Chapter 8
Adaptive Pelage Coloration in *Ctenomys*

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8.1 Introduction

In rodents, pelage color tends to resemble background habitat coloration, suggesting an adaptive significance (Sumner 1934; Dice and Blossom 1937; Cott 1940; Endler 1978; Krupa and Geluso 2000). But how does it work in subterranean lineages, in which individuals expend most of their lifetime in burrowing systems (Lacey et al. 2000)? Surprisingly, long stand studies in pocket gophers (*Thomomys bottae* and *Geomys bursarius*) and the Israeli subterranean mole rat (*Spalax ehrenbergi*) have demonstrated similar patterns to aboveground rodents, i.e., a strong correlation between dorsal pelage and soil coloration (Ingles 1950; Kennerly 1954, 1959; Krupa and Geluso 2000; Heth et al. 1988), presumably reflecting an influence of selective pressure when they are active on the surface. This concealment coloration is also substantiated in *Ctenomys* (Langguth and Abella 1970; Vassalo et al. 1994), in which pelage color varies continuously, both inter- and intraspecies (Langguth and Abella 1970; Freitas and Lessa 1984; Wlasiuk et al. 2003; Gonçalves and Freitas 2009; Gonçalves et al. 2012). Overall, coat coloration ranges from light to dark brown in tuco-tucos (Fig. 8.1). However, brown with white patterns, grayish, and melanic phenotypes are also present. Similarly, variation is found in the background environment, as species are spread throughout South America, including a variety– and vast areas – of habitats, e.g., pampas of Puna (above 4000 m), high mountain steppes, low valleys of the west, dunes of the Atlantic coast of the east, mesic and humid plains, desert or semi-deserts, open areas among subtropical

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Fig. 8.1 Inter- and intraspecific variation in pelage color of *Ctenomys*. (**a**) general view of a specimens' drawer of tuco-tucos from the Museum of Vertebrate Zoology (MVZ), revealing the typical brown pattern found. (**b**) *C. torquatus*; (**c**) *C. yolandae*; (**d**) *C. haigi*; (**e**) *C. bonettoi*; (**f**) *C. roigi*; (**g**) *C. dorbigny*; (**h**) *C. magellanicus*; (**i**) *C. maulinus*; (**j**) *C. sociabilis*; (**k**) *C. argentinus*; (**l**) *C. perrensi*; (**m**) *C. mendocinus*; (**n**) *C. fulvus*; (**o**) *C. peruanus*; (**p**) *C. opimus*. (Photographs (except b [from G. L. Gonçalves]) by T. R. O. Freitas – courtesy of mammal collection from the Museum of Vertebrate Zoology, UC Berkeley)

forests, and steppes of Terra del Fuego (Reig et al. 1990; Lacey et al. 2000; Bidau 2015; Freitas 2016).

In particular, two pairs of species that live in the Atlantic coast catches not only evolutionary biologists but anyone's eyes for its marked differences in pelage

associated with habitat background. The first is *Ctenomys australis* Rusconi, 1934 and *Ctenomys talarum* Thomas, 1989, occurring in a coastal dune region in southern Buenos Aires province of Argentina (Contreras and Reig 1965; Reig et al. 1990), and the second is *Ctenomys flamarioni* Travi, 1981 and *Ctenomys minutus* Nehring, 1887 (Freitas 1995a, b), which inhabit the southern Brazil coastal plain (Fig. 8.2a). *C. australis* and *C. flamarioni* have blonde coat color (light phenotype) and inhabit the sandy dunes, whereas *C. talarum* and *C. minutus* have brown pelage (dark phenotype) and inhabit sandy fields (Fig. 8.2b) that correspond to a continuum of coastal dunes toward the continent (Freitas 1995a, b; Busch et al. 2000) (Fig. 8.3a); these two habitats can be distinguished by soil color (Fig. 8.3b) and hardness, and plant cover (Malizia et al. 1991; Cutrera et al. 2010; Kubiak et al. 2015; Lopes et al. 2015; Kubiak et al. 2018). Phylogenetic relatedness between and within these pair of species also vary. *C. australis*, *C. talarum*, and C. *flamarioni* belong to the mendocinus species group, whereas *C. minutus* are placed in the torquatus species group (Parada et al. 2011; Chap. 2, this volume). In this context, the repeated phenotypes might represent convergence to similar habitats, in which ecological function is potentially cryptic anti-predation behavior (Langguth and Abella 1970; Vassalo et al. 1994), which has never been explored.

Two studies have investigated pelage variation in *Ctenomys* from an evolutionary genetics perspective. First, Wlasiuk et al. (2003) demonstrated that genetic drift underlies pelage forms in different populations of *Ctenomys rionegrensis* Langguth and Abella (1970) that include brown, dark-backed, and melanic phenotypes. Second, Gonçalves et al. (2012) performed a molecular approach targeting a key gene-driven of coatcolor – the Melanocortin 1 receptor (MC1R) –, including a wide range of species with distinct color pelages.

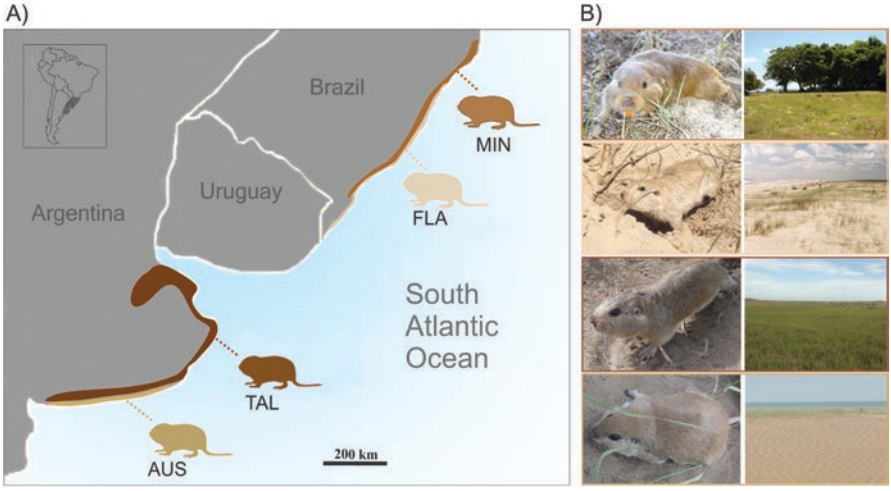

Fig. 8.2 (**a**) Geographic distribution of *Ctenomys flamarioni* (FLA), *Ctenomys minutus* (MIN), *Ctenomys australis* (AUS), and *Ctenomys talarum* (TAL) in the coastal plain of Argentina and southern Brazil with schematic shades of its pelage. (**b**) Convergence pattern of light-dark phenotypes (FLA-MIN and AUS-TAL) inhabiting contiguous habitats of sandy dunes and sandy fields

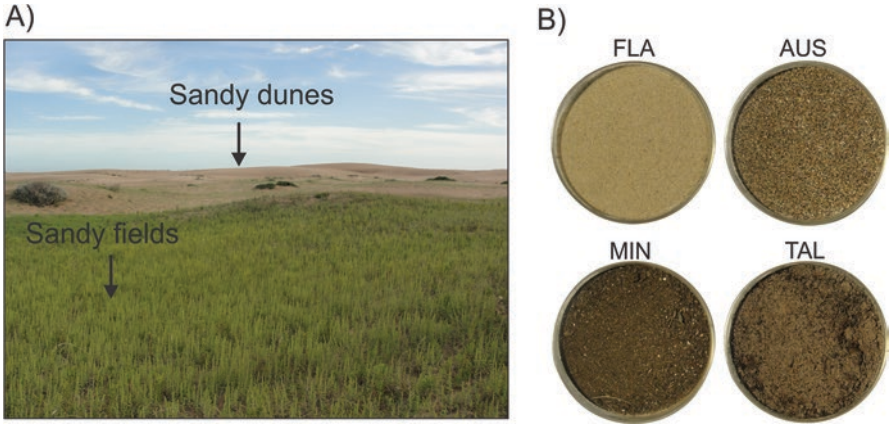

Fig. 8.3 (**a**) Habitats of *Ctenomys* in the coastal system: sandy dunes and sandy fields. (**b**) Soil coloration of each species' habitat. TAL, *C. talarum*; MIN, *C. minutus*; AUS, *C. australis*; FLA, *C. flamarioni*

Hair and skin color in rodents are largely determined by the amount, type, and distribution of melanin packaged in the melanosomes of epidermal cells and hair follicles (Jackson 1997). Mc1r acts as a pigmentary switch in the production of melanin: when activated by α-melanocyte stimulating hormone (α-MSH), it signals the production of black/brown pigment (eumelanin) and in the absence (or inhibition) of a-MSH, red/yellow pigment (pheomelanin) is synthesized (Jackson 1997). In mice, Mc1r dominant mutations are often associated with a hyperactive or constitutively active receptor resulting in predominantly black coat color (Jackson et al. 1994), whereas recessive loss-of-function mutations tend to trigger the production of pheomelanin, which leads to predominantly yellow or red coat color (Robbins et al. 1993). Similarly, in wild rodents several mutations were identified in Mc1r, and associated with the adaptive variation, e.g., the rock pocket mice (*Chaetodipus intermedius*; Nachman et al. 2003) and the beach mice (*Peromyscus maniculatus*; Hoekstra et al. 2006); also, melanism in British gray squirrel (*Sciurus carolinensis*) was linked to a 24-bp deletion in Mc1r (McRobie et al. 2009). In tuco-tucos, several coding substitutions were detected in Mc1r (Gonçalves et al. 2012), but none of them with plausible link to the phenotypes examined, especially the light pelage of *C. australis* and *C. flamarioni*, or melanic forms of *C. rionegrensis* and *C. torquatus*. Additionally, patterns of Mc1r expression were described for dorsal, flank, and ventral regions, but differences were not found between light and dark phenotypes; even though, the distinction among body regions was clear (Gonçalves et al. 2012).

8.2 Pelage Variation: From Genotype to Phenotype

Simple Mc1r mutations of large effect have not contributed to adaptive differences among species of tuco-tucos, thus the variation in coat-color among *Ctenomys* suggests that this trait might have a more complex or even polygenic basis. Finding the genes underlying this variation is probably a daunting task, which will require mapping and association studies involving more markers and defined populations. A suitable candidate gene is the Agouti signaling protein (Agouti), an antagonist of Mc1r; in mice, a local expression that varies both spatially and temporarily (Bultman et al. 1992; Siracusa 1994) results in suppression of synthesis of eumelanin and increased production of phaeomelanin. Agouti is the second most important gene linked with adaptive pelage color variation in rodents (e.g., beach mice (Steiner et al. 2007)), which remains to be explored, particularly in the blonde pelages of tuco-tucos, such as in *C. australis*, *C. flamarioni*, and *C. mendocinus* that also present an intraspecific variation of lighter pelage (see Fig. 8.1).

Typically, wild rodents have a pelage pattern of light ventral, which results from constitutive Agouti expression and associated production of phaeomelanin. In contrast, dorsal hairs have a banded pattern (commonly referred to as agouti hair): terminal and subterminal bands and a base. This banding derives from a pulse of Agouti expression during the intermediary phase of the hair cycle, resulting in the deposition of phaeomelanin during the middle of hair growth and deposition of eumelanin at the beginning and end of hair growth (Hoekstra and Nachman 2006). In the agouti-type pelage distinct variables may be target by the selection, as the distribution of pigment, i.e., bandwidth, and the density of pigment deposited in it, resulting in lighter or darker phenotypes. A few studies have dissected the pigment structure in the hair (e.g., *Peromyscus* (Linnen et al. 2009); *Spalax* (Singaravelan et al. 2010, 2013)) and ultimately inferred its contribution to overall appearance and convergence as well.

In this chapter, an original study on the pigmentation of *Ctenomys* is reported from a morphological perspective, hypothesizing an association of pelage and soil coloration. The hair pattern and pigment density are characterized in species of tuco-tucos from the Atlantic Coastal dune system that present repeated adaptive phenotypes, to test the existence of convergence.

8.3 Quantifying Hair, Pelage, and Soil Coloration

In vertebrates, the visible color spectrum typically ranges from 400 to 700 nm (blue to red) (Krupa and Geluso 2000). Therefore, it was used to measure the pelage and soil coloration. A total of 123 specimens of *C. talarum* (TAL = 20), *C. minutus* (MIN = 40), *C. australis* (AUS = 28), *C. flamarioni* (FLA = 35) from both field-caught and taxidermized specimens from scientific collections of the following institutions were used: Universidade Federal do Rio Grande do Sul (UFRGS),

Universidad Nacional de Mar del Plata (UNMDP), and Museo Municipal de Ciencias Naturales Lorenzo Scaglia (MCNLS) (Appendix). For each specimen, three body regions were analyzed: dorsal, flank, and ventral, determined to infer distinct selective pressures, since there are a differential influence on the individual's overall coloration (i.e., dorsal and flank are considered more relevant for evolution (see Linnen et al. 2009; Manceau et al. 2010)).

Quantification was obtained by the pixel densitometry method; the unit is defined as Gray for the RGB (red, green, and blue) system. Samples (pelage and soil) were photographed with the Munsell (X-Rite Inc.) universal color card to correct the value obtained in relation to the standardized black and white estimates, using the following formula:

$$\text{Calculated Color} = \frac{\text{Obtained Value} - \text{Black}}{\text{White} - \text{Black}}$$

The photographs were analyzed using the software AxioVision version 4.8 (Carl Zeiss Microimaging System Inc.); the central area was delimited using the outline spline tool, and the densitometry values were individually generated for red, green, and blue pixels. For each sample, three measurements were performed and averaged for each pixel. Then, the global average of RGB pixels was calculated.

Microscopic slides were prepared for the dorsal, flank, and ventral regions of each specimen, plucking 10 guard hairs per individual per region. Hairs were rinsed in 50% ethanol and immersed in colorless enamel under the coverslip. Each slide was photographed with a Sony® Cybershot DS20 camera attached to the Leica® M125 stereoscopic microscope using the 0.8X magnification for the whole hair, and 10X for the terminal and subterminal band images. The photographs were analyzed using the AxioVision, measuring hair width, and terminal and subterminal bandwidth. Also, the densitometry values of the pigment deposited in the terminal and subterminal bands were analyzed, zooming the same region analyzed (largest diameter) for all species.

For habitat characterization, soil samples were collected along an 80 m-transect, randomly delineated in each habitat. For *C. flamarioni* and *C. minutus* sampling was placed in Xangri-lá (29°47′S; 50°01′W) and Osório (29°31′S; 50°32′W) Municipalities, in southern Brazil. For *C. australis* and *C. talarum*, sampling sites were located in Necochea Municipality (38°03′S; 57°49′W and 38°02′S; 57°56′W, respectively), in Argentina. Soil samples were taken from the surface in every 10 m of transects and stored in 15 ml tubes. Additionally, eight samples were randomly taken from the burrowing system of each species for sampling comparison of underground vs aboveground. A total of 64 samples were individually placed in Petri dishes and dehydrated at 58 °C for 24 h. For plant coverage analysis, a specific area was photographed in each sampling stations of *C. minutus* and *C. flamarioni*, using a 1 m tape measure at the center of the image as a reference, in order to standardize the area (1 m^2). The percentage of plant coverage was estimated using the Braun-Blanquet method (1932). Previously published data from *C. australis* and *C. talarum* were taken from Cutrera et al. (2010).

Normal distribution of variables was tested using the Kolmogorov-Smirnov test, which is suitable for small sample sizes (Steinskog et al. 2007). Also, the heterogeneity of variance was tested with Bartlets test. Most of the data fit in a normal curve; however, significant heterogeneity variance was found. Thus, the data were treated as nonparametric. For comparisons in dorsal, flank, and ventral regions for differences in the distribution (bandwidth) and density (color) of pigment deposited in hair and pelage, the Kruskal-Wallis nonparametric test was used, followed by Dunn's multiple paired comparisons; the p-value (<0.05) was adjusted for multiple comparisons using the Bonferroni. Also, this test was used to compare microhabitat characteristics (soil coloration and plant cover) among species. To test the existence of an association between soil and pelage (dorsal, flank, and ventral) color, a simple linear regression analysis was used. Statistical analyzes were performed using the software XLSTAT (Addinsoft). Results of bandwidth/hair width, densitometry analysis, and substrate color are presented using the box-plots graphical method, including minimum and maximum values, mean, first, and third quartiles; other values are presented as mean (χ) ± standard error (SE).

8.4 Phenotypic Variation: Pigment Distribution and Density

In the dorsal pelage, tuco-tucos have the agouti hair type, presenting the banding pattern with black and yellow pigments alternately deposited (Fig. 8.4). In the flank and ventral hairs, the terminal band is absent. FLA has almost no pigment in ventral hairs; when present, it is composed only by pheomelanin. In the other three species, hairs from the flank and ventral regions have two-band patterns (subterminal and base), with pheomelanin in a lower density. Differences in the width of the terminal and subterminal bands were observed between light and dark phenotypes (Figs. 8.4, 8.5, and 8.6). TAL and MIN have the proportional widest terminal band and the shortest subterminal band in dorsal hairs; conversely, AUS and FLA present proportionally shortest terminal width and widest subterminal band in such region (Fig. 8.6). Contrary, the subterminal band in flank and ventral hairs did not vary significantly (also in proportion) between light and dark phenotypes (Fig. 8.6).

Differences in dorsal pelage coloration were identified among species of tuco-tucos, also within the light and dark phenotypes (Fig. 8.7): TAL and MIN presented significantly higher pigment density compared to AUS and FLA. Thus, TAL represents the darkest phenotype, whereas FLA the lightest. In the flank, the dark phenotypes significantly differ to the light ones; within phenotypes, differences were found only for light pelages (Fig. 8.7). In the ventral region, there were no significant differences between light and dark phenotypes (TAL, MIN, AUS). However, FLA showed marked distinction to all other species (Figs. 8.4 and 8.7). Significant differences in the pigment density within the terminal band were found between phenotypes (Fig. 8.7): dark species presented lower values compared to light ones. Similarly, dark phenotypes had distinct values for the subterminal band compared to the light ones.

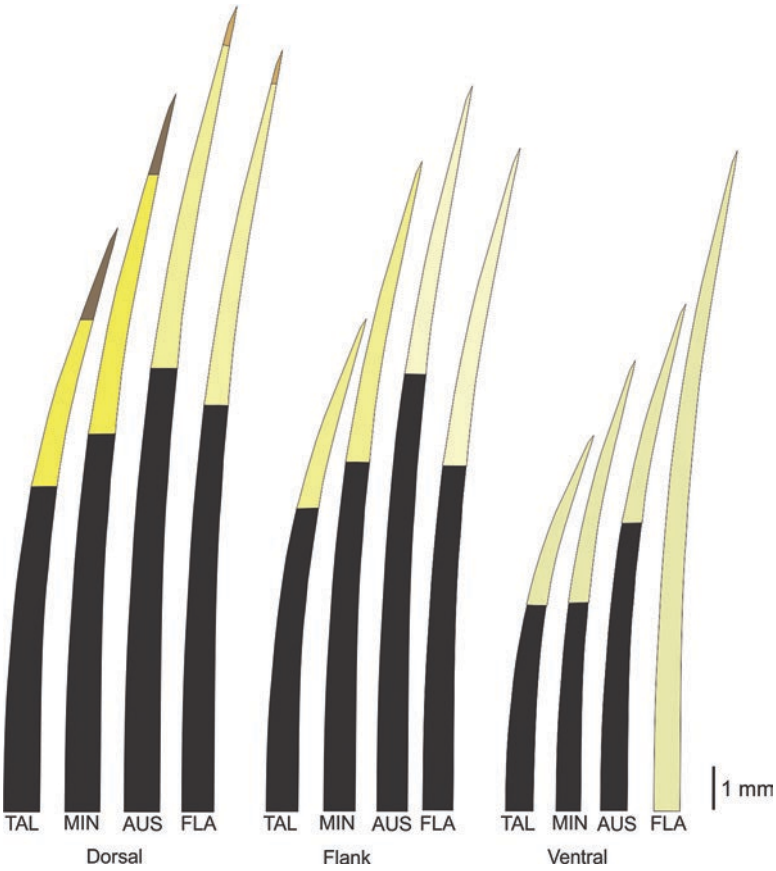

Fig. 8.4 Schematic representation of *Ctenomys* dorsal, flank, and ventral hairs, in scale. TAL, *C. talarum*; MIN, *C. minutus*; AUS, *C. australis*; FLA, *C. flamarioni*

These results suggest a remarkable influence of the density deposited in the terminal and subterminal bands on the overall coloration of an individual. Accordingly, the greatest functionality is supposed for the dorsal region in comparison to the flank (that is less intense) reinforced by the small variation. Results of pigment density in the ventral hairs corroborated this hypothesis, since no significant differences were found for the subterminal band and overall coloration between light and dark phenotypes (Fig. 8.7). Since the ventral region is relatively less exposed, the widest range of variation found might result from selective pressure relaxation. To test this assumption, the variances were estimated in several parameters analyzed (e.g., terminal and subterminal bandwidth, total hair width, pigment density within the terminal and subterminal bands in dorsal, flank, and ventral regions); eight of them presented heterogeneity among species. Not surprisingly, most occurred in parameters taken from the flank and ventral regions. In the dorsal, the terminal bandwidth shown the lowest values in light phenotypes. The dark phenotypes

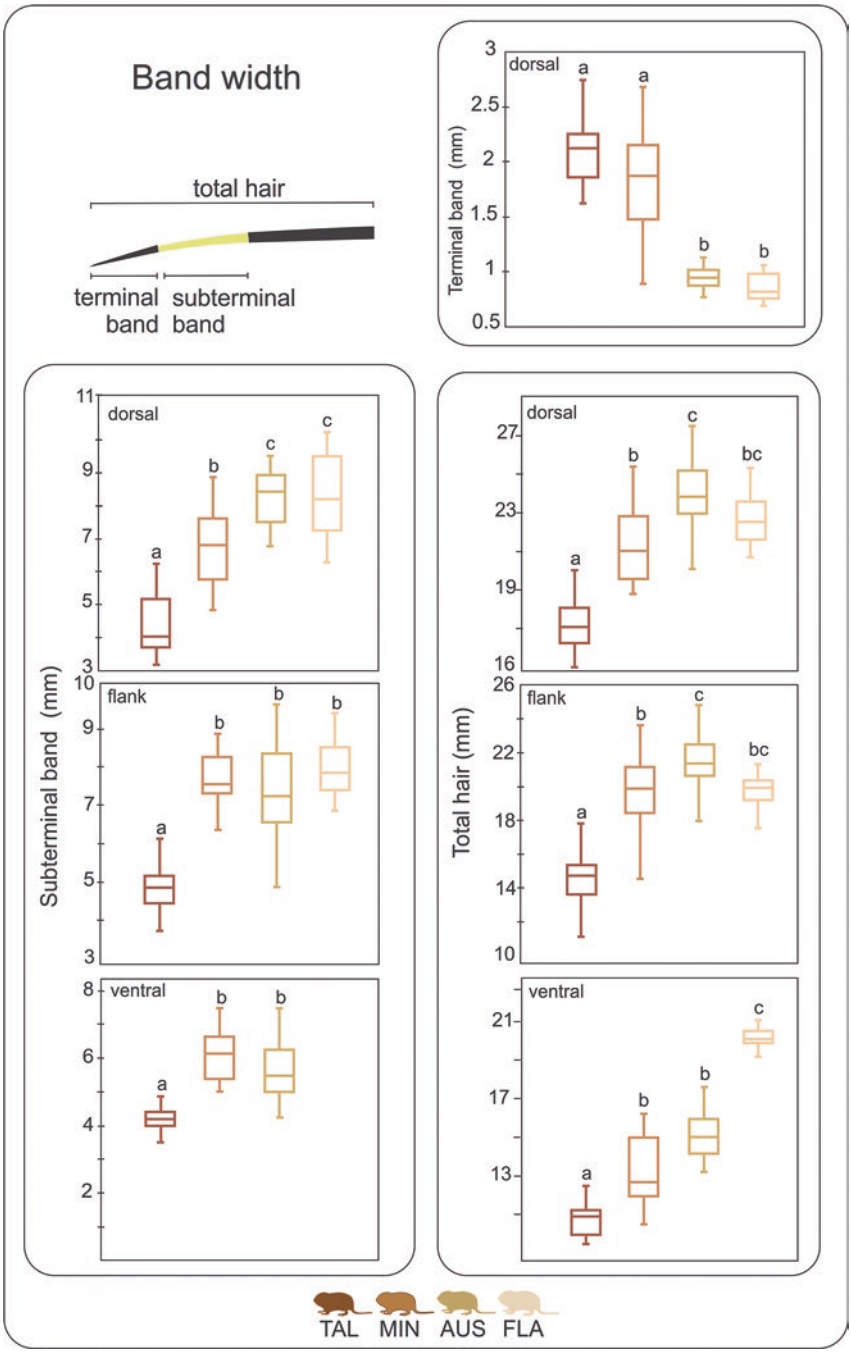

Fig. 8.5 Box-plots representing variability found in the terminal band (**a**), the subterminal band (**b**), and total hair (**c**) width in the four *Ctenomys* species: TAL, *C. talarum*; MIN, *C. minutus*; AUS, *C. australis*; FLA, *C. flamarioni*, showing the mean and first and third quartiles. Different letters over the box-plot indicate statistical significance between species, within each body region analyzed (dorsal, flank, and ventral). The colors indicate the phenotypes (see Fig. 8.2 and inlet schematic legend)

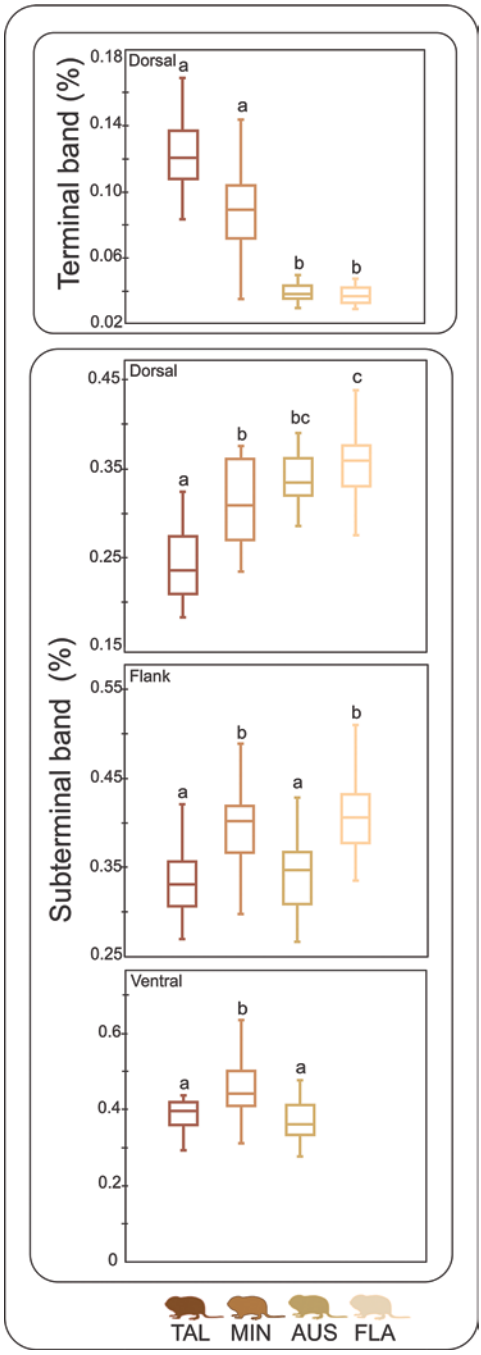

Fig. 8.6 Box-plots representing variability found in the proportional width of the terminal (**a**) and the subterminal bands (**b**) in the species of *Ctenomys*: TAL, *C. talarum*; MIN, *C. minutus*; AUS, *C. australis*; FLA, *C. flamarioni*, showing the mean, and the first and third quartiles. Different letters over the box-plot indicate statistical significance between species, within each body region analyzed (dorsal, flank, and ventral). The colors indicate distinct phenotypes (see Fig. 8.2 and inlet schematic legend)

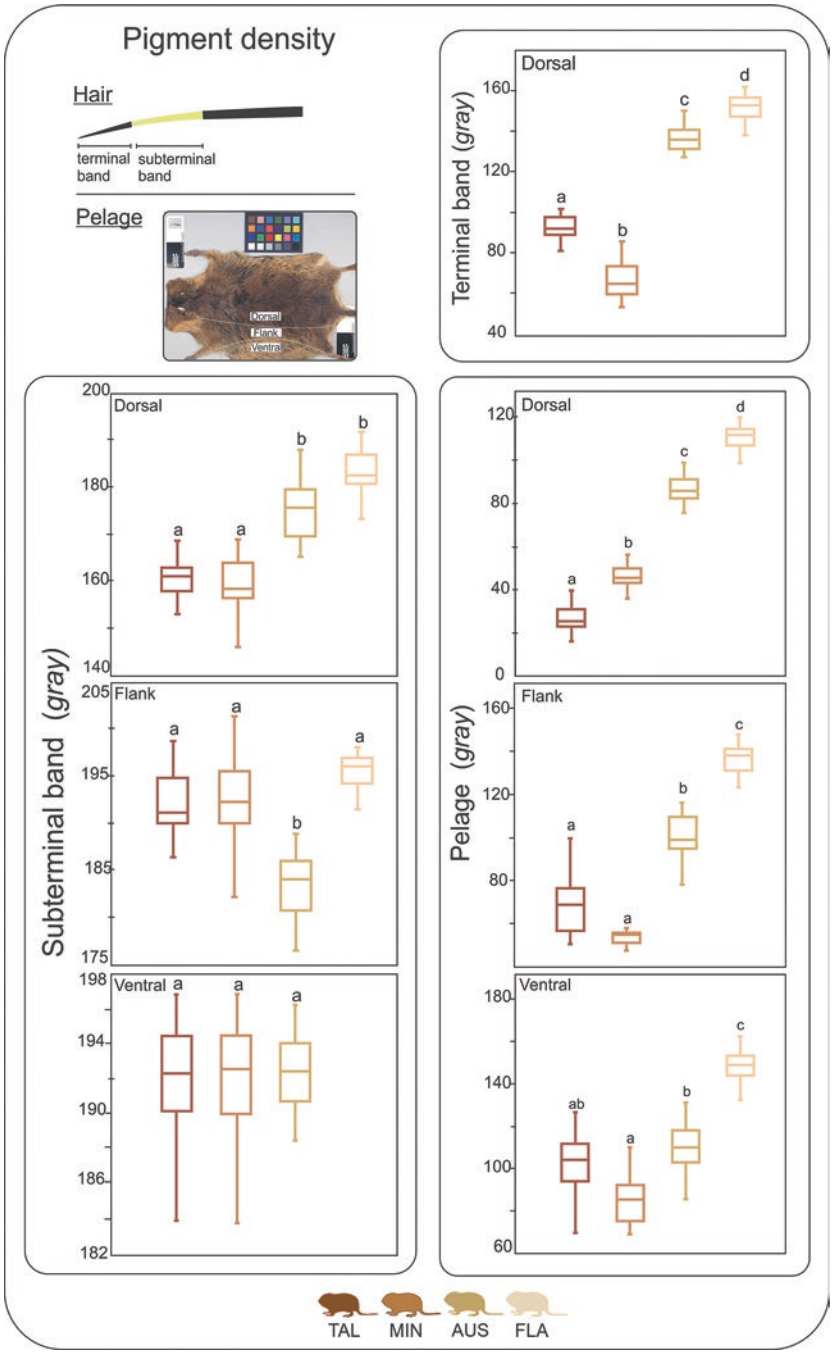

Fig. 8.7 Box-plots representing variability in hair density for the terminal (**a**) and subterminal (**b**) band and for the pelage (**c**) in the species of *Ctenomys*: TAL, *C. talarum*; MIN, *C. minutus*; AUS, *C. australis*; FLA, *C. flamarioni*, showing the mean and first and third quartiles. Different letters on the box-plot indicate statistical significance between species, within each body region analyzed (dorsal, flank, and ventral). The colors indicate distinct phenotypes (see Fig. 8.2 and inlet schematic legend)

showed 4–10 times the greatest variance compared to light for terminal bandwidth; therefore, the variation might be constrained in markedly cryptic light phenotypes, suggesting greater selective pressure (Table 8.1). Contrary, in the densitometry parameter the subterminal band showed similar variance in light and dark phenotypes, thus indicating less influence on the overall coloration comparatively to the terminal bandwidth. Significant differences in soil coloration were found between the two habitats (Fig. 8.8). No significant differences were observed between surface and burrow soil samples within sandy fields (TAL, $P = 0.48$; MIN, $P = 0.35$) and dunes (AUS, $P = 0.06$; FLA, $P = 0.06$), allowing sufficient representativeness of samples from transects. Similar to dorsal coat color, soil from TAL microhabitat had a higher density (i.e., lower values), whereas for FLA indicated the lowest density (i.e., higher values) (Table 8.2).

Additionally, linear regression analysis indicated a strong association ($R^2 = 0.87$; $P < 0.001$) of soil with dorsal and flank coat-color ($R^2 = 0.71$; $P < 0.001$), and moderate association with ventral ($R^2 = 0.57$; $P < 0.001$), which is mainly influenced by FLA pelage values in relation to other species (Fig. 8.9). For plant coverage, two markedly distinct groups of values were recovered, one representing sandy fields and the other sandy dunes (Table 8.2). Estimates from sandy fields were twice as high as those in sandy dunes, and did not differ significantly ($P > 0.05$) between similar microhabitats (Table 8.2).

8.5 Cryptic Coloration in *Ctenomys*

Tuco-tucos have a predominantly fossorial habit; though they are also active aboveground, particularly foraging in close to burrows (Comparatore et al. 1991; Busch et al. 2000). In particular, a high frequency of mobility was described for TAL (Busch et al. 1989), AUS (Vassalo et al. 1994), and FLA (Fernández-Stolz et al. 2007; Stolz 2006). Contrary to *Spalax*, in which aboveground exposure is recognized as accidental (Heth 1991), the regular activity in open areas indicates that predation might be more common in *Ctenomys* than any other subterranean lineage.

Ctenomids are often preyed on by several vertebrates, for example, burrowing owl (*Athene cunicularia*), pampas fox (*Pseudalopex gymnocercus*), lesser grison (*Galictis cuja*), white-eared opossum (*Didelphis albiventris*), Molina's hog-nosed skunk (*Conepatus chinga*), small hairy armadillo (*Chaetophractus vellerosus*), and Neuwied's lancehead (*Bothrops neuwidi*) (Busch et al. 2000).

Specifically, TAL and AUS represent 16% and 2%, respectively, of owl prey items (*Athene cunicularia*, *Asio flammeus*, and *Tyto alba*) (Vassalo et al. 1994), and such difference is attributed to markedly distinct body sizes (TAL ca. 118 g and AUS ca. 360 g). Predation in AUS occurred predominantly in subadult individuals, likely due to constrain of the predator in carrying out the prey (Vassalo et al. 1994). However, there is no data on the influence of cryptic behavior in these, or any other, *Ctenomys* species preventing predation (i.e., differential survival), linking to microhabitat selection.

Table 8.1 Analysis of significant variance among *Ctenomys talarum* (TAL), *Ctenomys minutus* (MIN), *Ctenomys australis* (AUS), and *Ctenomys flamarioni* (FLA) for different hair and pelage parameters

Parameter	Species	Var.	χ^2_{calc}	*P*
Terminal width – dorsal			45.72	<0.001
	TAL	8.24		
	MIN	26.16		
	AUS	1.19		
	FLA	2.83		
Total hair width – ventral			31.46	<0.001
	TAL	269.22		
	MIN	528.77		
	AUS	191.98		
	FLA	28.29		
Subterminal band width – flank			9.10	0.02
	TAL	22.25		
	MIN	28.27		
	AUS	31.85		
	FLA	76.89		
Subterminal band width – ventral			7.92	0.04
	TAL	15.31		
	MIN	15.41		
	AUS	4.68		
	FLA	8.83		
Subterminal band densitometry – dorsal			9.38	0.02
	TAL	16.85		
	MIN	57.85		
	AUS	55.32		
	FLA	25.74		
Subterminal band densitometry – flank			9.10	0.02
	TAL	22.25		
	MIN	28.27		
	AUS	31.85		
	FLA	76.89		
Subterminal band densitometry – ventral			7.92	0.04
	TAL	15.31		
	MIN	15.41		
	AUS	4.68		
	FLA	8.83		
Pelage densitometry – flank			16.58	0.001
	TAL	211.99		
	MIN	27.93		
	AUS	132.68		
	FLA	123.96		

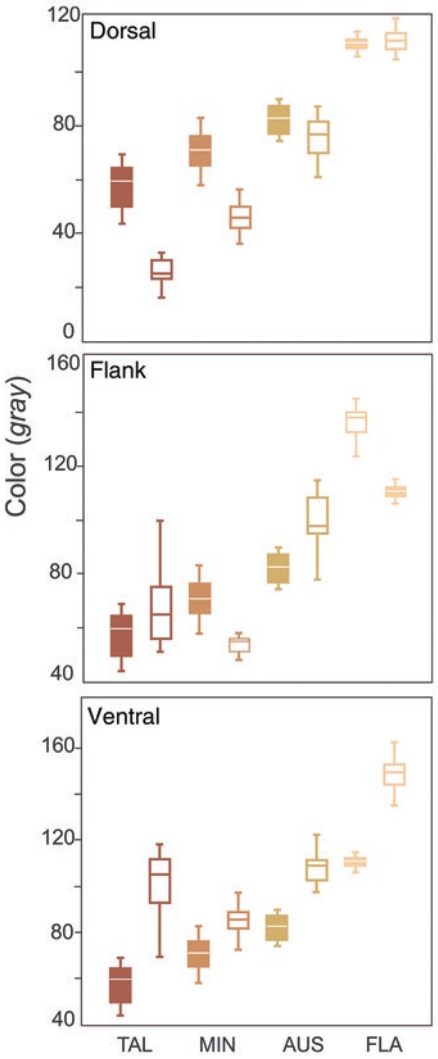

Fig. 8.8 Association of the color of the soil with an appearance in the species of *Ctenomys*: TAL, *C. talarum*; MIN, *C. minutus*; AUS, *C. australis*, FLA, *C. flamarioni*. (**a**) Box-plots representation of the variability found in the color of the soil (filled boxes) and dorsal pelage (non-filled boxes), showing the mean, and first and third quartiles. Different letters on the box-plot indicate statistical significance between species, within a given analyzed body region. The colors indicate distinct phenotypes (see Fig. 8.2 and inlet schematic legend)

Table 8.2 Estimates of mean ± standard error of soil color and plant cover in the microhabitats of *C. talarum* (TAL), *C. minutus* (MIN), *C. australis* (AUS), and *C. flamarioni* (FLA)

	Sandy fields		Sandy dunes		Kruskal–Wallis	
	TAL	MIN	AUS	FLA	K_{obs}	*P*
Soil coloration	58.14 ± 2.00	70.77 ± 1.78	82.03 ± 1.37	111.06 ± 0.85	53.37	< 0.001
Plant cover	57.18 ± 16.12	60.50 ± 12.31	25.31 ± 17.36	30.50 ± 10.72	43.56	< 0.001

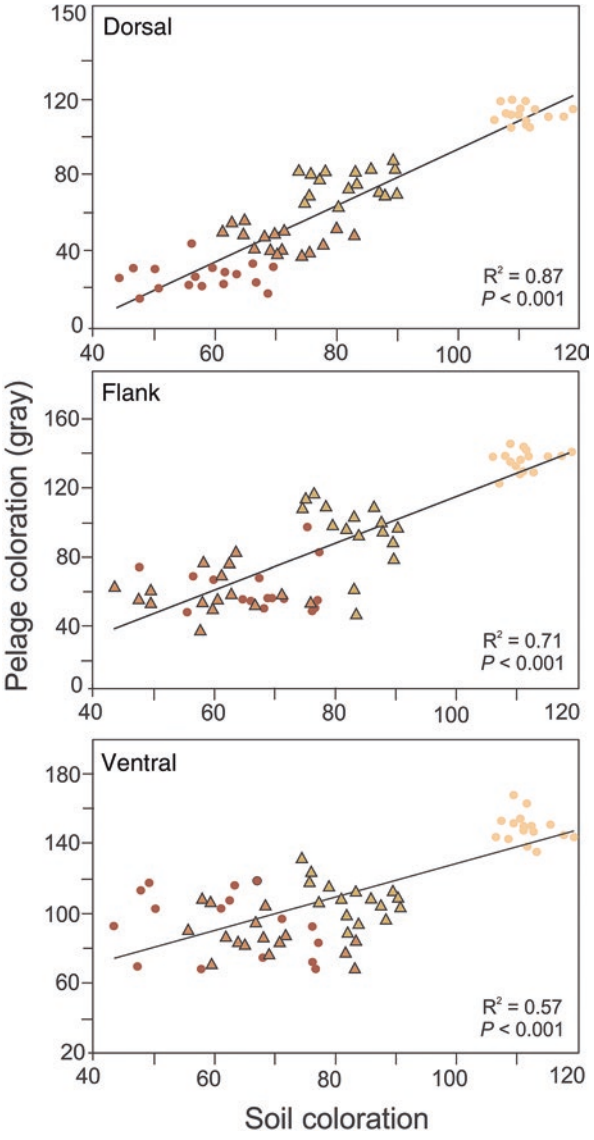

Fig. 8.9 Linear regression of soil color by coat color. The circles represent *Ctenomys talarum* and *Ctenomys flamarioni*, and the triangles *Ctenomys minutus* and *Ctenomys australis*. The colors indicate the phenotypes (see Fig. 8.2)

This study characterizes, for the first time, hair, pelage, and soil coloration in tuco-tucos. Specifically, data on distribution and density of pigment deposited in the terminal band of dorsal hairs highlights the biological relevance of such region also in this lineage. The smallest variances were found in the light phenotypes; therefore, dorsal coloration of AUS and FLA might be more restricted to vary. Accordingly, subtle changes in coat-color in these two species might contrast in pale dunes that present low plant cover (i.e., more exposed area), making them more susceptibility for predation capture, which remains to be tested. These species have the highest

dispersion rates in the genus (Stolz 2006; Vassalo 1998; Garcias et al. 2018), reinforcing the importance of cryptic behavior, assuming intense selective pressure in this system. In contrast, the widest range of variation was found in TAL and MIN, which may reflect the complexity of microhabitat with the highest plant cover, favoring camouflage despite an individual's overall coloration. Consequently, subtle changes in coloration of these dark phenotypes are unlikely to have an intense effect on differential survival. Moreover, a relevant aspect in MIN is the empirical observation that young individuals (2–3 months old) are lighter in color than adults (Fonseca 2003).

This corroborates the hypothesis of local adaptation, whose function is to protect young specimens that, in general, are the main target of predation (Vassalo et al. 1994; Lacey 2000). Although the species pairs occur in allopatry with areas of sympatry (Kubiak et al. 2015), they clearly present microhabitat selection, differing in relation to soil hardness, plant biomass, and plant cover (Vassalo 1998; Cutrera et al. 2010; Kubiak et al. 2015). AUS has a larger body size (Busch et al. 2000) and inhabits less resistant soils, whose primary productivity is reduced (Cutrera et al. 2010). Contrary, TAL occurs in rigid soils with dense and diverse plant cover (Malizia et al. 1991).

Previous studies have shown that the excavation energy cost is similar in these species, even in different soil types (Luna and Antinuchi 2007). Therefore, energy expenditure does not seem to be the main factor that might explain soil selection by TAL and AUS. Similarly, FLA inhabits less resistant sandy soils than MIN, and excavator activity and soil composition have non-significant differences between phenotypes (Rebelato 2006; Kubiak et al. 2015). Thus, the association in soil coloration with dorsal pelage observed suggests that crypsis is a potential factor influencing habitat dependence, with coat coloration being a significant variable, prior to selection by excavation activity and/or soil composition. Accordingly, each species might be constrained to its corresponding micro habitat due to the disadvantage of contrast with the background, especially given their high activity aboveground. Therefore, the similarity of ecological niches occupied by TAL-MIN and AUS-FLA are shreds of evidence of repeated local adaptation in dynamic habitats (e.g., Southern Brazil Coastal Plain; Tomazelli and Villwock 2000), in which population ecology and demography vary in time and space. In this context, the fixed ecological factor responsible for maintaining these local adaptations is potentially differential survival.

8.6 Convergent Evolution

AUS and FLA belong to the Mendocinus species group, defined by morphological characteristics (e.g., asymmetric sperm), karyotype ($2n$ = 47–48; similar G and C band patterns), and molecular data (Castillo et al. 2005; D'Elia et al. 1999; Freitas 1994; Lessa and Cook 1998; Massarini and Freitas 2005; Parada et al. 2011; Chapter 2, this volume). Such similarity of characters has raised questions such as whether

these two species, recognized as phylogenetically close related, share the same most recent common ancestor (Freitas 1994, 1995a; Fernández-Stolz 2007; Malizia et al. 1991; Mora et al. 2006). Comparative assessment of skull morphology revealed significant morphological differences (Fornel 2009; Massarini and Freitas 1995, 2005; Travi and Freitas 1984). Then, it was suggested that FLA might have split from an ancestral form from Argentina, by migration, isolation, and further differentiation of AUS (Freitas 1994; Massarini and Freitas 2005). This migration would have occurred in the Pleistocene when the coastal plain was under arid conditions, approximately 100 km wider than at present; thus, the River Plate was not a relevant geographical barrier (Corrêa et al. 1992). However, current evolutionary analysis of the Mendocinus group place AUS closer to *C. mendocinus* (Parada et al. 2011). In this context, the convergence observed in AUS and FLA in terms of body size and light coloration might represent repeated evolution in the occupation of coastal environments instead of the strict retention of an ancestral character (Fig. 8.10). Interestingly, a pale pelage coloration is found in museum specimens of *C. mendocinus* deposited in the Museum of Vertebrate Zoology (see Fig. 8.1). The Mendocinus species group might have a common genetic background that underlies the quality and quantity of pigment deposited in the hair, which should be further investigated. Since the phenotypic variation of *C. mendocinus* is intraspecific, it is a candidate species to explore the hair pattern together with the Agouti gene in populations of both forms, to understand the genetic basis of this adaptive phenotype in *Ctenomys*.

The increase in the subterminal band (and consequent reduction in the terminal band) of the hair, as well as the lower pigment density, provide the dilution of the overall color of the individuals, making them paler (i.e., the blond color of AUS and

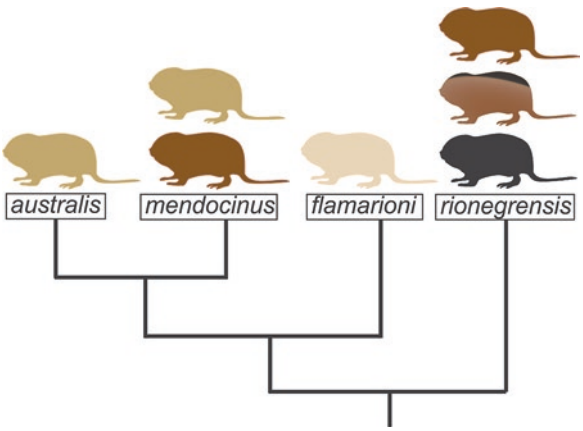

Fig. 8.10 Variation of pelage color within the mendocinus species group under a phylogenetic context (for details see D' Elia et al. Chapter 2, this volume), including *Ctenomys australis*, *Ctenomys flamarioni*, *Ctenomys mendocinus* and *Ctenomys rionegrensis*. Phenotypes observed are: blond (*australis* and *mendocinus*), brown (*mendocinus* and *rionegrensis*), pale blond (*flamarioni*), dark-backed (*rionegrensis*) and melanic (*rionegrensis*)

FLA) (Fig. 8.9). However, there are significant differences at fine-scale between light phenotypes. AUS has a dark-blond pelage compared to FLA, whose correspondence is directly reflected in its darker soil. Thus, the data generated in this study indicate convergent mechanisms of crypsis in the same ecological context. Similarly, the data showed parallel evolutionary trajectories in the generation of dark phenotypes in TAL and MIN. These species are phylogenetically distant (Parada et al. 2011), and converge in terms of body size, microhabitat, coat-color, and soil. Interestingly, mechanisms used to generate dark phenotypes are identical: increased eumelanin distribution at the tip of the hair (longer terminal width), and pheomelanin density in the subterminal band. These two small changes generate potentially advantageous phenotypes on dark soils, in which small variation is linked to the ability to turn into cryptic in a more complex environment.

8.7 Final Remarks

The determinants of color patterns in animals are still poorly understood, but three main functions are suggested: intraspecific communication, predator avoidance, and thermoregulation (Endler 1978). Tuco-tucos have a predominantly solitary habit and rarely are in direct contact with other individuals of the same species, suggesting that chemical and vocal communication rule the reproductive behavior in these animals (Francescoli 1999; Zenuto et al. 2004). Thus, it is assumed that coloration has little significant involvement in communication. Conversely, the results of this study suggest that pelage phenotypes of TAL, MIN, AUS, and FLA have an evolutionary significance of predator evasion, possibly also contributing to better thermoregulation (see Cutrera and Antinuchi 2004). The function of cripsis is reinforced by the differences in coat color in each of the four species, converging in parallel to two groups: light and dark phenotypes. Also, additional support comes from the strong association between *Ctenomys* dorsal pelage and soil coloration. Differences in plant cover of the four habitats corroborate this hypothesis, as they also show variation at the macroecological level, contributing to a fine-tuning of unique local adaptation of each species. Thus, the data allow to propose that natural selection may be the main evolutionary factor responsible for convergence in tuco-tucos. The existence of specific areas of sympatry in the distributional range of TAL-AUS (Reig et al. 1990; Contreras and Reig 1965) and MIN-FLA (Freitas 1995a; Kubiak et al. 2015) led to ask how the cryptic phenotypes behave when in contrasting habitat background, i.e., when opportunistically the dark phenotype occupy the sandy dunes, and light phenotype the sandy fields. The recent discovery of hybrids between FLA and MIN (Kubiak et al. 2015, 2020) reinforce the existence of admixture between phenotypes and habitats. Do the color phenotypes in contrasting soils have disadvantage comparatively to the cryptic ones? Will the disadvantage, if it exists, be higher in open habitats than in plant-covered fields? Quantitative studies involving controlled experiments, particularly using these natural laboratories of sympatry, are fundamental to evaluate rates of predation (i.e., prey capture) associated

with cryptic behavior in these species, which will clarify the effect of coloration on the differential survival of adaptive phenotypes.

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Appendix

Ctenomys specimens used:

C. talarum: UNMDP4; UNMDP5; UNMDP6; UNMDP7; UNMDP8; UNMDP9; UNMDP10; UNMDP11; UNMDP12; UNMDP13; UNMDP14; UNMDP15; UNMDP16; UNMDP17; MCNLS 93-1; MCNLS 93-3; MCNLS 93-2 UNMDP; MCNLS. *C. minutus*: TR579; TR639; TR640; TR641; TR642; TR643; TR644; TR645; TR646; TR647; TR648; TR649; TR650; TR651; TR652; TR653; TR654; TR655; TR656; TR657; TR1201; TR1202; TR1203; TR1207; TR1212; TR1219; TR1220; TR1221; TR1222; TR1225; LAMI2; TR1125; TR1126; TR1128; TR1129; TR1130; TR1132; TR1133; TR1137; TR1231; *C. australis*: UNMDP 1-1; UNMDP 1-2; UNMDP 1-3; MCNLS 81-1; MCNLS 82-22; MCNLS 82-67; MCNLS 82-68; MCNLS 82-69; MCNLS 82-71; MCNLS 82-238; MCNLS 82-239; MCNLS 82-240; MCNLS 82-241; MCNLS 82-242; MCNLS 82-243; MCNLS 82-244; MCNLS 82-245; MCNLS 84-20; MCNLS 84-23; MCNLS I-737; MCNLS I-740; MCNLS I-1044; MCNLS 1; MCNLS 2; MCNLS 4; UNMDP 37; UNMDP 38; UNMDP 39, UNMDP; MCNLS; *C. flamarioni*: PUC278; TR449; TR473; TR474; TR475; TR477; TR482; TR483; TR488; TR491; TR493; TR495; TR496; TR497; TR500; TR1152; TR1153; TR1154; DZRS01; G123; PUC408; TR476; TR478; TR479; TR480; TR484; TR485; TR489; TR490; TR494; TR498; TR499; TR1271; TR1272; TRNI1, TRNI2.

Literature Cited

Bidau CI (2015) Ctenomyidae. Ctenomys. In: Patton J, Pardiñas FU, D'Elía G (eds) Mammals of South America. Vol 2. Rodents. University of Chicago Press, Chicago, pp 818–877

Braun-Blanquet J (1932) Plant sociology: the study of plant communities. McGraw-Hill Publications in the Botanical Sciences, New York

Bultman SJ, Michaud EJ, Woychik RP (1992) Molecular characterization of the mouse agouti locus. Cell 71:1195–1204

Busch C, Malizia AI, Scaglia AO, Reig AO (1989) Spatial distribution and attributes of a population of *Ctenomys talarum* (Rodentia: Octodontidae). J Mammal 70:204–208

Busch C, Antinuchi CD, Valle CJ, Kittle MJ, Malizia AI, Vassalo AI, Zenuto R (2000) Population ecology of subterranean rodents. In: Lacey EA, Patton JL, Cameron GN (eds) Life underground, the biology of subterranean rodents. University of Chicago Press, Chicago, pp 183–226

Castillo AH, Cortinas MN, Lessa EP (2005) Rapid diversification of South American tuco-tucos (*Ctenomys*; Rodentia, Ctenomyidae): contrasting mitochondrial and nuclear intron sequences. J Mammal 86:170–179

Comparatore VM, Agnudsdei M, Busch C (1991) Habitat relations in sympatric populations of *Ctenomys australis* and *Ctenomys talarum* (Rodentia: Octodontidae) in a natural grassland. Mamm Biol 57:47–55

Contreras JR, Reig OA (1965) Datos sobre la distribución del gênero *Ctenomys* (Rodentia, Octodontidae) em la zona costera de la provincia de Buenos Aires comprendida entre Necochea y Bahía Blanca. Physis xxv(69):169–186

Correa ICS, Baitelli R, Ketzer JM, Martins R (1992) Translação horizontal e vertical do nível do mar sobre a plataforma continental do Rio Grande do Sul nos últimos 17.500 anos BP. Anais III Congresso ABEQUA, pp 225–240

Cott HB (1940) Adaptive coloration in animals. Methuen, London

Cutrera AP, Antinuchi CD (2004) Cambios en el pelaje del roedor subterráneo *Ctenomys talarum*: posible mecanismo térmico compensatório. Rev Chil Hist Nat 77:235–242

Cutrera AP, Mora MS, Antenucci CD, Vassallo AI (2010) Intra- and interspecific variation in home-range size in sympatric tuco-tucos, *Ctenomys australis* and *C. talarum*. J Mammal 91:1425–1434

D'Elia G, Lessa EP, Cook JA (1999) Molecular phylogeny of tuco-tucos, genus *Ctenomys* (Rodentia: Octodontidae): evaluation of the mendocinus species group and the evolution of asymmetric sperm. J Mamm Evol 6:19–38

Dice L, Blossom PM (1937) Studies of mammalian ecology in southwestern North America, with special attention to the colors of the desert mammals. Carnegie Inst Wash Publ 485:1–25

Endler JA (1978) A predator's view of animal color patterns. In: Hecht MK, Steere WC, Wallace (eds) Evolutionary biology, vol 11. Plenum Press, New York, pp 319–364

Fernández-Stolz GP (2007) Estudos evolutivos, filogeográficos e de conservação em uma espécie endêmica do ecossistema de dunas costeiras do sul do Brasil, Ctenomys flamarioni (Rodentia-Ctenomydae), através de marcadores moleculares microssatélites e DNA mitocondrial. Tese de doutorado, Universidade Federal do Rio Grande do Sul, Brasil. 193 pp

Fernández-Stolz GP, Stolz JFB, Freitas TRO (2007) Bottlenecks and dispersal in the tuco-tuco das dunas, *Ctenomys flamarioni* (Rodentia: Ctenomyidae), in Southern Brazil. J Mammal 88:935–945

Fonseca MB (2003) Biologia populacional e classificação etária do roedor subterrâneo tuco-tuco *Ctenomys minutus* Nehring, 1887 (Rodentia, Ctenomyidae) na planície costeira do Rio Grande do Sul, Brasil. Dissertação de mestrado, Universidade Federal do Rio Grande do Sul, Brasil. 110 pp

Fornel R (2009) Evolução na forma e tamanho do crânio no gênero *Ctenomys* (Rodentia: Ctenomydae). Tese de doutorado, Universidade Federal do Rio Grande do Sul, Brasil. 171 pp

Francescoli G (1999) A preliminary report on the acoustic communication in Uruguayan *Ctenomys* (Rodentia, Octodontidae): basic sound types. Bioacoustics 10:203–218

Freitas TRO (1994) Geographic variation of heterochromatin in *Ctenomys flamarioni* (Rodentia: Octodontidae) and its cytogenetic relationship with other species of the genus. Cytogenet Cell Genet 67:193–198

Freitas TRO (1995a) Geographic distribution and conservation of four species of the genus *Ctenomys* in southern Brazil. Stud Neotropical Fauna Environ 30:53–59

Freitas TRO (1995b) Geographic distribution if sperm forms in the genus *Ctenomys* (Rodentia: Octodontidae). Rev Bras Genét 18:43–46

Freitas TRO (2016) Family Ctenomyidae. In: Wilson DE, Lacher TEJ, Mittermeier RA (eds) Handbook of the mammals of the world: lagomorphs and rodents I. Lynx Editions, Barcelona, pp 499–534

Freitas TRO, Lessa EP (1984) Cytogenetics and morphology of *Ctenomys torquatus* (Rodentia, Octodontidae). J Mammal 65:637–642

Garcias FM, Stolz JFB, Fernández GP, Kubiak BB, Bastazini VAG, Freitas TRO (2018) Environmental predictors of demography in the tuco-tuco of the dunes (*Ctenomys flamarioni*). Mastozool Neotrop 25(2):293–304

Gonçalves GL, Freitas TRO (2009) Intraspecific variation and genetic differentiation of the collared tuco-tuco (Ctenomys torquatus) in Southern Brazil. J Mammal 90(4):1020–1031

Gonçalves GL, Hoekstra HE, Freitas TRO (2012) Striking coat colour variation in tuco-tucos (Rodentia: Ctenomyidae): a role for the melanocortin-1 receptor? Biol J Linn Soc 105:665–680

Heth G (1991) Evidence of above-ground predation and age determination of the prey in subterranean mole rats (*Spalax ehrenbergi*) in Israel. Mammalia 55:529–542

Heth G, Beiles A, Nevo E (1988) Adaptive variation of pelage color within and between species of the subterranean mole rat (*Spalax ehrenbergi*) in Israel. Oecologia 74:617–622

Hoekstra HE, Nachman M (2006) Coat color variation in Rock Pocket Mice (*Chaetodipus intermedius*): from genotype to phenotype. In: Lacey E, Myers P (eds) Mammalian diversification: from chromosomes to phylogeography (A celebration of the career of James L. Patton), vol I33. University of California Publications, Zoology, Berkeley

Hoekstra HE, Hirschmann RJ, Bundey RA, Insel PA, Crossland JP (2006) A single amino acid mutation contributes to adaptive beach mouse color pattern. Science 313:101–104

Ingles LG (1950) Pigmental variations in populations of pocket gophers. Evolution 4:353–357

Jackson IJ (1997) Homologous pigmentation mutations in human, mouse and other model organisms. Hum Mol Genet 6:1613–1624

Jackson IJ, Budd P, Horn JM, Johnson R, Raymond S, Steel K (1994) Genetics and molecular biology of mouse pigmentation. Pigment Cell Res 7:73–80

Kennerly TE Jr (1954) Local differentiation in the pocket gopher (*Geomys personatus*) in southern Texas. Tex J Sci 6:297–329

Kennerly TE Jr (1959) Contact between the ranges of two allopatric species of pocket gophers. Evolution 13:247–263

Krupa JJ, Geluso KN (2000) Matching the color of excavated soil: cryptic coloration in the plains pocket gopher (*Geomys bursarius*). J Mammal 81:86–96

Kubiak BB, Galiano D, Freitas TRO (2015) Sharing the space: distribution, habitat segregation and delimitation of a new sympatric area of subterranean rodents. PLoS One 10:e0123220

Kubiak BB, Maestri R, Almeida TS, Borges LR, Galiano D, Fornel R, Freitas TRO (2018) Evolution in action: soil hardness influences morphology in a subterranean rodent (Rodentia: Ctenomyidae). Biol J Linn Soc 125:766–776

Kubiak BB, Kretschmer R, Leipnitz LT, Maestri R, Almeida TS, Borges LR, Galiano D, Pereira JC, Oliveira EHC, Ferguson-Smith MA, Freitas TRO (2020) Hybridization between subterranean tuco-tucos (Rodentia, Ctenomyidae) with contrasting phylogenetic positions. Sci Rep 10:1502

Lacey EA (2000) Spatial and social systems of subterranean rodents. In: Lacey EA, Patton JL, Cameron GN (eds) Life underground: the biology of subterranean rodents. University of Chicago Press, Chicago/London, pp 257–293

Lacey EA, Patton JL, Cameron GN (2000) Life underground: the biology of subterranean rodents. University of Chicago Press, Chicago

Langguth A, Abella A (1970) Sobre una poblacion de tuco-tucos melanicos (Rodentia-Octodontidae). Acta Zool Lilloana 27:101–108

Lessa EP, Cook JA (1998) The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. Mol Phylogenet Evol 9:88–99

Linnen CR, Kingsley EP, Jensen JD, Hoekstra HE (2009) On the origin and spread of an adaptive allele in deer mice. Science 325:1095–1098

Lopes CM, De Barba M, Boyer F, Mercier C, da Silva Filho PJ, Heidtmann LM, Galiano D, Kubiak BB, Langone P, Garcias FM, Gielly L, Coissac E, de Freitas TRO, Taberlet P (2015) DNA metabarcoding diet analysis for species with parapatric vs sympatric distribution: a case study on subterranean rodents. Heredity 114:525–536

Luna F, Antinuchi CD (2007) Energy and distribution in subterranean rodents: sympatry between two species of the genus *Ctenomys*. Comp Biochem Physiol A Comp Physiol 147:948–954
Malizia AI, Vassallo AI, Busch C (1991) Population and habitat characteristics of two sympatric species of *Ctenomys* (Rodentia: Octodontidae). Acta Theriol 36:87–94
Manceau M, Domingues VS, Linnen CR, Rosenblum EB, Hoekstra HE (2010) Convergence in pigmentation at multiple levels: mutations, genes and function. Philos Trans R Soc B 365:2439–2450
Massarini AI, Freitas TRO (1995) Análise morfológica e citogenética de *C. flamarioni* e *C. australis* – duas espécies ecologicamente equivalentes (Rodentia: Octodontidae). Rev Bras Genet 18:487
Massarini AI, Freitas TRO (2005) Morphological and cytogenetics comparison in species of the mendocinus-group (genus *Ctenomys*) with emphasis in *C. australis* and *C. flamarioni* (Rodentia: Ctenomyidae). Caryologia 58:21–27
McRobie H, Thomas A, Kelly J (2009) The genetic basis of melanism in the Gray Squirrel (Sciurus carolinensis). J Hered 100:709–714
Mora MS, Lessa EP, Kittlein MJ, Vassallo AI (2006) Phylogeography of the subterranean rodent *Ctenomys australis* in sand-dune habitats: evidence of population expansion. J Mammal 87:1192–1203
Nachman MW, Hoekstra HE, D'Agostino SL (2003) The genetic basis of adaptive melanism in pocket mice. Proc Natl Acad Sci USA 100:5268–5273
Parada A, D'Elía G, Bidau CJ, Lessa EP (2011) Species groups and the evolutionary diversification of tuco-tucos, genus *Ctenomys* (Rodentia: Ctenomyidae). J Mammal 92:671–682
Rebelato GS (2006) Análise ecomorfológica de quatro espécies de *Ctenomys* do sul do Brasil (Ctenomyidae – Rodentia). Brasil. Dissertação de mestrado, Universidade Federal do Rio Grande do Sul, Brasil. 146 pp
Reig O, Busch C, Ortells M, Contreras J (1990) An overview of evolution, systematics, population biology, cytogenetics, molecular biology and speciation in Ctenomys. Prog Clin Biol Res 335:71–96
Robbins LS, Nadeau JH, Johnson KR, Kelly MA, Roselli-Rehfuss L et al (1993) Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. Cell 72:827–834
Singaravelan N, Pavlicek T, Beharav A, Wakamatsu K, Ito S et al (2010) Spiny mice modulate eumelanin to pheomelanin ratio to achieve cryptic coloration in "evolution canyon," Israel. PLoS One 5:e8708
Singaravelan N, Raz S, Tzur S, Belifante S, Pavlicek T et al (2013) Correction: adaptation of pelage color and pigment variations in Israeli subterranean blind mole rats, *Spalax Ehrenbergi*. PLoS One 8(8):e69346. https://doi.org/10.1371/annotation/27bebc65-09c5-4c58-be6c-4f22c4fe0919
Siracusa LD (1994) The agouti gene: turned on to yellow. Trends Genet 10:423–428
Steiner CC, Weber JN, Hoekstra HE (2007) Adaptive variation in beach mice produced by two interacting pigmentation genes. PLoS Biol 5:1880–1889
Steinskog DJ, Tjøstheim DB, Kvamstø NG (2007) A cautionary note on the use of the Kolmogorov–Smirnov test for normality. Mon Weather Rev 135(3):1151–1157
Stolz JFB (2006) Dinâmica populacional e relações espaciais do tuco-tuco das dunas (*Ctenomys flamarioni*) (Rodentia-Ctenomyidae) na Estação Ecológica do Taim-RS/Brasil. Dissertação de mestrado, Universidade Federal do Rio Grande do Sul, Brasil. 71 pp
Sumner FB (1934) Does 'protective coloration' protect? Results of some experiments with fishes and birds. Proc Natl Acad Sci USA 20:559–564
Tomazelli LJ, Willwock JA (2000) O Cenozóico no Rio Grande do Sul: geologia da planície costeira. In: Holz M, de Ros LF (eds) Geologia do Rio Grande do Sul. CIGO/UFRGS, Porto Alegre, pp 375–406
Travi VH, Freitas TRO (1984) Estudos citogenéticos e craniométricos de *Ctenomys flamarioni* e *Ctenomys australis* (Rodentia: Octodontidae). Ciên Cult 36:771

Vassallo AI (1998) Functional morphology, comparative behaviour, and adaptation in two sympatric subterranean rodents genus *Ctenomys* (Caviomorpha: Octodontidae). J Zool 244:415–427

Vassalo AI, Kittlein MJ, Busch C (1994) Owl predation on two sympatric species of tuco-tucos (Rodentia: Octodontidae). J Mammal 75:725–732

Wlasiuk G, Garza JC, Lessa EP (2003) Genetic and geographic differentiation in the Rio Negro tuco-tuco (*Ctenomys rionegrensis*): inferring the roles of migration and drift from multiple genetic markers. Evolution 57:913–926

Zenuto RR, Fanjul MS, Busch C (2004) Use of chemical communication by the subterranean rodent *Ctenomys talarum* (tuco-tuco) during the breeding season. J Chem Ecol 30:2111–2126