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Evolution of personality and locomotory performance traits during a late Pleistocene island colonization in a tree frog

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Abstract

Recent empirical and theoretical studies suggest that personality and locomotory performance traits linked to dispersal abilities are crucial components of the dispersal syndromes, and that they can evolve during range expansions and colonization processes. Island colonization is one of the best characterized processes in dispersal biogeography, and its implication in the evolution of phenotypic traits has been investigated over a wide range of temporal scales. However, the effect of island colonization on personality and performance traits of natural populations, and how these traits could drive island colonization, has been little explored. Noteworthy, no studies have addressed these processes in the context of late Pleistocene range expansions. Here, we investigated the contribution of island colonization triggered by postglacial range expansions to intraspecific variation in personality and locomotory performance traits. We compared boldness, exploration, jumping performance, and stickiness abilities in populations from 3 equidistant areas of the Tyrrhenian tree frog *Hyla sarda*, 2 from the main island (Corsica Island), and 1 from the recently colonized island of Elba. Individuals from Elba were significantly bolder than individuals from Corsica, as they emerged sooner from a shelter ($P = 0.028$), while individuals from Corsica showed markedly higher jumping and stickiness performance (both $P < 0.001$), resulting as more performing than those of Elba. We discuss these results in the context of the major microevolutionary processes at play during range expansion, including selection, spatial sorting, founder effects, and their possible interaction with local adaptation processes.

Key words: behavioral syndrome, colonization, dispersal, founder effect, *Hyla sarda*, insularity, personality.

Research on island populations has provided fundamental information on evolutionary diversification and adaptation (Losos and Ricklefs 2009). While it is well established that island populations differ predictably in morphology, size, and life history from their mainland counterparts, relatively few studies have documented insularity-driven changes in the behavioral phenotype (Gavriilidi et al. 2022). A known behavioral feature of island populations of reptiles, birds, and mammals is their "tameness", that is, the loss of the ability to recognize and respond to predators with competence, a phenomenon that has been associated to relaxed predation pressure and the costs of sustaining the machinery required to detect and escape from predators (Blumstein 2002; Blumstein and Daniel 2005; Rödl et al. 2007; Cooper et al. 2014; Brock et al. 2015; Baeckens and van Damme 2020). Moreover, in some islands, territoriality is reduced compared to mainland and individuals appear more tolerant toward intruders (Stamps and Buechner 1985; Gray and Hurst 1998). This reduced aggressiveness of island species would be explained by a number of factors operating in concert, such as higher available resource densities, reduced territory size, and

higher defense costs (Stamps and Buechner 1985). However, in other populations, intraspecific aggression on islands is more intense than on mainland (Vervust et al. 2007; Raia et al. 2010). These patterns are limited to reptiles, birds, and mammals, and it is unclear why some species/populations "follow the rule" and others less or not, while the mechanisms producing the phenotypic divergence—founder effect, genetic drift, selection, and plasticity—are also poorly known (Baeckens and van Damme 2020). Overall, the picture of the behavioral profiles of island populations is still incomplete (Gavriilidi et al. 2022).

The study of animal personality, defined as consistent interindividual variation in sets of behavioral traits with both genetic and epigenetic basis, provides an integrative approach to behavior (Carere and Maestripieri 2013). Animal personality can influence a wide range of population-level processes with significant ecological and evolutionary implications (Réale et al. 2007; Cote et al. 2010; Sih et al. 2012; Wolf and Weissing 2012; Canestrelli et al. 2016a). In particular, personality traits, such as boldness and exploration, have been linked to dispersal, range

expansion, and colonization processes in several theoretical and empirical studies (Fraser et al. 2001; Dingemanse et al. 2003; Cote et al. 2010; Chapple et al. 2012; Sih et al. 2012; Canestrelli et al. 2016a; Gruber et al. 2017). Notably, dispersal is a high-energy and resource-demanding process that imposes a number of physiological challenges and requires high-performance abilities (Phillips et al. 2006; Bonte et al. 2012; Kosmala et al. 2017; Jessop et al. 2018). However, despite the increasing number of studies on personality and dispersal-enhancing traits of animals in many contexts, particularly in invasive species, their role in shaping personality traits has not been explored (Canestrelli et al. 2016a). It is, therefore, paramount to delve into the expression of personality and other dispersal-enhancing traits in populations that have colonized islands, taking into account that any suite of traits that enhances departure, arrival, and settlement on a given island might, for several reasons (e.g., evolutionary inertia, novel predators, and/or competitors), be different from those that allow later generations to successfully survive on that island. For example, information on naturally colonized island populations is virtually absent. A recent study on *Rana temporaria* has actually suggested a differentiation of personality traits in relation to a recent colonization, but the ongoing flow of individuals between mainland and islands could not allow inferences about the causes and the temporal persistence of the observed divergence (Brodin et al. 2013).

Our main goal was to test whether island populations differ from their mainland counterparts in personality and locomotory performance traits, by comparing populations with a known evolutionary history from 3 different geographical areas (Foster 1999). We took advantage of the unique opportunity offered by the known colonization history of the Tyrrhenian tree frog (*Hyla sarda*), a species with an insular distribution in the Western Mediterranean Sea (Bisconti et al. 2011a, 2011b). We focused on 3 equidistant areas (ca 80 km), 2 from Corsica, where ancestral populations are located, and 1 from the recently colonized Elba island (Figure 1), distributed along the route of a postglacial range expansion (as inferred in Bisconti et al. 2011a,

2011b; Spadavecchia et al. 2021). The 2 Corsica areas were sequentially founded via diffusion dispersal, while the Elba populations originated via a jump dispersal event (Vences et al. 2003, 2004). Importantly, such expansion processes were recent (postglacial) and synchronous (Spadavecchia et al. 2021), allowing us to exclude effects due to long-term allopatric divergence. In fact, historical demographic reconstructions showed that this species underwent 2 sequential expansion steps: the first from Sardinia to southern Corsica, at the end of the late Pleistocene, and the second to the remaining part of the current range including northern Corsica and the Tuscan archipelago (Spadavecchia et al. 2021).

With the aim to investigate whether and how the island colonization affects phenotypic traits linked to dispersal, we compared personality (exploration and boldness) and locomotory performance (jumping performance at takeoff and stickiness) among populations from the 3 aforementioned areas. Boldness is a personality trait that is associated with both dispersal and risk of predation (e.g., Fraser et al. 2001; Hulthèn et al. 2017). Exploration reflects the individual response to a novelty (e.g., Verbeek et al. 1994; Cote et al. 2010). Locomotory performance is a key determinant of dispersal ability, and interindividual variation in jumping performance has been previously analyzed in the context of dispersal evolution during (invasive) range expansions (Phillips et al. 2006; Llewelyn et al. 2010; Kosmala et al. 2017; Louppe et al. 2017). In particular, jumping ability is expected to relate to locomotory performance in an insular context, and in turn to predator-escape ability. Moreover, the ability to adhere via an area-based wet adhesive mechanism using toe pads is crucial for a safe landing, since missing the target could have severe consequences in arboreal species (Bijma et al. 2016).

Material and Methods

Study species

The Tyrrhenian tree frog *H. sarda* is an amphibian species endemic to the Tyrrhenian islands of Sardinia and Corsica, and the Tuscan archipelago. This arboreal species is rather
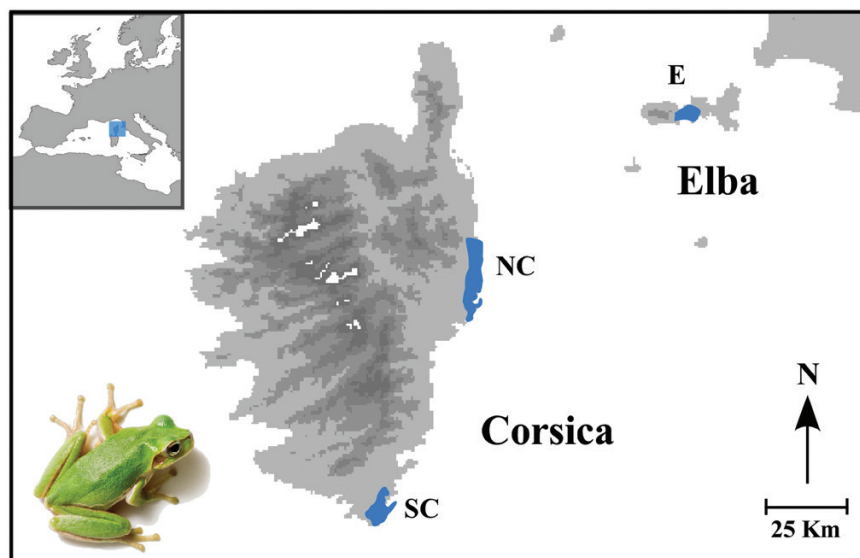
Figure 1. Geographical distribution of the 3 sampled areas. The study species *Hyla sarda* is shown in the lower left corner.

common at low and intermediate altitudes, with more abundant populations frequently found in coastal areas (Lanza et al. 2007). Bioclimatic niche models generated for *H. sarda* under both current and periglacial climatic conditions (Bisconti et al. 2011a) showed spatially and temporally homogeneous bioclimatic conditions in most coastal areas, including western Corsica and Elba Island. Compared to its closely related species *Hyla arborea*, the Tyrrhenian tree frog is more linked to aquatic habitats, where it breeds in pools and temporary ponds from spring to early summer (Lanza et al. 2007). During late Pleistocene, it colonized Corsica Island from Sardinia taking advantage of a wide and persistent land bridge between the 2 main islands, and then it reached Elba Island (see Introduction; Bisconti et al. 2011a, 2011b; Spadavecchia et al., 2021).

Sampling and housing

A total of 72 adults of *H. sarda* were sampled between April and July 2018 in Corsica (24 adults for the populations from North Corsica area and 24 adults for the populations from South Corsica area; see Figure 1) and Elba islands (24 adults; see Figure 1). After results from previous bioclimatic niche analyses (Bisconti et al. 2011a), all sampling sites in Corsica (Figure 1) were selected in strictly coastal areas (<10 m above sea level, within 1 km from the coastline), which allowed us to control for potential effects of bioclimatic differences on the geographic patterns of the investigated traits.

Individuals were sampled with hand nets at night after acoustic and visual localization, immediately stored in individual plastic boxes (13.1 cm × 10.2 cm × 4.9 cm) containing humid paper that was renewed daily. Within 3 days from sampling, individuals were transported to our lab facilities, where they were housed under controlled environmental conditions, at a temperature of 24/25°C, relative humidity of 60–80%, and natural photoperiod. They were kept in individual cages (25 cm × 25 cm × 25 cm) provided with a small, dechlorinated water tank (diameter 5 cm), a plant, and an oak wood shelter. The cages were not visually or acoustically isolated from each other and were placed randomly with respect to population of origin. Three times a week the cages were cleaned, the water renewed, and animals were fed *ad libitum* with crickets (*Acheta domestica*). Before starting the tests, the animals were left undisturbed for 2 weeks except for routine feeding and cleaning duties.

Sampling procedures were performed under the approval of the Institute for Environmental Protection and Research "ISPRA" (protocol # 5944), Ministry of Environment "MATTM" (protocol #8275), Regione Sardegna (#12144), and Corsica (#2A20180206002 and #2B20180206001). Permission to temporarily house amphibians was granted by the Local Health and Veterinary Centre, with license code 050VT427. All handling procedures were approved by the Ethical Committee of the Tuscia University for the use of live animals. During captivity, the animals were monitored daily. No adverse effects on the overall health of tree frogs were observed during the procedures. The animals were released in the original sampling locations at the end of the experimentation.

Personality traits

For the assessment of personality traits, 2 tests were performed, similarly as in previous studies on amphibians (e.g., Brodin et al. 2013; Kelleher et al. 2017, 2018): (1) arena test to investigate the propensity to explore (Figure 2A) and (2) emergence test to examine the boldness–shyness behavioral axis (Figure 2B). Both tests were repeated after 10 days to test for individual consistency over time. All tests were carried out under the same environmental conditions used for housing tree frogs.

Exploration: arena test

Considering the arboreal lifestyle of tree frogs, we used a cylindrical arena with vertical as well as horizontal dimension (diameter 145 cm × height 90 cm) enriched with a plant and a wooden shelter. Individuals were tested randomly with respect to the population of origin on the same day between 9:00 and 14:00. Each individual was gently introduced to the arena and left undisturbed in a dark jar (5cm × 3 cm) positioned along the border; after 2 min, the jar was opened and the animal was allowed to explore the arena for 10 min. After that, it was immediately put back in its home cage. The arena was cleaned after each test in order to remove any chemical cues or secretions left by previous individuals.

The tests were video recorded (Panasonic DMC-FZ300) and videos were analyzed by 1 operator using Boris 5.1.3 (Friard and Gamba 2016). The following variables were extracted: (1) latency to explore (s); (2) duration of activity (expressed as percentage of duration of test); (3) duration of time spent on the arena floor (expressed as percentage of duration of test); and (4) frequency of jumping events.
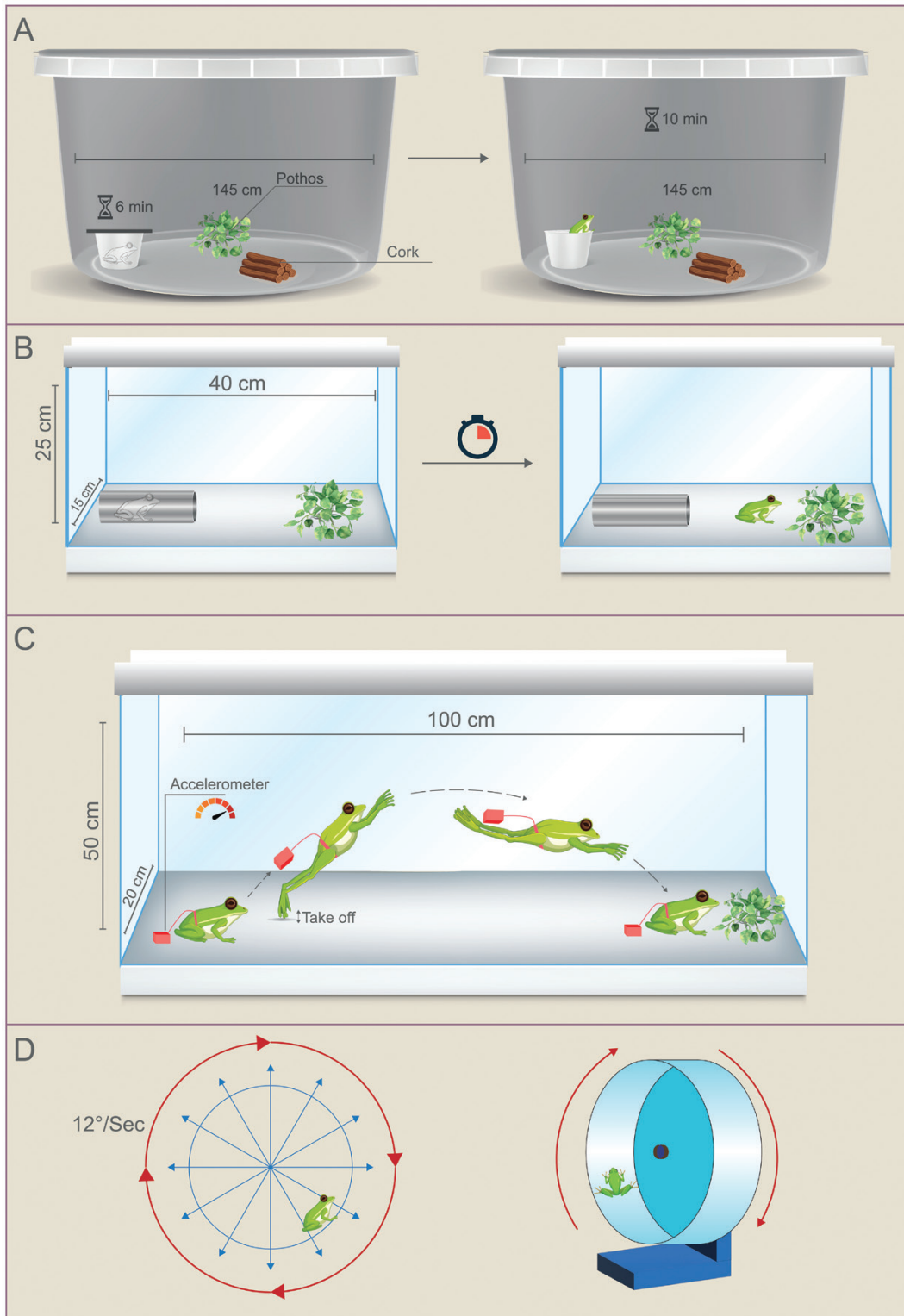
Boldness: emergence test

With the aim to test the boldness of individuals, we used the latency to exit from a shelter as a proxy for willingness to take risks. We used a rectangular arena (length 40 cm × height 25 cm) enriched with a plant. Individuals were tested randomly with respect to the population of origin on the same day between 9:00 and 14:00. Each individual was gently introduced to a cylindrical dark shelter (5cm × 3 cm) positioned along the arena's border; after 6 min, the shelter was opened, and the animal was given 10 min to exit from the jar. After that, it was immediately put back in its home cage and the arena was cleaned. Tests were video recorded, and videos were visually analyzed by 1 operator to measure the latency to exit from the shelter (s).

Locomotory performance

For the assessment of locomotory performance traits, we carried out 2 sets of tests. Firstly, we measured the maximum jumping performance at takeoff as a proxy of dispersal abilities (Figure 2C). The jumping force at takeoff was measured to characterize the propulsive phase of jump (e.g., Marsh and John-Alder 1994; Nauwelaerts and Aerts 2006; and reference therein), which is the predominant mode of locomotion in tree frogs. Secondly, we quantified the maximum adhesiveness force using a stickiness test (Figure 2D). Tree frogs have specialized toe pads, which adhere via an area-based wet adhesive mechanism (Emerson and Diehl 1980; Federle et al. 2006; Smith et al. 2006). This anatomical specialization is crucial for a safe landing and is intrinsically linked to jumping performance, as missing the target could have severe consequences in arboreal species, as compared to terrestrial species (Bijma et al. 2016).

All tests were carried out under the same environmental conditions used for housing the tree frogs.

Figure 2. Schematic representation of the tests conducted on *Hyla sarda*. (A) Arena test used to investigate the propensity to explore; (B) emergence test used to examine the boldness–shyness behavioral axis (as latency to exit from a refugium); (C) jumping test to measure the maximum jumping performance at takeoff; (D) stickiness test used to estimate maximum adhesiveness force (graphic design by Eustacchio Montemurno).

Jumping test

The jumping test was conducted in a rectangular arena of plexiglas (length 100 cm × height 50 cm), enriched with a plant placed at the opposite side of the arena, to obtain stand-ardized directionality of tree frog jumps. Individuals were tested randomly with respect to the population of origin on the same day between 9:00 and 14:00. The animals were equipped with an accelerometer, deployed by a pelvis-loop harness (Axy-4 units, Technosmart, Rome; 9.15 mm × 15 mm × 4 mm, 1 g weight including battery), and induced to jump by a slight stimulation in the pelvic region (Mitchell and Bergmann 2016). The logger was set to record triaxial

acceleration (0–4 g) at 100 Hz with 8-bit resolution. After that, all individuals were weighed (scale Acculab model ATILON ATL-224-I) and then gently put back in their home cages.

From the downloaded logger data, we extracted the dynamic body acceleration for each dimension (x, y, z) considering a running mean of 30 Hz (Wilson et al. 2006; Shepard et al. 2008). The x axis of the logger measured sway, the y axis measured surge, and the z axis measured heave (Halsey et al., 2008). After that, we summed these values to produce the vector sum of dynamic body acceleration (VeDBA = $\sqrt{[Ax2+Ay2+Az2]}$) by using Framework 4 software (version 2.5). For each individual, we quantified 5 VeDBA values, 1 for each jump, and the highest value of VeDBA was chosen to calculate the individual maximum jumping force at takeoff (VeDBA × body mass) and considered for the following analysis (Shepard et al. 2008).

Stickiness test

This test was designed to investigate the degree of adhesiveness, a functional adaptation to the arboreal lifestyle of tree frogs (Duellman and Trueb 1994). We used a transparent rotating wheel of plexiglas (diameter 20 cm × depth 20 cm) with a moderate and constant angular velocity (12°/s). Each individual was gently introduced in a water tank (dechlorinated tap water, at 25°C) for 1 min, to standardize hydration level among individuals. Then the individual was transferred to the wheel and 5 detachments were collected. We only considered detachments occurring when the head of the animal was oriented in the same direction of the rotation, to differentiate events reflecting the maximum stickiness ability of an individual from intentional detachment events. The whole procedure lasted about 2 min per individual. The tests were video recorded (Panasonic DMC-FZ300) and the videos analyzed by 1 operator using the Tracker software (version 4.11.0) to extract the angle of fall (radian). We calculated the maximum adhesion force (cosine alpha × body mass × g) (Barnes et al. 2006) for all the 5 detachments performed by each individual, and the best performance was retained for downstream analyses.

Data analysis

Generalized linear mixed effect (GLMM)-based repeatability models (rpt in package "rptR") were used to test the repeatability of each untransformed behavioral trait (Nakagawa and Schielzeth 2010). In each model, as a dependent variable, we entered behavioral traits singly and individuals as a random factor. Proportion function (rptProportion in package "rptR") with binomial error distribution, and logit link function was used for all the bounded variables (latency, activity, time spent on the floor, and latency to exit from shelter), while a Poisson error distribution with log link function (rptPoisson in package "rptR") was applied to count data (occurrence of jumping events; Stoffel et al. 2017). A parametric bootstrapping method (number of iterations = 1000) was used to calculate the confidence interval (CI) and the likelihood ratio test to estimate the p-value of the repeatability distribution. Behavioral variables were considered as personality traits and used for further analysis when their repeatability value was $R > 0.2$ and their CI excluded 0 (Table 1). Preliminary models showed that entering population as a fixed factor did not improve the fitting of the model, thus it was no longer considered.

The second set of analyses was performed to assess the population differences in both personality (boldness and jumping events) and performance traits (jumping and stickiness) by using Linear Models (LM in package "lme4"). Dependent variables were $\log_{10}$ transformed to meet the assumption of residuals normality. We fit 4 different models (1 for each trait) entering population as fixed factors and checking for all possible contrasts (Table 2). The same approach was used to test whether the same traits differ between the 2 islands. Preliminary models showed that entering animal body weight as covariate, both with and without interaction with population, did not improve the fitting of the model, thus it was no longer considered.

Post hoc comparisons were explored by calculating a standardized effect size Hedges' (mes in package "compute. es") for the following contrasts: Corsica versus Elba for both personality and performance traits. The forest plot (forest in package "metafor") was used to visualize estimates of effect size and their 95% CIs. Effect sizes were considered to be small (Hedges $g = 0.2$, explaining 1% of the variance), intermediate ($g = 0.5$, explaining 9% of the variance), or large ($g = 0.8$, explaining 25% of the variance) according to Cohen (1988).

Finally, we investigated the relationship between personality and performance traits. Generalized linear models (GLM in package "nlme") were run entering performance traits (jumping and stickiness) as dependent variable, population as fixed factor, and personality traits (shelter and occurrence of jumping events) as covariates. We fitted 4 different models including all trait combinations. The same approach was used to test the trait relationship between islands.

All statistical analyses were run using R software version 3.5.3 (R Core Team 2019).

Results

Repeatability of behavioral traits

GLMMs showed that boldness ($r = 0.39$), measured as the latency to exit from shelter, and the frequency of jumping events during exploration of the arena ($r = 0.30$) were significantly repeatable over time (Figure 3). On the contrary, latency to explore, activity, and time spent on the arena floor were not significantly repeatable and therefore they were excluded from the following analyses. The repeatability coefficient with its CI and the p-value of the measured behavioral traits are reported in Table 1.

Table 1. Summary of GLMM-based repeatability (R) estimates from multiplicative model. Parametric bootstrapping (number of iterations = 1,000) was used to calculate the CI interval and the likelihood ratio test to estimate the P-value of the repeatability distribution. Significant traits are shown in bold

Trait	Estimate	95 % CI	P-value
Latency to explore	0.03	0–0.074	0.149
Activity	0.03	0–0.798	0.110
Time on floor	0.11	0–0.254	0.107
Jumping events	0.30	0.035–0.522	**0.013**
Latency to exit from shelter	0.39	0.271–0.491	**<0.001**

Table 2. Linear models showing differences between Corsica (NC: north Corsica; SC: south Corsica) and Elba (E) populations in both personality (boldness and jumping events) and performance traits (jumping and stickiness). Coefficient estimates (±*SE*) of these models are shown with both personality and performance traits as dependent variable and population as fixed factor. Significant contrasts are shown in bold

| Dependent variable	Reference level	Level	Coefficient	SE	t-Value	Ps(>|t|)
Latency to exit from shelter	NC	SC	−0.071	0.287	−0.246	0.807
	NC	E	−0.576	0.287	−2.003	**0.049**
	SC	E	−0.505	0.273	−1.852	0.069
Jump occurrence	NC	SC	−0.199	0.344	−0.580	0.564
	NC	E	−0.267	0.341	−0.783	0.437
	SC	E	−0.067	0.332	−0.202	0.840
Jumping	NC	SC	−1.718	1.279	−1.343	0.188
	NC	E	−8.232	1.240	−6.639	**<0.001**
	SC	E	6.513	1.184	5.503	**<0.001**
Stickiness	NC	SC	0.066	0.065	1.029	0.312
	NC	E	−0.291	0.063	−4.635	**<0.001**
	SC	E	0.357	0.055	6.518	**<0.001**
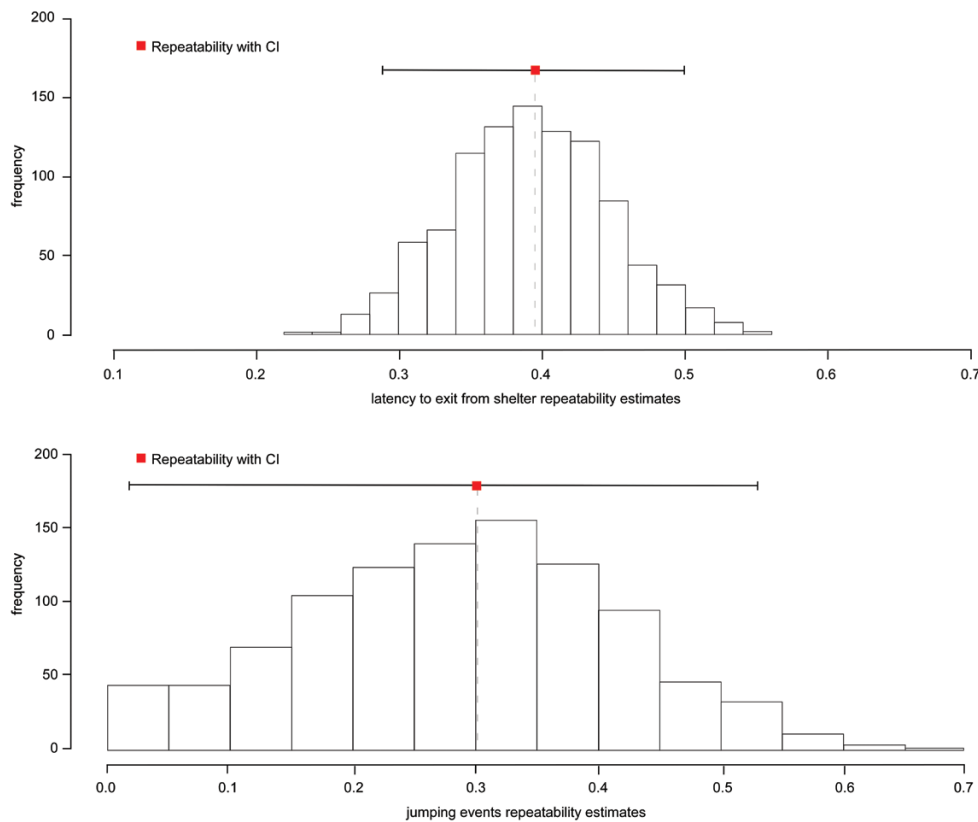
Figure 3. The repeatability estimates: on top: latency to exit from shelter of binomial distributed proportion data analyzed with logit link; on bottom: jumps occurrence of Poisson distributed count data analyzed with log link. Link scale repeatability are shown. CI, confidence interval.

Population differences in personality and performance traits

LMs revealed significant differences in personality traits between north Corsica and Elba populations for boldness (Table 2), which was significantly different also between the 2 islands pooling together the 2 Corsica populations ($P = 0.028$). Specifically, tree frogs from Elba Island were bolder (emerged sooner from a shelter) than those from Corsica. In fact, the estimated marginal means (EMM) from LMs showed that latency to exit from the shelter was higher in the Corsica populations (SC = 4.70 ± 0.193; NC = 4.77 ± 0.213) than in Elba (E = 4.19 ± 0.193) indicating a more prudent behavior for Corsica populations (Figure 3). Conversely, jumping frequency during the exploration test did not differ either among populations (Figure 3) as well as between the islands ($P = 0.575$). The parameter estimates of the LM used to test the differences in personality traits among populations are reported in Table 2. Estimates of the island's effect size in boldness, measured as latency to exit from shelter, were large and the 95% CI excludes 0 (Figures 4 and 5).

Both performance traits, jumping and stickiness, differed markedly among populations ($P < 0.0001$), except between
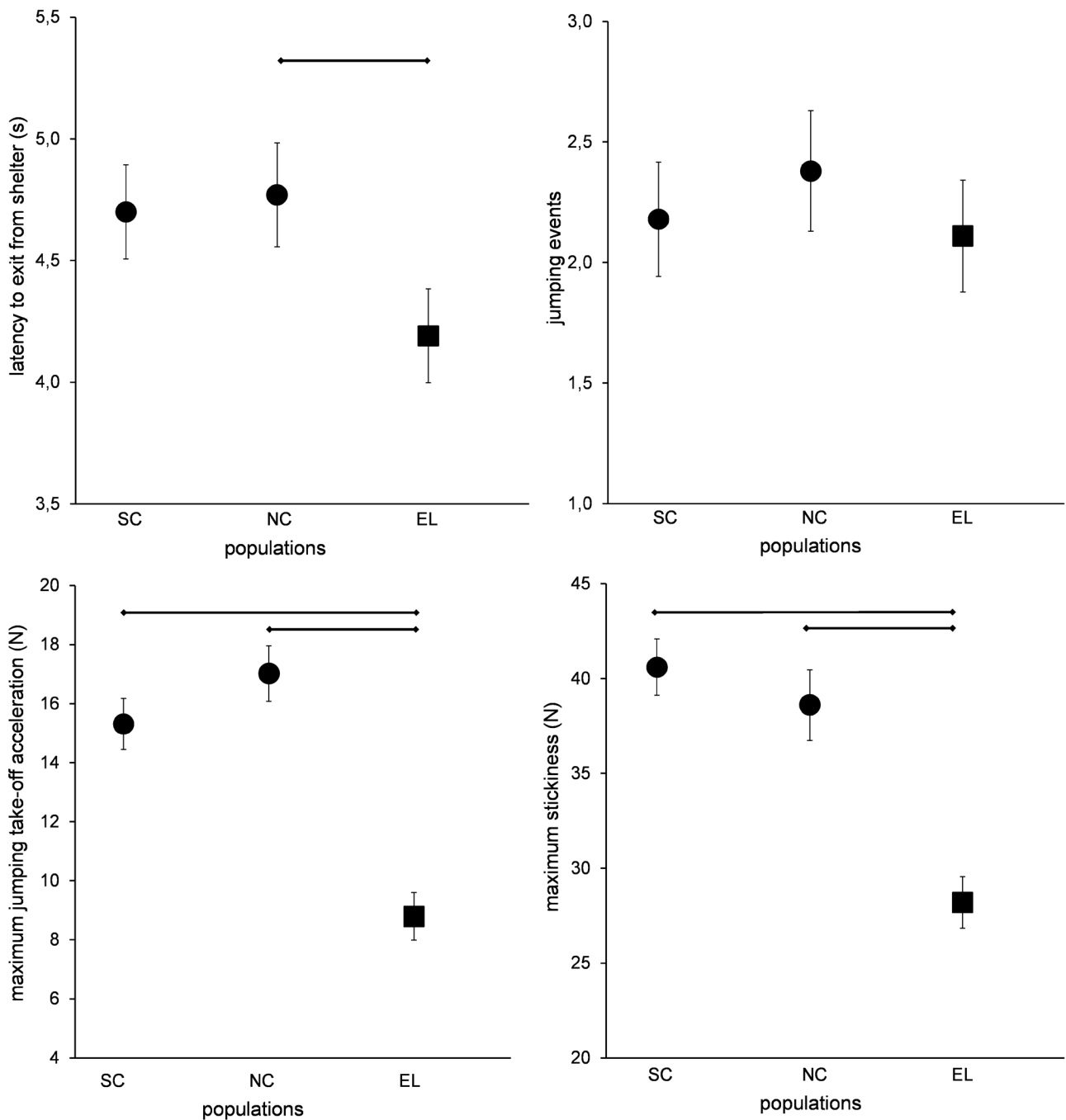
Figure 4. Estimated marginal means and standard errors from linear models for personality traits (on top) and performance traits (on bottom). SC, South Corsica; NC, north Corsica (black dot); EL, Elba Island (black square). Significant contrasts are indicated with bar.

south Corsica and north Corsica (Table 2). Both Corsica populations showed greater jumping takeoff performance than Elba populations, as well as higher stickiness. Moreover, both performance traits were significantly different between the 2 islands ($P < 0.001$). The EMM values of the maximum jumping takeoff performance (N) were twice higher in the north Corsica (NC = 17.02 ± 0.942) compared to those of Elba (E = 8.79 ± 0.807), while those of the south Corsica lied in the middle (SC = 15.31 ± 0.866). Similarly, values of stickiness, measured as the maximum adhesion force (N), were significantly higher in Corsica (SC = 40.6 ± 1.48; NC =38.6 ± 1.86) than in Elba (E = 28.2 ± 1.36). A summary of the coefficient estimates used to investigate population differences in performance traits

is reported in Table 2. Estimates of the island's effect size in performance differences were remarkable and the 95% CI excludes 0 for both maximum jumping forces at takeoff and stickiness (Figures 4 and 5).

Results did not change if 7 females out of 72 frogs were removed from all the models performed.

Relationship between personality and performance

Significant evidence for association between personality and performance traits, at population level, was found between jumping frequency during the exploration test and the maximum jumping performance. All 3 group of populations showed a significant association between these 2 traits (Table 3).

Table 3. Summary of general linear models used to validate the association between performance (jumping and stickiness) and personality traits (jumping events and latency to exit from shelter) at population level. Significant factors are shown in bold, indicating significant evidence between jumping frequency during the exploration test and the maximum jumping performance

Variable	Effect	*df*	*F*-value	*P*-value
Jumping	Population	39	33.067	**<0.001**
	Jumping events	38	1.206	0.279
	Population × jumping events	36	4.292	**0.0213**
Stickiness	Population	32	18.876	**<0.001**
	Jumping events	31	1.222	0.278
	Population × jumping events	29	0.358	0.702
Jumping	Population	40	28.917	**<0.001**
	Latency to exit from shelter	39	0.340	0.563
	Population × latency to exit from shelter	37	1.696	0.197
Stickiness	Population	33	21.472	**<0.001**
	Latency to exit from shelter	32	1.586	0.217
	Population × latency to exit from shelter	30	1.583	0.221
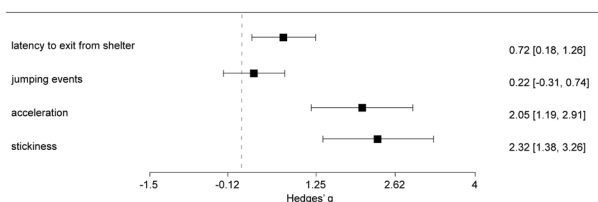
Figure 5. Forest plot estimating the effect size and 95% confidence interval calculated from all test statistics for both personality and performance traits between islands (Corsica vs. Elba).

Discussion

The present study provides evidence of a substantial differentiation in personality and locomotory performance traits of tree frog populations residing on a recently colonized island (late Pleistocene). We found that tree frogs from Elba Island were significantly bolder than individuals from Corsica (Table 2), which suggests a less prudent behavior. Moreover, individuals from Elba Island showed a markedly lower performance in both jumping and stickiness abilities than those from Corsica (Table 2). To our knowledge, this is the first study to provide empirical data that could relate to the evolution of personality and dispersal-related traits to past biogeographic events, as previously hypothesized (Canestrelli et al. 2016a). Such significant differentiation could not be explained by long-term allopatric divergence due to the isolation of Elba from Corsica populations, since Elba island and the north of Corsica were colonized as the last step of a single and recent range expansion event (less than 10 ka; Spadavecchia et al. 2021). While this island colonization event likely triggered the observed phenotypic differentiation, it might imply a number of different eco-evolutionary processes at play during and after the

colonization, molding spatial patterns of behavioral and performance traits, as detailed below.

The first one is selection in favor of dispersal-enhancing traits by spatial sorting (Shine et al. 2011). This process has been invoked to explain changes in dispersal-related phenotypes along an expansion route, in particular, at the range front of an expanding population (Lowe et al. 2015; Canestrelli et al. 2016b; Phillips and Perkins 2019). Mounting evidence on invasive species suggests that spatial sorting processes could promote directional changes in the phenotypic and genotypic makeup of populations during range expansions (e.g., Travis and Dytham 2002; Phillips et al. 2010; Shine et al. 2011; Brown et al. 2014). In the case of Corsica and Elba populations of *H. sarda*, spatial sorting should have promoted the highest degree of phenotypic differentiation between the south of Corsica (the starting point) and the northern populations, that is, northern Corsica and Elba, which were almost simultaneously colonized during the last range expansion (Spadavecchia et al. 2021). On the contrary, the highest degree of differentiation emerged between Elba and both Corsica populations. Although we cannot exclude a role of gene flow between northern and southern Corsica populations in smoothing putative phenotypic differences, the observed pattern is unlikely to be the product of spatial sorting.

Another hypothesis is the possible role played by ecological release in Elba Island. This process is the expansion of range, habitat, and/or resource use by an organism after arrival in a new community; it is often invoked for populations on initial island colonization (Gillespie 2009). Ecological release may have caused a shift in the behavioral repertoire of the founding population, leading to an increase in the expression of boldness (Blondel 2000; Novosolov et al. 2013; Baeckens and Van Damme 2020). The results of our work show that Elba individuals are bolder and less performing than Corsica individuals, which is surprising given their common origin and the similar ecological communities of the 2 sites. A bolder attitude could indicate a role of selection pressure due to, for example, a lower abundance of predators that could make this behavioral strategy advantageous (Gavriilidi et al. 2022). In a study on fire-bellied toads (*Bombina orientalis*), predation was a major driver of geographical differences in activity levels, which were lower in an island with high predation pressure (Kang et al. 2017). On the other hand, a lower performance in dispersal abilities might imply a role in habitat coverage or in fragmentation of suitable habitats that would favor dispersion. Elba is a continental island, with ecological communities that do not show obvious differences from neighboring mainland areas, and there is no evidence that it did undergo any massive change in animal and plant communities in the recent past. In particular, no differences could be considered for what concerns the presence of other predators for this species within Elba Island, such as other amphibians, birds, and snakes communities. However, in the absence of a formal comparative analysis of the respective ecological communities (and their variation over time), this scenario remains speculative, and a role played by local adaptation processes cannot be excluded.

Bioclimatic differences between islands certainly affect important habitat variables and might have promoted the observed differentiation on colonization of the Elba Island because they have a crucial role in the microhabitat cover, which could affect the probability of being detected from

predators and/or the relative safety in finding potential refuges (e.g., Martìn et al. 2005). However, results from a bioclimatic niche modeling showed a comparable situation between Corsica and Elba, leading us to exclude a role of past/present climatic conditions in explaining such a pattern of differentiation (Bisconti et al., 2011b). Likewise, we could also exclude any latitudinal effect that should have caused a differentiation between southern and northern Corsica, and Elba Island.

A crucial role in explaining the origin of the observed differences could be linked to a founder event associated with the colonization of Elba Island. The lower genetic diversity of the Elba Island populations, as compared to the rest of the species' range (Bisconti et al. 2011a), is a typical outcome of genetic drift after island colonization (Wright 1931; Waters et al. 2013) and could support a scenario of phenotypic divergence via founder event on colonization. Indeed, this study shows a substantial divergence in personality and performance traits in an isolated environment compared to the original population. At the same time, despite this being a parsimonious explanation of the observed pattern, it does not rule out a role of environmental filtering exerted by island recurring conditions or other factors. For example, since dispersal-related phenotypic traits might be affected by land-cover features (Brown et al. 2006; Shine et al. 2021), and vegetation patterns are plausibly relevant for a partially arboreal species like *H. sarda*, a comparative and retrospective analysis of the past and current vegetation coverage of the 2 islands Corsica and Elba could contribute to a better explanation of the observed differentiation.

Finally, we would like to emphasize that, to the best of our knowledge, our study is one of the few so far showing repeatable interindividual differences in jumping frequency, a trait plausibly related to locomotory performance in an insular context. Jumping frequency in tree frogs could relate to predator-escape ability, a trait which would be therefore under selection, as hypothesized for sprint speed in lizards (Hoskins et al. 2017). Interestingly, in all sampled populations, the jumping frequency was positively correlated with the jumping performance and this association between traits could be the inheritance of the past range expansion process, because it would have favored dispersal from Corsica to Elba.

The study of island populations has the potential to provide fundamental insights into the evolution of personality, one of the most studied aspects of the individual phenotype, because these traits might be part of the "island syndrome" (Gavriilidi et al. 2022). Studies on aggressive and acoustic behavior, as well as on flight distance, indicator of risk-taking behavior (e.g., Møller 2008; Hamao et al. 2021), and other specific antipredatory responses on this and other island species and populations, together with an analysis of local ecological and demographic factors, especially predation pressure and population density, should be the following steps to achieve a more complete picture of the causes of the peculiar behavioral phenotype of island populations. In this respect, this study by considering the legacy of a past range expansion event highlights the possible role of historical biogeographic processes in shaping insular phenotypic differentiation.

Conflict of Interest

No conflict of interests.

Author Contributions

D.Can., C.C., and R.B. designed research; D.Can., R.B., A.C., and A.L. performed research; A.L., D.Cos., and C.C. analyzed data; R.B. and C.C. wrote the paper with inputs from the other authors.

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