



Distribution of genetic diversity within and between Western Mediterranean island populations of the black rat *Rattus rattus* (L. 1758)

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Genetic diversity was estimated by allozyme analysis at 26 loci in black rat populations (*Rattus rattus*) from 15 western Mediterranean islands (Hyères, Corsica, Sardinia and related islets). Although overall variability levels were low ($H=0.025$), the mean heterozygosity values for the islands were similar to those for three reference mainland populations. Within the islands, however, genetic diversity varied in relation to island size and geographic isolation. In particular, most small insular populations were significantly more variable than those on both large and isolated islands. The genetic relationships between island populations were established by F_{ST} analyses indicating possible geographic origins and patterns of colonization. The maintenance of unexpectedly high levels of variability in the small island populations is discussed in relation to changes in the demographic and social structure observed in these populations. These island populations of black rat illustrate how genetic diversity may be efficiently maintained in a series of interconnected spatially fragmented populations.

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ADDITIONAL KEY WORDS—allozymes – genetic differentiation – colonization – meta-population – island syndrome.

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INTRODUCTION

Loss of genetic variability and increased differentiation are expected features of island populations. This pattern can occur through different stochastic processes related to geographic isolation (Nei, Maruyama & Chakraborty, 1975; Sirkkoma, 1983), the most common of which are the founder effect and genetic drift (Mayr, 1942; Kilpatrick, 1981; Frankel & Soulé, 1981; Motro & Thomson, 1982; Sirkkoma, 1983; Lande & Barrowclough, 1987). Theoretical models of subdivided populations predict that the extent of change in genetic parameters will be related to extinction/recolonization patterns, migration rates and colonist origin (Slatkin, 1977; Wade & McCauley, 1988; McCauley, 1991, 1993; Hastings & Harrison, 1994). In these models, genetic differentiation between local populations may in fact decrease under certain conditions (Slatkin, 1977, 1985, 1987; Wade & McCauley, 1988), while maintenance of genetic diversity within local populations is critically dependent on their extinction probabilities and migration patterns (McCauley, 1991; Gaggiotti, 1996). Most studies dealing with island samples of vertebrates have supported a decrease in genetic variability (Frankham, 1997) and an increase in differentiation among island populations (Kilpatrick, 1981; Boag, 1988; Grant, 1994; Grant & Grant, 1994), the more so the smaller and more isolated the island. For example, significant correlations have been obtained between heterozygosity levels of protein loci and the surface area of islands in lizards (Soulé & Yang, 1973; Gorman *et al.*, 1975) and rodents (Patton, Young & Myers, 1975; Berry & Peters, 1977; Navajas y Navarro & Britton-Davidian, 1989). These correlations have been ascribed to the higher rate of genetic drift and subsequent loss of variation due to the lower carrying capacity in small islands.

The black rat (*Rattus rattus*) is a widespread rodent species in islands worldwide (Atkinson, 1985) and as such is an appropriate model to investigate patterns of genetic variability and differentiation in relation to colonization processes and geographic isolation. Although extensively studied from a karyological point of view, few estimates of genetic variability in black rats are available. Overall, levels of intrapopulation allozyme polymorphism are relatively low ranging from 0% to 8% in mainland populations (Baverstock *et al.*, 1983; Gemmeke & Niethammer, 1984; Patton *et al.*, 1975), but reach values of up to 16% in more thoroughly investigated island populations (Pasteur *et al.*, 1982; Gemmeke & Niethammer, 1984; Patton *et al.*, 1975). In the present study, the effects of island geography (isolation and size) on the level and distribution of allozymic diversity were studied in black rat populations from Western Mediterranean islands and islets where this species is by

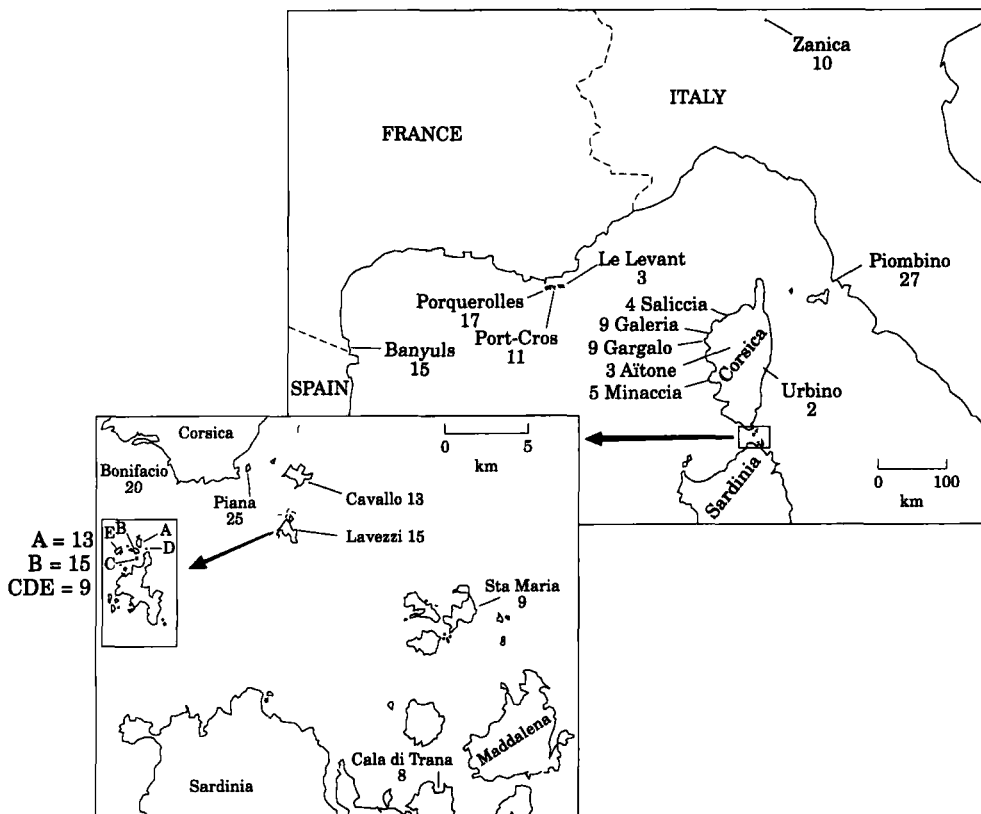


Figure 1. Map of the western Mediterranean area indicating the localities studied and their sample size. A, B, C, D and E refer to the islets of the Lavezzi island.

far the most common mammal present (Cheylan, 1984). The influence of colonization patterns on the genetic structure of these populations is discussed in relation to the relatively accurately dated expansion of the black rat into this region (Vigne, 1992). Additionally, its well documented island population dynamics and ecology (Cheylan & Granjon, 1985, 1987; Cheylan, 1988; Granjon & Cheylan, 1990a, 1993) provide a background for discussion of the consequences of island population structure on the apportionment of genetic variance within and between islands.

MATERIAL AND METHODS

Two hundred and forty-two individuals were live-trapped in 15 different Mediterranean islands as well as three mainland reference localities (Fig. 1). Two of the latter (Banyuls, Pyrénées Orientales, France and Piombino, Tuscany, Italy) are coastal Mediterranean localities where free-ranging black rats are common, whereas in the third (Zanica, Lombardy, Northern Italy), rats occur solely inside man-made constructions (commensal populations) due to the more continental climate. All island samples consisted of wild-living populations from two geographical areas, a Tyrrhenian group of islands and islands off the coast of Provence in France. The

TABLE 1. Geographical parameters of the islands studied. Remoteness indicates distance to the closest mainland coastline or major Tyrrhenian island (Corsica or Sardinia)

| Locality | Surface area | Remoteness |
|---------------------------|------------------------|------------|
| Corsica | 8680 km ² | |
| Sardinia | 24 089 km ² | |
| Corsico-Sardinian islands | | |
| Sta Maria | 186 ha | 4000 m |
| Cavallo | 115 ha | 2350 m |
| Lavezzi | 66 ha | 9500 m |
| Gargalo | 23 ha | 50 m |
| Piana | 6.4 ha | 300 m |
| Lavezzi A | 1.7 ha | 15 m |
| Lavezzi B | 1.5 ha | 75 m |
| Lavezzi CDE | 0.2–1.3 ha | 35–215 m |
| Hyères islands | | |
| Porquerolles | 1250 ha | 2300 m |
| Le Levant | 1010 ha | 9150 m |
| Port-Cros | 640 ha | 1000 m |

Tyrrhenian group included samples from Corsica (several coastal sites and one in the mountain range: Aitone, elevation 1000 m), Sardinia (Cala di Trana near Palau), seven islands from the Bouches-de-Bonifacio Straits between Corsica and Sardinia, and the island of Gargalo (North-western Corsica). The Provence sample was taken from three Hyères islands: Porquerolles, Port-Cros and Le Levant. The size and the isolation of these islands are indicated in Table 1.

Starch gel electrophoresis was performed according to Pasteur *et al.* (1987) using blood and tissue samples (kidney and liver). Twenty-one proteins corresponding to 26 loci were analysed: acid phosphatase (*Acp*), adenosine deaminase (*Ada*), albumin (*Alb*), amylase (*Amy*), esterase (*Est-1*, *Est-2*), guanine deaminase (*Gda*), glyoxalate (*Glo*), glutamate oxaloacetate transaminase (*Got-1*, *Got-2*), alpha-glycerophosphate dehydrogenase (*Gpd*), glucose-phosphate-isomerase (*Gpi*), haemoglobin (*Hbb*), isocitrate dehydrogenase (*Id-1*, *Id-2*), lactate dehydrogenase (*Ldh-1*, *Ldh-2*), NAD-dependent malate dehydrogenase (*Mdh-1*, *Mdh-2*), NADP-dependent malate dehydrogenase (*Me-1*), mannose-phosphate-isomerase (*Mpi*), nucleoside phosphorylase (*Np*), phosphoglucosylase (*Pgm-1*), phosphoglucuronate dehydrogenase (*Pgd*), sorbitol dehydrogenase (*Sdh*) and superoxide dismutase (*Sod*). Genic variability was measured using BIOSYS-1 (Swofford & Selander, 1981) by the unbiased mean heterozygosity (Nei, 1978), the mean number of alleles per locus, and the percentage of polymorphic loci using the 0.05 frequency level (i.e. a locus is considered polymorphic when the frequency of alleles is at least 5%). This criterion was applied only to unique alleles and not to those present at a level higher than 5% in one or more populations but at a lower level in others. Due to the small sizes of some of the samples from Corsica, several localities were pooled according to geographic and genetic proximity (see Table 3). Statistical tests for departure from Hardy-Weinberg expectations and differentiation among populations were performed using GENEPOP 2.0 (Raymond & Rousset, 1995). The amount of interpopulation and inter-island heterogeneity was estimated using standardized genetic variance, F_{ST} (Wright, 1965) computed according to Weir & Cockerham (1984), using GENEPOP 2.0. To limit the

occurrence of type I error, the sequential Bonferroni correction was applied to all series of tests (Sokal & Rohlf, 1995). Phylogenetic relationships between populations were constructed using the FITCH option of PHYLIP (Felsenstein, 1985) which allows for unequal evolutionary rates. The phenogram was drawn with the TreeView software distributed by Page (1996) using as input data the F_{ST} values estimated between pairs of populations. The isolation by distance model (Slatkin, 1993) between islands was tested within the seven islands from the Bouches-de-Bonifacio Straits between Corsica and Sardinia by a Mantel test performed using GENEPOP 3.0 (10 000 permutations were performed).

RESULTS

Genetic diversity

Of the 26 loci analysed, 11 were polymorphic in at least one population. The monomorphic systems included: *Acp*, *Alb*, *Est-1*, *Glo*, *Got-1*, *Gpd*, *Gpi*, *Id-1*, *Id-2*, *Ldh-1*, *Mdh-1*, *Me-1*, *Pgm-1*, *Sdh* and *Sod*. Allelic frequencies at the polymorphic loci and genic variability parameters are presented in Tables 2 and 3. All samples conformed to Hardy–Weinberg expectations. Variability levels ranged from $H=0.00$ in the Zanica locality to $H=0.049$ in the Levant island sample. Although overall values were low, differences were apparent within island groups: a low mean value was present in the large islands ($H=0.019$ for Corsica and Sardinia), while it reached higher levels in all smaller islands (mean $H=0.028$).

The relationship between island size and genetic diversity was investigated within all small island samples (≤ 1250 ha), excluding the large islands of Corsica and Sardinia. A significant positive correlation was found between mean heterozygosity and size of the island (Pearson correlation test, $r=0.717$, $P=0.013$ for 11 islands, Fig. 2). The lowest values were present in the smallest islands (the Lavezzi islets, <2 ha), whereas those larger than 100 ha (Cavallo, Santa Maria and the three Hyères islands) showed the highest variability values. One exception was noted which indicated that geographic isolation was also involved in determining variability levels: the genetic diversity present in the larger Lavezzi island matched more closely that of its islets than of similar-sized islands. The islands were then grouped into three sets according to size and geographic isolation: large (Corsica and Sardinia), small (from 6 to 1250 ha, excluding the Lavezzi island), and the Lavezzi island and islets (see Table 3). Heterozygosity levels were compared between islands within these three sets and with the Mediterranean mainland samples using Spearman's rank correlation. Results indicated that the small island group had significantly higher mean heterozygosity values than those in the large island one and the Lavezzi complex ($R=-0.7769$, $P<0.005$ and $R=0.8367$, $P<0.005$ respectively). All other comparisons including island-mainland ones, were not significant ($-0.1559<R<0.6211$, $P>0.15$).

Genetic differentiation

Genetic differentiation between samples was measured by F_{ST} values (Table 4). The phenogram produced by the FITCH procedure showed that populations were

TABLE 3. Genetic variability at 26 loci. N =sample size; A =mean number of alleles per locus; p =% polymorphism; H =expected heterozygosity (Nei, 1978); weighted means and standard errors are indicated; * excluding the Lavezzi islands, see text

| Locality | N | A | p | H |
|-------------------------------------|----------------------|------------------|-----------------|-------------------|
| TYRRHENIAN ISLANDS | | | | |
| Corsica | mean 14.3 ± 5.13 | 1.10 ± 0.00 | 7.7 ± 0.00 | 0.019 ± 0.009 |
| 1. Saliccia, 2. Galeria | 13 | 1.1 ± 0.1 | 7.7 | 0.030 ± 0.021 |
| 3. Aitone, 4. Minaccia, 5. Urbino | 10 | 1.1 ± 0.1 | 7.7 | 0.018 ± 0.012 |
| 6. Bonifacio | 20 | 1.1 ± 0.1 | 7.7 | 0.013 ± 0.011 |
| Sardinia | | | | |
| 7. Cala di Trana | 8 | 1.0 | 3.8 | 0.015 ± 0.015 |
| Large island mean | 12.8 ± 5.25 | 1.08 ± 0.05 | 7.1 ± 1.95 | 0.019 ± 0.008 |
| Corsico-Sardinian islands | | | | |
| 8. Gargalo | 9 | 1.1 ± 0.1 | 11.5 | 0.033 ± 0.019 |
| Bouches-de-Bonifacio Straits | | | | |
| 9. Lavezzi | 15 | 1.1 ± 0.1 | 3.8 | 0.009 ± 0.009 |
| 10. Lavezzi A | 13 | 1.1 ± 0.1 | 7.7 | 0.021 ± 0.014 |
| 11. Lavezzi B | 15 | 1.1 ± 0.1 | 11.5 | 0.017 ± 0.010 |
| 12. Lavezzi CDE | 9 | 1.1 ± 0.1 | 7.7 | 0.018 ± 0.015 |
| Lavezzi mean | 13.0 ± 2.83 | 1.10 ± 0.00 | 7.7 ± 3.14 | 0.016 ± 0.005 |
| 13. Piana | 25 | 1.2 ± 0.1 | 11.5 | 0.026 ± 0.016 |
| 14. Cavallo | 13 | 1.2 ± 0.1 | 15.4 | 0.032 ± 0.016 |
| 15. Sta Maria | 9 | 1.1 ± 0.1 | 11.5 | 0.035 ± 0.022 |
| PROVENCE ISLANDS | | | | |
| Hyères islands | | | | |
| 16. Port-Cros | 11 | 1.1 ± 0.1 | 7.7 | 0.039 ± 0.027 |
| 17. Le Levant | 3 | 1.1 ± 0.1 | 11.5 | 0.049 ± 0.028 |
| 18. Porquerolles | 17 | 1.1 ± 0.1 | 11.5 | 0.034 ± 0.019 |
| Small island mean* | 12.4 ± 7.00 | 1.14 ± 0.05 | 11.6 ± 2.22 | 0.033 ± 0.007 |
| MAINLAND | | | | |
| 19. Piombino | 27 | 1.2 ± 0.1 | 15.4 | 0.035 ± 0.021 |
| 20. Zanica | 10 | 1.0 ± 0.0 | 0 | 0.000 ± 0.000 |
| 21. Banyuls | 15 | 1.1 ± 0.1 | 7.7 | 0.027 ± 0.019 |
| Mediterranean mainland mean | 21 ± 8.49 | 1.16 ± 0.071 | 12.7 ± 5.44 | 0.032 ± 0.006 |
| Overall island mean | 12.7 ± 5.35 | 1.12 ± 0.046 | 9.3 ± 3.18 | 0.024 ± 0.011 |
| Overall mainland mean | 17.3 ± 8.74 | 1.13 ± 0.100 | 10.2 ± 7.70 | 0.026 ± 0.018 |
| Overall mean | 13.4 ± 5.98 | 1.12 ± 0.054 | 9.5 ± 3.96 | 0.025 ± 0.012 |

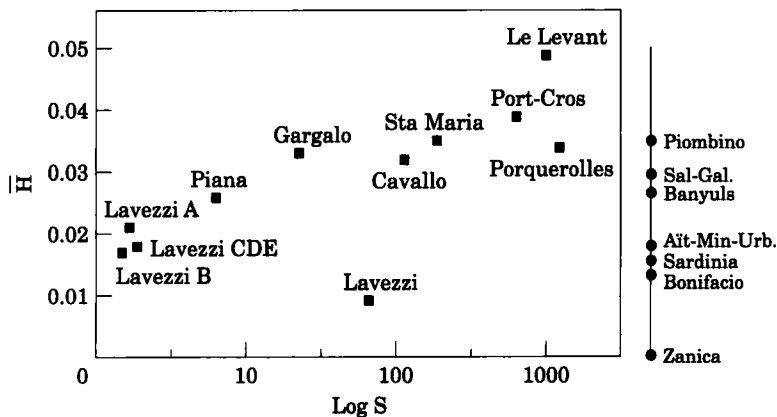


Figure 2. Relationship between surface area in hectares of islands ($\log S$) and mean heterozygosity (H). Mean heterozygosity in the samples from Corsica, Sardinia and the mainland are indicated for comparison. Sal-Gal and Ait-Min-Urb refer to pooled Corsican samples as shown in Table 3.

TABLE 4. F_{ST} values between pairs of populations. SalGal and AiMinUr refer to pooled Corsican samples as shown in Table 3

| Locality | 1-2 | 3-5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|------------------|--------|--------|--------|---------|--------|--------|---------|---------|--------|---------|--------|--------|--------|--------|--------|--------|--------|
| 1-2. SalGal | | | | | | | | | | | | | | | | | |
| 3-5. AiMinUr | 0.0782 | | | | | | | | | | | | | | | | |
| 6. Bonifacio | 0.0570 | 0.0305 | | | | | | | | | | | | | | | |
| 7. Sardinia | 0.1613 | 0.1292 | 0.1250 | | | | | | | | | | | | | | |
| 8. Gargalo | 0.0994 | 0.0981 | 0.1071 | 0.1439 | | | | | | | | | | | | | |
| 9. Lavezzi | 0.1863 | 0.1035 | 0.1114 | 0.1690 | 0.0880 | | | | | | | | | | | | |
| 10. Lavezzi A | 0.1679 | 0.0250 | 0.1410 | 0.1546 | 0.1454 | 0.1283 | | | | | | | | | | | |
| 11. Lavezzi B | 0.1474 | 0.0187 | 0.0884 | 0.1125 | 0.1181 | 0.0691 | -0.0069 | | | | | | | | | | |
| 12. Lavezzi CDE | 0.1423 | 0.0680 | 0.1331 | 0.1345 | 0.1197 | 0.1297 | 0.0898 | -0.0057 | | | | | | | | | |
| 13. Piana | 0.1111 | 0.0659 | 0.0792 | -0.0303 | 0.1195 | 0.0792 | 0.1047 | 0.0645 | 0.0970 | | | | | | | | |
| 14. Cavallo | 0.0841 | 0.0080 | 0.0491 | -0.0254 | 0.0779 | 0.0828 | 0.0562 | 0.0157 | 0.0244 | -0.0068 | | | | | | | |
| 15. StaMaria | 0.0314 | 0.0629 | 0.0950 | 0.2047 | 0.0949 | 0.1883 | 0.0734 | 0.2606 | 0.1675 | 0.1617 | 0.1884 | | | | | | |
| 16. Port-Cros | 0.4430 | 0.4797 | 0.5508 | 0.4732 | 0.3978 | 0.5160 | 0.4362 | 0.5111 | 0.4838 | 0.5016 | 0.4704 | 0.4616 | | | | | |
| 17. Levant | 0.4627 | 0.5961 | 0.6564 | 0.5906 | 0.4861 | 0.5901 | 0.4899 | 0.7153 | 0.5860 | 0.6196 | 0.5807 | 0.4644 | 0.2225 | | | | |
| 18. Porquerolles | 0.0860 | 0.0658 | 0.0425 | 0.0332 | 0.0808 | 0.0450 | 0.0220 | 0.1062 | 0.1215 | 0.0941 | 0.1103 | 0.1092 | 0.3768 | 0.4334 | | | |
| 19. Tuscany | 0.2035 | 0.1877 | 0.2046 | 0.2064 | 0.0464 | 0.2151 | 0.1712 | 0.2295 | 0.2279 | 0.2095 | 0.2044 | 0.1766 | 0.4717 | 0.5140 | 0.1582 | | |
| 20. Lombardy | 0.1548 | 0.0783 | 0.0721 | 0.1786 | 0.1281 | 0.0361 | 0.0483 | 0.0579 | 0.0982 | 0.0048 | 0.0842 | 0.2600 | 0.5260 | 0.7731 | 0.0781 | 0.2004 | |
| 21. Banyuls | 0.4843 | 0.5181 | 0.5605 | 0.4870 | 0.4654 | 0.5107 | 0.4370 | 0.5765 | 0.5236 | 0.5316 | 0.5177 | 0.5023 | 0.2763 | 0.2460 | 0.3406 | 0.4723 | 0.5796 |

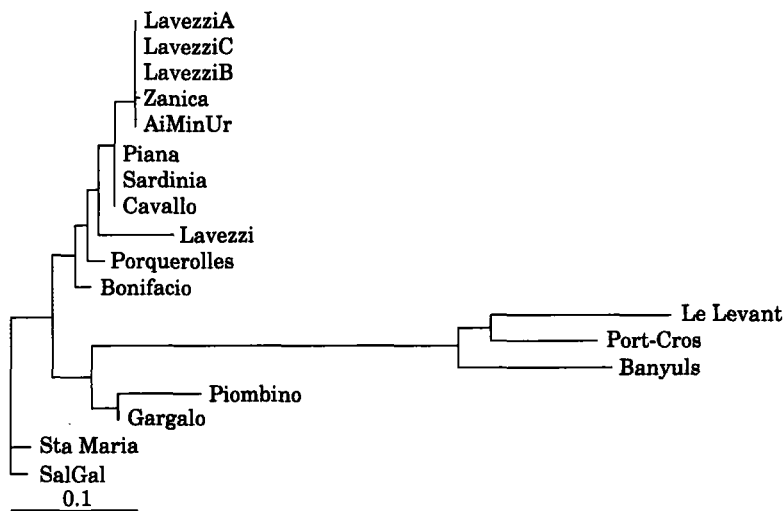


Figure 3. Similarity network between samples produced by the least-square method (FITCH) using estimates of F_{ST} (average percent standard deviation = 39.6903). SalGal and AiMiUr refer to pooled Corsican samples as shown in Table 3.

structured into two main clusters (Fig. 3). The first cluster grouped two of the Hyères islands (Port-Cros and le Levant) with Banyuls in France, and the island of Gargalo with Piombino. The relatively high level of differentiation of the former group was illustrated by the considerable branch length separating them. The second cluster was subdivided in two, with Santa Maria and a sample from Corsica on the one hand, and all other island and mainland samples on the other. Within this cluster, genetic affinities seemed to bear little relation to geographic proximity. It should be noted that the Hyères islands formed a heterogeneous group since the Porquerolles sample appeared more related to the Corsican one than to its neighbouring islands.

The genetic structure of the island and mainland populations was analysed by calculating F_{ST} levels between different sets of nested populations (Fig. 4). The samples from Corsica behaved as a panmictic population ($P(F_{IS}) = 0.085$). The islands from the Bouches-de-Bonifacio Straits yielded a similar but significant F_{ST} level. The diversity level then increased up to 0.09 for the whole group of Tyrrhenian islands. No further increase was observed with the inclusion of Porquerolles and Zanica but a slight step in diversity was reached when the Piombino sample was added. Although closely related as shown previously, the differentiation level between the two Hyères islands and Banyuls was high and similar to that for the whole set of populations studied.

To analyse the pattern of genetic diversity in relation to inter-island migration dynamics, F_{ST} values were calculated between island populations within the Tyrrhenian and the Hyères complexes. A relationship with geographic proximity was apparent yielding two patterns. The first one was present in the Hyères island complex in which populations from neighbouring islands showed significant levels of differentiation ($0.0004 < P < 0.02$, for the three pairwise comparisons; global $F_{ST} = 0.364$, $P < 10^{-4}$). The other was observed in the seven island sample from the Bouches-de-Bonifacio Straits within which all pairwise comparisons except Lavezzi and Santa Maria ($P = 0.003$) yielded non-significant F_{ST} values. Independence

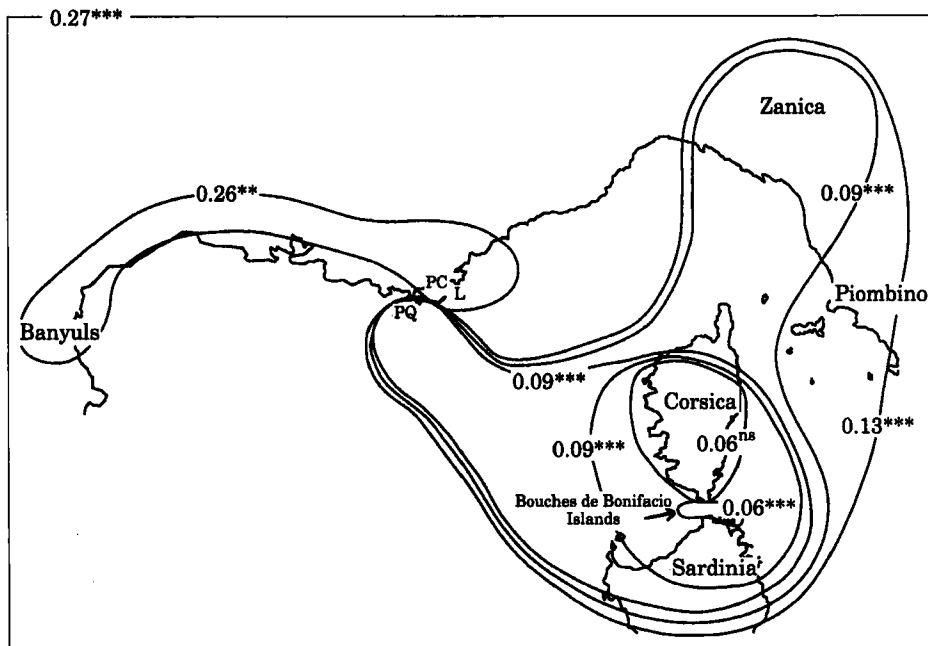


Figure 4. Contour map illustrating levels of genetic differentiation within sets of nested populations. F_{ST} values and levels of significance are indicated on the contours for all populations included within them: ns = non-significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. L = Le Levant; PC = Port-Cros; PQ = Porquerolles.

between geographic distance and genetic differentiation within this group of islands was tested and was not rejected ($P = 0.07$). These results showed that although overall F_{ST} values were significant in the Bouches-de-Bonifacio Straits islands ($F_{ST} = 0.06$, $P = 10^{-4}$), the level of differentiation between islands was slight with a low contribution of inter-island distance.

DISCUSSION

Genetic diversity patterns

The analysis of allozymic diversity performed in this study confirmed the overall low level of genetic polymorphism in *Rattus rattus* published in previous surveys: California (level of polymorphism [p] = 8%; Patton *et al.*, 1975), Greece and Bulgaria (4% and 8% respectively; Cheylan, 1986), Egypt and Italy (p = 0%; Gemmeke & Niethammer, 1984), although island populations tended to show higher levels of variability (Maldives Islands: p = 15%, Gemmeke & Niethammer, 1984 and *in litt.*; Galapagos: p = 10%, Patton *et al.*, 1975). A much higher rate of polymorphism was recorded in an Eastern population from Thailand (p = 38%, Gemmeke & Niethammer, 1984), which is included in the area of origin of the black rat and where populations may have higher levels of variability (see Din *et al.*, 1996). Alternatively, this sample may correspond to the chromosomally differentiated form

($2n=42$). The mean genetic diversity observed in the black rat populations studied here ($p=9.5\%$, $H=0.025$) was less than half that measured in another widespread rodent species (*Mus musculus domesticus*, $p=25\%$, $H=0.09$, Britton-Davidian, 1990), but fell within the range scored for above ground rodents (Nevo, Filipucci & Beiles, 1990). The heterogeneous values of the mainland populations may reflect differences in population structure related to the habitat occupied, i.e. continuous in wild-living Mediterranean populations (Piombino = 15%, Banyuls = 8%) and dispersed in commensal non-Mediterranean ones (Zanica = 0%).

Even though levels of genetic variability were low in the black rat, the study of these Mediterranean island populations clearly showed that compared to mainland populations, no reduction in genetic diversity has occurred in the islands. Within the latter, however, large differences were observed and only one group of very small and/or geographically isolated islands (Lavezzi island and islets) showed reduced allozyme variability most likely due to founder effects and genetic drift. Populations from the remaining islands had contrasting values of mean heterozygosity, the levels being unexpectedly higher in the small island group (6–1250 ha) than in the large one (Corsica and Sardinia). These results are not in agreement with the predicted genetic consequences of island population structure leading to a decrease in genetic variability within islands and an increase in inter-island genetic differentiation (Kilpatrick, 1981). As this is clearly not the case in most of the Mediterranean islands studied here, additional features such as colonization processes, population structure and dynamics likely contribute to such a genetic structure.

Differentiation and colonization patterns

The analysis of allozyme divergence showed that populations of black rats from Corsica, Sardinia and their neighbouring islets formed a genetically homogeneous group, which in turn was closely related to the Italian population of Zanica (Fig. 4). This Tyrrhenian island group is clearly differentiated from the Hyères islands (with the exception of the Porquerolles population). Although Port-Cros and Le Levant are more closely related to the mainland Banyuls population than to any other sample, the level of divergence is higher both between these two islands and with the mainland than within the Tyrrhenian group. Colonization and/or migration patterns may account for these geographical differences.

The geographically remote position of the Tyrrhenian islands from the mainland suggests that colonization by the black rat most likely occurred by human introduction. The first traces of human occupation in these islands date back to the 9th millennium BP in Corsica and probably somewhat before that in Sardinia (Vigne, 1992). Subsequent colonizations are recorded from around 800 BC to 238 BC (Phoenicians, Carthaginians and Romans). Finally, Corsica was integrated into the Republic of Genoa (Italy) in the fifteenth century, then to France in 1758. The first clues to the presence of *R. rattus* in Corsica are documented in the Monte di Tuda cave in layers dated 393–151 BC (Audoin-Rouzeau & Vigne, 1994), but the frequent sea traffic in the Mediterranean Sea since the Iron Age may well have led to an earlier introduction, resulting in an Eastern and/or African origin. In Sardinia, Sanges and Alcover (1980) found rat bones in layers dated 3500 BC, but the dating of this site is still under debate (Audoin-Rouzeau & Vigne, 1994). Colonization of the Corsico-Sardinian islets by the black rat most likely occurred at the same time

or very rapidly after the establishment of rat populations in the main islands (Corsica and Sardinia), as traces of human occupation are known on Cavallo since Roman times, and since the neolithic period in the Lavezzi islands (Agostini, 1978) where rat bone remains are documented from the fourteenth to the seventeenth centuries (Vigne *et al.*, 1994). Support for the relatedness of these latter islands to both of the large ones is indicated by the distribution of alleles in the Lavezzi islands which occur either in Corsica (*Got-2⁷⁰*) or in other Sardinian islands (*Np¹⁷⁰* present on the Maddalena island, unpublished data). A pattern involving colonization from multiple geographic origins would have considerably reduced founder effects and contributed to an initially high level of variability and homogeneity in the rat populations of the Tyrrhenian islands.

The group formed by the Hyères islands shows a large inter-island differentiation and the relatedness of two of these islands to the westernmost mainland sample. These results suggest that the populations from Port-Cros and le Levant most likely originated from southern France. The clustering of Porquerolles with the Tyrrhenian islands may be evidence of recent exchanges between the two, but more likely results from stochastic processes due to the overall low level of variability and small sample size. Indeed, this genetic similarity is not supported by the presence of Tyrrhenian-specific alleles.

Both sets of islands (Tyrrhenian and Hyères) are likely the sites of continued passive introductions, either through resort yachting and/or commercial traffic. However, the genetic differentiation observed between the Hyères islands and between these and the Corsico-Sardinian complex argues for a low extant long-distance effective migration rate. Thus, early multiple introductions and geographic origin of colonization best explain the differentiation patterns between the Tyrrhenian and Hyères groups of islands. These processes alone, however, cannot account for the maintenance or increased levels of genetic variability in some of the smaller islands, which suggests that additional factors such as reduced rates of extinction/recolonization and/or high inter-island migration rates are occurring (McCauley, 1991; Gaggiotti, 1996).

Population structure of island rats

The genetic diversity pattern in the insular rat populations showed unexpectedly high levels in the small island group (excluding the Lavezzi islands) compared to that in the large one (Corsica and Sardinia). This is particularly true in the Tyrrhenian islands where not only do the small island populations show no reduction in variability due to stochastic processes but their variability is increased compared to that in the large ones by the addition of several new alleles.

Within this small island group, the relationship between island size and mean heterozygosity of its population suggests that demographic characteristics may well play a role in maintaining genetic variability. Ecological and behavioural analyses have shown that social modifications have occurred in these populations involving increased inter-individual tolerance and leading to stable and high population densities (Granjon, 1987; Granjon & Cheylan, 1990a) such as is known to occur in other rodent species (for reviews, see Stamps & Buechner, 1985 and Adler & Levins, 1994). On the other hand, these demographic adaptations are much less pronounced in Corsica and Sardinia where interspecific competition and predator diversity are

important, whereas they are absent or reduced in the small islands (Cheylan, 1984; Granjon & Cheylan, 1988; Thibault, Patrimonio & Torre, 1992; Vigne, 1992). Differences in selective pressures between small and large island populations are further documented by body size which is considerably reduced in rats from Corsica compared to that in small island and mainland individuals (Orsini & Cheylan, 1988; Granjon & Cheylan, 1990b). Thus, on small islands, the establishment of stable and high density populations would limit the extent of fluctuations in abundance, thereby reducing the probability of extinction and contribute to maintain genetic diversity more efficiently than in the large islands characterized by more unstable and smaller local population sizes.

If reduced extinction probabilities due to demographic adjustments best explain the size-related diversity patterns in these islands, the disparity in differentiation levels between the Hyères and the Tyrrhenian sets of islands indicates that differences in migration patterns may exist. Whereas low inter-island migration rates would adequately account for the highly differentiated Hyères populations, the genetic relatedness within the island populations from the Bouches-de-Bonifacio Straits suggests that effective inter-island movement occurs, resulting in a metapopulation structure. Although aggressive interactions exhibited by residents towards invading individuals have been observed (Granjon & Cheylan, 1989), effective migration could take place during low density periods. Recolonization after the experimental removal of the entire rat population from some small islands was shown to occur within a few months in the least isolated islands (<100 m), to slightly more than one year in the more isolated ones (>100 m) (Cheylan, 1986). These results suggest that relatively high migration and/or recolonization rates may lead to intermittent gene flow among populations inhabiting geographically close islands, and contribute to counteract losses of variability and reduce differentiation (Gaggiotti, 1996). The study of black rat populations from the Tyrrhenian islands thus provides empirical evidence suggesting that genetic diversity may be more successfully and efficiently maintained in an interconnected series of spatially fragmented and high density populations (Bouches-de-Bonifacio Straits islands) than in one large one (Corsica).

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