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Living on the edge: morphological and behavioral adaptations to a marginal high-elevation habitat in an arboreal mammal

Claudia TRANQUILLO,[1] Lucas Armand WAUTERS,[1,2] Francesca SANTICCHIA,[1]
Damiano PREATONI[1] and Adriano MARTINOLI[1]

[1]Environment Analysis and Management Unit - Guido Tosi Research Group - Department of Theoretical and Applied Sciences, Università degli Studi dell'Insubria, Varese, Italy and [2]Evolutionary Ecology Group, Department of Biology, University of Antwerp, Wilrijk, Belgium

Abstract

Habitats are characterized by different local environmental conditions that influence both behavior and morphology of species, which can result in habitat-dependent phenotypic differences among animals living in heterogeneous environments. We studied 3 alpine populations of Eurasian red squirrels (*Sciurus vulgaris*), 1 living in a marginal high-elevation habitat at the edge of the species' altitudinal distribution, and 2 occurring in higher-quality habitats. Here, we investigated whether squirrels living in the marginal area differed in 2 morphological parameters (body size and body mass) and/or in the expression of 4 personality traits estimated with an open field test and a mirror image stimulation test (activity, exploration, activity-exploration, and social tendency). Furthermore, we tested whether within-individual variance of the traits (behavioral plasticity) was higher in the edge habitat. Male squirrels in the edge habitat were smaller and weighed less than in the other study areas, while among females, size–habitat relationships were less marked. These sex-specific patterns were explained by a strong association between body mass and reproductive success in female squirrels. Squirrels in the marginal habitat were more active, explorative, and had a more social personality than in the other habitats. However, in contrast to our predictions, behavioral plasticity was smaller in the marginal habitat, but only for the trait exploration. Our results suggest that animals choose the habitat that best fits their personality, and that habitat-related differences in selective pressures may shape animals' morphology.

Key words: behavioral plasticity, marginal edge-habitat, morphology, personality, *Sciurus vulgaris*

Correspondence: Claudia Tranquillo, Environment Analysis and Management Unit - Guido Tosi Research Group - Department of Theoretical and Applied Sciences, Università degli Studi dell'Insubria, via J. H. Dunant 3, 21100 Varese, Italy.
Email: claudia.tranquillo@uninsubria.it

INTRODUCTION

Local environmental conditions, and their temporal changes, can exert selective pressures on animals, resulting in phenotypic variation among populations living in different habitats (Kawecki & Ebert 2004). These local phenotypic adaptations often refer to physiological or morphological traits subject to natural selection, but also to behavioral traits, that are assumed to be more flexible with animals capable of adjusting their behavior in response to fluctuations in environmental conditions (Bolnick *et al.* 2003; Holtmann *et al.* 2017). However, both theoretical and empirical studies on animal personality, defined as behavioral differences among individuals of a species that are consistent over time and across situations (e.g. Réale *et al.* 2007; Carter *et al.* 2013), suggest that behavioral plasticity may be restrained by an individual's personality type, and that animals select habitats that best fit their personality (Dall *et al.* 2004; Sih *et al.* 2004; Holtmann *et al.* 2017). In other words, personality traits may reduce an individual's ability to cope with certain environmental conditions and, consequently, result in non-random distribution of behavioral phenotypes across different habitats (Cote *et al.* 2010; Holtmann *et al.* 2017; Wauters *et al.* 2019).

For mammals living in marginal habitats on the edge of a species' distribution range, environmental conditions can be extreme and exert strong selective pressure on individuals, different from those acting in more central, higher-quality habitats, characterized by more abundant resources and, in some cases, less extreme weather conditions (Endler 1977; Rodrigues *et al.* 2010). This could result in habitat-dependent phenotypic differences in body size and/or personality for mammals living in heterogeneous environments (Wauters *et al.* 2007; Hewes *et al.* 2017; Brehm & Mortelliti 2021; Boone *et al.* 2022). Also, phenotypic differences between animals from marginal and high-quality areas may be due to individuals showing higher behavioral plasticity in populations living at the distribution edge (Dingemanse *et al.* 2010).

Depending heavily on tree seeds as their main food resource throughout most of the year, arboreal squirrels in temperate regions typically occur in resource-limited producer–consumer systems (Boutin & Larsen 1993; Koprowski 1994a; Boutin *et al.* 2006; Wauters *et al.* 2008). In heterogeneous landscapes, spatio-temporal variation in tree seed abundance varies greatly, creating a mosaic of habitats that differ in their quality for tree squirrel populations in terms of overall food availability as well as temporal fluctuations in the size of tree-seed crops (Koprowski 1994a,b; Salmaso *et al.* 2009; Di Pierro *et al.* 2011; Tranquillo *et al.* 2022). Moreover, morphological and/or behavioral differences and variation in personality traits are related to space and/or habitat use (Wauters *et al.* 2007, 2021; Boon *et al.* 2008) and to variation in survival and/or reproductive success (McAdam *et al.* 2002; Wauters *et al.* 2007; Santicchia *et al.* 2018). Therefore, tree squirrels are particularly interesting models to study habitat-related phenotypic differences in body size and/or personality for mammals living in heterogeneous environments.

Here, we used the Eurasian red squirrel (*Sciurus vulgaris*) to investigate if phenotypic variation differs between individuals from a population living in a marginal low-quality habitat on the edge of the elevational distribution and animals occurring in moderate to high-quality conifer forests at similar or lower elevations in the Italian Alps. As explained above, habitat quality refers to average seed-crop size and annual fluctuations in seed production of conifers, the main food resource for red squirrels in Alpine forests (Wauters *et al.* 2008; Salmaso *et al.* 2009; Rodrigues *et al.* 2010; Tranquillo *et al.* 2022 for detailed measurements of food abundance in these study sites).

Eurasian red squirrels occupying extreme, high-elevation habitat differ from conspecifics in higher quality habitats at lower elevations in population dynamics (Mari *et al.* 2008; Rodrigues *et al.* 2010) and space use (Romeo *et al.* 2010). Space use differs between high-elevation marginal habitat of dwarf mountain pine (*Pinus mugo*) and high-quality Scots pine (*Pinus sylvestris*) forest at lower-elevations (Romeo *et al.* 2010). In fact, home ranges and core areas are larger in the marginal habitat, with more pronounced sex differences in core area size (Romeo *et al.* 2010). In contrast, patterns of core area overlap and intrasexual territoriality are similar as in other populations from various habitat types (Wauters & Dhondt 1992; Lurz *et al.* 2000; Wauters *et al.* 2001; Di Pierro *et al.* 2011). However, low densities in the edge population (Rodrigues *et al.* 2010) result in higher female by males overlap in spring–summer, when males expand their home ranges during the mating season to increase access to estrus females (Romeo *et al.* 2010). Moreover, squirrels' spacing behavior responds more strongly to variation in food availability and/or density fluctuations in marginal than in high-quality habitats (Romeo *et al.* 2010; Wauters *et al.* 2021), suggesting apparent behavioral plasticity to respond to the ecological constraints in marginal habitats.

We measured morphological (body mass and hind foot length) and behavioral (exploration, activity, activity-exploration and social tendency) traits of red squirrels. Hind foot length is strongly correlated with total head and body length and thus is considered a reliable proxy for body size (Wauters & Dhondt 1989a). Behavioral traits have been shown to determine an animal's personality (Mazzamuto *et al.* 2019; Wauters *et al.* 2019; Santicchia *et al.* 2020a, 2021).

In particular, we predicted: (i) Differences in body size and mass between the habitats, with smaller squirrels of lower body mass in the marginal, poorer-quality

Table 1 Red squirrel density estimated using MNA and food abundance expressed in energy-content of conifer seeds ($MJ\cdot ha^{-1}$, mean ± SE), in the 3 study areas per year

	Cancano (54 ha) (0.35 $traps\cdot ha^{-1}$) ($n = 68$; ID = 34)		Bormio (92 ha) (0.22 $traps\cdot ha^{-1}$) ($n = 55$; ID = 36)		Valfurva (77 ha) (0.26 $traps\cdot ha^{-1}$) ($n = 226$; ID = 92)	
Year	Red squirrel density ($ind\cdot ha^{-1}$)	Food abundance ($MJ\cdot ha^{-1}$)	Red squirrel density ($ind\cdot ha^{-1}$)	Food abundance ($MJ\cdot ha^{-1}$)	Red squirrel density ($ind\cdot ha^{-1}$)	Food abundance ($MJ\cdot ha^{-1}$)
2016	0.17	128 ± 13	0.15	2301 ± 299	0.40	2863 ± 328
2017	0.20	294 ± 35	0.13	1605 ± 208	0.49	1361 ± 103
2018	0.20	243 ± 58	0.11	1939 ± 216	0.38	1559 ± 270
2019	0.07	234 ± 32	0.10	1273 ± 149	0.35	170 ± 19
2020	0.11	496 ± 84			0.25	5404 ± 597
2021	0.09	108 ± 23			0.19	47 ± 14

Size of the study areas (ha) and trap density ($traps\cdot ha^{-1}$) between brackets. Arena test sample sizes (n) and number of different animals (ID) between brackets, for each study areas.

habitat, where (based on earlier studies of habitat-related differences in foot length and body mass of red squirrels, Wauters *et al.* 2007) smaller squirrels may be advantaged since they have lower daily energy-requirements than larger and heavier individuals. Alternatively, squirrels may be smaller and weigh less in the poorer-quality habitat because of lower food intake. (ii) Differences in the expression of behavioral traits, with squirrels in the edge population having higher average levels of activity and exploration than in high-quality habitats, since active-explorative personality types should be better adapted at exploring low-abundance food resources (see also Spiegel *et al.* 2017). (iii) A more pronounced advantage of higher behavioral plasticity in edge than in high-quality habitats, resulting in higher within-individual variance of behavioral traits in the edge population (e.g. Dingemanse & Wolf 2013).

MATERIALS AND METHODS

Study areas, trapping, and handling

As part of a long-term research project on spatio-temporal variation in producer-consumer dynamics in pulsed resource systems, tree-seed abundance, the population dynamics, life-history, personality traits and space use of red squirrels were studied in 3 populations occupying different types of conifer forests (Wauters *et al.* 2007, 2008; Salmaso *et al.* 2009; Rodrigues *et al.* 2010; Santicchia *et al.* 2021; Tranquillo *et al.* 2022). These study areas, located in Lombardy, Northern Italy, are characterized by subalpine mixed conifer forests that differ in the dominant tree species. Cancano (CAN; 46°33′N, 10°15′E, elevation from 1940 m to 1970 m a.s.l.) extends over 54 ha in a high-elevation valley, and it is part of a monospecific forest of dwarf mountain pine, *Pinus mugo*, whose seeds are the only major food resource for red squirrels (Rodrigues *et al.* 2010; Romeo *et al.* 2010). Bormio (BOR; 46°27′N, 10°30′E, elevation from 1950 m to 2130 m a.s.l.; size 92 ha) is dominated by Arolla pine, *Pinus cembra* (73.2%), with larch, *Larix decidua* (17.8%) and scarce Norway spruce, *Picea abies* (8.0%), and dead trees (1.0%) (Wauters *et al.* 2007; Santicchia *et al.* 2021). Valfurva (VAL; 46°27′N, 10°31′E, elevation from 1650 m to 1870 m a.s.l.) is located on an east-north-east slope in Valfurva valley and covers 78 ha. It is characterized by Norway spruce (88.9%) with a lower presence of Arolla pine (6.2%), dead trees (3.0%), and larch (1.9%) (Wauters *et al.* 2007; Santicchia *et al.* 2021).

Based on elevation and habitat quality (average seed-crop size and annual fluctuations in seed production, see also Table 1), Cancano is a marginal edge-habitat, Bormio is a high-elevation habitat of moderate habitat-quality with harsh winter conditions, and Valfurva is a high-quality habitat, typical for the dominant subalpine conifer forest-type of the central Italian-Alps (Wauters *et al.* 2007; Rodrigues *et al.* 2010).

Population dynamics and life-history traits were studied by Capture-Mark-Recapture (CMR), and food abundance was estimated by annual cone counts in Cancano (2005–2021), Bormio (2002–2019), and Valfurva (2002–2021). Squirrel density was calculated by dividing the minimum number of animals known to be alive (MNA) by study area size (ha) (Wauters *et al.* 2008). Details of CMR methods and conifer seed-crop size are given elsewhere (Wauters *et al.* 2008; Salmaso *et al.* 2009; Rodrigues *et al.* 2010). Briefly, CMR sessions were carried out each year in May–June (spring–summer) and in September–October (autumn), using live traps (Tomahawk trap model 202, Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) placed with baling wire on tree trunks at breast height or on the ground (Wauters *et al.* 2008; Tranquillo *et al.* 2022). We pre-baited traps with hazelnuts once per week for about 1 month prior to trapping; trapping sessions lasted 4 to 5 days, and we checked traps 2 to 3 times per day to reduce the time a squirrel was confined in the trap. A trapped squirrel was flushed from the trap in a zipper-tube handling bag, and, at first capture, individually marked with a numbered metal ear tag (Monel 1005 1L1 National Band and Tag Co, Newport, Kentucky, USA). Sex and reproductive condition were determined based on external genitalia (details in Wauters *et al.* 2007; Tranquillo *et al.* 2022). Males were classified based on testes size and position (testes abdominal, testes scrotal); females as non-breeding, post-estrus (pregnant), or lactating. At each capture, an animal was weighed to the nearest 5 g with a spring balance (Pesola AG, Baar, Switzerland), and the length of the right hind foot (claws excluded) was measured (0.5 mm precision) with a thin wing ruler.

Personality quantification: arena test

We studied the personality of red squirrels performing the open field test (OFT; 4 min), to estimate an animal's activity and exploration in a novel environment, followed by the mirror image stimulation test (MIS; 3 min) to determine the degree of sociability or avoidance (social tendency), aggressiveness, and being alert toward a conspecific, as well as the tendency for expressing behaviors that define a combined activity-exploration trait (Mazzamuto *et al.* 2019; Wauters *et al.* 2019; Santicchia *et al.* 2020a, 2022). Both tests were carried out during the same capture event in a portable arena, placed within 20 m from the trap location, where a previously identified squirrel was released by opening a sliding door and allowing the animal to move from the handling bag into the arena (Mazzamuto *et al.* 2019; Santicchia *et al.* 2021, 2022). At the end of the experiment, the squirrel was released and the arena was cleaned with 90% ethylic alcohol. More details on the arena test are given in previous studies (Santicchia *et al.* 2021, 2022). The time that an individual spent in each behavioral state (behaviors defined in Table S1, Supporting Information) was calculated using the CowLog 3.0.2 software (Hänninen & Pastell 2009). Next, we applied the expert-based method, defined and validated by Mazzamuto *et al.* (2019), to obtain new scores for 7 personality-linked variables (Ethogram description and personality traits in Table S1, Supporting Information).

To check the assumptions of repeatability of the traits, squirrels were tested multiple times in different CMR sessions to have repeated measures (individuals tested multiple times: $n = 78$, mean ± SE = 2.63 ± 0.10, range = 2–9). Nevertheless, to increase the power of the test, squirrels with only one arena test were also included in the adjusted repeatability estimates (Martin *et al.* 2011; Santicchia *et al.* 2022).

Body size and body mass analyses

A total of 193 individuals (114 males and 79 females) were sampled to assess the differences in morphological traits (see also Results, *Differences in body size and body mass*). Squirrels were classified as juveniles or adults based on external genitalia and body mass (<225 g; Wauters *et al.* 2007 and references therein), and juveniles were excluded from the analyses to avoid any bias in relation to our predictions.

We explored the effects of study area and sex on hind foot length with a general linear model (GLM). Mean foot length of each individual was used as dependent variable ($n = 193$ different squirrels), adding study area and sex as categorical variables and the study area by sex interaction.

Next, we investigated whether study area affected variation in body mass. Since body mass fluctuates seasonally and with reproductive condition, and since effects of reproductive condition differ between the sexes (Wauters & Lens 1995; Wauters *et al.* 2007), we used Linear Mixed Models (LMM), with multiple body mass measurements for individual squirrels, for each sex separately. Body mass was used as dependent variable, hind foot length as explanatory continuous variable, and study area, season, and reproductive condition as fixed effects. We further included all 2-factor interactions with study area in the full model, and added squirrel identity as a random intercept to account for repeated measures over time. A model with compound symmetry correlation structure best fitted the data (based on Schwarz's Bayesian Information Criteria). We tested interactions and fixed effects using Type-III SS and performed model selection using a backward procedure based on *P*-values (eliminating parameters with partial $P > 0.15$). Degrees of freedom and standard errors of *F*- and *t*-tests were obtained using the Kenward–Rogers method (Verbeke & Molenberghs 2000; Wauters *et al.* 2007), and pairwise comparisons were based on differences between least square means (DLSM).

Personality analyses

A total of 162 squirrels (100 males and 62 females) were behaviorally tested (see also Results, *Behavioral differences*). To account for possible confounding effect due to modification in personality with developmental changes (Cabrera *et al.* 2021; see also Discussion for details), we excluded juvenile individuals from this dataset.

Since personality traits are defined as behaviors consistent over time and under different contexts (Réale *et al.* 2007; Carter *et al.* 2013) we first calculated the adjusted repeatability (R) of each expert-based group (see Table S2, Supporting Information). Repeatability was estimated using a LMM through the function *rpt*, with the R package *rptR* (Stoffel *et al.* 2017). We performed a model for each square-root-transformed value of expert-based group (activity, exploration and immobility from OFT and sociability, avoidance, alert and activity-exploration from MIS) as dependent variable. Likewise, we estimated the adjusted repeatability also for social tendency, a variable that combines avoidance and sociability scores (Pearson correlation coefficient sociability-avoidance: $r = -0.87$; $n = 349$; $P < 0.0001$) and measures the tendency to react more or less social or amenable to the mirror image (*Social tendency* = *log*[(*sociability score* + (1 − *avoidance score*))/2], Santicchia *et al.* 2020a).

Each model had squirrel identity (ID) as random intercept and sex, arena test order (first test was coded as 1, subsequent tests were coded as 0), year (from 2016 to 2021), and number of days since the previous arena test as fixed effects. As in previous studies (Santicchia *et al.* 2020a,b, 2021, 2022), we included in the models arena test order and number of days since the previous arena test, to account for habituation and temporal proximity between 2 arena tests (Dingemanse *et al.* 2012; Mazzamuto *et al.* 2019). The likelihood ratio test (LRT) was used to test for significance of the random intercept of each model (Martin & Réale 2008). Full repeatability model outputs are provided in Table S2, Supporting Information.

We then investigated whether behavioral traits expression changed between study areas using a Bayesian generalized linear mixed effects model based on a Markov Chain Monte Carlo algorithm with the R package *MCMCglmm* version 2.32 (Hadfield 2010). This model included 4 behavioral traits (activity and exploration from the OFT, activity-exploration and social tendency from MIS) as dependent variable, that were previously centered and scaled [(x_i – mean x)/SD x] with a Gaussian residual error distribution. Squirrel identity (ID) was added as random effect since repeated observations were present, and to estimate both among-individual and within-individual variation of the dependent variables. Study area, sex, body mass (centered and scaled), season (spring–summer [May–June–July]; autumn [September–October]) and arena test order were included as fixed effects. Posterior distribution of the model was based on 1 050 000 iterations with a burn-in of 50 000 iterations and thinning of 40, such that 25 000 iterations were used to obtain point estimates and 95% credibility intervals (CIs). We used an inverse-gamma uninformative prior, for the random effect and the residual variation (Wilson *et al.* 2010). We applied the Gelman–Rubin statistic (Gelman & Rubin 1992) and Geweke diagnostic (Geweke 1992) to confirm model consistency and convergence. Full model output is provided in Table S3, Supporting Information.

In a final step, to obtain the among-individual and within-individual variances and the estimates of the correlations among the behavioral traits for each study area, we performed other 3 MCMCglmm models using 3 restricted dataset (one for each study area), in which we included the same dependent variables, fixed effects and random effect as in the main model. This approach allowed us to estimate the differences between study areas in the among- and within-individual variances (Royauté & Dochtermann 2021; Santicchia *et al.* 2022). Statistical analyses were carried out using the software R (R Core Team 2020).

Ethical note

Trapping, marking and handling procedures and arena test experiments complied with the Guidelines for the treatment of animals in behavioral research and teaching (*Animal Behaviour*, 2020, 159, I–XI; https://doi.org/10.1016/j.anbehav.2019.11.002). Legal requirements according to the Italian Wildlife Protection and Hunting Law L.N. 157 from 1992 and fieldwork was approved by authorization decrees n. 11190 of 29/11/2013, n. 9523 of 15/10/2014, and n. 198 of 13/01/2017 from Direzione Generale Agricoltura, Regione Lombardia; and the permission Protocol N. 414 of 28/02/2014 of the Stelvio National Park.

Table 2 (a) Mean right hind foot length (±SD) of red squirrels in 3 populations in the Italian Alps; data by sex and for the sexes combined; sample size is given between brackets. (b) Mean body mass (±SD) of male and female red squirrels in 3 populations in the Italian Alps; sample size is given between brackets

	Females	Males	Both sexes
a) Foot length			
Valfurva	(46) 57.8 ± 1.1	(60) 58.0 ± 1.1	(106) 57.9 ± 1.1
Bormio	(17) 57.3 ± 0.9	(31) 57.5 ± 1.0	(48) 57.4 ± 0.9
Cancano	(16) 57.5 ± 0.9	(23) 56.5 ± 1.0	(39) 56.9 ± 1.1
b) Body mass			
Valfurva	(140) 315 ± 27	(275) 305 ± 18	(415) 308 ± 22
Bormio	(25) 299 ± 26	(74) 290 ± 19	(99) 293 ± 21
Cancano	(50) 311 ± 31	(100) 285 ± 16	(150) 294 ± 25

RESULTS

Differences in body size and body mass

Hind foot length in red squirrels ($n = 193$) varied between 54 and 61 mm (overall mean ± SD = 57.6 ± 1.1 mm). Foot length differed between study areas and there was a significant study area × sex interaction (Area effect $F_{2,187} = 11.24$; $P < 0.0001$; Sex effect $F_{1,187} = 1.27$; $P = 0.26$; area × sex $F_{2,187} = 4.88$; $P = 0.009$). Female squirrels in Valfurva were slightly larger than in Bormio (Table 2a, DLSM $P = 0.049$, all other pairwise comparisons $P > 0.30$). Males in Valfurva were larger than in Bormio (DLSM $P = 0.041$) while males in Cancano were the smallest (DLSM VAL-CAN $P < 0.0001$; BOR-CAN $P = 0.0008$; Table 2a). Consequently, there was no size difference between the sexes in the populations in Valfurva and Bormio, while in Cancano females were significantly larger than males (Table 2a: DLSM $P = 0.004$).

Body mass of subadult and adult red squirrels ($n = 664$, with repeated measures of 193 different animals) varied between 235 and 380 g (overall mean ± SD = 303 ± 24 g). For females we obtained 215 measures of 79 individuals (full LMM reported in Table S4, Supporting Information). In the final model, female body mass increased with foot length (estimate ± SE = 25.2 ± 4.7; $t_{79} = 5.38$; $P < 0.0001$), but the slope differed between study areas (foot length × study area $F_{2,92} = 4.37$; $P = 0.015$). The increase in body mass with foot length was stronger in the marginal habitat Cancano than in the high-quality habitat Valfurva (difference in slope 15.2 ± 5.2; $t_{82} = 2.91$; $P = 0.005$; Fig. 1). Overall, females weighed slightly more in Valfurva than in Bormio, with Cancano intermediate (Table 2b, area effect $F_{2,92} = 4.38$; $P = 0.015$; Fig. 1), and lactating females had a higher body mass than animals in post-estrus or non-breeding ($F_{2,191} = 25.9$; $P < 0.0001$, DLSM all $P < 0.01$).

For male red squirrels, we used 449 body mass measures of 114 individuals (full LMM reported in Table S4, Supporting Information). In the final model, male body mass increased with foot length (estimate ± SE = 7.4 ± 1.3; $t_{128} = 5.78$; $P < 0.0001$; Fig. 2) and on average males were heavier in Valfurva than in the other 2 areas (Table 2b; area effect $F_{2,100} = 6.32$; $P = 0.003$; DLSM VAL > BOR $P = 0.005$; VAL > CAN $P = 0.005$; BOR and CAN $P = 0.77$). Males in breeding condition weighed more than non-breeding males (estimate ± SE = 9.6 ± 2.7; $t_{426} = 3.57$; $P = 0.0004$), and body mass increased from spring–summer to autumn (estimate ± SE = 9.5 ± 2.7; $t_{421} = 3.57$; $P = 0.0005$).

Behavioral differences

We estimated the adjusted repeatability of the 7 expert-based traits and of social tendency, using a total of 349 arena tests on 162 different red squirrels (100 males and 62 females). Immobility, activity, and activity-exploration had moderate repeatability (R range: 0.28 to 0.37; see also Bell *et al.* 2009), while exploration and social tendency had low repeatability ($R = 0.14$ and 0.15, respectively; Table S2, Supporting Information). The repeatability of sociability, avoidance, and alert was low and not significant (Table S2, Supporting Information). Immobility was strongly and negatively correlated with activity ($r = -0.93$; $n = 349$; $P < 0.0001$); therefore, this trait was not included in the multivariate models.

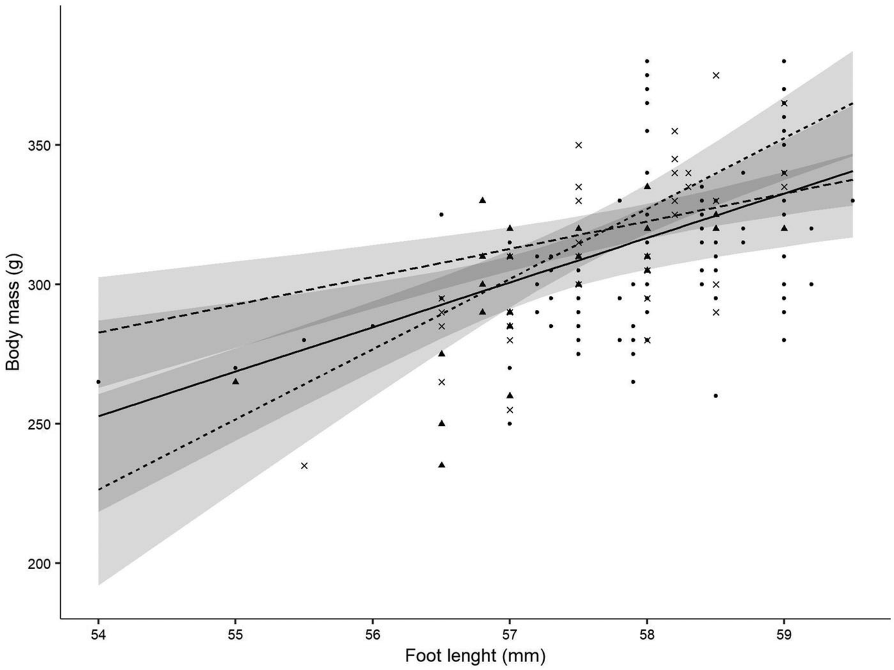

Figure 1 Relationship between foot length and body mass of female red squirrels in Bormio (solid line, triangles), Cancano (dotted line, crosses), and Valfurva (dashed line, circles). The lines represent the predicted relationships, shaded areas represent the 95% confidence intervals, and symbols represent observed values.

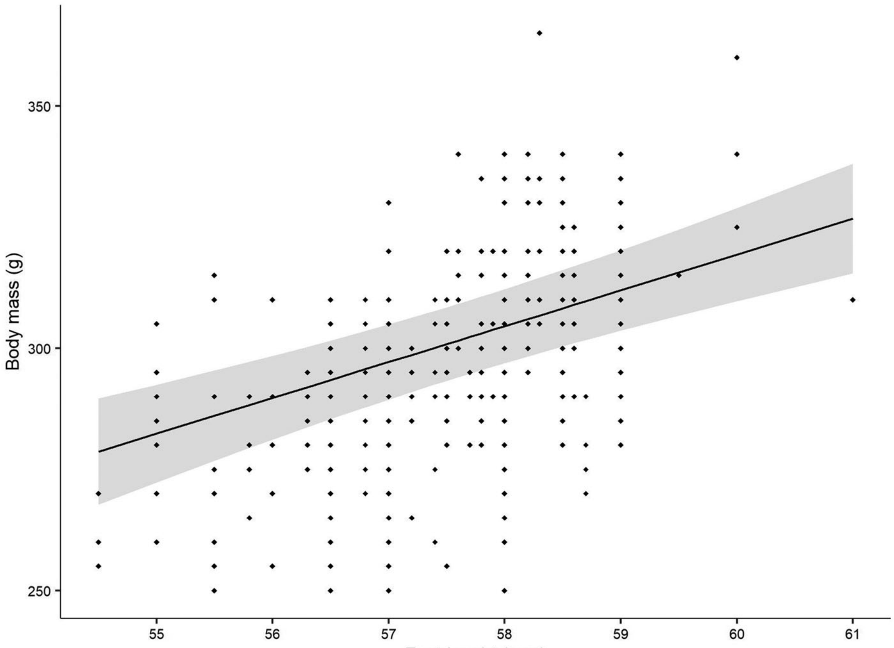

Figure 2 Relationship between foot length and body mass of male red squirrels. The solid line represents the predicted relationship, shaded areas represent the 95% confidence intervals, and diamonds represent observed values.

Table 3 Posterior means and 95% credibility intervals of the comparisons between the 3 study areas (BOR, Bormio; CAN, Cancano; VAL, Valfurva) for each behavioral trait, obtained from the posterior distribution of the full MCMCglmm model

		Differences		
Dependent variable		CAN–BOR	VAL–BOR	CAN–VAL
OFT	Activity	**0.62 (0.24 to 1.01)**	0.27 (−0.05 to 0.59)	**0.35 (0.03 to 0.66)**
	Exploration	0.19 (−0.16 to 0.55)	−0.27 (−0.57 to 0.03)	**0.46 (0.19 to 0.74)**
MIS	Activity-exploration	**0.55 (0.15 to 0.94)**	0.10 (−0.24 to 0.44)	**0.45 (0.12 to 0.77)**
	Social tendency	**0.51 (0.11 to 0.90)**	0.17 (−0.15 to 0.51)	**0.34 (0.03 to 0.65)**

Significant results in bold.

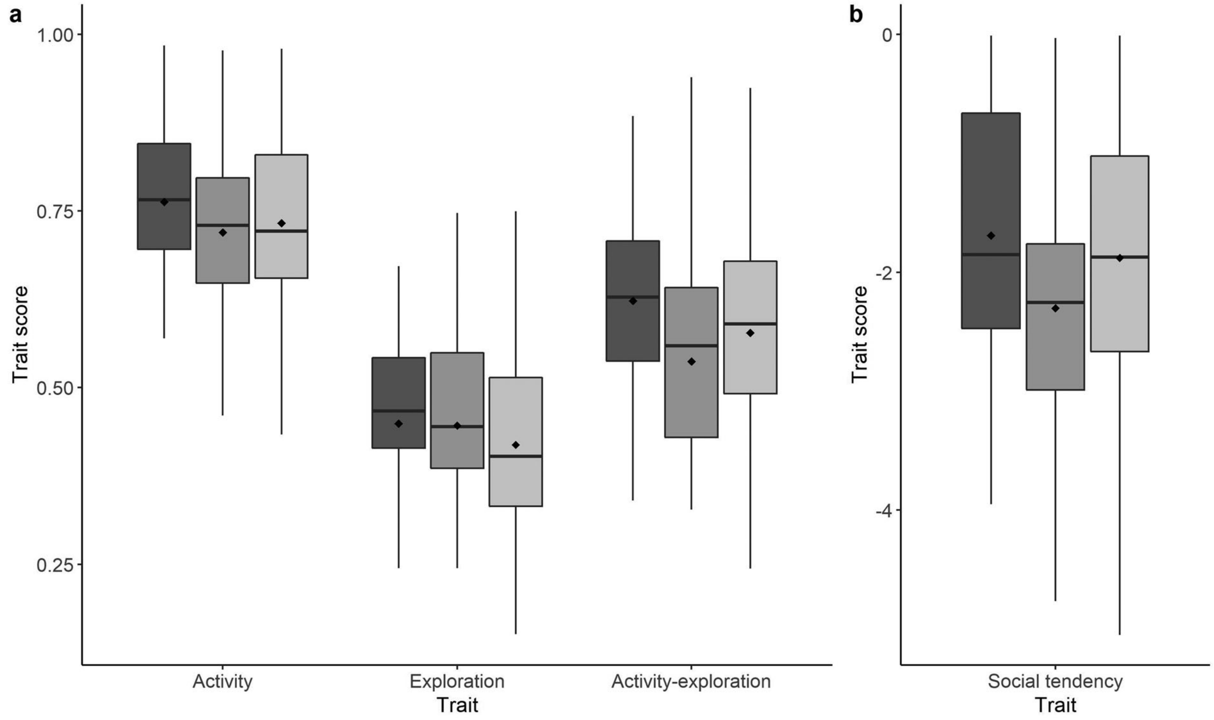

Figure 3 Comparisons of the behavioral scores between the 3 study areas (Cancano, dark grey; Bormio, grey; Valfurva, light grey). (a) Activity, exploration, and activity-exploration square root transformed. (b) Social tendency true values. Boxplots show median (solid horizontal line), mean (black diamond), and 1st (25%) and 3rd (75%) quartiles.

We found several significant differences in personality traits between study areas. Squirrels had higher activity, activity-exploration and social tendency in Cancano than in Bormio and Valfurva (Table 3; Fig. 3). Squirrels living in Cancano were also significantly more explorative than conspecifics living in Valfurva (Table 3; Fig. 3). There was no significant difference in activity, exploration, activity-exploration or social tendency between Bormio and Valfurva (Table 3; Fig. 3). Male squirrels have a lower active-explorative score than female squirrels ($\beta = -0.29$, 95% CI = −0.56 to −0.01, pMCMC = 0.041; Table S3, Supporting Information), and heavier animals were more explorative than individuals with lower body mass ($\beta = 0.12$, 95% CI = 0.01 to 0.23, pMCMC = 0.028). In spring–summer, red squirrels were significantly more active ($\beta = 0.29$, 95% CI = 0.12 to 0.47, pMCMC = 0.001), explorative ($\beta = 0.58$, 95% CI = 0.39 to 0.78, pMCMC < 0.0001),

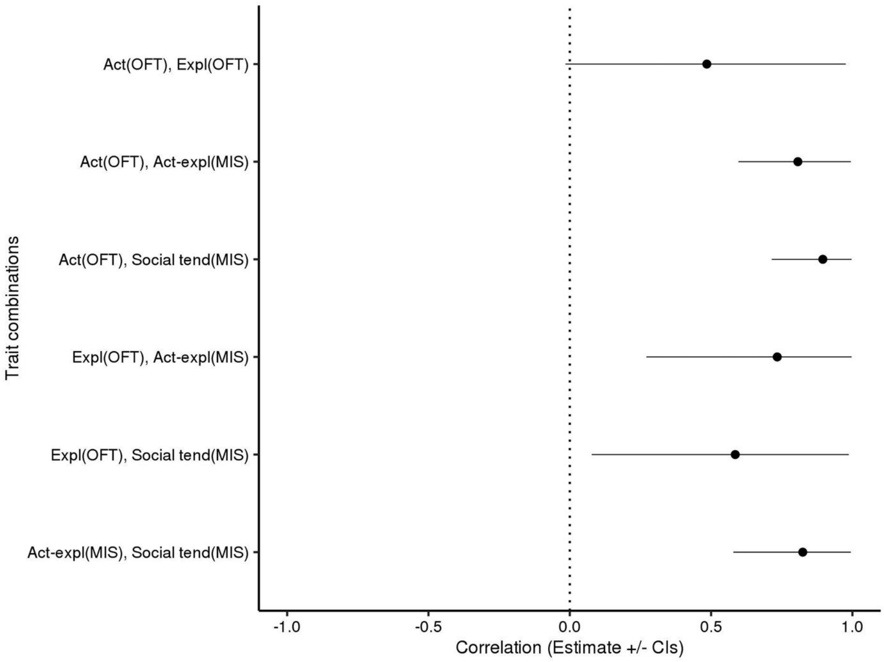

Figure 4 Correlations (estimate ± 95% credibility intervals) between the dependent variables derived from the full MCMCglmm model. Significant results (0 not included in the 95% CIs).

and also active-explorative ($\beta = 0.41$, 95% CI = 0.21 to 0.60, pMCMC < 0.0001) than in autumn. Finally, activity and exploration were higher during the first arena test than during subsequent ones (activity: $\beta = 0.75$, 95% CI = 0.56 to 0.94, pMCMC < 0.0001; exploration: $\beta = 0.52$, 95% CI = 0.32 to 0.72, pMCMC < 0.0001), contrary to social tendency, that was lower during the first experiment ($\beta = -0.33$, 95% CI = −0.54 to −0.11, pMCMC = 0.003).

Among- and within-individual variances and behavioral syndrome

Most of the posterior estimates of the correlations among the personality traits were significant (Fig. 4). There were positive correlations of activity with activity-exploration, and with social tendency; of exploration with activity-exploration, and with social tendency; and between activity-exploration and social tendency (Fig. 4). This suggests a behavioral syndrome (a close association of different behavioral traits; Sih *et al.* 2004, 2012; Réale *et al.* 2007) whereby more active squirrels being also more explorative and more social.

Comparing among- and within-individual variances between the study areas (Table S5, Supporting Information), we found that the among-individual variance of exploration was higher in Cancano than in Valfurva (Table 4), suggesting more phenotypic variation in exploration behavior in red squirrels living in the marginal study area. In contrast, the within-individual variances of exploration were lower in Cancano than in Bormio and Valfurva (Table 4), suggesting a higher plasticity of this behavioral trait in Bormio and in Valfurva than in Cancano, the marginal habitat.

DISCUSSION

In this study, we investigated whether there were morphological and/or behavioral differences between 3 populations of Eurasian red squirrels in alpine study areas. Our results demonstrate that squirrels in the edge habitat of their altitudinal distribution showed both morphological and behavioral adaptations to the extreme weather and food conditions. First, we found that males in the marginal habitat were smaller and weighed less than in the high-quality Norway spruce dominated forest. In contrast, females living in the edge habitat did not differ in body size and body mass from females living in the other 2 study areas. Secondly, squirrels living in the edge habitat had higher scores of behavioral traits, confirming our hypothesis that more active-explorative types are advantaged in

Table 4 Mean differences ± SD (95% credibility intervals) in between-individual variances (a) and within-individual variances (b) of the dependent variables in the study areas (BOR, Bormio; CAN, Cancano; VAL, Valfurva) based on the 3 MCMCglmm models

	Differences		
Dependent variable	BOR–CAN	VAL–CAN	BOR–VAL
a) *Between-individual*			
Activity	0.11 ± 0.22 (−0.28 to 0.59)	0.16 ± 0.15 (−0.17 to 0.44)	−0.05 ± 0.21 (−0.39 to 0.42)
Exploration	−0.19 ± 0.37 (−0.89 to 0.62)	**−0.49 ± 0.25 (−1.06 to −0.11)**	0.30 ± 0.29 (−0.04 to 1.03)
Activity-exploration	0.15 ± 0.50 (−0.72 to 1.22)	−0.34 ± 0.28 (−0.96 to 0.11)	0.49 ± 0.43 (−0.16 to 1.47)
Social tendency	0.04 ± 0.28 (−0.49 to 0.66)	−0.08 ± 0.19 (−0.52 to 0.22)	0.12 ± 0.24 (−0.24 to 0.70)
b) *Within-individual*			
Activity	−0.07 ± 0.23 (−0.51 to 0.40)	−0.07 ± 0.16 (−0.42 to 0.21)	0.003 ± 0.20 (−0.32 to 0.44)
Exploration	**0.67 ± 0.31 (0.14 to 1.35)**	**0.36 ± 0.14 (0.04 to 0.60)**	0.31 ± 0.29 (−0.17 to 0.97)
Activity-exploration	0.46 ± 0.35 (−0.11 to 1.27)	0.29 ± 0.16 (−0.08 to 0.57)	0.17 ± 0.34 (−0.33 to 0.96)
Social tendency	−0.0001 ± 0.25 (−0.47 to 0.52)	0.27 ± 0.18 (−0.12 to 0.60)	−0.27 ± 0.23 (−0.66 to 0.23)

Significant results in bold.
For estimates of the between-individual and the within-individual variances by study area, see Table S5, Supporting Information.

low-quality habitats. Finally, and contrary to our predictions, animals living in the marginal habitat did not have a higher behavioral plasticity, but their within-individual variance of exploration was significantly lower than in the other 2 study areas.

Morphological differences

Between-habitat variation in body mass and body size, measured by hind-foot length, revealed in this study confirmed earlier findings based on 6 populations (Wauters *et al.* 2007), 2 of which were also included here (Valfurva and Bormio). As in the previous study, there was no size difference between the sexes in Valfurva and Bormio, but in the marginal habitat (Cancano) hind foot length of females was on average 1 mm longer than males. Moreover, among males, the body size – body mass relationship did not differ between the study areas and males at Cancano weighed on average 20 g less than in the high-quality habitat Valfurva. Among females, patterns were more complicated: body mass increased more strongly with size (hind foot length) in the marginal than in the high-quality habitat and, consequently, there was little difference in the body mass of adult females between the study areas; on average only 4 g between Cancano and Valfurva. These sex-specific patterns differ from previous findings on this and other tree squirrel species over most of their range (Nash & Seaman 1977; Boutin & Larsen 1993; Koprowski 1994a,b; Wauters & Dhondt 1989a; Lurz & Lloyd 2000), and suggest differential selective pressures for male and female red squirrels in the lowest-quality marginal habitat Cancano than in neighboring populations living in the same geographic area (see also Hoffman & Merilä 1999; McAdam *et al.* 2002; McAdam & Boutin 2003). Hence, our data suggest that in the marginal, high-elevation mountain-pine habitat, smaller and lighter males have an advantage over larger, heavier ones. In mammals, animals of smaller size/body mass can meet daily energy requirements with a lower food-intake than bigger individuals. In Cancano, red squirrels feed mainly on the small seeds from cones of mountain pine from late summer to early spring and on its buds and male flowers in spring–early summer (Romeo *et al.* 2010). Species-specific energy content of all seeds contained in a cone of mountain pine is only 6.0 kJ against the 51 and 121 kJ, respectively, of a Norway spruce cone and an Arolla pine cone, the main food resources in the other 2 study areas. Thus, smaller size among male red squirrels in the marginal habitat could be an adaptation to a reduced availability of high-energy food resources characterized by relatively long handling times (unpublished data; see also data on Scots pine in Wauters *et al.* 1992).

However, this trend was not observed among female red squirrels. We believe this is due to the differential energy investment in reproduction between the sexes. Reaching a high body mass is essential for female red squirrels to enhance their reproductive success. Indeed, the probability to enter estrus (Wauters & Dhondt 1989b), lactation success (Wauters & Dhondt 1995; Wauters & Lens 1995; Wauters *et al.* 2007), offspring weaning mass and early survival (Wauters *et al.* 1993), and individual variation in lifetime reproductive success (Wauters & Dhondt 1995) are all strongly and positively related to body mass. Also in this study, lactating females weighed more than non-breeding ones. Moreover, in the marginal habitat, persistency of adults is low and most females will have only a single reproductive event in their lifetime (Rodrigues *et al.* 2010); hence having a high body mass should aid females to maximize reproductive output in terms of litter size and early offspring survival (Mari *et al.* 2008).

Finally, heavier males tend to be more successful in mating than males of lower body mass, which should favor larger and heavier animals (Wauters *et al.* 1990). However, these observations were carried out in high-density populations where up to 4 males competed for access to the estrus female. In Cancano, densities were so low that, probably only 1 or 2 males took part at a mating chase, which could reduce sexual selection for larger males. Finally, these sex-specific differences between habitats argue against the hypothesis that squirrels are smaller and weigh less in the poorer-quality habitat because of having an overall lower food intake.

Personality traits and habitat selection

Red squirrels in the marginal edge habitat were more active, active-explorative and social than in the other 2 higher-quality areas. Moreover, animals in Cancano were also more explorative than those in Valfurva but not than those living in the Arolla pine forest of Bormio, which is characterized by a higher overall tree-seed abundance than the mountain pine forest of Cancano, but also by a higher patchiness (forest heterogeneity) (Table 1; see also Salmaso *et al.* 2009). Hence, individuals that behave more explorative might have an advantage in finding food-rich patches and optimize home range use (Wauters *et al.* 2021), explaining why the expression of exploration was similar in Bormio as in the marginal habitat of Cancano. Several studies on wild animals found a non-random distribution of individuals' personality traits between heterogeneous habitats (Cerqueira *et al.* 2016; Holtmann *et al.* 2017; Brehm & Mortelliti 2021; Rabdeau *et al.* 2021). For example, in dunnocks (*Prunella modularis*), personality has been observed having an impact on habitat choice, bolder individuals settled in areas where human disturbance is high, contrary to shier ones that prefer sites with less human disturbance (Holtmann *et al.* 2017). As suggested in these studies, and in agreement with the "personality-matching habitat choice" hypothesis (Edelaar *et al.* 2008), an individual settles in the habitat that is the most suitable for its personality and that best matches the animal's capacity to use this habitat (Edelaar *et al.* 2008; Holtmann *et al.* 2017). These authors also suggest that behavioral adjustments over time contribute little to the personality-habitat relationship (Edelaar *et al.* 2008; Holtmann *et al.* 2017). This could explain why, in contrast with our prediction, we did not find a higher plasticity (within-individual variance) of the personality traits in the marginal habitat compared to the other ones. In our study system, we believe that increased activity and activity-exploration, but also high exploration scores, result in higher success in acquiring resources, and thus enhance the probability to settle in marginal areas of the distribution range, characterized by poorer habitat quality (Biro & Stamps 2008; Wauters *et al.* 2021). Similarly, a study by Boone and colleagues (2022) investigating the interaction between the personality of another scatter-hoarding mammal (deer mice, *Peromyscus maniculatus*), and seed (food) availability, found that more active/explorative mice choose lower-quality seeds when food availability was reduced by 50%, in contrast with less active/explorative individuals that continued to select high-quality seeds both at high and low food availability (Boone *et al.* 2022). Hence, this study suggests that more active and explorative individuals usually tend to use a wider variety of food resources than less active/explorative ones (Toscano *et al.* 2016; Boone *et al.* 2022). Therefore, the presence of more active, explorative and active-explorative individuals in the edge habitat may also be due to the fact that these personality types can more easily switch and adapt to other, lower-quality food resources (in our case the seeds of mountain pine cones but also male flowers and buds of mountain pine, rather than the more energetic Norway spruce and Arolla pine seeds) than less active/explorative ones.

Together with the personality traits related to habitat- and space use, we also found a higher social tendency in the marginal habitat than in other study areas. This could be due to the behavioral syndrome we described (see also Santicchia *et al.* 2022). In other words, if higher activity and exploration may benefit animals in the marginal habitat and both are associated with higher social-tendency in a behavioral syndrome, then the squirrels in the marginal habitat will express more social behavior during the MIS test, without there being a selective advantage of behaving more social.

Alternative hypotheses: early experience or behavioral plasticity

An alternative explanation of the differences in personality between the study areas may be due to the shaping of squirrels' behavior by the different pressures that characterized each habitat. In fact, changes in personality may occur throughout the individual's life (Stamps & Groothuis 2010), leading to a weakening of the personality-matching habitat choice hypothesis. In our study, we did not specifically examine this behavioral plasticity throughout an individual's life, but in general, considerable modifications in personality seem to happen after drastic developmental changes (e.g. sexual maturation) (Cabrera *et al.* 2021). To account for this, we removed juveniles from our analyses and we performed multiple arena tests in different sessions (over several months to several years) of the same individual to test the repeatability of the trait over time. Moreover, it is reported that an animal's experience during early development could have an effect on its personality as adult (Stamps & Groothuis 2010). However, the majority of red squirrels disperse when between 4 and 10 months old, while some disperse as adults (>1-year old), over 1 up to 5–10 km, and philopatry is rare (Wauters & Dhondt 1993; Wauters *et al.* 1994, 2011; Di Pierro *et al.* 2010). There is genetic evidence that dispersal occurs between 2 of our study sites (Di Pierro *et al.* 2010) and that also in the marginal site most of the newly caught animals are immigrants (our unpublished data). Hence, the animals monitored here arrived in a given study site coming from several directions and distances, guaranteeing differences in the early experiences of animals settling in that area. Therefore, we think that behavioral plasticity or early life experience did not have a great effect on the differences in personality that we found between the different habitats.

A further point to consider is the possibility that squirrels rapidly change their behavior (rapid behavioral change; e.g. within a few days from the settlement in the new area) in response to the environmental pressures (Holtmann *et al.* 2017). For this study, we did not examine this rapid behavioral plasticity, due to the difficulties to observe personality in the first days of establishment in the new area. However, a deep investigation of behavior before and after the settlement is necessary to examine this alternative.

Other factors affecting the expression of personality traits

Female red squirrels tended to have a higher score for the activity-exploration trait than males. This could be related to the higher energy investment by females in reproduction (see *Morphological differences*), producing an advantage in resource acquisition for more active-explorative individuals. In line with this and with previous studies on red squirrels, heavier squirrels were more explorative than smaller ones (Santicchia *et al.* 2021, 2022). This association is found also in other tree squirrel species (*Tamias striatus*: Martin & Réale 2008; *Sciurus carolinensis*: Santicchia *et al.* 2019, 2020b).

We also observed a seasonal effect on behavioral traits: squirrels in spring–summer were more active, explorative and active-explorative than in autumn. Animals may show some degree of behavioral plasticity among seasons to cope with predictable abiotic and biotic environmental conditions (e.g. changes in photoperiod, rainfall, temperature, tree-seed availability), especially at high latitudes and altitudes (Zhang & Buck 2022; see also Beaman *et al.* 2016). However, only few studies investigated seasonal fluctuations in behavior in free-living Sciurids (Arctic ground squirrels, *Urocitellus parryii*: Chmura *et al.* 2020; Abert's squirrels, *Sciurus aberti*: Zhang & Buck 2022). In our studied species, the seasonal variation in trait expression could be linked to tree phenology; new fresh cones with mature seeds are available from the end of summer and during autumn and winter (Romeo *et al.* 2010; Zong *et al.* 2010). Therefore, augmented activity and exploration in spring–early summer might be linked to higher foraging needs when feeding on low-energy food sources such as male flowers, buds, or cached seeds that need long searching times (Zong *et al.* 2010).

In conclusion, the evidence from this study suggests that personality has an important role in habitat selection with consequences on animals' morphology. This study was carried out in red squirrels populations in the altitudinal limit of their distribution range, but the same study system could be replicated in different contexts with different environmental stressors (Wauters *et al.* 2019; Santicchia *et al.* 2022) to better understand an individual's choice of habitat (e.g. differences among urban-rural gradient). Such future studies should also measure fitness components of individuals (survival and/or reproductive success) which would allow to investigate whether natural selection favors different personalities in relation to habitat type (e.g. Santicchia *et al.* 2018). Also, the habitat-related patterns in animal personality and the comparisons of among- and within-individual variance in personality traits documented here, contributes to our still limited knowledge about behavioral plasticity differences between populations occurring in different habitats in vertebrates (Dingemanse & Wolf 2013; Hewes *et al.* 2017). Particularly, our findings that only for exploration, and not for the other behavioral traits, the within-individual variance differed among populations strengthens the hypothesis that it is primarily the animal's personality that determined the individual's choice of habitat, and that behavioral plasticity was not strongly associated with habitat selection.

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SUPPLEMENTARY MATERIALS

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Ethogram for the open field test (OFT) and mirror image stimulation (MIS) test. Description of the single behaviors and indication of the expert-based grouping into categories that represent personality traits.

Table S2 Repeatability of OFT and MIS behaviors estimated with rptR function, calculated on the entire dataset including also individuals without repeated arena test measures.

Table S3 Full MCMCglmm model: parameters, prior and outputs.

Table S4 Linear mixed models explaining variation in body mass for female and male red squirrels in 3 populations in the Italian Alps.

Table S5 Mean ± SD (95% credibility intervals) of between-individual variances (a) and within-individual variances (b) of the dependent variables in each study area based on the 3 MCMCglmm models.

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