Unexpected and spatially structured genetic diversity of the relict population of the endangered corsican land snail Tyrrhenaria ceratina

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RESEARCH ARTICLE

Unexpected and spatially structured genetic diversity of the relict population of the endangered corsican land snail *Tyrrhenaria ceratina*

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Abstract
Population genetics of threatened species provides information about evolutionary pressures over those populations and thus may inform conservation management strategies. However, conservation genetics still has a low impact on conservation practices. This study's aim is to integrate genetics in the conservation management of the only-known population of an extremely narrow-range endemic Corsican snail – *Tyrrhenaria ceratina* –, whose distribution area is restricted to the Ricantu site in Corsica. Using non-invasive DNA samples of 210 individuals, we amplified 13 microsatellites loci to assess the population viability, genetic structure and demographic history of the population, along with the estimation of the historical and contemporary gene flow between identified genetic clusters. We also estimated the dispersal ability of the species. Our results showed a surprisingly high genetic diversity, along with a pattern of isolation by distance (IBD) and a strongly spatialized genetic structure. Furthermore, we underlined a low functional connectivity, along with evidence of a recent decline in the population size, which are both likely due to a historical fragmentation between the sampled areas, caused by anthropization. Overall, this study allows to provide a first insight about the functioning of the population, to guide future conservation actions for the species.

Keywords Isolation by distance · Population genetics · Conservation biology · Genetic connectivity · Habitat fragmentation

Introduction

The present day biodiversity results from a combination of evolutionary processes such as natural selection, especially at the intraspecific level, that helps expand or decrease it, ultimately leading to speciation and extinction events. In addition to natural extinction events that shape the history of life (Raup 1994), anthropogenic impacts are at present the major factors responsible for the erosion of biodiversity (e.g. Lande 1998). Amongst direct drivers of biodiversity loss, the reduction and fragmentation of habitats, overexploitation, climate change and the introduction of invasive species are leading to a sharp decline in the numbers of natural populations (e.g. Bender et al. 1998). Then many species are endangered along with low or declining population sizes that may be an indirect consequence of a species' range decline or in turn may secondarily cause a range reduction (IUCN, 2021). These threatened populations can be studied through the prism of two complementary paradigms: effect of small population size on population persistence

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and causes of the declining population to provide adaptive management framework (Caughley 1994).

Small and isolated populations, which furthermore have a restricted range, are more sensitive to demographic stochasticity, environmental and genetic effects, so that they can be trapped in an extinction vortex (Fagan and Holmes 2006). Because of reduced genetic diversity in small or fragmented populations compared to larger and connected populations (Frankham 1996), genetic factors have a major influence on the survival of these small populations (Gaggiotti 2003). This loss of genetic diversity can lead to a decrease in the average fitness of populations (Reed and Frankham 2003) and a lower capacity to adapt to environmental changes (Lande and Shannon 1996). Furthermore, inbreeding and increased genetic drift exacerbate the extinction vortex, by reducing the fitness of inbred individuals (Crnokrak and Roff 1999), and by increasing the risk of fixation of weakly deleterious mutations (Gabriel and Bürger 1994).

Using genetic data to assess the genetic potential of threatened populations is therefore a key approach in conservation biology (Willi et al. 2022) as it can help delimiting conservation and management units (Coates et al. 2018; Schmidt et al. 2018), ruling reintroductions (O'Brien et al., 2017) and ex-situ conservation programs (Witzenberger and Hochkirch 2011), and identifying gene exchange corridors between threatened populations (Sharma et al. 2013).

The land snail *Tyrrhenaria ceratina* (Syn. *Helix ceratina*, Shuttleworth 1843; Gargominy et al. 2021) is a helicid snail endemic to Corsica, whose emergence may date back to the late Miocene (5 million years ago; Korábek et al. 2021). Its current range is restricted to the Ricantu site, located south-east of Ajaccio on a surface of habitat less than 2 ha (Fig. 1). Considered as extinct since 1930, the population of this narrow strip of land between Ajaccio airport and the sea was rediscovered in 1994 at Ajaccio and nowhere else in Corsica (Bouchet et al. 1997). Empty shells and fossil records found in several geographical localities distant from the current distribution are tangible evidence of a past much broader distribution. Quaternary shells have been collected from the north near Bastia in the Brèches of Toga (Bouchet et al. 1997; Caziot 1903, 1911 [in French]), to the south at Bunifazziu (Neolithic shells, 5600 – 5000 BC), to the west in fossil dunes near Piana on Arone beach (Upper Pleistocene; Ferrandini , pers. comm., 2017), more than sixty kilometres far from the Ricantu site.

The remnant population of Ricantu is potentially the world's only surviving population of *T. ceratina*. Since 2011, this species has been assessed as critically endangered in the IUCN Red list (Charrier et al. 2013; IUCN, 2021) according to:

(i) the putative poor quality of its coastal habitat submitted to human frequentation and moreover subject to natural hazards increasing the risk of extinction of the species;
(ii) a small distribution area, estimated at 0.34 km² well under the 100 km² threshold below which a species is theoretically considered critically endangered (IUCN, 2012). The current distribution of *T. ceratina* is therefore a very pronounced example of micro-endemism. In addition, its distribution is discontinuous as individuals are observed in three areas more or less distant on the Ricantu site. The population, likely fragmented in space into three isolated sub-populations, would persist thanks to metapopulation function that relies on dispersal among subpopulations.
(iii) its small and potentially declining population size. In 2010, Charrier et al. (2013) estimated between 3800 and 5200 mature individuals while in 2018 and 2021 the total number of individuals was between 830 and 5525 respectively (data unpublished).

Despite the strong threats to non-marine mollusks (Lydeard et al. 2004), mollusks are often overlooked in conservation biology (Régnier et al. 2009). However, *T. ceratina* is an exception because it is a protected since 1992 under the French law and its habitat has been strongly protected since 1997. Moreover, the Ricantu site is classified in the Natura 2000 network and is in the domain of the Coastal Conservation Authority (Conservatoire du littoral). Since 2014, *T. ceratina* is the subject of 5-year National Action Plans for Threatened Species (NAP, n.d.) which aims at assessing the conservation status of threatened species and their habitats, and outlines conservation priorities. Among key measures implemented to achieve the "favorable" status of a threatened population according to IUCN criteria, one is to describe the genetic structure of the Ricantu population, assess the genetic diversity, and put forward hypotheses on the origin of the species' decline. More broadly, genetic studies on natural populations of invertebrates with very pronounced micro-endemism are still extremely rare. Understanding the genetic mechanisms involved in the extinction of such small population with low colonization capacity may be of great interest in conservation biology. Beyond these more formal considerations, the results of this first genetic study of *T. ceratina* will serve as a basis for guideline of the conservation actions of the NAP, including connectivity restoration projects and in situ ex-situ breeding for future reintroduction and/or translocation.

The objectives of the study and underlying hypotheses, made possible by the recent development of microsatellite markers (Geneoscreen, Lille, France), are:

(1) to assess population viability using parameters classically estimated in conservation genetics (i.e. genetic diversity, inbreeding, effective size) and reveal the evolutionary mechanisms that shape the genetic diversity. Due to the small size of the population and the potential fragmentation into several subpopulations, we expect low genetic diversity as a result of increased genetic drift, inbreeding and reduced gene flow (**H1**);
(2) to define the genetic structure of the population in order to explore the hypothesis of fragmentation into three subpopulations (**H2**);
(3) to estimate historical and contemporary gene flow to interpret the current spatial pattern of genetic diversity through the prism of temporal changes in the landscape. Due to recent habitat fragmentation caused by human activities from the 1950s onwards (Paradis et al., 2011[in French]), as well as the reduction in the species' range, we expect a higher historical gene flow than contemporary one (**H3**);
(4) to infer an evolutionary scenario to formulate hypotheses about the origin of the Ricantu population. As it is a relict population, we anticipated that the most likely scenario is that the population originated from an ancestral population that underwent a demographic contraction (**H4**).

Results obtained aims to improve recommendations for the various actors involved in the NAP.

Materials and methods

Study area and sampling collection

Samples of *T. ceratina* were collected between November 2018 and April 2021 from three zones of the Ricantu site referred to as Northwest (formerly Tahiti 1, Tahiti 2 and Natural localities), Centre (formerly Military locality), and Southeast (formerly Gravona locality) where the presence of the species was already established (Cucherat 2019) (Fig. 1). These five localities have been delineated on the basis of the site's management history and restoration actions. From each individual sampled and georeferenced, we used a dry sterilized cotton swab to collect epithelial cells present in the mucus. Swabs were then air-dried, placed in sterile tubes and sent as quickly as possible to Université Picardie Jules Verne (UPJV) for DNA extraction. A total of 324 individuals were sampled.

Studied species

In the daylight, *T. ceratina is* a nocturnal psammophile living burrowed more or less deeply in sand, under plant or not, and is active by night, under wet conditions, just in a narrow strip above the higher tide, colonized by *Genista salzmannii* var. *salzmannii* and *Scrophularia ramosissima.* For a more detailed description of its macrohabitat see Bouchet et al. (1997) and Charrier et al. (2013) [in French]. Two other Helicid species co-occur with *T. ceratina* (*Massylea vermiculata* and *Cantareus apertus*), with no evidence of interspecific competition. To date, two seasons of growth and reproduction (spring and autumn) are known, alternating with two periods of inactivity (aestivation and hibernation in summer and winter respectively). Individuals reach sexual maturity between 1.5 and 2 years and the longevity of the individuals would be of 4 to 5 years (Chevalier and Charrier 2002).

DNA extraction and polymerase chain reaction

DNA was extracted using QIAamp® DNA Blood Mini (Qiagen) kit following manufacturer's protocol (Spin Procotol). Two 50µL elutions were carried out to increase DNA concentration. Samples were stored at -20 °C. A set of 15 microsatellites loci, developped by Genoscreen (Lille), were amplified using primers described Table S1 (Appendix A). Reactions varied according to the field sampling. For older samples (2018–2020), simplex PCR reactions were carried out in a total volume of 15µL per reaction, containing 8µL of MyTaqMix (Bioline ®), 2 × 0.2 µL primers (5mM), 1.6µL of H2O, and 5 µL of genomic DNA. For recent samples (2021), multiplex PCR reactions were carried out in a total volume of 15µL per reaction, containing 8µL of MyTaqMix (Bioline ®), 2 × 0.2 µL of each primers (5mM), 0.8µL of H_2O, and 5 µL of genomic DNA. PCR cycling conditions consisted of an initial denaturation step of 95 °C for 10 min, 40 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, then a final extension step of 72 °C for 10 min. Fragments analysis was performed with a 16 capillary sequencer ABI3130 XL. GENEMAPPER v4.0 software (APPLIED BIOSYSTEMS) was used for alleles identification and genotyping.

Data analysis

Genetic diversity and genetic structure

Genetic diversity was investigated by computing allelic richness (Ar), observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{is}) with "*hierfstat*" package (Goudet 2005) in R (4.0.3). A set of

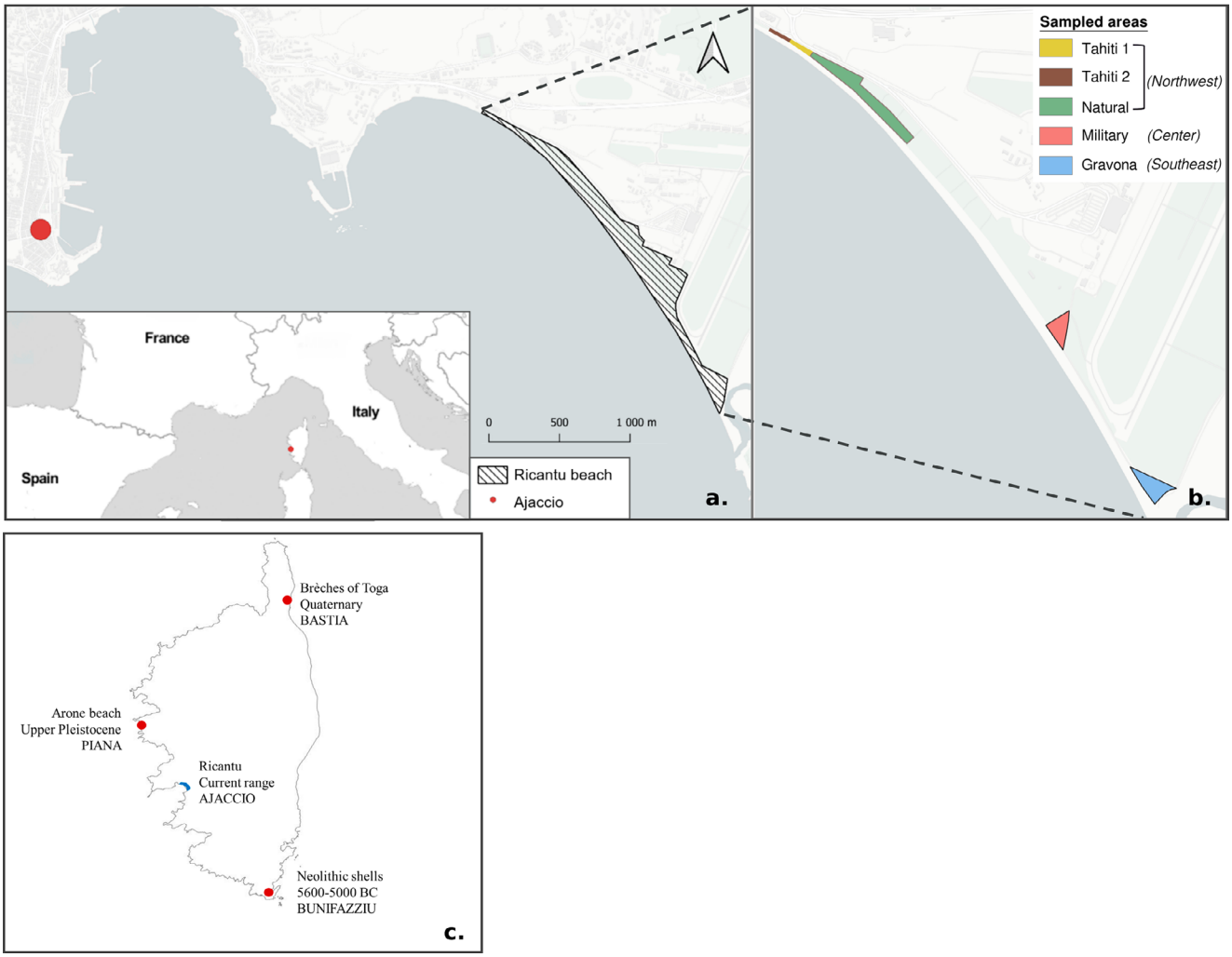

Fig. 1 Maps showing the range of *T. ceratina* (**a**), sites sampling for this study (**b**) and (**c**) collection sites of fossil shells recorded. The Ricantu site, currently the only known distribution area of the species, is adjacent to Ajaccio airport. Five localities (Tahiti 1, Tahiti 2, Natural, Military and Gravona) were sampled in three distinct areas (Northwest, Centre and Southeast respectively)

1000 bootstrap replicates were used to calculate the 95% confidence interval for F_{is}. A Hardy-Weinberg equilibrium test on loci was performed using "*pegas*" package (Paradis 2010). For all statistical tests, the maximum type I error rate was set at $\alpha = 0.05$.

Bayesian clustering inference method implemented in STRUCTURE software (v.2.3.4) (Pritchard et al. 2000) was used to assess the most likely number of genetic clusters (K). The model selected for computation allowed admixture, assumed a correlation between allele frequencies, and used the sampling area as prior for clustering. For each K from 1 to 6, 20 repetitions of 140 000 Markov Chain Monte Carlo (MCMC) were performed, after a burn-in of 40 000 iterations. Structure Harvester Software (Earl and vonHoldt 2012) was used to define the optimal number of K by comparing two methods : the computation of log probabilities of the data (ln[Pr(X|K)]) and the ΔK statistic (Evanno et al. 2005). Assignment probabilities of individuals and populations to each K were averaged between the 20 iterations with CLUMPP software (Jakobsson and Rosenberg 2007). A hierarchical clustering analysis was then performed to detect an eventual finer genetic structure (Vähä et al. 2007).

Genetic differentiation and isolation by distance

Genetic structure observed for species with low dispersal abilities can often be explained with an Isolation by Distance (IBD) model (Wright 1943) in which spatially close individuals will tend to be more genetically similar, resulting in a positive correlation between geographical and genetic distances. IBD model was tested between the five localities using the linear regression method for population in two dimensions developed by Rousset (1997). The mean coordinates of all quadrats of one locality were used

to assign coordinates to each area. To assess genetic differentiation between areas, pairwise Fst (Weir and Cockerham 1984) were computed.

Genetic distance (Dgen) was calculated as:

$$Dgen = \frac{F_{st}}{(1 - F_{st})}$$

Natural logarithms of geographical distances between localities (Dgeo) were used to build the linear regression. IBD significance was assessed with a Mantel test, using 1000 permutations. Based on these results, mean population density (De) and mean dispersal distance per generation (δ) were estimated using the following formulas (Rousset 1997):

$$De = \frac{Ne}{\pi * \left(\frac{S}{2}\right)^2}$$

$$\delta = 2\sqrt{\frac{1}{4\pi Deb}}$$

where *Ne* is the mean effective populations size of each locality, *S* the smallest distance between two localities, and *b* the slope of the linear regression between genetic and geographical distances. *Ne* estimator v.2.1 (Do et al. 2014) was used to calculate effective population size of each locality. We used the linkage disequilibrium method as recommended for small population (Waples and Do 2010) and its potential bias are relatively well known (Araki et al. 2007). Rare alleles (i.e. with a frequency inferior to 0.02) were excluded to avoid Ne overestimation (Do et al. 2014).

Historical and contemporary gene flow

Historical and contemporary gene flow between pairs of genetic clusters (inferred with clustering approach previously described) were estimated with Bayesian inference methods, using respectively MIGRATE v 4.4.3 (Beerli 2006) and BAYEASS v.3.0.4 (Wilson and Rannala 2003) softwares (see Appendix A1 for detailed description of both methods used).

Evolutionary scenario

To infer demographic history of Ricantu population, we used the Approximative Bayesian Calculation (ABC) algorithm implemented in DIYABC v 2.1.0 software (Cornuet et al. 2008). This algorithm is particularly adapted to complex datasets studied in population genetics because it allows posterior probabilities of different models to be compared by means of summary statistics (Beaumont et al. 2002). Default parameters were kept for analysis. Chosen summary statistics were: mean number of alleles, mean genetic diversity, mean variance of allelic size for each area, along with pairwise Fst, pairwise mean number of alleles, and pairwise mean genetic diversity. A million simulated datasets were obtained for each scenario, which were hierarchically compared to lead to the most likely one using the direct approach of estimation of posterior probabilities. DIYABC also allows an estimation of some parameter values, such as mutation rate. Type I and Type II errors of the final most likely scenario were computed with the method based on *a priori* distributions.

Results

Quality control

Individuals with more than 20% of non-amplified loci were excluded from the multicolocus genotype dataset. Loci 13 and 45 were also removed due to a low success of amplification. Consequently, 210 individuals and 13 polymorphic loci were kept for analysis.

Genetic diversity and genetic structure

From the 210 individuals kept for analysis, the number of alleles per locus ranged from 4 alleles (locus 36) to 11 alleles (loci 4 and 5). Genetic diversity estimates per areas and overall areas are shown in Table 1. There was no significant difference between sampled areas for standardized allelic richness (Ar) ($\chi^2 = 2.11$; $p = 0.714$), observed heterozygosity (H_o) ($\chi^2 = 1.58$; $p = 0.811$), expected heterozygosity

Table 1 Table displaying sampled area and overall areas (Total), the number of individuals used for analysis (N), mean allelic richness (Ar), mean observed heterozygosity (Ho), mean expected heterozygosity (He), and inbreeding coefficient (Fis). For Ar, Ho and He standard deviation (sd) is displayed. For Fis, 95% confidence interval values are given

Areas	Localities	N	Ar ± sd	H_o ± sd	H_e ± sd	F_{is} [CI 95%]
Northwest	Tahiti 1	51	4.028 ± 1.283	0.532 ± 0.167	0.622 ± 0.134	0.151 [0.0675–0.2373]
	Tahiti 2	14	3.952 ± 1.394	0.497 ± 0.201	0.631 ± 0.117	0.212 [0.0876–0.3432]
	Natural	57	4.063 ± 1.477	0.560 ± 0.170	0.630 ± 0.140	0.125 [0.0495–0.1736]
Centre Southeast	Military	35	4.346 ± 1.494	0.491 ± 0.209	0.618 ± 0.180	0.226 [0.1210–0.3007]
	Gravona	53	4.487 ± 1.136	0.489 ± 0.133	0.643 ± 0.135	0.242 [0.1853–0.2923]
	Total	210	6.724 ± 2.191	0.510 ± 0.148	0.666 ± 0.144	0.226 [0.1655–0.2712]

(H_e) ($\chi^2 = 0.311$; $p = 0.989$) and inbreeding coefficient (F_{is}) ($\chi^2 = 4.82$; $p = 0.305$). Mean allelic richness overall areas was 6.724 ± 2.191, and inbreeding coefficient overall areas was 0.226, indicating an excess of homozygotes due to non-random mating. Mean observed and expected heterozygosity overall areas were respectively 0.510 ± 0.148 and 0.666 ± 0.144. Loci showing deviation from Hardy Weinberg equilibrium varied depending on the areas (Appendix A, Fig. A1).

The optimal number of clusters (K) to describe the genetic structure of Ricantu population was $K = 3$ (Appendix A, Fig. A2). A spatial genetic differentiation on a Northwest / South-East axis was observed (Fig. 2a). Thus, individuals sampled in the Northwest area were mostly assigned to cluster 1 with most of the assignment probabilities reaching more than 90% (Fig. 2b). It must be noted, however, that a few individuals sampled in Centre area also have strong assignment probabilities to cluster 1 (reaching up to 70%). Individuals originating from the Centre were predominantly assigned to cluster 2. Finally, individuals of Southeast locality mainly had strong assignment probabilities to cluster 3 even though this area was less genetically homogeneous than the two areas previously described, with several individuals having assignment probabilities to cluster 2 superior to 50%.

Hierarchical genetic structure analysis on Northwest locality did not allow to detect a finer genetic structure (Appendix A, Fig. A3).

Genetic differentiation and isolation by distance

We found a significant isolation by distance (IBD) pattern between areas (Appendix A, Fig. A4). The estimated population density (De) was $De = 0.001$ individuals/m^2. Estimated mean dispersal distance per generation was $\delta = 71.76$ m / generation.

Historical and contemporary gene flow

According to credibility intervals, higher contemporary than historical gene flow were observed for the two gene flow measures towards the Northwest area, issued from the Centre and Southeast areas respectively (Appendix A, Fig. A5). All other historical and contemporary gene flow estimates were overlapping. No cluster pair was more connected than another. All estimates of gene flow were very low (< 0.1). However, it must be noted that the mean overall historical gene flow was 0.0112, that is almost ten times less than mean overall contemporary gene flow (0.0011). Every contemporary gene flow estimate was higher than its contemporary analogue.

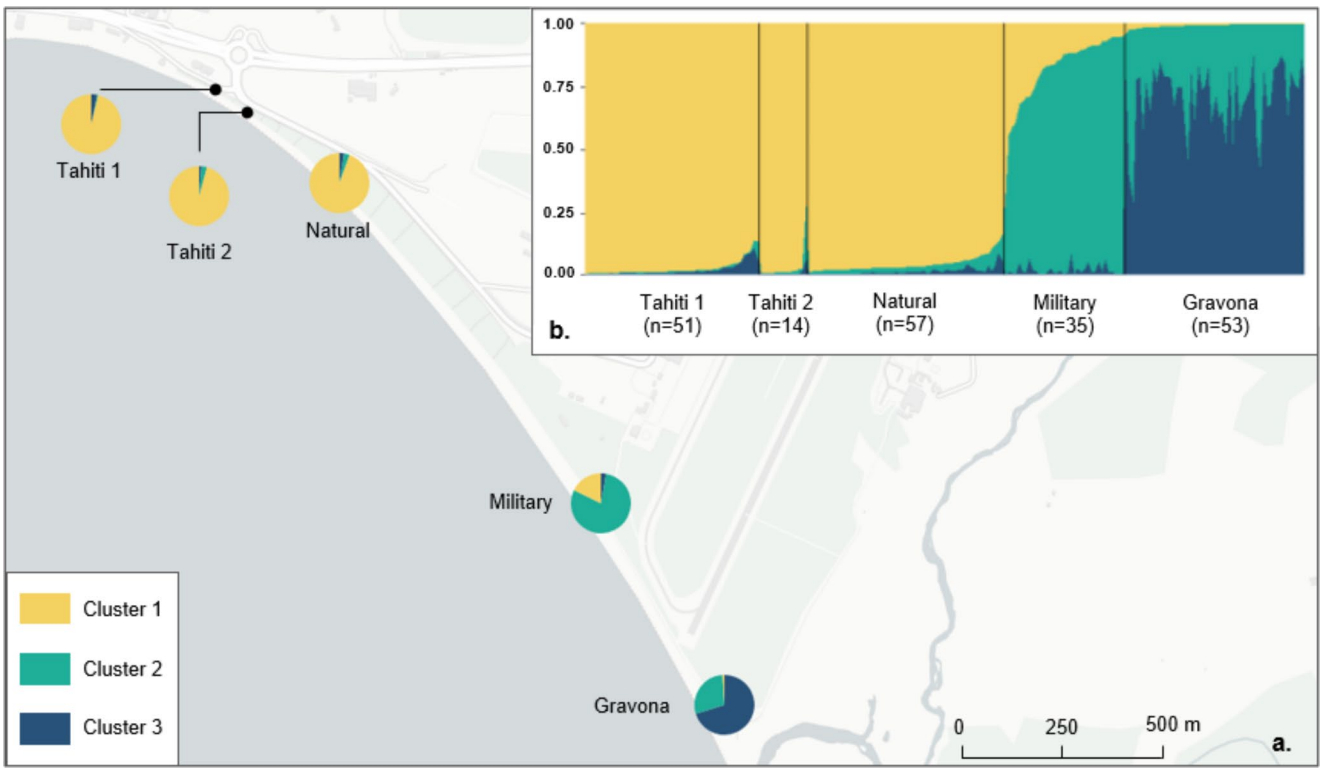

Fig. 2 **(a)** Map of relative importance of each genetic cluster per sampled areas, inferred with STRUCTURE software **(b)** Assignment probabilities of individuals to each genetic cluster, computed with STRUCTURE software, and plotted with strataG package in R (Archer et al., 2017). Individuals are gathered according to their sampling areas, and the number of individuals for each area (n) is displayed

Evolutionary scenarios

Ricantu population origin's scenarios were elaborated based on the results of genetic differentiation, indicating three genetic clusters. All effective population size in tested scenarios were equal to avoid an unnecessary complexification of models. Hierarchical progression leading to the final most likely scenario can be find in Fig. A7 (Appendix A). For every tested scenario, mutation rate estimate was 10^{-4}.

The final most likely scenario is shown in Fig. A6 and had a relative probability of 0.4920 compared to the two other scenarios it was tested against. Type I error estimate was 0.423 and type 2 error estimate was 0.127.

This scenario described that an ancestral population with an effective population size N_0 (with a median over all simulated data set of 7.44×10^3 individuals) would have gone through a population shrinkage and thus reached an effective population size of N_0-ret (with a median over all simulated data set of 2.45×10^3 individuals). The reduced ancestral population would then have been divided into two subpopulations, accounting for two hypothetical entities that subsequently contributed to the emergence of the three genetically distinct clusters identified from the 5 sampled localities.

Discussion

Genetic health of the ricantu population

Surprisingly, our results do not support the H1 hypothesis of low genetic diversity. The relict population of *T. ceratina* showed a quite high genetic diversity, with values of allelic richness and expected heterozygosity similar to those observed -with also microsatellite markers- in non-threatened Helicidae species (e.g. Arnaud et al. 2001). Mechanisms already observed in *Helicidae*, such as multiple paternity (Evanno et al. 2005), or inbreeding avoidance (Dahirel et al. 2013) could explain this high neutral genetic diversity. This rather high genetic variability does not prevent high inbreeding at some loci, a result that is rather consistent with a fragmented and small population. Although the population may likely suffer from inbreeding, homozygotes excess, common in gastropods (e.g. Wiehn et al. 2002), may only be the result of sampling bias, due to unsampled heterozygote genotypes. The high values of both allelic richness and observed heterozygosity support this hypothesis.

As expected for a threatened species, the effective population size (Ne) estimated is extremely low, and far below the population size (N) recently estimated (Cucherat, unpublished), which indicates a strong effect of demographic and genetic stochasticity (Palstra and Ruzzante 2008). Nevertheless, Ne value estimated using linkage disequilibrium (LD) across pairs of loci corresponds to the lowest size through which the population has passed over time following a contraction, although a rapid demographic recovery may have occurred afterwards (Slatkin 2008). This hypothesis seems sound due to the high genetic diversity of the population and the size reduction of the ancestral population inferred by our evolutionary scenario. Similar estimates of Ne carried out with the same method on bottlenecked populations of Helicidae support this hypothesis (Qiu et al. 2019). A possible cause of this seventy years long demographic crash is the heavy anthropization of the Ricantu site since the 1950s (Paradis et al. 2011 [in French]). The habitat restoration of two main areas of Ricantu in 2000 and 2014 (Fig. A8) (Galleras and Freytet 2014) could have contributed to an increase in population size, although this was not reflected in Ne estimates. In addition, there is an overlap of generations, as *T. ceratina* produces two generations of individuals per year, and an adult lives between three and four years after its first reproduction. Given genotypes from several generations were used (Waples et al. 2014) and the concept of effective population size (Ne) was developed under a discrete-generation mode, the linkage disequilibrium (LD) method used may have also led to an underestimation of Ne.

At first sight, the genetic health of *T. ceratina* would therefore not be of too much concern. However, we must be aware that the neutral genetic diversity estimated here may not reveal adaptive genetic diversity (Hall et al. 2012), and therefore does not predict population persistence. The use of neutral markers to determine the genetic diversity of a population and to assess its extinction risk is being questioned (Teixeira and Huber 2021; Väla et al., 2008). Thanks to the recent development of genomic techniques, functional genetic diversity can now be quantified and compared to potentially neutral diversity, which may be of great interest in conservation genetics (Allendorf et al. 2010; Kohn et al. 2006).

High genetic structuring linked to low connectivity

Our results show that the entire population is spatially organized with a genetic pattern that appears to be driven by isolation by distance (IBD). Moreover, the population is subdivided into three spatially distinct genetic groups, supporting the hypothesis of fragmentation into sub-populations (**H2**). As shown for other land snail species (e.g. Arnaud et al. 2003), such IBD pattern resulting is consistent with the species' low dispersal ability and fragmented habitat. Given that high level of genetic diversity is maintained in samples studied, we assume that local gene exchanges including indirect gene flow over several generations (van Strien et al.

2015) would be sufficient to counteract the stochastic effects of genetic drift. The low average dispersal distance of 71m per generation supports this hypothesis. With a generation time of *T. ceratina* of 1.5 years, an adult would travel an average distance of 47 m/year. This value is consistent with those estimated for other terrestrial gastropod species (Kramarenko 2014). Therefore, in the Northwest area, snails can possibly cross in one generation the 3m and 6m wide sand paths with no vegetation between Tahiti 1 and Tahiti 2, and Tahiti 2 and Natural localities respectively. This would explain the genetic homogeneity of these areas. By contrast, at the metapopulation level, the linear distances of 935m and 580m separating respectively the Northwest area and Centre one, and the Centre and Southeast areas, would not be easily crossed by individuals, explaining the clustering of the metapopulation into three distinct genetic clusters. Such short-range gene flow would contribute to the persistence of subpopulations through the movement of alleles trapped within the three identified clusters, corresponding globally to Northwest, Centre and Southeast areas. This retention of "ancestral" alleles could therefore explain both the sharp spatial genetic structure observed and the relatively high level of estimated genetic diversity. Non-deterministic factors such as physical barriers to dispersal would have further accentuated these spatial and temporal genetic discontinuities. This is evidenced by the very low values of functional connectivity (gene flow) estimated between the three genetic clusters. The Ricantu site has been strongly disturbed after the World War II. Figure 3 summarises main disturbances of the site on the basis of analysis of aerial photography of the French National Institute of Geography (pictures available since 1950). Various landscape barriers over time would have prevented functional connectivity between areas of T. *ceratina* occurence. Firstly, the Northwest stretch of the Gravona River has been disconnected from the Southeast one for the construction of the Ajaccio airport in 1950' years. At this period, the landing runway was longer, separating the Southeast site from the others. Up to 2000, the development of the airport was followed by construction of a car park, a military summer camp and plantations of non-indigenous plant species, between the present-day Northwest and the Central areas. In the same lap time, remnant habitats were disturbed by diverse car traffic and sand withdrawal. A sea high tide protection of the

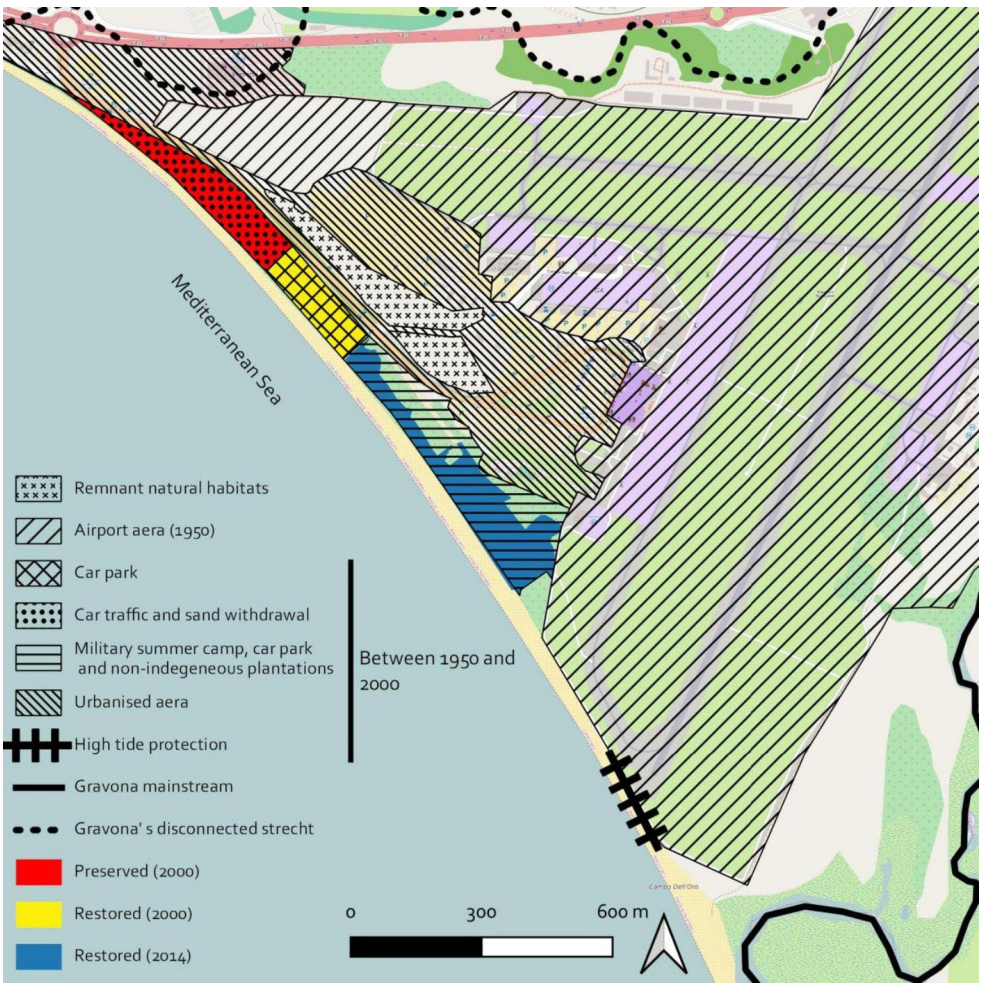

Fig. 3 Mapping of putative landscape barriers that may have limited the dispersal of *T. ceratina* on the Ricantu site adjacent to Ajaccio airport, based on aerial photographs from the Institut National de l'Information Géographique et Forestière (IGN), dating respectively from 1951, 1966, 2002 and 2007

landing runway has been built against sea storms. Since the rediscovery of the species, all remnant habitats where the snail was found have been protected, the car park and military summer camp have been destroyed and natural habitats of the fluvio-marine terrace have been restored, respectively in 2000 and 2014. Currently, there is no anthropogenic habitats between the Northwest area and the Central one, but only a few pioneer individuals has been detected so far. Based on credibility intervals, most historical and contemporary gene flow cannot be differentiated. Therefore, these results suggest that the low functional connectivity has not significantly changed through time. However, even if confidence intervals are large and mostly non-significant, we suggest a beneficial effect of protection and restoration on functional connectivity, given that all contemporary gene flow estimates are higher than the historical ones. Unlike our hypothesis **H3** which states a stronger past than recent gene flow, such finding may suggest that recent habitat changes over time could have promoted the movement of individuals. However, this conclusion is supported by fragile results because the historical migration rates, performed with MIGRATE, vary depending on the mutation rate used. Here, the mutation rate of 10^{-4} seems justified as this is the value inferred in all scenarios tested with DIYABC. Nevertheless, higher mutation rates of 10^{-3} or even 10^{-2} have been used in other gastropod species (e.g. Jarne and Theron, 2001).

All these temporal and spatial shifts between unfavorable (fragmentation) and favorable (protection and restoration) habitats would explain why such subdivided population exhibit a surprisingly high level of genetic diversity. Just as quaternary refugia can contribute to preserve the genetic diversity of a species, remnant habitats may have served as reservoirs of diversity from which secondary contact between alleles when the habitat became favorable promoted genetic innovations. Rather than causing gradual population genetic erosion, anthropogenic disturbances that impacted *T. ceratina* habitat may have yielded complex and unexpected genetic patterns due to possible local recolonizations (Millette et al. 2020). Although this hypothesis requires that disturbances do not affect the viability of the population, it is supported by other studies specifically highlighting the resilience of land snail species in fragmented habitats (Triantis et al. 2009), which can be explained because many mollusk species only need very small areas to persist for long periods.

A non-exclusive hypothesis is the one of a delayed habitat fragmentation effect on the population response. Such asynchronous response appears as a kind of apparent reprieve to populations that will inexorably disappear. This time delay in extinction, called "relaxation time" (Diamond 1972), may affect the dynamics of diversity estimates in delaying the joint impact of genetic drift, inbreeding, and gene flow on populations. Indeed, several studies (e.g. Landguth et al., 2010) suggest that the duration of fragmentation would then be too short to detect any genetic effect in the population. The high level of genetic diversity estimated in the unique population of *T. ceratina* would therefore only be an apparent biodiversity surplus (Halley et al. 2017), an example of the well-known phenomenon of the extinction debt (Hylander and Ehrlen 2013). Such allelic excess would be the signature of alleles conserved from pre-fragmentation times and currently trapped in the fragmented population.

Towards a restoration of connectivity?

Although possibly misestimated, especially if the population has experienced a recent decrease in connectivity (Samarasin et al. 2017), the very low and similar gene flow values measured at two different time scales between the three genetic clusters identified does not allow to underline a significant effect of the site restoration on functional connectivity. However, higher contemporary gene flows estimates than historical ones may be the expression of a restoration of landscape connectivity, linked to the ecological restoration of certain areas. This hypothesis is consistent with the individual assignment tests, which show a high probability that some individuals belong to non-dominant genetic clusters in their sample areas. Moreover, individuals were observed moving near the airport runway, which has gradually recovered a low vegetation after the 1950s. Gene migration via the dispersal of individuals is therefore both possible and probable between Centre and Southeast areas. Thus, the historically fragmented population of *T. ceratina* in Ricantu would function as a metapopulation due to the restoration of connectivity between previously isolated habitats. Still, this hypothesis is not statistically supported, given the overlap of historical and contemporary gene flows, and must be verified through time by the genetic and demographic follow-up of the study population.

Population history of the Ricantu

The most likely history scenario inferred from DIYABC software is consistent with the hypothesis of a demographic contraction of an ancestral population that gave rise to the current metapopulation (**H4**). The estimated decrease to about one third of the effective size of the original ancestral population is substantial. As mentioned previously, this sharp decline did not result in a total erosion of neutral genetic diversity, yet this is often observed in species with low dispersal ability and pronounced micro-endemism (e.g. Johnson 2005). Unfortunately, data on the current and past occurrence of the species are so limited and poor that it is

impossible to identify potentially suitable habitats areas that would allow to hindcast or forecast the species' versus distinct lineages' distribution in response to climate change.

However, being able to date and quantify demographic events of population contraction versus expansion is essential to unravel the causes that led the Ricantu population to the risk of extinction. Knowledge of the temporal and spatial dynamics of these past events would help refine hypotheses explaining the contraction in the species' range and population size, including those that could be linked to anthropogenic fragmentation of its habitat, or to Quaternary (Pleistocene) climate change which influence is well known on the distribution of many Corsican endemic species (e.g. Salvi et al. 2010).

Concluding remarks

We showed that the genetic diversity of the relict population of *T. ceratina* was high and structured even though it is a microendemic and endangered species. These unexpected results are promising for future translocation of populations, that must be envisioned to lessen the extiction risk of a unique population and given that the effects of habitat restoration on connectivity does not seem to be substantial, or need more time to be effective. However, we need to investigate whether the three distinct genetic groups identified exhibit variations in fitness for specific life history traits and adaptive potential. Such a functional assessment of diversity would allow the most suitable individuals to be selected for successful translocation. One limitation is that at this spatial scale, it is unlikely to identify relevant genetic variation that could reflect local adaptations. Furthermore, inference of demographic and historical events that affected the Ricantu population should be supported by a phylogeographic approach. Mitochondrial sequences of cytochrome oxidase I (COI) and 16 S genes of *T. ceratina* have already been produced from a small number of individuals but unfortunately did not provide sufficient nucleotide variation to complete the phylogeographic analysis. Further analyses are planned based on a larger number of individuals selected according to their genetic groups to assess the geographical patterns of genetic diversity in order to (i) improve our understanding of the demographic history of the species in relation to its distribution changes (ii) estimate the time of divergence between lineages, (iii) infer the historical factors that might explain the resulting phylogeographic structure, thereby eliminating the potentially confounding impact of history and correctly assessing the genetic consequences of habitat fragmentation.

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Handling and sampling of this protected species were performed under permits: Arrêtés préfectoraux 2 A 2018-01-29-001, 2 A 2020-03-25-001 and 2 A-2021-02-25-003.

Author Contribution AG, MD and PP conceived the idead and designed methodology; CN, XC, MD, LC and AG collected the data, LC and SD did the laboratory work. LC, AC and PP analysed and interpreted the data; LC and AG led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Declarations

Competing interests The authors declare no competing interests.

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