Does biogeographic history shape spatial patterns of intraspecific variation in personality, performance and morphology?

Roberta Bisconti[1], **Andrea Chiocchio**[1,*], **David Costantini**[1], **Claudio Carere**[1], **Daniele Canestrelli**

[1] Department of Ecological and Biological Sciences, Tuscia University, Largo dell'Università s.n.c., 01100 Viterbo, Italy
*Address corresponding to Andrea Chiocchio, E-mail: a.chiocchio@unitus.it

Abstract:

Addressing how individual variation within populations drives the evolution of biodiversity patterns is a major challenge in ecology and evolutionary biology. Historical biogeographic processes have had dramatic consequences on the structure of biodiversity. However, while the interplay between historical processes and genotypic diversity within populations has been widely investigated, the effects of such processes on phenotypic diversity remain poorly explored. Here, we investigate whether dispersal-driven processes of historical biogeographic relevance, such as late Pleistocene range dynamics, have contributed to shape the geographic patterns of phenotypic trait variation. We focus on dispersal-related personality, morphological and performance traits in the Tyrrhenian tree frog, *Hyla sarda,* which underwent a northward range expansion from the Sardinia Island to the Corsica Island during the Last Glacial Maximum, when a temporary land-bridge connected these islands. We collected tree frogs from four geographic areas along the past expansion route, controlling for altitude, local habitat, demographic factors, and bioclimatic differences between geographic areas. Then, we scored intraspecific variation in two personality traits, two performance traits, along with morphological traits likely involved in the dispersal process. Tree frogs from the northern area in Corsica were more prudent in a novel environment, they had significantly larger body size, longer limbs, wider heads, and displayed stronger take-off and adhesion performances compared to individuals from the source area in Sardinia. Overall, these results suggest a non-random spatial sorting of the intraspecific variation in multiple phenotypic traits during the range expansion phase. In turn, they also suggest that population differentiation in phenotypic traits associations might be a legacy of past biogeographic dynamics, identifying an overlooked driver of the current phenotypic architecture of animal populations.

KEYWORDS: Pleistocene range expansion, dispersal, personality, locomotory performance, biogeography

1. INTRODUCTION

Understanding the processes shaping the geographic patterns of intraspecific variation is a major challenge in ecological studies (Holt 2003; Des Roches et al., 2018; Raffard et al., 2019). In the last decades, considerable efforts have been focused on investigating the processes shaping the spatial distribution of genetic diversity within and among populations (Hewitt 2000, 2004; Schmitt, 2007; Weiss & Ferrand, 2007; Fonseca et al., 2023). Pleistocene glacial–interglacial cycles have been identified as a major factor in this respect, as they triggered periodic expansions and contractions of populations, which have left multiple imprints on their current genetic structure (Hewitt, 2004; Schmitt, 2007; Weiss & Ferrand, 2007; Fonseca et al., 2023). Although the link between historical biogeographic processes and intraspecific genetic diversity has been widely investigated, the contribution of these processes to the evolution of intraspecific phenotypic diversity is still virtually unexplored. At the same time, mounting evidence from studies on the ongoing range expansions (*e.g.* biological invasions) is showing that behavioural, morphological, physiological, and life history traits, play a crucial role in determining the ability of organisms to face new environmental conditions (Simmons & Thomas, 2004; Cote et al., 2007; Phillips et al., 2010; Shine et al., 2011). Therefore, investigating the interplay between biogeographic processes and intraspecific phenotypic variation is of great value to our understanding of how biodiversity evolves and is maintained.

Dispersal plays a main role in shaping the spatial pattern of biodiversity (Clobert et al., 2001; Travis et al., 2002; Ronce, 2007; Shine et al., 2011; Lowe & McPeek, 2014; Canestrelli et al., 2016a; Bonte & Dahirel, 2017). Recent studies have shown that inter-individual differences in dispersal ability can directly affect the success of colonisation into new areas and the establishment of new populations (Bowler & Benton, 2005; Cote et al., 2007; Clobert et al., 2009; Canestrelli et al., 2016a). In particular, literature on invasive species is showing that dispersers could be a non-random sample of individuals characterised by specific phenotypic profiles (Fraser et al., 2001; Phillips et al., 2008; Cote et al., 2010; Canestrelli et al., 2016a; Louppe et al., 2017). These studies suggest that evolutionary and ecological processes experienced by any expanding population drive the directional evolution of phenotypic traits at the range-edge of an expansion (Pintor et al., 2008; Atwell et al., 2012; Brodin et al., 2013; Myles-Gonzalez et al., 2015; Gruber et al., 2017). Behavioural traits can influence individual dispersal propensity (Cote et al., 2010; Canestrelli et al., 2016a). Individuals from the range-edge of an expansion would be characterized by a specific suite of personality traits, showing a more exploratory, more active, and bolder behaviour than those living in long established areas (Atwell et al., 2012; Myles-Gonzalez et al., 2015; Canestrelli et al., 2016a; Gruber et al., 2017). These traits are predicted to influence all dispersal stages (*i.e.,* departure, transience, and settlement; Dingemanse et al., 2003; Cote and Clobert, 2007; Cote et al., 2010; Canestrelli et al., 2016a), resulting in a rapid evolution of divergent personalities along an expansion route. Locomotory performance is also a key factor for expansion dynamics. During an expansion individuals would be spatially sorted by their locomotor abilities (Phillips, et al., 2010; Shine et al., 2011), and evidence showed how this process has often driven a rapid evolution in the locomotory traits (Travis & Dytham, 2002; Phillips et al., 2010; Shine et al., 2011) associated with morphological characteristics that would improve the dispersal rate compared to individuals living in long-invaded areas (Phillips et al., 2006; Phillips et al., 2008; Llewellyn et al., 2010; Louppe et al., 2017). For example, in the cane toad (*Rhinella marina*), a fast dispersal rate with straighter paths and longer legs has been documented at the edge of the invaded Australian range (Phillips et al., 2008; Alford et al., 2009; Llewellyn et al., 2010). However, while evidence on the impact of differential dispersal profiles and personality during contemporary expanding populations is growing, how phenotypic polymorphisms linked to dispersal abilities would have contributed to key past processes structuring biogeographic patterns remains substantially unexplored.

In this study we aim to bridge this gap by exploring how dispersal-driven processes may have shaped the contemporary geographic patterns of intraspecific variation in personality, performance, and morphological traits along the past expansion route of the Tyrrhenian tree frog (*Hyla sarda*; Bisconti et al., 2011a, Spadavecchia et al., 2021). Previous phylogeographic investigations on this amphibian have provided relevant insights into its historical range expansion (Bisconti et al., 2011a; Spadavecchia et al., 2021). During the last Glacial Maximum, when the sea levels were lower, a temporary land-bridge connected the islands of

Sardinia and Corsica (Van Andel & Shackleton, 1982; Shackleton et al., 1984), allowing a northward range expansion of the Tyrrhenian tree frog from Sardinia to Corsica (Bisconti et al., 2011a; Spadavecchia et al., 2021). However, after the last Glacial Maximum, the sea levels rose, separating the newly established populations in Corsica from their source populations in Sardinia, thus impeding the subsequent flow of individuals from the rear. Recent studies investigating physiological traits of tree frog individuals from these two islands clearly showed a spatial pattern of variation, likely linked to this historical population expansion. Specifically, populations of the two islands showed a clear differentiation in rates of physiological ageing owing to oxidative stress, different telomere dynamics, and differences in anti-predator strategies (Liparoto et al., 2020; Canestrelli et al., 2021; Spadavecchia et al., 2023).

We explored the hypothesis that historical biogeographic events of dispersal contribute to shape patterns of phenotypic diversity. We expect that Sardinia and Corsica treefrogs would differ in dispersal-related phenotypic traits, such as personality, morphology, and locomotor performance. We also tested the hypothesis that this legacy of past events should be detected in the geographic pattern of these dispersal-related phenotypic traits. Specifically, if dispersers are a non-random sample of individuals, we predict that, compared to Sardinia tree frogs, Corsica tree frogs should be bolder, and have longer limbs and better jumping performance.

2. MATERIAL AND METHODS

2.1 Sampling and housing

Sample collection of tree frogs was carried out along a latitudinal transect from the northern part of the island of Corsica to the ancestral area of the glacial refuge in central-eastern Sardinia (Figure 1 and Table 1). A total of 93 tree frogs were collected in spring 2018 from four areas (Figure 1; Table 1). Individuals were captured with hand nets during the first hours of the night, after acoustic and visual localization and then transported to the housing facilities at Tuscia University (Ichthyogenic Experimental Marine Centre, CISMAR:), Italy. Housing conditions are described in detail in Bisconti et al., 2023.

The sampling design was accurately planned to reduce possible confounding effects linked to local habitat, kinship or inbreeding between individuals: a) we sampled two areas per island, and two breeding sites for each area, collecting individuals from different ponds within each site (see Table 1 and Figure 1); b) all the sampling areas were located on the coasts on the eastern side of species distribution, to avoid both current and past bioclimatic differences among sampling sites, as showed by previous species distribution model (Bisconti et al., 2011a); c) we collected tree frogs from ponds exclusively located to coastal areas to avoid any effect linked to environmental conditions correlated to altitude; d) finally, to avoid any effect due to ecological and evolutionary effects of colonisation through jump dispersal rather than through a spatial diffusion process, we intentionally excluded other islands (Elba and Capraia) inhabited by this species (Bisconti et al., 2011a,b; Bisconti et al., 2023).

2.2 Ethical note

All the 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 Prefecture of Corsica (#2A20180206002 and #2B20180206001).

Permission to temporarily house amphibians at Tuscia University (Ichthyogenic Experimental Marine Centre, CISMAR:), Italy. All handling procedures outlined in the present study were approved by the Ethical Committee of the of the Tuscia University for the use of live animals. During captivity the animals were monitored daily. No adverse effects on the overall health condition of the tree frogs were observed during the procedures. The animals were released in the original sampling locations at the end of the experimentation.

2.3 Behavioural tests

To investigate personality traits variation along the post-glacial range expansion route of *H. sarda* we focused on two personality traits that are commonly associated to the dispersal propensity: exploration attitude in a novel environment and boldness (Canestrelli et al., 2016a). Exploratory activity is considered crucial in promoting dispersal, as individuals with a greater propensity toward exploration were generally found at the expanding range edge (Dingemanse et al., 2003; Cote et al., 2010). Exploration activity was investigated measuring the latency in exploring a novel environment, and the time spent in exploration. Propensity to disperse was also assessed by investigating the individual’s boldness, *i.e.* the individual's propensity to take risks (Réale et al., 2007; Canestrelli et al., 2016a). Bold individuals are considered willing to accept the intrinsic risks of dispersal (Stamps, 1985; Bonte et al., 2012), thus dispersing earlier than shyer conspecific.

To assess these personality traits, we performed two distinct behavioural tests as described below and each test was repeated after 10 days to measure temporal consistency. All the tests were performed in the housing room, to avoid any changes in humidity and temperature that could affect the analysed behavioural features (Duellman & Trueb, 1994).

2.3.1 Exploration in a novel environment

Considering the arboreal lifestyle of *H. sarda*, a cylindric arena developed in both vertical and horizontal dimensions was employed, enriched with plants (*Epipremnum aureum*) and an oakwood that allows the individuals to hide, eliminating the possibility that the measured behaviours reflected abnormal fear or anxiety rather than spontaneous exploratory behaviour (see Bisconti et al., 2023). The test is accurately described in Bisconti et al., (2023). Briefly: after a 5-minute acclimatation period, individuals were left to explore the arena for 10 minutes, and the individual exploration behaviour was recorded using an HD video camera (Nova Germany model DVR AHD-7908). We extracted the following variables using Boris 5.1.3 (Friard & Gamba, 2016): 1) latency to explore (s); 2) duration of activity (expressed as percentage of duration of test); 3) duration of time spent on the arena ground floor; 4) jumping activity, expressed as percentage of time in activity (moving) spent jumping.

2.3.2 Boldness

This test was performed to assess the individual behaviour across the boldness-shyness behavioural axis. We considered the latency to exit from a shelter as a proxy of the individual propensity to take risk. A HD video camera (Nova Germany model DVR AHD-7908) was used to record a rectangular arena where all animals were caged into a cylindrical dark shelter for a 6-minute acclimatation period and then left free to exit (Bisconti et al., 2023). The software Boris 5.1.3 was used to analyse the individual behaviour, measuring the time it took to exit the shelter.

2.4 Locomotory performance tests

To investigate the variation in locomotory performances along the past range expansion route of *H. sarda* we assessed two performance traits that could have a crucial role in the species dispersal. Considering jumping as the predominant mode of locomotion in tree frogs, we explored two performance traits linked to jumping abilities: the jumping force, and the adhesion force. The jumping force was inferred evaluating the force at take-off, that may be considered a crucial trait in determining the dispersal capacity in a tree frog since the distance reached with jumps is a function of the propulsion force generated at take-off (*e.g.,* Marsh & John-Alder 1994; Nauwelaerts & Aerts 2006 and references therein). The adhesion force is also considered a proxy of dispersal abilities because it plays a main role during the landing phase (Duellman & Trueb, 1994; Bisconti et al., 2023). Indeed, tree frogs have specialised toe pads used during the landing phase (Emerson & Diehl 1980; Federle et al. 2006; Smith et al. 2006). Therefore, the differential ability to adhere to the substrate after a jump would improve the safety of the landing, avoiding missing the target (Bijma et al., 2016).

The jumping force was quantified by means of a jumping test, while the adhesion force was quantified by a stickiness test, as described below. Before each test, all the individuals were weighted using a scale Acculab model (ATILON ATL-224-I).

2.4.1 Jumping test

A rectangular arena was videotaped with a video camera (Panasonic model no. DMC-FZ300) placed laterally to the setup. Each tree frog was fitted with an accelerometer data logger (two Axy-4 units, Technosmart, Rome, 9.15 x 15 x 4 mm, 1 gr weight including battery) set to record triaxial acceleration (0 - 4 g) at 100 Hz, and the tree frog was induced to jump by stimulating its caudal region (Mitchell & Bergmann, 2016). Five jumps for individuals were collected (see Bisconti et al., 2023 for further details). The dynamic body acceleration for each dimension was extracted from the downloaded data (x, y, z; Wilson et al., 2006; Shepard et al., 2008). The logger's x axis recorded sway, the y axis recorded surge, and the z axis recorded heave (Halsey et al., 2008). By using Framework 4 software (version 2.5), we added these values to get the Vector sum of Dynamic Body Acceleration [VeDBA = (Ax2+ Ay2+ Az2)], quantifying five VeDBA values for each individual, one for each jump, and the highest value of VeDBA was chosen to calculate the individual maximum jumping force at take-off [VeDBA x mass] and considered for the analysis.

2.4.2 Stickiness test

A smooth plastic rotating wheel with a moderate and constant angular speed (3 revolutions per minute) was used to test the maximum adhesiveness force. We recorded five falls for each individual using Nova Germany model DVR AHD-7908. The software Tracker (version 4.11.0) was used to extract the angle of fall (radian) for each of the five falls, and the largest value was selected for the subsequent analyses. The maximum adhesion force was calculated [cos (α)× mass × g] using Barnes et al. (2006)'s standard protocol (see Bisconti et al., 2023, for further details).

2.5 Morphological traits

Locomotion in anurans with an arboreal lifestyle is characterised by individuals with morphological adaptations to better perform during jumps and landings (Kamada et al., 2017; Bijma et al., 2016). Thus, we investigate those morphological traits that directly play a role in these two phases of locomotion, such as body size and limb lengths (Phillips et al., 2006). We also collected data on another morphological trait that is intrinsically linked to jumping performance: the head size. Indeed, there is evidence suggesting that the head plays a crucial role improving the ability of tree frogs in performing jumps, especially during the jumping phase by maintaining the posture, and during the landing phase by hitting the substrate first (Reilly et al., 2016; Kamada et al., 2017).

We collected measures of the following morphological traits: (1) snout–vent length (SVL; mm); (2) head size (mm); (3) forelimb length (mm); (4) hindlimb length (mm). To obtain standardised measures, the tree frogs were anaesthetised by a two-minute submersion in a solution of MS-222 (0.05% m/v), placed in the prone position on 1x1 cm grid paper, parallel to the plane of a camera lens, and photographed using a Panasonic LUMIX (DMC-FZ300) digital camera. All photographs were taken with the same camera settings and under the same lighting conditions. After the photographs, each individual was placed in a humid box until it fully recovered, and then reintroduced into its own fauna box. The measures were then extracted by the photographs and analysed by using ImageJ 1.52 (National Institutes of Health, USA). The measurement of head size, forelimb length and hindlimb length have been standardised by extracting the residual of the regression between each single trait and the SVL.

2.6 Data analysis

We performed all statistical analyses using R software version 4.3.1 (R Core Team 2023). To evaluate the repeatability of the behavioural traits, we ran Generalized Linear Mixed effect (GLMM)-based repeatability models using the "rpt" function of the *rptR* package (Schielzeth & Nakagawa, 2011; Stoffel et al., 2017), setting as Poisson distribution datatype; duration of activity and duration of time spent on the arena floor level were treated as Proportion datatype. We entered each behavioural trait as a dependent variable and individual as a random factor. We considered behavioural variables as personality traits, and used them in the subsequent analyses, when their repeatability value R > 0.2, and the lower bound of the CI was > 0.0 (Brodin et al., 2013; Kelleher et al., 2017; Kelleher et al., 2018 and references therein). Preliminary models showed that entering sampling location within each island as a random factor did not improve the fit of the model (i.e. the AIC value was not reduced beyond 2); thus, it was not subsequently considered. Also, running the models with or without females did not change at all both the model fit and the results, and thus we retained females for all the subsequent analyses.

We tested the hypothesis of a phenotypic change across the past expansion route of *Hyla sarda* from Sardinia to Corsica. First, we tested the association of phenotypic variation with the distance from the estimated area of origin of the range expansion from Sardinia to Corsica as estimated in Spadavecchia et al. (2021). We set a distinct linear regression model between each behavioural, performance and morphological variable and the distance from the area of origin, measured in km using the Google Earth (Google Inc.) "ruler" function.

Then, we assessed the differences in personality, performance, and morphological traits between the two islands by running Generalized Linear Models (GLMs; "lm" function in the basic package *stats*) entering each variable singly as dependent variables, and island as fixed factor. We also run the analyses including sampling location within each island as a random factor in generalized linear mixed-effect models (GLMMs; "lmer" function in the *lme4* package) and selected the best-fit model using the AIC (lowest AIC). For repeatable personality traits, we used the mean between the two repetitions; the variables were square-root (frequency of jumping events) or log10 (all the others) transformed to meet the assumption of residuals normality. To exclude the effect of the body size on the morphological traits, for each measured trait we extracted the residuals of the regression with the SVL. Normality and homoscedasticity of model residuals were evaluated to ascertain the goodness of model fit. For the selected best-fit models, we contrasted the estimated marginal means and standard errors from the models as computed using the *emmeans* r package.

3. RESULTS

The repeatability coefficients with its CI and the p-value of are reported in Table 2. Three out of the five investigated behavioural traits resulted significantly repeatable: boldness, measured as the latency to exit from the shelter (r = 0.40), the frequency of jumping events during the arena exploration (r = 0.35), and the latency to explore a novel environment (r = 0.31). Conversely, activity and duration of time spent on the arena floor were not significantly repeatable and thus they were not considered as personality traits and excluded from the subsequent analyses.

Results from all the models showed a phenotypic change across the past expansion route of *Hyla sarda* from Sardinia to Corsica. We found significant associations between phenotypic variation and the distance from the estimated area of origin of the range expansion from Sardinia to Corsica for all the analysed traits but two (hind limb length, and head width; see Supplementary Figure S1). Also, we found substantial and significant differences between Sardinia and Corsican populations for all the analysed traits but one (maximum jumping force at take-off). The AIC model comparison (Supplementary Table 1) showed that entering sampling location within each island as a random factor did not improve the fit of the model (*i.e.* the AIC value was not reduced) for all the traits but two (performance traits), supporting the independence of the pattern found from local sampling site conditions.

We found significant differences in personality traits between Sardinia and Corsica individuals (Table 3), with Corsica individuals showing a shyer profile and higher jumping frequencies. The latency to exit from the shelter and the latency to explore were longer for Corsica individuals than those showed by Sardinia individuals ($p = 0.032$ and $p < 0.001$, respectively). The jumping frequency during exploration was significantly higher in Corsica than in Sardinia ($p = 0.001$). The estimated marginal means and standard errors for personality differences between Sardinia-Corsica are shown in Figure 2.

Sardinia and Corsica populations differed in both jumping force and stickiness, with tree frogs from Corsica showing higher performance values than individuals from Sardinia (Table 3 and Figure 3). However, only stickiness showed significant differences (p <0.001), whereas the differences in jumping force resulted not significant (p = 0.094). The estimated marginal means and standard errors for personality differences between Sardinia-Corsica are shown in Figure 3.

All morphological traits linked to dispersal ability showed significant differences between the two populations (Table 4 and Figure 4). The investigated morphological traits showed that Corsica tree frogs were larger in body size (p <0.001), with longer forelimbs (p = 0.044) and hindlimbs (p = 0.026), and wider head size (p = 0.011).

4. DISCUSSION

We found substantial and significant differences between individuals from the two islands in all the investigated phenotypic traits, with the Corsica tree frogs showing a shyer behavioural profile, longer decision-making time, a significant attitude to jump during exploration, and more efficient dispersal-related performance and morphological traits.

The spatial phenotypic pattern observed could be explained by several main scenarios. Variation in bioclimatic conditions between the two islands could contribute to shape the differences founded. Climatic conditions could, indeed, affect habitat variables and the microhabitat cover, that in turn, could differentially influence the dispersal and the ability in avoiding predators especially for an arboreal species (Martin et al. 2005). However, previous studies showed a comparable bioclimatic condition between the two islands that, coupled with a negligible latitudinal effect, contribute to excluding a role of variation of climatic condition in explaining such a pattern of differentiation (Bisconti et al., 2011a). An alternative scenario could be attribute to differences in ecological community between the two islands. In particular, the presence of different interacting species within the two islands could contribute to mould local adaptations, for example in avoiding predation (Kang et al. 2017; Gavriilidi et al., 2022). Yet, Corsica and Sardinia are continental islands, they share geological origin and history, and no differences in their animal communities had never been reported, contributing to rule out a possible role of these ecological forces to explain the pattern emerged. Lastly, a genetic basis of diversity among populations of the two islands could be invoked to discuss the phenotypic differences showed. Therefore, divergent populations could show different phenotypic traits even within the species level (Lipshutz, et al., 2017; Stamp et al., 2020). However, previous phylogeographic and population genetic investigations showed the absence of remarkable differences in the levels of genetic variability and the lack of spatial genetic structure between Sardinia and Corsica populations, thus excluding a genetic influence in explaining the pattern observed (Bisconti et al., 2011a,b). Our data may suggest that the past range expansion has been promoted by a non-random sample of individuals, and the evolution of the observed differences would be the result of different selection pressure along the colonisation route. Individuals that successfully colonised Corsica Island crossing the land bridge during the expansion phase would be sorted by their dispersal propensity, and this spatial sorting processes would have promoted directional changes in the phenotypic profiles of individuals during this past range expansions. This scenario would be further supported by the observed non-random spatial pattern in the geographical cline of all phenotypic traits studied. All these traits would show a clear latitudinal gradient of variation that perfectly mirrors the putative route of range expansion for this species (see Supplementary Figure 1). In the following sections, we will discuss our findings for each group of traits studied, evaluating their putative implications during range expansion and the colonization of new environments.

The shyer behavioural profile showed by the Corsica individuals could appear counterintuitive, as dispersers at the range edge have frequently been described as bolder and more exploratory than those living in long-established areas (*e.g.* Pintor et al., 2008; Liebl & Martin, 2012; Atwell et al., 2012; Myles-Gonzalez et al., 2015; Canestrelli et al., 2016a; Gruber et al., 2017). However, recent empirical studies have been showing that a shy and more prudent behavioural profile is an efficient alternative strategy to navigate in a novel environment (Gavriilidi et al., 2022; Mazza & Eccard, 2023; Eccard et al., 2023). On the contrary, a shyer profile in expanding populations could allow a more careful assessment of the surrounding environment, thereby reducing risks and ultimately increasing the chances of survival (Groothuis & Carere 2005; Réale et al. 2010; Mazza & Eccard, 2023). By requiring less energy investment in exploration, this behavioural profile could favour metabolic resource allocation to other critical functions pivotal for a successful colonisation, such as foraging, growth, and reproduction (Verbeek et al., 1994; Deerenberg et al., 1998; Cockrem, 2007; Coppens et al., 2010). Besides, the higher propensity of Corsica tree frogs to jump during their overall activity duration, compared to individuals from the source island, may have facilitated a cost-benefit optimization for these dispersers. Despite the energetic costs associated with a jumping locomotory mode, it could allow individuals to cover longer distances with a single movement, interspersed with pauses for energy recovery and for predatory and environmental context assessment, before exploring again. The higher propensity to jump shown by tree frogs from Corsica is reflected in their better locomotory performance than those from Sardinia. As key traits in determining species dispersal ability, locomotory performance traits play a direct role in dispersal processes and, consequently, in the overall success of expansion (Phillips et al., 2006; Phillips et al., 2008; Llewellyn et al., 2010; Louppe et al., 2017). The performance trait analysis revealed that Corsica tree frogs exhibit greater jumping and adhesion forces than those from Sardinia. In such an arboreal species, both traits are expected to significantly enhance dispersal ability, playing a crucial role during both the initial jumping phase and the subsequent landing phase. This may contribute to covering longer distances with each jump while minimizing the risk of missing the target during landing (see material and methods; Duellman and Trueb, 1994; Kosmala et al., 2017). More performant profiles showed by Corsica individuals during dispersal are in line with findings from several other studies exploring locomotory performance traits in contemporary expanding populations (Phillips et al., 2008; Alford et al., 2009; Llewellyn et al., 2010). In fact, recent studies on invasive species have investigated the evolution of locomotory performance traits at the range-edge of expanding populations, revealing a significant improvement in the locomotor abilities of dispersers, thus suggesting that, during an expansion, individuals may be spatially sorted based on their dispersal-related performance ability (Phillips et al., 2008, Alford et al., 2009; Llewellyn et al., 2010; Shine et al., 2011; Louppe et al., 2017).

Morphological analyses showed that Corsica tree frogs have a larger body size, longer limbs, and a wider head size than those from Sardinia. All these features imply a better performance of these individuals during the dispersal and the colonisation of new environments. A larger body size would confer several advantages to individuals during an expansion. Studies on amphibians showed that larger individuals have a better locomotory performance both on land and in water (Cabrera-Guzman et al., 2013). Moreover, a larger size plays an important role in other essential functions particularly crucial for an amphibian during colonisation, such as avoiding dehydration (Kosmala et al., 2020), allowing to be more performant in capturing prey (Cabrera-Guzman et al., 2013) and also increasing the reproductive success, whereby the larger male would be more successful both in intraspecific competition and during the amplexus (Bowcock et al., 2013; Herrel et al., 2012; Clarke et al., 2019). Similarly, longer limbs improve locomotion, and, thus, the dispersal ability (Phillips et al., 2006; Clarke et al., 2019). Longer legs allow a greater propulsive force in jumping

(Kamada et al., 2017; Bijma et al., 2016). For example, in the invasive *Rhinella marina*, individuals at the edge of the Australian invasion front exhibited longer legs, a trait significantly enhancing invasion success (Phillips et al., 2006). In the context of jumping amphibians like tree frogs, longer forelimbs could also contribute to improving landings, while dampening the energy of jumps upon touchdown (Bijma et al., 2016). Lastly, colonising individuals with a wider head would likely adapt better to landings after jumps. The increased propulsion resulting from longer legs might lead to more forceful landings and given that the head is the first part of the body to arrive on a surface, a larger head size could confer better management of shock absorption during collisions.

In conclusion, this study provides novel evidence that historical range expansion could have played a role in explaining the geographic patterns of intraspecific phenotypic variation in natural populations. Rapid, substantial, and directional evolution in dispersal-enhancing traits had been already documented in populations currently undergoing range expansion (Phillips et al., 2006; Liebl and Martin, 2012; Gruber et al., 2017; Kosmala et al., 2017). However, although studies of ongoing invasion provide an excellent opportunity to investigate the causes and consequences of dispersal dynamics, they do not allow the investigation of their long-term legacies, in evolutionary perspective (Canestrelli et al., 2016a, b). The selective advantages associated with adaptive, dispersal-enhancing, traits during an expansion phase may be transient, as suggested by several empirical evidence (Travis & Dytham, 2002; Brown et al., 2007; Phillips et al., 2008; Brown et al., 2014). Recently established populations may indeed restore variation (phenotypic and genotypic) through a slower flow of individuals from the rear or may evolve novel trait states in response to demographic density increases in newly colonised areas (Cobben et al., 2015; Canestrelli et al., 2016a; Perkins et al., 2016). To date, we are not aware of studies addressing if the predicted directional changes in dispersal-enhancing traits may persist in populations long after the expansion stops, and the demographic conditions promoting spatial sorting processes vanish (Cobben et al., 2015; Canestrelli et al., 2016a; Perkins et al., 2016). In this frame, the expansion history of the Tyrrhenian tree frogs could offer the opportunity to learn about the long-term legacy of non-neutral processes at play during a range expansion. Finally, to investigate the genomic underpinnings of the phenotypic evolution promoted by historical range expansions appears the next mandatory step to reach a more complete understanding of the eco-evolutionary processes moulding spatial and temporal patterns of variation of biological diversity.

REFERENCES

Alford, R. A., Brown, G. P., Schwarzkopf, L., Phillips, B. L., & Shine, R. (2009). Comparisons through time and space suggest rapid evolution of dispersal behaviour in an invasive species. *Wildlife Research*, *36* (1), 23–28. https://doi.org/10.1071/WR08021

Atwell, J. W., Cardoso, G. C., Whittaker, D. J., Campbell-Nelson, S., Robertson, K. W., & Ketterson, E. D. (2012). Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. *Behavioral Ecology*, *23* (5), 960–969. https://doi.org/10.1093/beheco/ars059

Barnes, W. J. P., Oines, C., & Smith, J. M. (2006). Whole animal measurements of shear and adhesive forces in adult tree frogs: insights into underlying mechanisms of adhesion obtained from studying the effects of size and scale. *Journal of Comparative Physiology A*, *192* (11), 1179–1191. https://doi.org/10.1007/s00359-006-0146-1

Bijma, N. N., Gorb, S. N., & Kleinteich, T. (2016). Landing on branches in the frog *Trachycephalus resinifictrix* (Anura: Hylidae). *Journal of Comparative Physiology A*, *202* (4), 267–276. https://doi.org/10.1007/s00359-016-1069-0

Bisconti, R., Canestrelli, D., Colangelo, P., & Nascetti, G. (2011a). Multiple lines of evidence for demographic and range expansion of a temperate species (*Hyla sarda*) during the last glaciation. *Molecular Ecology*, *20* (24), 5313–5327. https://doi.org/10.1111/j.1365-294X.2011.05363.x

Bisconti, R., Canestrelli, D., & Nascetti, G. (2011b). Genetic diversity and evolutionary history of the Tyrrhenian treefrog *Hyla sarda* (Anura: Hylidae): adding pieces to the puzzle of Corsica–Sardinia biota. *Biological Journal of the Linnean Society*, *103* (1), 159–167. https://doi.org/10.1111/j.1095-8312.2011.01643.x

Bisconti, R., Carere, C., Costantini, D., Liparoto, A., Chiocchio, A., & Canestrelli, D. (2023). Evolution of personality and locomotory performance traits during a late Pleistocene island colonization in a tree frog. Current zoology, 69(5), 631-641. https://doi.org/10.1093/cz/zoac062

Bonte, D., Van Dyck, H., Bullock, J.M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., Saastamoinen, M., Schtickzelle, N., Stevens, V.M., Vandewoestijne, S., Baguette, M., Barton, K., Benton, T.G., Chaput-Bardy, A., Clobert, J., Dytham, C., Hovestadt, T., Meier, C.M., Palmer, S.C., Turlure, C. & Travis, J.M.. 2012. Costs of dispersal. Biological Reviews 87:290–312. htpps:// doi.org/10.1111/j.1469-185X.2011.00201.x.

Bonte, D., & Dahirel, M. (2017). Dispersal: a central and independent trait in life history. *Oikos, 126* (4), 472–479. https://doi.org/10.1111/oik.03801

Bowcock, H., Brown, G. P., & Shine, R. (2013). Sexual selection in cane toads *Rhinella marina*: A male's body size affects his success and his tactics. *Current Zoology*, 59(6), 747-753. https://doi.org/10.1093/czoolo/59.6.747

Bowler, D. E., & Benton, T. G. (2005). Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews*, *80* (2), 205–225. https://doi.org/10.1017/s1464793104006645

Brodin, T., Lind, M.I., Wiberg, M.K., & Johansson, F. (2013). Personality trait differences between mainland and island populations in the common frog *Rana temporaria*. *Behavioral Ecology and Sociobiology*, 67:135–143. https://doi.org/10.1007/s00265-012-1433-1

Brown, C., Burgess, F., & Braithwaite, V. A. (2007). Heritable and experiential effects on boldness in a tropical poeciliid. *Behavioral Ecology and Sociobiology*, 62 (2), 237–243. https://doi.org/10.1007/s00265-007-0458-3

Brown, G. P., Phillips, B. L., & Shine, R. (2014). The straight and narrow path: the evolution of straight-line dispersal at a cane toad invasion front. Proceedings of the Royal Society B: Biological Sciences, 281 (1795), 20141385. https://doi.org/10.1098/rspb.2014.1385

Cabrera-Guzmán, E., Crossland, M. R., Brown, G. P., & Shine, R. (2013). Larger body size at metamorphosis enhances survival, growth and performance of young cane toads (*Rhinella marina*). *PLoS One*, *8*(7), e70121. https://doi.org/10.1371/journal.pone.0070121

Canestrelli, D., Bisconti, R., & Carere, C. (2016a). Bolder Takes All? The Behavioural Dimension of Biogeography. *Trends in Ecology and Evolution*, *31* (1), 35–43. https://doi.org/10.1016/j.tree.2015.11.004

Canestrelli, D., Porretta, D., Lowe, W. H., Bisconti, R., Carere, C., & Nascetti, G. (2016b). The tangled evolutionary legacies of range expansion and hybridization. *Trends in Ecology & Evolution*, 31(9), 677-688. https://doi.org/10.1016/j.tree.2016.06.010

Canestrelli, D., Bisconti, R., Liparoto, A., Angelier, F., Ribout, C., Carere, C., & Costantini, D. (2021). Biogeography of telomere dynamics in a vertebrate. *Ecography*, 44: 453–455. https://doi.org/ /10.1111/ecog.05286

Clarke, G. S., Shine, R., & Phillips, B. L. (2019). May the (selective) force be with you: spatial sorting and natural selection exert opposing forces on limb length in an invasive amphibian. *Journal of Evolutionary Biology*, *32*(9), 994-1001. https://doi.org/10.1111/jeb.13504

Clobert, J., Danchin, E., Dhondt, A. A., & Nichols, J. (2001). *Dispersal.* Oxford University Press, New York. https://doi.org/10.1046/j.1365-2540.2001.0963a.x

Clobert, J., Le Galliard, J. F., Cote, J., Meylan, S., & Massot, M. (2009). Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology letters*, *12* (3), 197–209. https://doi.org/10.1111/j.1461-0248.2008.01267.x

Cobben, M. M. P., Verboom, J., Opdam, P. F. M., Hoekstra, R. F., Jochem, R., & Smulders, M. J. M. (2015). Spatial sorting and range shifts: consequences for evolutionary potential and genetic signature of a dispersal trait. *Journal of Theoretical Biology*, *373*, 92–99. https://doi.org/10.1016/j.jtbi.2015.03.019

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology*, 148(Suppl 2), 169-178. https://doi.org/10.1007/s10336-007-0175-8

Coppens, C. M., de Boer, S. F., & Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: towards underlying mechanisms. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1560), 4021-4028. https://doi.org/10.1098/rstb.2010.0217

Cote, J., & Clobert, J. (2007). Social personalities influence natal dispersal in a lizard. *Proceedings of the Royal Society B: Biological Sciences*, *274* (1608), 383–390. https://doi.org/10.1098/rspb.2006.3734

Cote, J., Clobert, J., Brodin, T., Fogarty, S., & Sih, A. (2010). Personality–dependent dispersal: Characterization, ontogeny and consequences for spatially structured populations. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365* (1560), 4065–4076. https://doi.org/10.1098/rstb.2010.0176

Deerenberg, C., Overkamp, G. J. F., Visser, G. H., & Daan, S. (1998). Compensation in resting metabolism for experimentally increased activity. *Journal of Comparative Physiology B*, 168, 507-512. https://doi.org/10.1007/s003600050171

Des Roches, S., Post, D.M., Turley, N.E. Bailey, J. K., Hendry, A. P., Kinnison, M. T., Schweitzer, J. A. & Palkovacs, E. P. (2018). The ecological importance of intraspecific variation. *Nature Ecology & Evolution* 2, 57–64. https://doi.org/10.1038/s41559-017-0402-5

Dingemanse, N. J., Both, C., van Noordwijk, A. J., Rutten, A. L., & Drent, P. J. (2003). Natal dispersal and personalities in great tits (*Parus major*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270* (1516), 741–747. https://doi.org/10.1098/rspb.2002.2300

Duellman, W. E., & Trueb, L. (1994). *Biology of amphibians*. JHU press.

Eccard, J. A., Mazza, V., Holland, C., & Stuart, P. (2023). The timid invasion: behavioural adjustments and range expansion in a non-native rodent. *Proceedings of the Royal Society B*, *290*(2003), 20230823. https://doi.org/10.1098/rspb.2023.0823

Emerson, S.B., Diehl, D. (1980). Toe pad morphology and mechanisms of sticking in frogs. *Biological Journal of the Linnean Society*, 13:199–216. https://doi.org/10.1111/j.1095-8312.1980.tb00082.x

Federle, W., Barnes, W.J.P., Baumgartner, W., Drechsler, P., & Smith, J.M. (2006). Wet but not slippery: boundary friction in tree frog adhesive toe pads. *Journal of the Royal Society Interface*, 3:689–697. https://doi.org/10.1098/rsif.2006.0135

Fonseca, E. M., Pelletier, T. A., Decker, S. K., Parsons, D. J., & Carstens, B. C. (2023). Pleistocene glaciations caused the latitudinal gradient of within-species genetic diversity. *Evolution Letters*, 7(5), 331-338. https://doi.org/10.1093/evlett/qrad030

Fraser, D. F., Gilliam, J. F., Daley, M. J., Le, A. N. & Skalski, G. T. (2001). Explaining leptokurtic movement distributions: intrapopulation variation in boldness and exploration. *The American Naturalist*, *158* (2), 124–135. https://doi.org/10.1086/321307

Friard, O., & Gamba, M. (2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, 7:1325–1330. https://doi.org/10.1111/2041-210X.12584

Gavriilidi, I., De Meester, G., Van Damme, R., & Baeckens, S. (2022). How to behave when marooned: the behavioural component of the island syndrome remains underexplored. *Biology Letters*, 18(4), 20220030. https://doi.org/10.1098/rsbl.2022.0030

Groothuis, T. G., & Carere, C. (2005). Avian personalities: characterization and epigenesis. *Neuroscience & Biobehavioral Reviews*, 29(1), 137-150. https://doi.org/10.1016/j.neubiorev.2004.06.010

Gruber, J., Brown, G., Whiting, M. J., & Shine, R. (2017). Geographic divergence in dispersal–related behaviour in cane toads from range–front versus range–core populations in Australia. *Behavioral Ecology and Sociobiology*, *71,* (2), 38. https://doi.org/10.1007/s00265-017-2266-8

Halsey, L. G., Green, J. A., Wilson, R. P., & Frappell, P. B. (2008). Accelerometry to estimate energy expenditure during activity: best practice with data loggers. *Physiological and Biochemical Zoology*, *82* (4), 396–404. https://doi.org/10.1086/589815

Herrel, A., Gonwouo, L. N., Fokam, E. B., Ngundu, W. I., & Bonneaud, C. (2012). Intersexual differences in body shape and locomotor performance in the aquatic frog, *Xenopus tropicalis*. *Journal of Zoology*, 287(4), 311-316. https://doi.org/10.1111/j.1469-7998.2012.00919.x

Hewitt, G. M. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, *405* (6789), 907. https://doi.org/10.1038/35016000

Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359* (1442), 183–195. https://doi.org/10.1098/rstb.2003.1388

Holt, R. D. (2003). On the evolutionary ecology of species' ranges. *Evolutionary Ecology Research* 5, 159–178

Kamada, K., Tachibanagi, R., & Nakagawa, H. (2017). Strategy of landing behavior of the tree frog *Hyla japonica*. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 327(10), 600-610. https://doi.org/10.1002/jez.2148

Kang, C., Sherratt, T.N., Kim, Y. E., Shin, Y., Moon J., Song, U., Kang J.Y., Kim, K., & Jang Y. (2017). Differential predation drives the geographical divergence in multiple traits in aposematic frogs. Behavioral Ecology 28:1122–1130. https://doi.org/10.1093/beheco/arx076

Kelleher, S.R., Silla, A.J., & Byrne, P.G. (2018). Animal personality and behavioral syndromes in amphibians: a review of the evidence, experimental approaches, and implications for conservation. *Behavioral Ecology and Sociobiology*, 72: 79. https://doi.org/10.1007/s00265-018-2493-7

Kelleher, S. R., Silla, A. J., Dingemanse, N. J., & Byrne, P. G. (2017). Body size predicts between-individual differences in exploration behaviour in the southern corroboree frog. *Animal behaviour*, 129, 161-170. https://doi.org/10.1016/j.anbehav.2017.05.013

Kosmala, G. K., Brown, G. P., Shine, R., & Christian, K. (2020). Skin resistance to water gain and loss has changed in cane toads (*Rhinella marina*) during their Australian invasion. *Ecology and Evolution*, 10(23), 13071-13079. https://doi.org/10.1002/ece3.6895

Kosmala, G., Christian, K., Brown, G., & Shine, R. (2017). Locomotor performance of cane toads differs between native-range and invasive populations. *Royal Society Open Science*, 4(7), 170517. https://doi.org/10.1098/rsos.170517

Liebl, A. L., & Martin, L. B. (2012). Exploratory behaviour and stressor hyper-responsiveness facilitate range expansion of an introduced songbird. *Proceedings of the Royal Society B: Biological Sciences*, 279(1746), 4375-4381. https://doi.org/10.1098/rspb.2012.1606

Liparoto, A., Canestrelli, D., Bisconti, R., Carere, C., & Costantini, D. (2020). Biogeographic history moulds population differentiation in ageing of oxidative status in an amphibian. *Journal of Experimental Biology*, 223(21), jeb235002. https://doi.org/10.1242/jeb.235002

Llewellyn, J., Phillips, B. L., Alford, R. A., Schwarzkopf, L., & Shine, R. (2010). Locomotor performance in an invasive species: cane toads from the invasion front have greater endurance, but not speed, compared to conspecifics from a long-colonised area. *Oecologia*, *162*, 343-348. https://doi.org/10.1007/s00442-009-1471-1

Louppe, V., Courant, J., & Herrel, A. (2017). Differences in mobility at the range edge of an expanding invasive population of *Xenopus laevis* in the west of France. *Journal of Experimental Biology*, *220*(2), 278-283. https://doi.org/10.1242/jeb.146589

Lowe, W. H., & McPeek, M. A. (2014). Is dispersal neutral? *Trends in ecology and evolution*, *29* (8), 444–450. https://doi.org/10.1016/j.tree.2014.05.009

Marsh, R.L., & John-Alder, H.B. (1994). Jumping performance of hylid frogs measured with high-speed cine film. *Journal of Experimental Biology*, 188:131–141. https://doi.org/10.1242/jeb.188.1.131

Mazza, V., & Eccard, J. A. (2023). Expanding through the Emerald Isle: exploration and spatial orientation of non-native bank voles in Ireland. Current Zoology, zoad038. https://doi.org/10.1093/cz/zoad038

Mitchell, A., & Bergmann, P. J. (2016). Thermal and moisture habitat preferences do not maximize jumping performance in frogs. *Functional Ecology*, *30*, 733–742. https://doi.org/10.1111/1365-2435.12535

Myles–Gonzalez, E., Burness, G., Yavno, S., Rooke, A., & Fox, M. G. (2015). To boldly go where no goby has gone before: boldness, dispersal tendency, and metabolism at the invasion front. *Behavioral Ecology*, *26* (4), 1083–1090. https://doi.org/10.1093/beheco/arv050

Nauwelaerts, S., & Aerts, P. (2006). Take-off and landing forces in jumping frogs. *Journal of Experimental Biology*, 209:66–77. https://doi.org/doi:10.1242/jeb.01969

Perkins, T. A., Boettiger, C., & Phillips, B. L. (2016). After the games are over: Life-history trade-offs drive dispersal attenuation following range expansion. *Ecology and Evolution*, 6, 6425–6434. https://doi.org/10.1002/ece3.2314

Phillips, B. L., Brown, G. P., and Shine, R. (2010). Evolutionarily accelerated invasions: The rate of dispersal evolves upwards during the range advance of cane toads. *Journal of Evolutionary Biology*, *23* (12), 2595–2601. https://doi.org/10.1111/j.1420-9101.2010.02118.x

Phillips, B. L., Brown, G. P., Travis, J. M., & Shine, R. (2008). Reid's paradox revisited: the evolution of dispersal kernels during range expansion. *The American Naturalist*, *172* (S1), S34–S48. https://doi.org/10.1086/588255

Phillips, B. L., Brown, G. P., Webb, J. K., & Shine, R. (2006). Invasion and the evolution of speed in toads. *Nature*, *439*, 803. https://doi.org/10.1038/439803a

Pintor, L. M., Sih, A., & Bauer, M. L. (2008). Differences in aggression, activity and boldness between native and introduced populations of an invasive crayfish. *Oikos*, *117* (11), 1629–1636. https://doi.org/10.1111/j.1600-0706.2008.16578.x

Raffard, A., Santoul, F., Cucherousset, J., & Blanchet, S. (2019). The community and ecosystem consequences of intraspecific diversity: A meta-analysis. *Biological Reviews*, *94*(2), 648-661. https://doi.org/10.1111/brv.12472

Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, 82:291–318. 10.1111/j.1469-185X.2007.00010.x

Réale, D., Dingemanse, N. J., Kazem, A. J., & Wright, J. (2010). Evolutionary and ecological approaches to the study of personality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1560), 3937-3946. https://doi.org/10.1098/rstb.2010.0222

Reilly, S. M., Montuelle, S. J., Schmidt, A., Krause, C., Naylor, E., & Essner Jr, R. L. (2016). Functional evolution of jumping in frogs: Interspecific differences in take-off and landing. *Journal of Morphology*, 277(3), 379-393. https://doi.org/10.1002/jmor.20504

Ronce, O. (2007). How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annual Review of Ecology, Evolution and Systematics*, *38*, 231–253. https://doi.org/10.1146/annurev.ecolsys.38.091206.095611

Schielzeth, H., & Nakagawa, S. (2011). rptR: Repeatability for Gaussian and non-Gaussian data. R package.

Schmitt, T. (2007). Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. *Frontiers in zoology*, *4* (1), 11. https://doi.org/10.1186/1742-9994-4-11

Shackleton, J. C., Van Andel, T. H., & Runnels, C. N. (1984). Coastal paleogeography of the central and western Mediterranean during the last 125,000 years and its archaeological implications. *Journal of field Archaeology*, *11* (3), 307–314. https://doi.org/10.2307/52928

Shepard, E. L., Wilson, R. P., Quintana, F., Laich, A. G., Liebsch, N., Albareda, D. A. and Newman, C. (2008). Identification of animal movement patterns using tri–axial accelerometery. *Endangered Species Research*, *10*, 47–60. https://doi.org/10.3354/esr00084

Shine, R., Brown, G. P., & Phillips, B. L. (2011). An evolutionary process that assembles phenotypes through space rather than through time. *Proceedings of the National Academy of Sciences*, *108*(14), 5708-5711. https://doi.org/10.1073/pnas.1018989108

Simmons, A. D., and Thomas, C. D. (2004). Changes in dispersal during species' range expansions. *The American Naturalist*, *164* (3), 378–395. https://doi.org/10.1086/423430

Smith, J.M., Barnes, W.J.P., Downie, J.R., Ruxton, G.D. (2006). Structural correlates of increased adhesive efficiency with adult size in the toe pads of hylid tree frogs. *Journal of Comparative Physiology A*, 192:1193–1204. https://doi.org/10.1007/s00359-006-0151-4

Spadavecchia, G., Chiocchio, A., Bisconti, R., & Canestrelli, D. (2021). Paso doble: a two-step Late Pleistocene range expansion in the Tyrrhenian tree frog (*Hyla sarda*). Gene 780:145489. ttps://doi.org/10.1016/j.gene.2021.145489

Spadavecchia, G., Chiocchio, A., Costantini, D., Liparoto, A., Bisconti, R., & Canestrelli, D. (2023). Spatial differentiation of background matching strategies along a Late Pleistocene range expansion route. *Evolutionary Ecology*, 37(2), 291-303. https://doi.org/10.1007/s10682-022-10216-2

Stamps, J.A., & Buechner, M. (1985). The territorial defense hypothesis and the ecology of insular vertebrates. Q Rev Biol. 60:155–181. https://doi.org/10.1086/414314

Stoffel, M.A., & Nakagawa, S., & Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 8(11): 1639–1644. https://doi.org/10.1111/2041-210X.12797

Thiede, J., 1978. A glacial Mediterranean. *Nature* 276, 680–683. https://doi.org/10.1038/276680a0

Travis, J. M., & Dytham, C. (2002). Dispersal evolution during invasions. *Evolutionary Ecology Research*, *4* (8), 1119–1129.

Van Andel, T. H., and Shackleton, J. C. (1982). Late Paleolithic and Mesolithic coastlines of Greece and the Aegean. *Journal of field archaeology*, *9* (4), 445–454. https://doi.org/10.1179/009346982791504454

Verbeek, M. E., Drent, P. J., & Wiepkema, P. R. (1994). Consistent individual differences in early exploratory behaviour of male great tits. *Animal Behaviour*, 48(5), 1113-1121. https://doi.org/10.1006/anbe.1994.134

Weiss, S., & Ferrand, N. (2007). Phylogeography of southern European refugia. Dordrecht (Netherlands): Springer.

Wilson, R. P., White, C. R., Quintana, F., Halsey, L. G., Liebsch, N., Martin, G. R., & Butler, P. J. (2006). Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant. *Journal of Animal Ecology*, *75* (5), 1081–1090. https://doi.org/10.1111/j.1365-2656.2006.01127.x.

FIGURES AND TABLES

Table 1. Geographic coordinates and sample size of the sites sampled to study phenotypic variation within *Hyla sarda* populations.

Island	Sampled site	Latitude	Longitude	N
Corsica	Aleria	42.1132	9.5221	14
	San Giuliano	42.2677	9.5184	10
	Etagn de Canettu	41.4456	9.2035	10
	T10	41.4603	9.2170	13
Sardinia	Stazzo Pulcheddu	41.1639	9.3620	8
	Porto Pollo	41.1844	9.3303	15
	Cala Ginepro	40.4481	9.7920	14
	Siniscola	40.5814	9.7691	9

Table 2. 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.

Variable	Estimate	95 % CI	P-value
Latency to explore	0.31	0.09-0.50	**0.002**
Activity	0.04	0.00-0.10	0.051
Time on floor	0.07	0.00-0.21	0.183
Jumping events	0.35	0.14-0.53	**0.001**
Latency to exit	0.40	0.20-0.55	**<0.001**

Table 3. Linear models showing differences between Corsica and Sardinia Island populations in behavioural and performance traits. Coefficient estimates (±*SE*) of these models are shown with both behavioural and performance traits as dependent variable and island as fixed factor. Significant contrasts are shown in bold.

Dependent variable	Reference	Level	Coefficient	*SE*	t-Value	P
Latency to explore	C	S	-0.526	0.131	- 4.028	**<0.001**
Jumping events	C	S	-1.256	0.358	-3.504	**0.001**
Latency to exit	C	S	-0.396	0.181	-2.185	**0.032**
Jumping	C	S	-3.050	1.552	-1.965	0.094
Stickiness	C	S	-7.731	1.969	-3.926	**<0.001**

Table 4. Linear models showing differences between Corsica and Sardinia Island populations in morphological traits. Coefficient estimates (±*SE*) of these models are shown with morphological traits as dependent variable and population as fixed factor. Significant contrasts are shown in bold.

Dependent variable	Reference Level	Level	Coefficient	*SE*	t-Value	P
SVL	S	C	-0.238	0.062	-3.844	**<0.001**
Fore limb	S	C	-0.062	0.030	-2.059	**0.044**
Hind limb	S	C	-0.100	0.044	-2.288	**0.026**
Head width	S	C	-0.032	0.0124	-2.595	**0.011**

Figure 1. Geographical distribution of the 8 sampled populations. The dashed line shows the approximate location of the coastline during the last glacial maximum (Thiede, 1978).

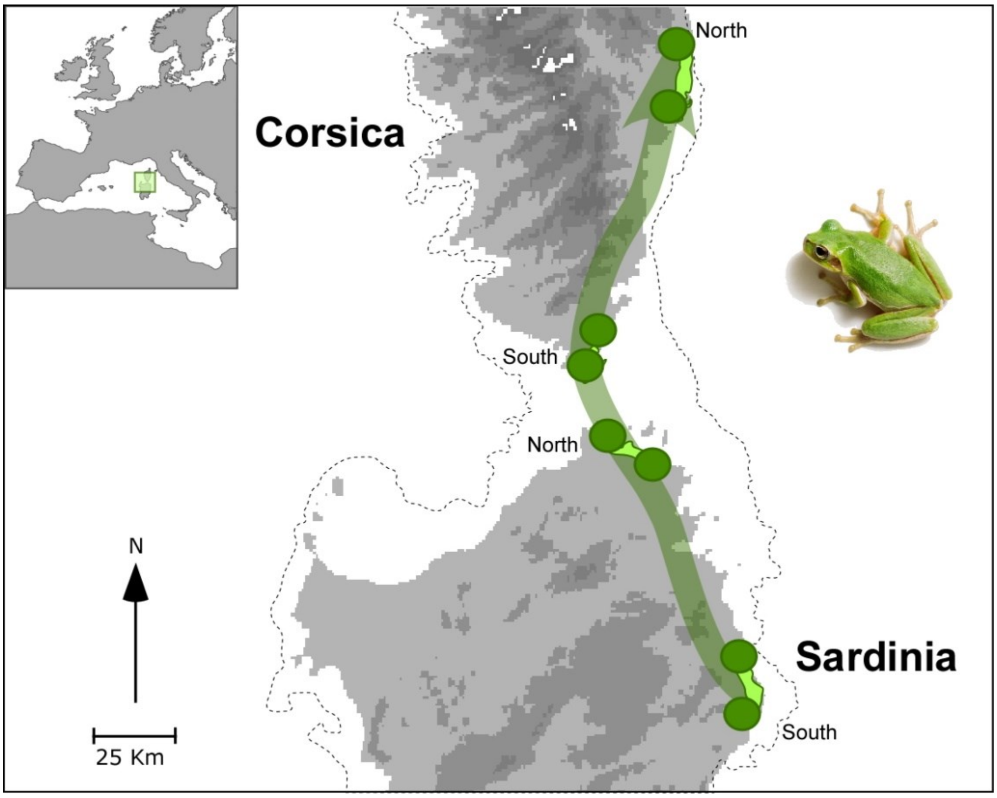


Figure 2. Estimated marginal means and standard errors from linear models for personality traits for each island (A: latency to explore; B: latency to exit; C: frequency of jump event).

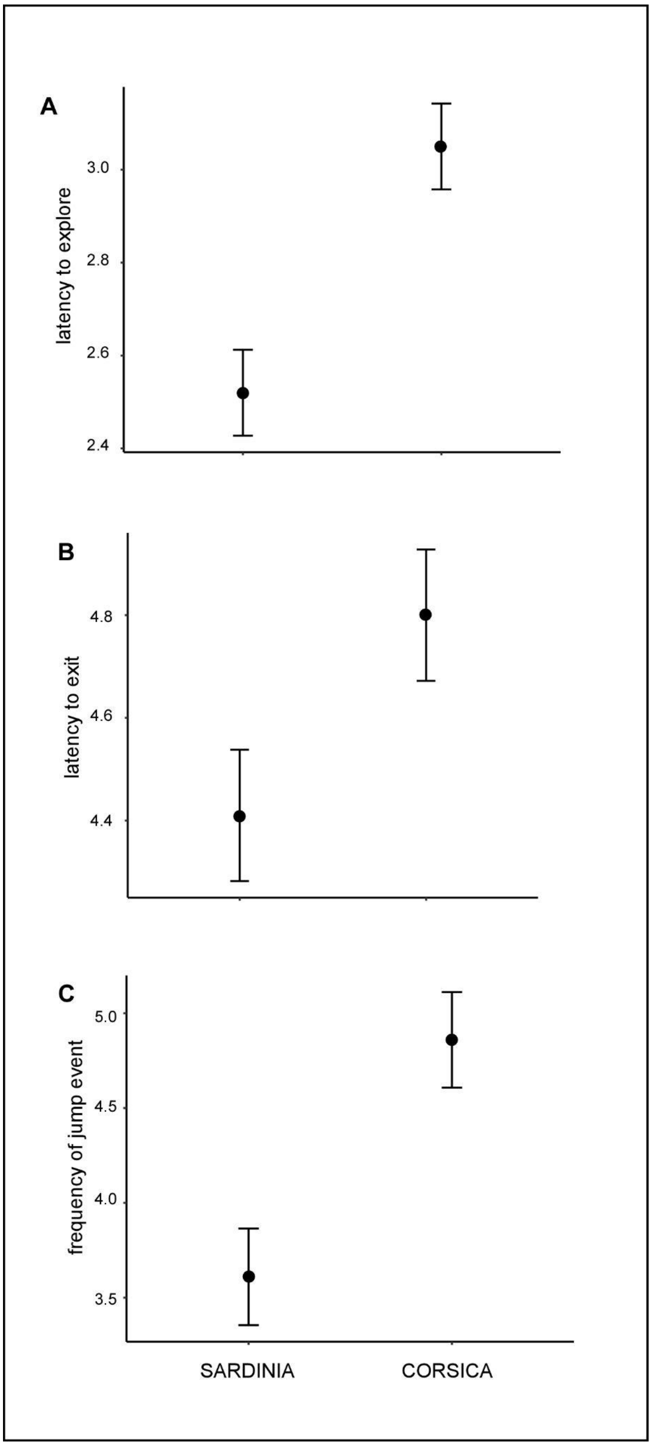


Figure 3. Estimated marginal means and standard errors from linear models for performance traits for each island (A: jumping force at take-off; B: adhesion force).

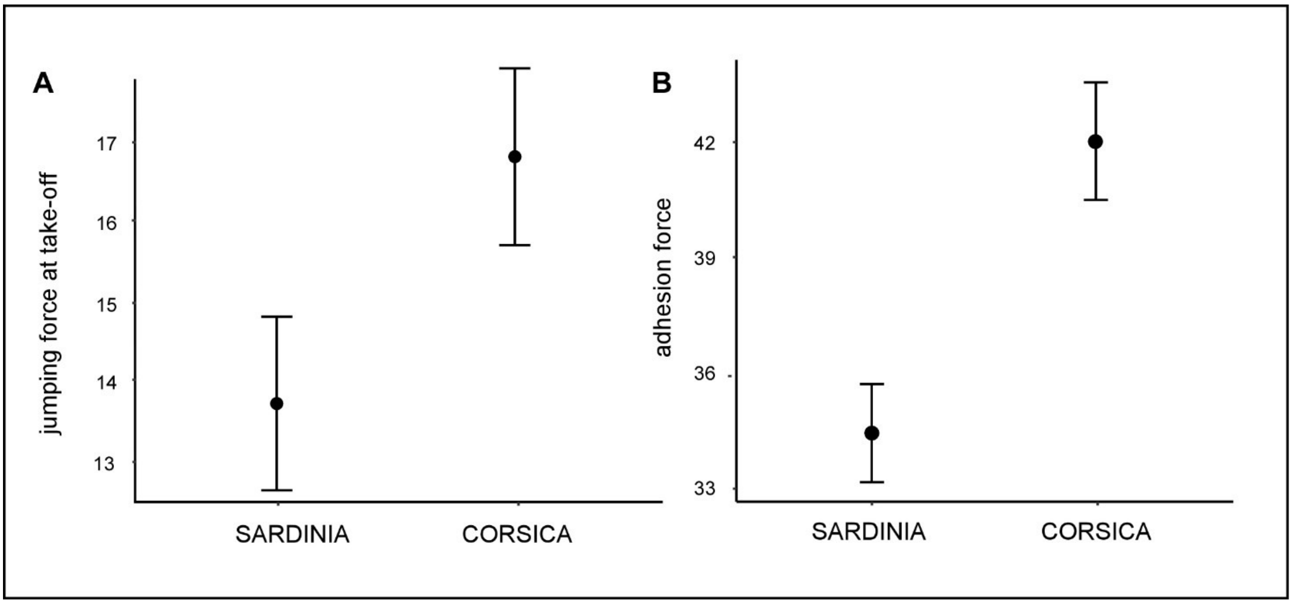


Figure 4. Estimated marginal means and standard errors from linear models for morphological traits for each island (A: SVL; B: hind limb; C: fore limb; D: head size).

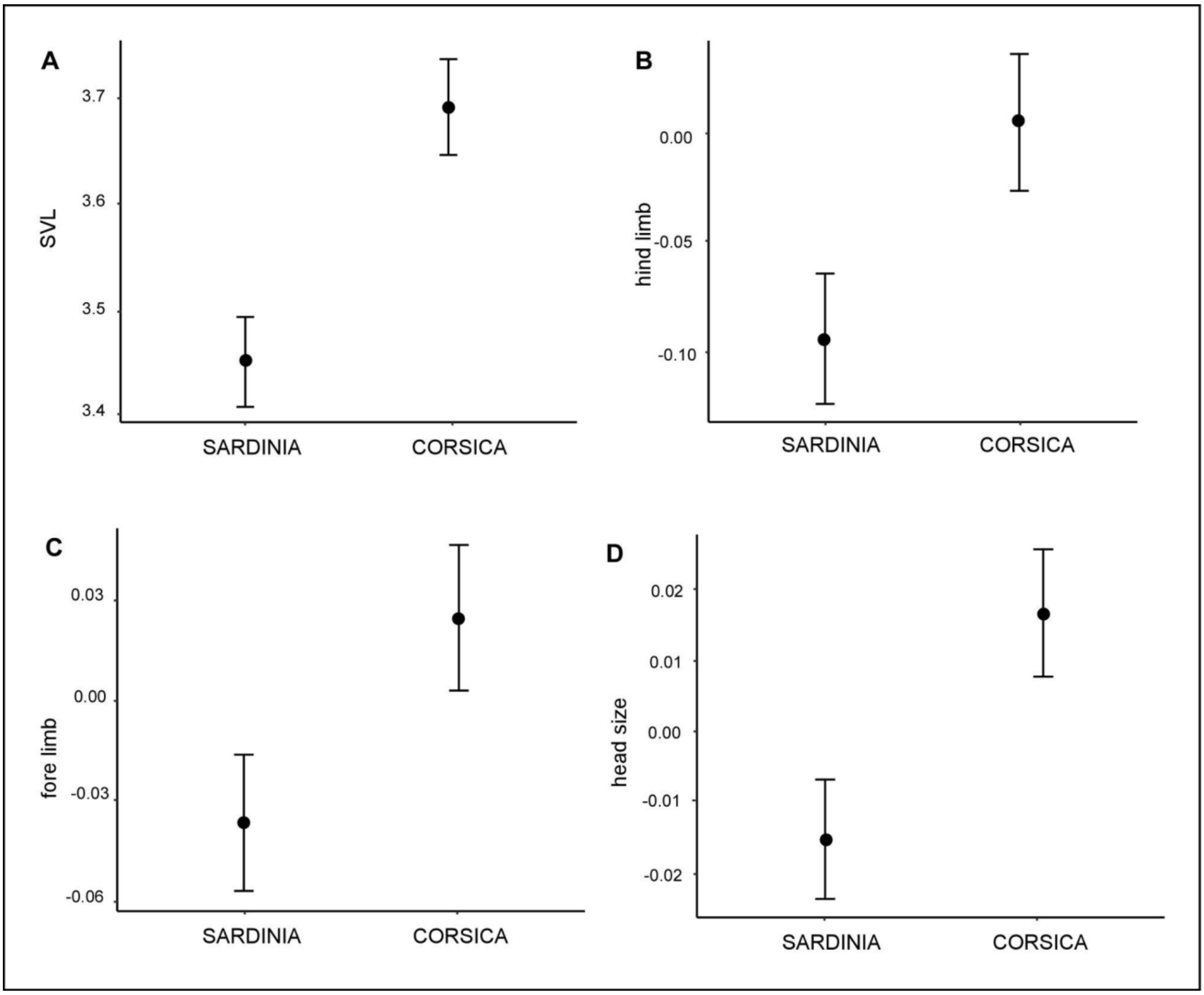
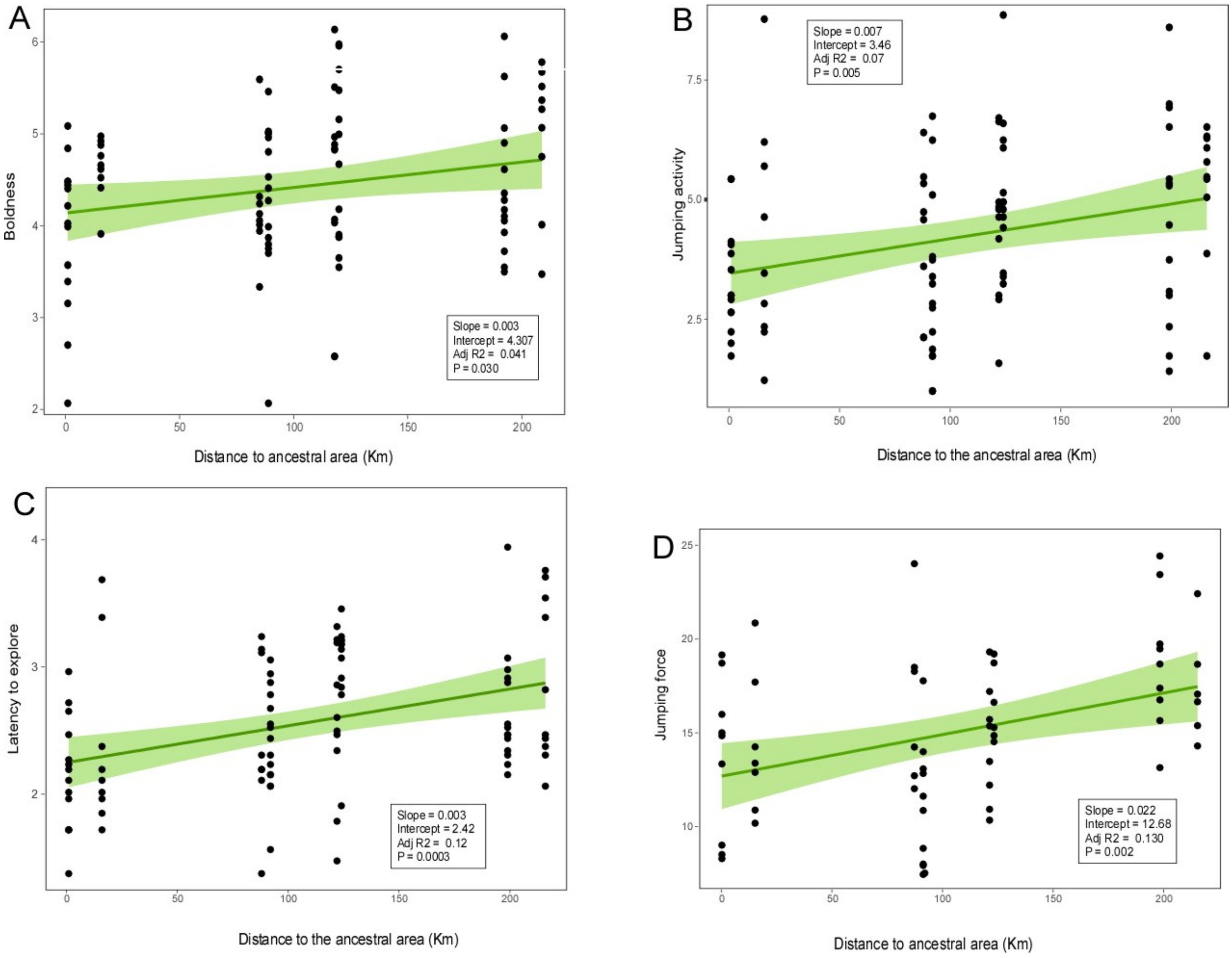
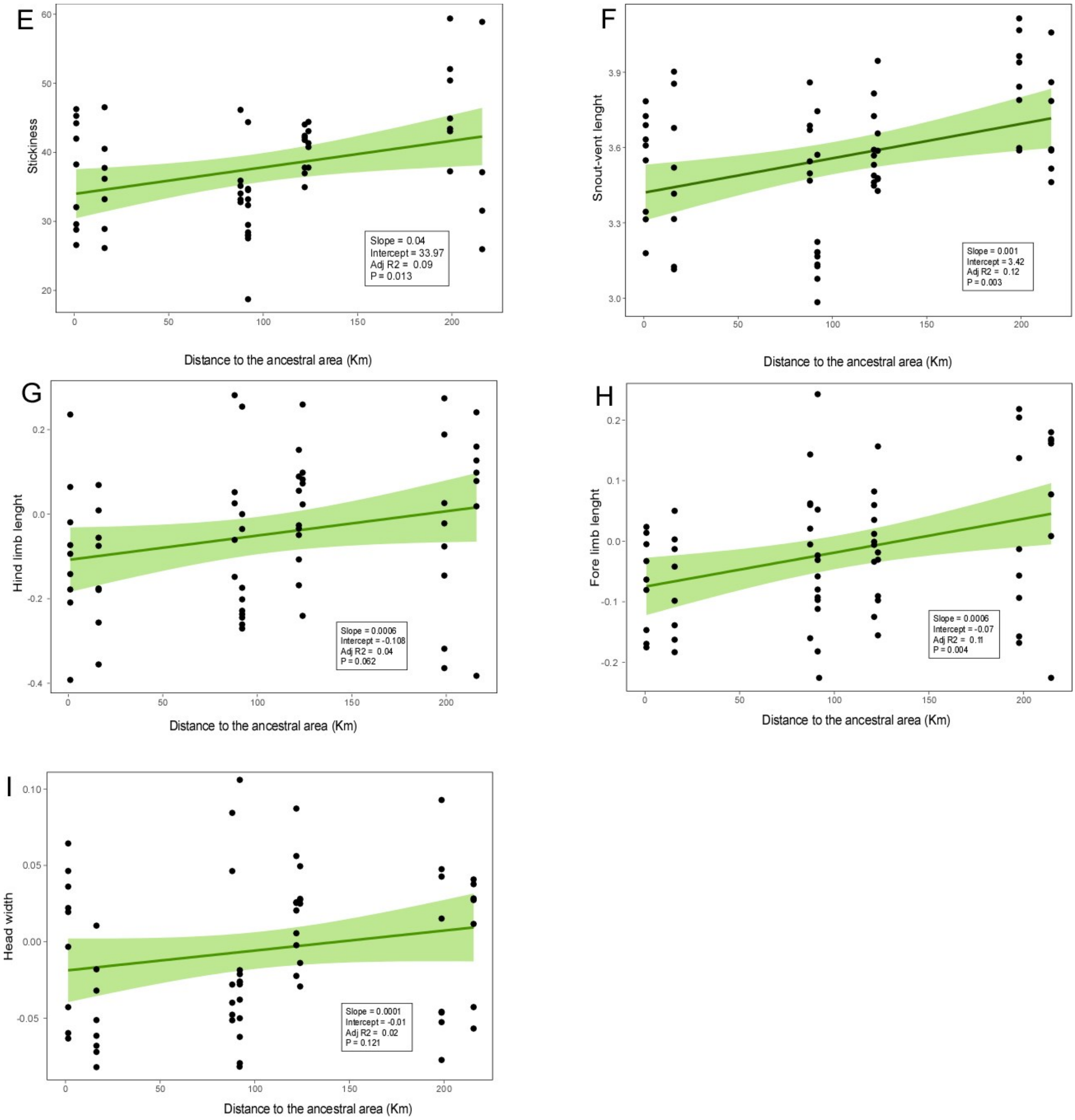


Table S1. AIC values of the alternative models run entering each variable singly as dependent variables, and island as fixed factor (Model 1) or including sampling location as a random factor (Model 2); the selected best fit models, i.e. lowest AIC, are reported in bold.

Phenotypic trait		AIC
Behaviour		
Latency to explore		
	Model 1	**179.06**
	Model 2	186.93
Boldness		
	Model 1	**239.34**
	Model 2	245.79
Jumping activity		
	Model 1	**369.65**
	Model 2	373.49
Locomotory performance		
Jumping force		
	Model 1	348.02
	Model 2	**343.07**
Stickiness		
	Model 1	384.76
	Model 2	**380.61**
Morphology		
Snout–vent length		
	Model 1	**4.42**
	Model 2	7.44
Head size		
	Model 1	**-194.23**
	Model 2	-177.15
Forelimb length		
	Model 1	**-83.85**
	Model 2	-70.10
Hindlimb length		
	Model 1	**-38.92**
	Model 2	-26.64

Figure S1. Phenotypic trait variation across the inferred past range expansion route in the Tyrrhenian tree frog, *Hyla sarda*: results from the linear regression model between the distance from the area of origin (in km) and each behavioural, performance and morphological trait investigated in this study.




E
Stickiness
60
50
40
30
20
0
50
100
150
200
Distance to the ancestral area (Km)
Slope = 0.04
Intercept = 33.97
Adj R2 = 0.09
P = 0.013
F
Snout-vent lenght
3.9
3.6
3.3
3.0
0
50
100
150
200
Distance to the ancestral area (Km)
Slope = 0.001
Intercept = 3.42
Adj R2 = 0.12
P = 0.003
G
Hind limb lenght
0.2
0.0
-0.2
-0.4
0
50
100
150
200
Distance to the ancestral area (Km)
Slope = 0.0006
Intercept = -0.108
Adj R2 = 0.04
P = 0.062
H
Fore limb lenght
0.2
0.1
0.0
-0.1
-0.2
0
50
100
150
200
Distance to the ancestral area (Km)
Slope = 0.0006
Intercept = -0.07
Adj R2 = 0.11
P = 0.004
I
Head width
0.10
0.05
0.00
-0.05
0
50
100
150
200
Distance to the ancestral area (Km)
Slope = 0.0001
Intercept = -0.01
Adj R2 = 0.02
P = 0.121