thermal constraints. For example, *Vipera berus* populations in cold, mountainous regions exhibit melanistic coloration to enhance thermal absorption, thereby increasing reproductive success (Andrén & Nilson, 1981; Luiselli, 1992). However, melanism in ectotherms also serves other adaptive functions, including predator evasion—as seen in the industrial melanism of *Biston betularia* (Cook & Saccheri, 2013)—aposematism (Turner, 1977), UV protection (Gunn, 1998), disease resistance (Wilson et al., 2001), and sexual selection (Wiernasz, 1989).

In endothermic vertebrates, conversely, diverse hypotheses explain the adaptive significance of melanism. While primarily associated with mimicry and camouflage in mammals (Caro, 2005), exceptions such as small-bodied rodents may still evolve melanism due to thermal constraints (Zevgolis et al., 2022). Although documented in various rodent species (e.g., Kingsley et al., 2009; Kambe et al., 2011), experimental validation remains limited (e.g., Nachman et al., 2003). Current evidence largely supports the camouflage hypothesis (Majerus & Mundy, 2003), with classic experiments on *Peromyscus* mice demonstrating that nocturnal raptors capture individuals more frequently when their coloration contrasts with the background (Dice, 1947; Kaufman, 1974).

The bank vole (*Clethrionomys glareolus*) is a species in the Cricetidae family with a broad Palearctic distribution, from the Pyrenees to the Altai Mountains, and as far north as Scandinavia and the Arctic Circle, including Britain and Ireland (Pardiñas et al., 2016). Although Musser & Carleton (2005) renamed the genus to *Myodes* based on presumed precedence, recent research by Krystufek et al. (2020) supports the use of *Clethrionomys*, a name used for over a century. Bank voles prefer forest areas rich in litter and ground material like stumps and dead logs. In Italy, there are five distinct evolutionary lineages (Bertolino et al., 2023). In other part of the bank vole distribution range scientific literature features descriptions of abnormal coloration, sometimes melanistic. Bobek & Bartke (1967) described a melanistic specimen of bank vole in Poland. Subsequent captures allowed other melanistic specimens to be found and bred in captivity, showing that the gene capable of producing black coloration was recessive (Drozdz, 1971). Other melanistic vole have been described: for instance, other studies have focused on the water vole (e.g. Bazhan et al., 1996).

This paper presents a case study of a bank vole population exhibiting a high frequency of melanistic individuals. This population was sampled from 2017 to 2020 using different survey methods (i.e live and camera trapping, visual observation). The objective of this paper is describing the evidence found, also briefly discussing, based on genetic analysis data, the taxonomic position of studied specimens within known and genetically described populations of *C. glareolus*.

MATERIALS AND METHODS

Study area

The study was conducted in the Lepini Mountains, a pre-Apennine limestone range located in southern Latium (Central Italy). The area is geographically distinct, situated between the Tyrrhenian coastal plains and the central Apennine chain. Notably, the range is separated from the main Apennines by the Valle del Sacco, a highly anthropized industrial zone that effectively isolates the Lepini Mountains from the continuous forested areas of the peninsula.

The landscape is characterized by extensive forest cover, predominantly composed of Mediterranean and deciduous oaks (particularly Quercus cerris L.), as well as chestnut and beech forests at higher altitudes. The highest elevation in the range is Mount Semprevisa (1536 m a.s.l.). This extensive forest cover supports a diverse flora and fauna, including a rich community of aerial and terrestrial predators of small mammals (for a detailed species list, see Corsetti & Marozza, 2020).

Data presented in this study were collected between January 2017 and May 2020. Sampling was carried out within the framework of the field surveys for the Atlas of Animal Biodiversity of the Lepini Mountains (Corsetti & Marozza, 2020). The climate ranges from a Mediterranean climate on the lower slopes exposed to the Tyrrhenian Sea (mild winters, dry summers) to a more continental climate at higher altitudes, with harsher winters and greater temperature variations in the inland and north-eastern areas, but influenced by the proximity to the sea, which also brings significant rainfall, especially in autumn and winter (see Corsetti & Marozza, 2020).

Camera trapping

Data collection took place between February 2019 and March 2020. The study area was stratified into 52 grid cells, each measuring 12.25 km^2 (3.5 X 3.5 km). Camera traps were installed as close as possible to the centroid of each cell, avoiding urbanized locations. The sampling design consisted of three sessions using two sequential arrays of 26 traps each. Each array remained active for 21 consecutive days before being rotated. The total sampling effort amounted to 3,276 trap-days, with inter-session intervals of 77 and 182 days, respectively. Details are in Angelini et al. (2023).

We used camera traps equipped with PIR and invisible IR flash (Apeman H68 [n = 16]), Apeman H45 [n = 2], Acorn 5310A, Boskon Guard BG529, Crenova rd1000, HC-800A Trail Camera, Trail Camera 3G 3.0CG HD, Suntek HC 700G, Toguard H45, Victure HC400; nominal trigger times range from 0.3 to 0.8 sec; cameras were never used for the same location across different sessions), set for recording 20'' videos during the whole day.

Cameras were mounted at a height of 20–30 cm to effectively target small mammals and were set to operate continuously in video mode. Microsite selection prioritized clear visibility, avoiding vegetation or debris that might obstruct the field of view at ground level (e.g. see McCleery et al. 2021). Every two weeks, we extracted SD cards and reviewed the footage for mammal presence.

Direct visual observations

Data collection was supplemented by direct visual observations made opportunistically during fieldwork activities. Although these sightings were non-systematic, they allowed for the reliable identification of phenotypic traits, specifically distinguishing between melanistic and non-melanistic individuals.

Live trapping

Live trapping was conducted opportunistically at locations where melanistic individuals had been detected via camera trapping. We deployed 10 Sherman traps baited with a mixture of oatmeal and hazelnut cream. Upon capture, tissue samples were collected from the ear pinna using a biopsy punch and preserved in 95% ethanol. Non-disposable instruments were utilized for the procedure. In total, samples were obtained from two individuals, a sample size considered sufficient for the aims of the analysis. Individuals were temporarily handled for photographic documentation and subsequently released at the point of capture.

Genetic analyses

One of the aims of this paper was to verify the taxonomic position of target population within other populations of *C. glareolus*. More in detail, in order to i) correctly assign the captured animals to an existing genetic lineage (see Colangelo et al, 2012) and ii) check if melanistic bank voles could represent a differentiated lineage, we sequenced and amplified *cytochrome b* fragment of two individuals (one melanistic and one non-melanistic) following the protocol of Colangelo et al. (2012). As in Colangelo et al. (2012) primers of Deffontaine et al. (2005) were used. A comprehensive search was conducted in the GenBank database, filtering entries by species and geographic origin. Successively we downloaded *cytochrome b* sequences from GenBank of individuals belonging to different mitochondrial lineages described for Italy and Europe including three outgroups (n=53). The phylogenetic tree was built using Maximum Likelihood (Tamura-Nei model) in MEGA 7.0 (Kumar et al., 2016). Robustness of phylogenetic reconstruction was tested by bootstrap resampling (500 replicates). We used the AIC test with the software Model Test (Version 2.1.10). The two sequences have been deposited in GenBank under the accession numbers PX663954 and PX663955.

RESULTS AND DISCUSSION

Camera trap surveys provided the first evidence of melanistic bank voles in the study area. The species was detected on eight occasions across three localities (Table 1; Figure 1). In total, combining camera trapping and direct captures, 16 individuals were recorded, with a striking prevalence of the melanistic morph (n = 12) over the wild-type reddish morph.

Phenotypically, the melanistic individuals are distinct, characterized by a uniformly black coat including the extremities (auricles, tail, feet) and nose (Figures 2a and 2b). Despite this morphological differentiation, phylogenetic analysis places these individuals firmly within the Mediterranean clade (Colangelo et al., 2012), showing minimal genetic divergence (distance = 0.001) compared to the sample average (0.02; Figure 3).

These findings highlight a population with an exceptionally high frequency of melanism, a phenomenon appearing unique to *Clethrionomys glareolus* in Italy and Southern Europe. Significantly, the persistence of melanistic individuals in the same area reported by Amori et al. (2002) suggests a stable, long-term local selection rather than a transient anomaly.

Reports of abnormal coloration, including melanism, also exist elsewhere in the species' distribution range. For instance, Bobek & Bartke (1967) described a melanistic specimen in Poland. Subsequent captive breeding of these individuals demonstrated that the phenotype is controlled by a recessive allele (Drozdz, 1971).

The underlying drivers of this phenomenon remain speculative. While melanism in cold, mountainous environments is often linked to thermal efficiency, this hypothesis appears ill-suited to the study area, which exhibits a distinct Mediterranean climate near the Tyrrhenian coast. Conversely, populations in inland Apennine regions, which face more severe temperature fluctuations, have not yielded any melanistic individuals to date. A second hypothesis suggests cryptic coloration as an adaptation to the dark volcanic soil found in the area. The hypothesis that melanism evolves as an adaptation to dark substrates—thereby enhancing crypsis—has been recently supported by evidence across multiple taxa (including small mammals) collected by Lakušić et al. (2026) in a volcanic environment. However, this trait is notably absent in other mountainous regions with similar volcanic substrates. While the role of sexual selection warrants further investigation (Cassini, 2022), the most plausible explanation involves the random fixation of traits due to isolation (genetic drift). The Lepini Mountains are surrounded by intensively cultivated and industrialized lowlands, leaving only limited potential connectivity on the northern side. This isolation hypothesis is supported by extensive regional surveys; despite intense efforts to map Latium mammals (e.g., Capizzi et al., 2012), no melanistic voles were captured in the surrounding areas, reinforcing the uniqueness of the Lepini population.

However, the high genetic similarity between these voles and those in neighbouring areas challenges the hypothesis of strict population isolation. Consequently, future investigations should prioritize the analysis of site-specific variables, such as substrate coloration and microhabitat structure. In conclusion, while this study documents a unique prevalence of melanism in this bank vole population, the current sample size limits the ability to draw definitive conclusions. Comprehensive research utilizing larger datasets and multifactorial analyses is required to fully elucidate the evolutionary and ecological drivers of this phenotype. Specifically, deeper analysis is needed to determine how the anthropogenic barriers surrounding the Lepini Mountains—characterized by intensive agriculture and industry—interact with selective processes and gene flow.

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Table 1 – Sampled localities in the Lepini Mountains between 2017 and 2020, with number of detected bank voles according to different sampling methods (I.e. camera trapping, live trapping, and direct observation). M: melanistic; R: reddish.

Locality	Elevation	Habitat	Sampling method	Number of individuals and colour (M or R)
Pian della Faggeta	1070	Beech forest	Direct observation	1M
Bosco di Cori	700	Oak forest	Camera trap	1M
Piani di Montelanico	750	Oak and chestnut forest	Camera trap	2M 1R
Piani di Montelanico	750	Oak and chestnut forest	Direct observation	1M
Pianoro S. Serena	1070	Beech forest	Camera trap	3M 1R
Pianoro S. Serena	1090	Beech forest	Direct observation	1 M 1R
M. Malaina	1350	Beech forest	Direct observation	1 M
La Torre, Supino	400	Mixed forest	Direct observation	1 M
Monte Ardicara	1300	Scrubland	Direct observation	1M 1R

FIGURE CAPTIONS

Figure 1 – Geographic distribution of *Clethrionomys glareolus* observations in the study area (see Table 1). Black circles indicate localities where only melanistic individuals were observed; white circles denote sites where melanistic and reddish morphs co-occurred. No localities yielding exclusively reddish individuals were recorded. The inset shows the location of the study area within the Italian peninsula.

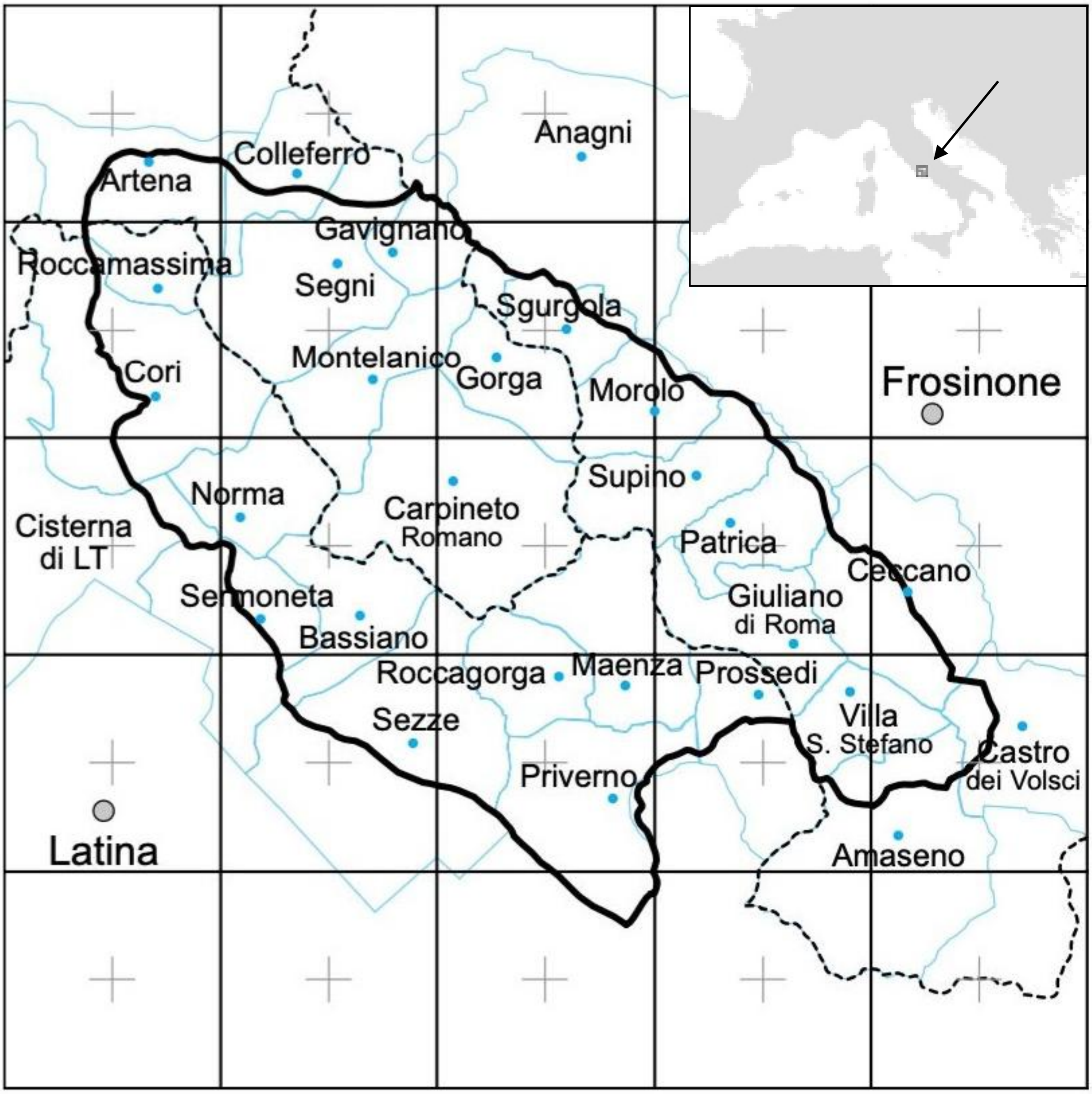

Figure 2 – External appearance of melanistic (a) and reddish wild-type (b) bank voles (*Clethrionomys glareolus*) captured at the same locality in the Lepini Mountains. Note the marked phenotypic difference between the two morphs (photos by G. Mastrobuoni)

Figure 3 – Dendrogram showing the relationship between the two individuals subjected to the analyses (Myo1 and Myo2, deposited in GenBank under the accession numbers PX663954 and PX663955) and the cyt b sequences of individuals belonging to different mitochondrial lineages described for Italy and Europe including three outgroups (n=53), downloaded from GenBank