Research Article

Population genetic analysis of invasive black rats: Defining eradication units in the Tuscan Archipelago National Park

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Abstract

Invasive species are one of the main causes of biodiversity loss, and rodents in particular are regarded as a real threat worldwide, especially to island ecosystems. The Tuscan Archipelago National Park is the largest in the Mediterranean basin, it harbours a large number of autochthonous endemic species, mostly reptiles and insects, and hosts many migratory birds during their seasonal movements. Although a number of sites in the Archipelago are under strict protection regimes, the invasive black rat *Rattus rattus* has significantly affected survival of local wildlife. As part of an eradication campaign conducted in 2012 and 2017, we assessed genetic diversity and population differentiation of black rats from a total of six locations on the largest Elba Island, a possible source of invasion, and the southern, small islands of Pianosa and Montecristo using six nuclear DNA microsatellite loci. We recorded a strong population structure and identified the islands of Elba, Pianosa and Montecristo as three distinct eradication units. Despite some degree of admixture was recorded on Elba, the largest island of the archipelago was unlikely the main source of invasive rats to Pianosa and Montecristo. We also recorded evidence of past reduction in population size, particularly in Montecristo, probably due to repeated past founding events. Biodiversity management plans should consider monitoring vessels arriving to the Tuscan Archipelago from the mainland and the major Tyrrhenian islands in order to limit alien invasion. Moreover, as reinvasion can occur a few years after eradication, regular monitoring should be conducted thus to rapidly intercept the arrival of new invaders.

Introduction

During the last century, exponential growth of the global market and subsequent increase in marine freight transports resulted in a dramatic proliferation of invasive species worldwide (Di Castri, 1989; Mack et al., 2000; Pascal et al., 2003, 2005). Invasive species have been described as the second main cause of biodiversity loss after habitat destruction and the predominant threat to island endemics (Vitousek et al., 1996; Alonso et al., 2001; Clout and Veitch, 2002). Insular ecosystems are particularly vulnerable to biological invasions. Rates of extinction of isolated, autochthonous species due to introduction of allochthonous taxa may in fact be much greater than in mainland areas (Reaser et al., 2007; McCreless et al., 2016). Eradication of alien fauna is therefore one of the most direct answer to protect island biodiversity and restore original ecosystem dynamics (Veitch et al., 2011).

The Tuscan Archipelago lies off the western coast of Italy, between the Ligurian and the Tyrrhenian Sea. It consists of seven major islands (Gorgona, Capraia, Elba, Pianosa, Montecristo, Giglio and Giannutri) and several minor islets most of which are entirely or in part included in the marine and terrestrial Tuscan Archipelago National Park, established in 1999. The Tuscan Archipelago is the largest European marine park and includes areas under different levels of protection. Pianosa and Montecristo, in particular, have strict regulations governing public access. The Park harbors a large number of autochthonous endemic terrestrial species, particularly insects and reptiles, and is an important stepping stone for migratory birds. The Tuscan Archipelago is also

a Tyrrhenian island biodiversity hotspot (Médail and Quézel, 1999), is listed in the European Habitats Directive and the Birds Directive (the Council Directives 92/43/EEC and 97/49/EC, respectively), and includes several Sites of Community Importance and Special Protection Areas.

Nevertheless, the Tuscan Archipelago is not exempt from the presence of invasive plant and animal species, one of the most threatening being the black rat *Rattus rattus* (Genovesi and Carnevali, 2011). Invasive black rats are considered a pest worldwide (Lowe et al., 2000; King et al., 2011). They are nocturnal omnivores and key predators of invertebrates, lizards, and ground nesting birds (Sultana and Borg, 2006a,b; Towns et al., 2006; Gibbs, 2009). The detrimental effects of such invasive rodents on island ecosystems are well documented (e.g. Moors and Atkinson, 1984; Atkinson, 1985; Courchamp et al., 2003; Lorvelec and Pascal, 2005; Abdelkrim et al., 2009). Over the last few decades, many rat eradication campaigns have been carried out worldwide, particularly to protect insular biocenoses (e.g. Towns et al., 2001; Graham and Veitch, 2002; Kerbiriou et al., 2004; Pascal et al., 2005; Sposimo et al., 2008; Amaral et al., 2010; Capizzi et al., 2010; Ragionieri et al., 2013; Tabak et al., 2015). Most of these conservation plans resulted in successful restoration of biodiversity just a few years after completion of the eradication programs (Jones et al., 2016). However, there are examples of eradication campaigns that failed because of survival of a few alien individuals and/or recolonization of eradicated areas from nearby islands or the mainland (Thorsen et al., 2000; Courchamp et al., 2003; Parkes et al., 2011; Savidge et al., 2012). When a few individuals survive eradication, high reproduction rates typical of black rats and absence of predators that limit dispersal of aliens may lead to a relatively

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fast increase in population size (Abdelkrim et al., 2007; Russell et al., 2009a,b). If recolonization occurs, then absence of intraspecific competition allows the newly arrived individuals to rapidly spread across the eradicated area (Russell et al., 2010).

According to the eradication unit concept (*sensu* Abdelkrim et al., 2007), recolonization of eradicated areas can be prevented if the interconnected populations are eradicated simultaneously (e.g. Robertson and Gemmell, 2004; Abdelkrim et al., 2005a,b, 2007, 2010; Capizzi et al., 2010). It is therefore important to define the geographic boundaries of an eradication unit, and to this purpose DNA profiling has proven an invaluable tool for describing levels of distinctiveness among target populations of invasive species (Abdelkrim et al., 2010; Russell et al., 2010; Savidge et al., 2012). From a genetic perspective, an eradication unit consists of a group of populations interconnected by a high rate of gene flow and therefore genetically homogeneous. A common approach to uncover patterns of either natural or human-driven migration of invasive rat populations is by looking at nuclear DNA microsatellite allele variation (Fewster et al., 2011). Once the genetic profile and structure of eradication units are characterized, then simultaneous eradication can be implemented to prevent re-colonisation from nearby populations (Abdelkrim et al., 2007).

Using eight polymorphic autosomal microsatellite loci for a total of 135 individuals of *R. rattus*, we assessed patterns of genetic diversity and population differentiation of black rats in the Tuscan Archipelago National Park. We collected samples from the two strictly protected islands of Pianosa and Montecristo and from two harbors of Elba Island, where private and tourist boats set sail regularly to Pianosa. Landing on Montecristo is permitted to very few small vessels only (Fig. 1). The main aims of the study were: i) to estimate differences in genetic diversity among populations from the three islands; and ii) to assess evidence of past reduction in population size due to either bottleneck or founding events. We also investigated whether Pianosa and Montecristo were to consider distinct eradication units resulting from either a single or multiple introduction from Elba Island. Considering the geographic distance among islands of the Tuscan Archipelago, we excluded the hypothesis of natural migration of individuals (i.e. swimming) between islands and focused on possible human-driven translocation events. By assessing patterns of connectivity among alien island populations, our work, as part of a comprehensive eradication program of invasive species of plants and animals (www.montecristo2010.it; www.restoconlife.eu), provided background information to set up rat eradication plans on two of the most important islands for biodiversity conservation of the Tuscan Archipelago (Sposimo et al., in press) and contributed to an assessment of possible sources of invasions of one of the biodiversity hotspots of the Mediterranean area.

Materials and methods

Study area and sample collection

A total of 135 male and female adult samples of *R. rattus* were captured using traps from three islands of the Tuscan Archipelago, Montecristo (MC, n=30), Pianosa (PS, n=64) and Elba (EL, n=41) islands. On Pianosa Island, rats were collected in the village near the harbor (PS_V, n=17) and in two uninhabited areas, one lying to the South-East of the village near Punta Brigantina and Punta Secca (PS_SE, n=26), and a second one located to the North-West of the village, close to Punta Grottone (PS_NW, n=21) (Fig. 1). Private and tourist boats depart from Elba to the islands of Pianosa, daily in summer and weekly in winter. Small boats can now land on Montecristo under restricted permits only. The ports of Marina di Campo (EL_MC, n=23) and Rio Marina (EL_RM, n=18) on Elba Island were sampled as possible sources of invasive black rats (Fig. 1). For each captured rat, 10–50 g of tail muscle tissue was collected and preserved in absolute ethanol.

Laboratory analysis

DNA was extracted by overnight digestion of tissues at 55 °C in a lysis buffer containing 0.1 m Tris buffer, 0.005 m EDTA, 0.2 m NaCl and
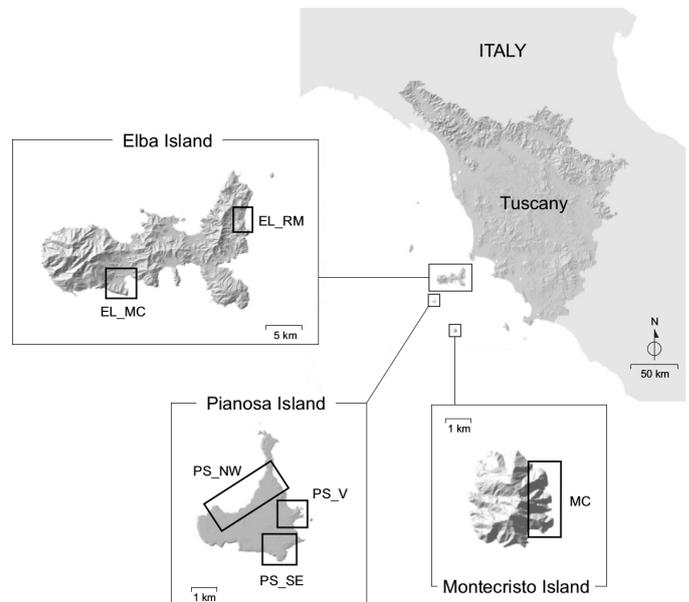
Figure 1 – Map of the study sites. Black rat samples were collected in Rio Marina (EL_RM) and Marina di Campo (EL_MC) on Elba Island, in the North-West (PS_NW), South-East (PS_SE) and the main village near the harbour (PS_V) of Pianosa Island, and on the Island of Montecristo (MC).

0.4‰ SDS, pH 8.0, and 0.1 mg proteinase K, followed by isopropanol-ethanol precipitation (Sambrook and Russell, 2001). Samples were resuspended in DNAase-free water and preserved at −80 °C. A total of 135 samples were screened for polymorphisms at eight microsatellite loci described for *R. norvegicus* by Jacob et al. (1995) and successfully tested in *R. rattus* by Ragionieri et al. (2013). Each locus was separately amplified by polymerase chain reaction (PCR) according to conditions and thermal profiles described in Ragionieri et al. (2013). PCR products were then pooled into two multilocus sets: R1 including D10Rat20, D5Rat83, D7Rat13 and D19Mit2, and R2 including D11Mgh5, D16Rat81, D10Mit5 and D9Rat13. Amplicons were resolved by capillary electrophoresis in an Applied Biosystems 3130xl Genetic Analyzer and allele sizes scored against a GeneScan500 LIZ size standard using GeneMapper 5.0 (Applied Biosystems).

Statistical analysis

Genetic diversity

We used MICROCHECKER 2.2.3 (van Oosterhout et al., 2004) to test loci for scoring errors due to stuttering, allele dropout and evidence of null alleles. The number of alleles and allelic richness for each locus and population were calculated using FSTAT 2.9.3.2 (Goudet, 1995). Linkage equilibrium among loci and Hardy-Weinberg equilibrium (HWE) were assessed for each population using GENEPOP 4.2.1 (Rousset, 2008). Significance levels were adjusted for multiple tests by using a sequential Bonferroni correction (Rice, 1989). Statistical significance of the estimator f of the inbreeding coefficient FIS was evaluated after 10000 allele permutations performed in Genetix 4.05 (Belkhir et al., 1996–2004).

Since rats can form social and familiar groups (Granjon and Cheylan, 1989), individuals from a sampling site can be more related than individuals from different sampling sites if gene flow is negligible. We therefore used GenAlex 6.5 (Peakall and Smouse, 2006) to compare mean pairwise relatedness calculated for each sampling site to average relatedness estimated across sampling sites using the Lynch and Ritland (1999) estimator. Statistical significance was obtained after 1000 permutations of relatedness values.

Population structure

Genetic divergence among sampling sites was estimated by the exact test for population differentiation implemented in GENEPOP 4.2.1. This test verifies the existence of differences in allele frequencies at each locus and for each population. Single locus significance *p*-values

were calculated using a Markov chain with 1000 batches and 1000 iterations per batch combined over loci using the Fisher method. Genetic differentiation was also assessed by the FST estimator Θ using ARLEQUIN 3.5 (Excoffier and Lischer, 2010). Statistical significance of Θ values under the null hypothesis of no differentiation among sampling sites was assessed after 10000 allele permutations.

A spatial analysis of molecular variance (SAMOVA) was performed using the simulated annealing procedure as implemented in SAMOVA 2.0 (Dupanloup et al., 2002) to define groups of sampling sites that maximized the percentage of total variance explained by variation among groups. We used 100 simulated annealing processes for K values from 2 to 6. We also performed a Principal Coordinates Analysis (PCoA) based on genetic distances between individuals assessed by multivariate analysis of loci and samples to investigate the presence of distinct groups. The analysis was conducted using the covariance-standardized method implemented in GenAlex 6.5.

We applied the Bayesian clustering method implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) to infer the most likely number of genetically distinct clusters (populations) given the observed genotypes and to evaluate the proportion of each individual's genotype belonging to each inferred population. We used the admixture model as the most appropriate for populations that may have recent ancestors from more

than one population (Pritchard et al., 2000). We run 1000000 Markov Chain Monte Carlo iterations without prior population information for a number of populations K ranging from 1 to 6 using a burn-in period of 20000 iterations. We calculated the mean likelihood over three runs for each K with correlated allele frequencies and estimated the most likely number of clusters as described in Evanno et al. (2005). The K value with the highest ΔK was then used as prior information to estimate the proportion of membership of each genotype in each of the K populations. Results were graphically visualized using STRUCTURE PLOT (Ramasamy et al., 2014).

Demographic patterns

Evidence of bottlenecks was assessed for the six *R. rattus* sampling sites using BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996). Populations that have experienced a recent reduction in their effective population size are expected to exhibit a corresponding reduction in the number of alleles and gene diversity. In particular, the number of alleles decreases faster than gene diversity so that, in a recently bottlenecked population, the observed gene diversity is higher than the average gene diversity expected from the observed number of alleles in a population of constant size at mutation-drift equilibrium (Luikart et al., 1998). The average gene diversity expected at equilibrium was calcu-

Table 1 – Genetic diversity measures in *R. rattus* from six sampling sites in the Tuscan Archipelago. N_A, number of alleles; A_R, allelic richness; A_P, number of private alleles; H_O, observed heterozygosity; H_E, expected unbiased heterozygosity; f, F_{IS} index estimator. Means±SE values. *, $p<0.008$ (after Bonferroni correction). Abbreviations as in Fig. 1.

Sampling site		Locus	Allele range	N_A	A_R	A_P	H_O	H_E	f
Elba	EL_MC n=23	D10Rat20	106–132	12	10.8	3	0.61	0.90*	0.33*
		D19Mit2	195–237	9	8.6	2	0.89	0.89	−0.10
		D5Rat83	175–179	3	3.0	–	0.85	0.68	−0.26
		D7Rat13	157–185	8	7.2	–	0.68	0.75	0.10
		D11Mgh5	232–280	5	4.4	–	0.59	0.63	0.06
		D16Rat81	136–188	10	8.6	4	0.68	0.83	0.18
		All loci		7.80 ± 1.35	7.1	9	0.72 ± 0.04	0.77 ± 0.05*	0.08*
	EL_RM n=18	D10Rat20	120–132	6	6.0	–	0.40	0.51	0.22
		D19Mit2	215–231	8	7.7	1	0.94	0.87	−0.03
		D5Rat83	171–179	3	3.0	–	0.47	0.59	0.20
		D7Rat13	171–185	6	5.7	–	0.78	0.74	−0.06
		D11Mgh5	232–280	8	7.9	3	0.88	0.83	−0.06
		D16Rat81	136–188	6	6.0	1	0.76	0.85	0.10
		All loci		6.20 ± 0.75	6.0	5	0.71 ± 0.06	0.73 ± 0.09	0.03
Montecristo	MC n=30	D10Rat20	106–120	2	2.0	–	0.04	0.12	0.66
		D19Mit2	189–219	5	4.9	3	0.47	0.56	0.16
		D5Rat83	177–187	5	4.9	2	0.52	0.67	0.23*
		D7Rat13	163–181	7	5.8	1	0.73	0.72	−0.02
		D11Mgh5	242–274	4	3.8	2	0.30	0.40	0.25*
		D16Rat81	156–168	6	5.4	1	0.63	0.69	0.09
		All loci		4.80 ± 0.70	4.5	9	0.45 ± 0.09	0.53 ± 0.10*	0.15*
Pianosa	PS_V n=17	D10Rat20	108–126	7	6.9	–	0.75	0.84	0.11
		D19Mit2	203–241	8	7.8	–	0.88	0.79	−0.12
		D5Rat83	171–185	4	3.9	–	0.47	0.58	0.19
		D7Rat13	157–181	6	5.9	–	0.63	0.71	0.12
		D11Mgh5	232–286	6	5.9	–	0.65	0.72	0.10
		D16Rat81	156–186	2	2.0	–	0.18	0.17	−0.07
		All loci		5.50 ± 0.88	5.4	–	0.59 ± 0.10	0.63 ± 0.10	0.07
	PS_SE n=26	D10Rat20	108–126	8	7.4	–	0.65	0.78	0.16
		D19Mit2	205–241	10	8.4	1	0.92	0.77	−0.20
		D5Rat83	171–185	3	3.0	–	0.38	0.41	0.07
		D7Rat13	157–185	8	6.7	–	0.58	0.66	0.12*
		D11Mgh5	232–286	7	6.0	1	0.72	0.75	0.04*
		D16Rat81	156–166	2	2.0	–	0.23	0.21	0.11
		All loci		6.30 ± 1.28	5.6	2	0.58 ± 0.10	0.60 ± 0.10	0.03
	PS_NW n=21	D10Rat20	108–126	8	7.6	–	0.71	0.83	0.15
		D19Mit2	205–241	7	6.6	–	0.90	0.82	−0.10
		D5Rat83	171–185	5	4.5	–	0.40	0.57	0.30
		D7Rat13	159–181	8	6.8	1	0.71	0.72	0.01
		D11Mgh5	232–286	8	7.6	1	0.81	0.85	0.05
		D16Rat81	156–166	2	1.7	–	0.05	0.05	0.00
		All loci		6.30 ± 0.99	5.8	2	0.64 ± 0.13	0.60 ± 0.13	0.07
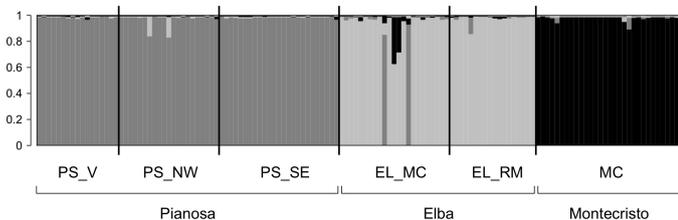
Figure 2 – Plot of proportions of black rats' genome belonging to each of the three clusters considered most likely to account for the observed *R. rattus* genotypes. Each individual is represented by a vertical line partitioned into K=3 segments (dark grey, light grey, black) with lengths corresponding to the proportion of its genome originating from each of the three clusters inferred by a model-based Bayesian method. Black vertical bars define distinct island sampling sites. Location acronyms as in Fig. 1.

lated from a distribution of 10000 simulated gene diversities under the two-phase mutation model (TPM) and the step-wise mutation model (SMM) of microsatellite evolution. For the TPM, we set 70 multi-step mutations with a 12% variance among multi-steps (Piry et al., 1999), and obtained statistical significance based on 1000 replications. Gene diversity excess was assessed using a Wilcoxon sign-rank test (Luikart et al., 1998). We also assessed whether the observed allele frequencies at each locus deviated from an L-shaped distribution expected under mutation-drift equilibrium (Luikart et al., 1998).

We also calculated the Garza and Williamson (2001) index (M) to test for a reduction in rat population size using ARLEQUIN 3.5. The M index is a ratio of the number of alleles observed in a sample divided by the number of alleles expected under the observed allele size range. Garza and Williamson (2001) reported that M values greater than 0.82 should be representative of stable populations that have not suffered a known reduction in size, whereas values of the M index lower than 0.68 indicate a bottleneck or a founder event.
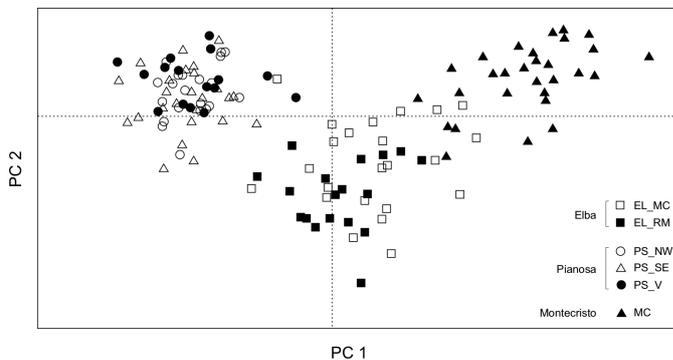
Results

Genetic diversity

Loci D10Mit5 and D9Rat13 were excluded from the analyses because of the occurrence of a high number of null alleles. All remaining loci were polymorphic, with number of alleles ranging from 7 (locus D5Rat83) to 22 (locus D19Mit2). Relatively high levels of molecular variation were recorded at all sampling sites. Allelic diversity varied from 4.83±0.70 to 7.83±0.35 and average allelic richness ranged from 4.47 to 7.09 (Tab. 1). High values of expected and observed heterozygosity were recorded for Elba Island, while comparatively lower diversity was recovered for Pianosa and Montecristo in particular (Tab. 1). Number of private alleles ranged from 0 to 9 with the lowest values recorded in Pianosa and the highest number assessed in the islands of Elba (EL_MC) and Montecristo (Tab 1).

No significant linkage disequilibrium was recorded across populations. One sampling site from Elba Island (EL_MC) and the Montecristo location deviated from HWE after Bonferroni correction (Tab. 1). Both locations had relatively high and significant *f* values (Tab. 1).

Average pairwise relatedness based on the Lynch and Ritland (1999) estimator for the Pianosa Island sampling sites were r=0.089 (PS_V), r=0.069 (PS_NW) and r=0.089 (PS_SE). Relatedness values recorded

Figure 3 – Principal coordinates analysis (PCoA) plot based on six microsatellite loci showing patterns of genetic divergence among black rats sampled on the Islands of Elba, Pianosa and Montecristo. Principal component (PC) 1 and PC 2 explain 26.19% and 10.41% of genetic variation, respectively. Names of sampling sites as in Fig. 1.

on the island of Elba were r=0.049 (EL_MC) and r=0.089 (EL_RM), while an average relatedness among individuals of r=0.138 was recorded in Montecristo (MC). All values were higher than the average relatedness calculated among all individuals across locations (r=-0.006±0.001, *p*<0.001).

Population structure and demographic trends

There was a strong genetic differentiation among sampling sites. The Fisher exact test rejected the hypothesis of genetic homogeneity of allele frequency distributions (χ^2=∞, df=12, *p*<0.001). Significant differentiation was also recorded by F-statistics performed across populations (Θ=0.23, *p*<0.001). All pairwise comparisons revealed significant Θ values, except between the three Pianosa Island sampling sites (Tab. 2).

The spatial analysis of molecular variance recorded the highest, significant F_{CT}=0.24 for K=3 and clustered the Elba Island sampling sites (EL_RM and EL_MC) into group 1, Pianosa locations (PS_V, PS_NW and PS_SE) into group 2 and assigned Montecristo to group 3.

Population structure Bayesian analysis mirrored the SAMOVA results and revealed that the most probable number of clusters for interpreting the observed genotypes was K=3 based on the highest modal value of ΔK=5012.37 estimated using the Evanno et al. (2005) method (Supplemental Fig. S1). Three main partitions were used as prior population information for calculating the posterior probability of individual assignment (Fig. 2). Individuals from the three Pianosa locations were nearly entirely assigned to cluster 1 (99%). Black rats from the EL_RM location on Elba Island were assigned to cluster 2 (98%), while samples from Montecristo were assigned to the third cluster (98%). The Elba Island sampling site EL_MC was the most admixed, with 87.3% of individuals assigned to cluster 2 and 8.7% and 4% assigned to cluster 1 and 3, respectively.

Table 2 – Pairwise comparison matrix of the F_{ST} estimator Θ between black rat sampling sites (below diagonal) and corresponding *p* values (above diagonal). Significant *p* values <0.003 after Bonferroni correction. Abbreviations as in Fig. 1.

| | ELBA | | MONTECRISTO | PIANOSA | | |
	EL_MC	EL_RM	MC	PS_V	PS_SE	PS_NW
EL_MC	–	<0.001	<0.001	<0.001	<0.001	<0.001
EL_RM	0.06	–	<0.001	<0.001	<0.001	<0.001
MC	0.18	0.20	–	<0.001	<0.001	<0.001
PS_V	0.25	0.24	0.35	–	0.07	0.05
PS_SE	0.24	0.24	0.36	0.03	–	0.009
PS_NW	0.24	0.23	0.35	0.05	0.04	–

Table 3 – Results of the heterozygosity excess and M-ratio tests to assess evidence of population bottleneck in black rats from Elba, Montecristo and Pianosa islands. TPM, two-phase model of microsatellites mutation; SMM, stepwise model of microsatellites mutation; N_{exc}, expected number of loci with heterozygosity excess under mutation-drift equilibrium; Ratio, number of microsatellite loci exhibiting heterozygosity deficiency vs. excess; p_{TPM} and p_{SSM} are probability values for the Wilcoxon test for heterozygote excess under the TPM and SMM models, respectively. Mode indicates a L-shaped normal distribution or a shifted distribution. Location acronyms as in Fig. 1.

| | Heterozygosity excess test | | | | | | | |
| | TPM | | | SMM | | | M-ratio test | |
Location	N_{exc}	Ratio	p_{TPM}	N_{exc}	Ratio	p_{SMM}	Model	M-ratio
EL_MC	3.60	3:3	0.22	3.56	3:3	0.92	L-shaped	0.30
EL_RM	3.56	3:3	0.22	3.54	6:0	0.42	L-shaped	0.32
MC	3.53	5:3	0.58	3.52	6:0	1.00	L-shaped	0.28
PS_V	3.47	5:1	0.92	3.41	5:1	0.98	L-shaped	0.23
PS_SE	3.43	5:1	0.99	3.42	6:0	1.00	L-shaped	0.25
PS_NW	3.46	3:3	0.92	3.45	3:3	0.92	L-shaped	0.26

Principal component 1 and 2 explained 26.19% and 10.41% of genetic variation, respectively, and confirmed a clear genetic separation of the Montecristo black rat population from the other islands (Fig. 3). Both components also clustered *R. rattus* individuals from Pianosa Island, while principal component 2 separated, with a few exceptions, the Elba islands population from Pianosa and Montecristo.

The heterozygosity excess approach performed under the stepwise and two-phase models of microsatellite mutation reported no evidence of reduction in effective population size (Tab. 3). The Montecristo population and two sampling sites of Pianosa Island (PS_V and PS_SE) showed evidence of heterozygosity deficiency. Moreover, all six locations had a clear L-shaped allele frequency distribution. On the other hand, the M ratio index varied from 0.23 to 0.32 suggesting a bottleneck event in all island populations. The highest M values were recorded for black rats from Elba Island (Tab. 3).

Discussion

Genetic diversity

Overall, black rat populations from Pianosa, Montecristo and Elba islands were characterized by values of genetic variability similar to those reported for other insular populations of *R. rattus* (Abdelkrim et al., 2005a, 2009; Savidge et al., 2012; Ragionieri et al., 2013). The Elba Island populations had the highest allelic richness and heterozygosity values, while significantly lower levels of genetic variability were recorded for Pianosa and Montecristo. Low genetic variation on such small island populations can be due to a founder effect, bottleneck event, genetic drift and/or limited gene flow (Frankham et al., 2010). The Montecristo population was also not in HWE equilibrium, which, beside small population size, may be due to the presence of different family groups and inbreeding, a pattern suggested by a significant *f* value and the high average pairwise relatedness estimator recorded in the island. Conversely, urban environments such as harbours may offer abundant, distinct colonization sites, food resources, and allow a relatively large number of rat populations to cohabit in small areas (Banks and Smith, 2015; Mangombi et al., 2016).

Eradication units and no evidence of source populations

We investigated genetic diversity and populations divergence of *R. rattus* sampled on three islands in the Tuscan Archipelago National Park. Difference in allele frequency distribution, analysis of variance and principal components as well as Bayesian clustering techniques all recorded a strong population structure across the study area. The islands of Elba, Montecristo and Pianosa were defined as three distinct populations with no or very limited gene flow. Such a subdivision would designate the three black rat island populations of our study as three separate eradication units. No significant differences were, in fact, recovered among sampling sites from Pianosa island. Similarly, black rats sampled on Montecristo were strongly differentiated from *R. rattus* collected on other island locations and showed a relatively high number of private alleles. On the other hand, a few admixed individuals were recovered on the islands of Elba by Bayesian structure analysis. Moreover, the first of the principal components and F-statistics recovered a pattern of mild differentiation between the Elba locations of Rio Marina (EL_RM) and Marina di Campo (EL_MC). These could indicate that rats from the two sampling sites on Elba Island originated by the same source black rat population/s.

The three islands included in this study are separated by a much larger distance than what could be covered by a black rat swimming in sea water. This is no more than 1 km according to Russell et al. (2008). Passive translocation among islands and from the mainland may instead be possible thanks to private and touristic boats to and across the Tuscan Archipelago. The two sampling sites of EL_MC and EL_RM on Elba Island were indeed selected for our study because they are the two harbours in the Tuscan Archipelago from where most private and touristic ships depart to the southern islands. They may therefore represent possible source of invasion by black rats to Montecristo and Pianosa islands. Our results, however, suggests that this may not be the

case given the strong degree of divergence among all three islands and the absence of genetically admixed individuals on either Montecristo or Pianosa that could be assigned to Elba Island. Although evidence of mild admixture was recorded by the second of the two principal components, that was not clear enough to point to recurring passive translocation that may define a source-sink pattern of gene flow.

Establishing a possible source of rat invasion is complex and should consider the social behavior of *R. rattus*. Black rats, in fact, tend to form social and familiar groups, which are very aggressive towards unrelated individuals such that newcomers are generally excluded from their territory or killed (Granjon and Cheylan, 1989). Such an intrinsic demographic control may therefore mask the actual level of gene flow among populations. Although the two Elba harbours are unlikely the source of rat populations found on Pianosa and Montecristo, we cannot exclude that, after an eradication campaign, rats from Elba Island or other sites may invade Pianosa and Montecristo by passive translocation and, free of intraspecific competition, establish new alien populations on the southern islands (Oppel et al., 2011). Therefore, in order to minimize such a risk, bio-security measures have already been in force on both southern islands.

Evidence of past changes in population size

We tested for evidence of a population size declines due to bottleneck or founder events using three different methods (heterozygosity excess, shifts in allele frequencies and low ratios of allelic number to allelic size range) and obtained different results. The M-ratio test only recovered evidence of past reduction in population size. Following a bottleneck or a founder event, heterozygosity and allele frequency distribution are quickly re-established to values and patterns typical of a population in mutation-drift equilibrium. On the other hand, the M-ratio shows a relatively longer recovery time so that it can be used to detect past reduction in population size (Garza and Williamson, 2001). For that reason, populations that have experienced a recent decline will have evidence of bottleneck recovered by all three methods used in our study, whilst an historical bottleneck will be most likely inferred by the M-ratio test only (Marshall et al., 2009). Garza and Williamson (2001) also showed that the magnitude of the decrease in M is positively correlated with the severity and duration of the reduction in size, and that the rate of recovery in M is positively correlated with post-reduction population size. The very low M-ratio values recorded in our study therefore suggests that Pianosa, Elba and Montecristo in particular experienced a drastic reduction in population size in the past. This scenario may be due to a founder event whereby a few individuals established the present black rat populations far back in time. Similar results were recorded by Abdelkrim et al. (2005a) in Guadeloupe Island, southern Caribbean Sea, and interpreted as signature of an old founder effect, probably related to the first introduction of *R. rattus* in the Guadeloupe Archipelago back in the 1700s.

The time of arrival of *R. rattus* in the Tuscan Archipelago is currently unknown, but it is presumably very ancient. Black rats dispersed into the western Mediterranean basin from Asia approximately 2100 years before present (Vigne and Valladas, 1996), presumably by a single colonization event (Colangelo et al., 2015), and was first recorded in central Italy 600 years later (De Grossi Mazorin, 1987). Archeological studies show evidence of human colonization in the Tuscan Archipelago 6000 years ago (Patton, 1996). Under the Roman Empire, due to its strategic position, the Tuscan Archipelago became a hub for commercial maritime routes to the Iberic peninsula and North Africa. Human activities in the Tuscan Archipelago reached an apex in the XIV century and continued ever since. It is therefore possible that, because of the long history of dispersal and colonization, rat populations of the Tuscan Archipelago experienced several fluctuations in size due to repeated extinction and recolonization events.

Conclusions

Invasive black rats can significantly affect Mediterranean island ecosystems, and pose direct threats to endemic wildlife (Baccetti et al., 2009; Capizzi et al., 2016). Their impact on Montecristo and Pi-

anosa island natural environment is well documented. For instance, predation upon birds resulted in the extinction of the yelkouan shearwater (*Puffinus yelkouan*) and a decrease in the Cory's shearwater (*Calonectris diomedea*) population size on Pianosa Island (Arcamone and Sposimo, 2001; Palmer and Pons, 2001). In Montecristo, predation events led to virtually no annual productivity in the entire yelkouan shearwater population estimated at 400–700 fertile pairs (Baccetti et al., 2009).

The eradication campaign conducted on Montecristo in 2012 has been successful so far, as demonstrated by population size increase of autochthonous species (Sposimo et al., in press). Similar results are expected for Pianosa, where rat eradication is still ongoing (see Lorvelec and Pascal, 2005; Croll et al., 2016; Jones et al., 2016). However, rat eradication can sometime fail because of 1) re-invasion from nearby islands and/or the mainland and 2) rapid colonization thanks to the absence of intraspecific competition (Howald et al., 2007). The presence of black rats in the Tuscan Archipelago is linked to human settlement and maritime movements rather than natural dispersal. In our study, we showed that Elba, Pianosa and Montecristo islands harbour genetically distinct rat populations and that the larger island of Elba is unlikely to be a main source of invasive rats to the southern islands. For the campaigns on Pianosa and Montecristo islands to succeed in the long term, post eradication bio-security measures should be planned in order to control and prevent inadvertent movements of rat individuals, as recommended by Russell et al. (2008). We strongly suggest to check the possible arrival of new invaders using approaches that are able to detect populations at very low densities, for baiting traps are inadequate in such a situation (see Russell et al., 2008). We also encourage to consider regular monitoring of vessels arriving from the mainland and the major Tyrrhenian islands of Corsica and Sardinia, which are likely to be source populations of invasive rats. ✍

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Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Supplemental Figure S1 Plot of ΔK for a number of clusters K ranging from 1 to 6.