

Genetic characterization of black rat (*Rattus rattus*) of the Canary Islands: origin and colonization

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Abstract In the Canary Islands two invasive rat species, *Rattus rattus* and *Rattus norvegicus* are present, but little is known about the origin and colonization. To this end, a molecular study was performed on *R. rattus* from the Archipelago and from the nearest continents. Partial cytochrome *b* gene sequencing offered very low levels of haplotype and nucleotide diversities, with only seven haplotypes identified. All of them belong to the European Lineage I, specifically to the “ship rat” cluster. The haplotype network showed a star-like topology. Haplotype distribution showed a genetic subdivision between eastern and central/western islands, suggesting a double colonization event. This hypothesis is congruent with historical human colonization and it is similar to that proposed for the rodent parasite *Hymenolepis diminuta*. In addition, a possible role of the Canary

Islands as a faunal link with the European and American continents is discussed.

Keywords *Rattus rattus* · Canary Islands · Cytochrome *b* · Colonization

Introduction

Rodents (especially the rat species *Rattus exulans*, *Rattus rattus* and *Rattus norvegicus*) may have been the most widely introduced vertebrates to accompany humans in our history of global dispersal (Drake and Hunt 2009). They are present in many regions of the world, including islands, where invasive species are the primary drivers of extinction and ecosystem change (Townsend et al. 2006).

The Canary Islands consist of seven main volcanic islands and several islets, off the Atlantic coast of northwest Africa. These islands were already known to Mediterranean Classical cultures, but they were rediscovered and visited by Genoese, Majorcan, Portuguese and French sailors during the fourteenth century (Fregel et al. 2009). At that time an indigenous human population already inhabited the Canaries. According to archaeological (Onrubia-Pintado 1987) and molecular data (Rando et al. 1999; Flores et al. 2003), the oldest human settlement dates back to the first millennium BC, and most likely originated from North Africa.

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Today, two invasive *Rattus* species, the black rat (*R. rattus*) and the Norway rat (*R. norvegicus*) are known to occur in the Canary Islands, and several paleontological studies have documented their presence. Recent findings suggest that the introduction of *R. rattus* on Lanzarote occurred before Middle Age European contact (Rando et al. 2011) whilst *R. norvegicus* introduction is more recent, eighteenth century (Nogales et al. 2006). Multiple researches about the impacts on native biota have been published, and at present there is a notable increase in knowledge about parasitic and bacterial fauna of *Rattus*. In contrast, little is known about the origin and colonization of the Canary Islands by rats.

Over the past few years, many studies about the origin and genetic composition of black rat populations of several geographic areas around the world based on nuclear and mitochondrial DNA data have been published (for example Tollenaere et al. 2010; Bastos et al. 2011; Aplin et al. 2011).

Our aim is to describe the genetic diversity and phylogeographic patterns of the black rat in the Canaries based on cytochrome *b* (*cyt b*) partial sequences. In addition, we compare the patterns of colonization between rats and their endoparasites, given the recently published results about the distribution of cestode species on muridae from the Canary Islands (Foronda et al. 2011).

Results

Our study included *R. rattus* from Canary Islands, Europe and Africa, and *R. norvegicus* as outgroup from Canaries and Finland (Table 1). All the haplotypes obtained have been deposited in the GenBank with Accession Numbers HE820119-HE820125 for *R. rattus*, and HE820126- HE820127 for *R. norvegicus*.

Base composition of sequences obtained was comparable to *cyt b* sequences from other European murid rodents (Martin et al. 2000) with an average of 31.0 % A, 28.6 % C, 27.3 % T and 13.0 % G. The complete dataset contained 79 variable and 71 parsimony-informative sites across the 706 nts resulting in a total of 9 haplotypes. For *R. rattus*, nucleotide and haplotype diversities were very low ($\pi = 0.00118$, $h = 0.647$) (Table 1); only seven variable positions were found, three of which were parsimony-informative. Combinations of these variants resulted in seven

haplotypes (Rr1–Rr7), all of them belonging to same lineage as mentioned above.

ML and Bayesian trees of the haplotypes obtained for both species together with those of different lineages/groups found in other world regions, showed identical topology (Fig. 1). All the Canarian, European and African black rat haplotypes found in this study clustered with those belonging to “Group A” described by Tollenaere et al. (2010) and specifically to the “ship rat” cluster from the “Lineage I” (Aplin et al. 2011).

The haplotype network (Fig. 2) showed a star-like topology. In this network Rr1 haplotype occupies the central position differing from the others by a unique mutational step, except for the Rr6 haplotype found only in the Spain (Iberian Peninsula) sample, which shows three substitutions. Rr1 was present in all Canarian populations excepting Fuerteventura, and it was the unique haplotype found in Senegal, Benim and France samples. Rr2 and Rr3 haplotypes were found only in Tenerife and El Hierro samples, respectively. Haplotype Rr4 was detected in El Hierro and Lanzarote populations, being in the last population the most frequent haplotype. Finally, Rr5 was the only haplotype found in Fuerteventura and was also detected in Lanzarote and Spain, while Rr7 was found in Tunisia (Table 1; Fig. 2).

Discussion

The values of haplotype and nucleotide diversities were similar to those found in black rats from Madagascar (Hingston et al. 2005; Tollenaere et al. 2010) and South Africa (Bastos et al. 2011), in spite of the fact that these authors analyzed a longer fragment (more than 1,000 bp). The reduced diversity found in our samples agrees with the results obtained by Aplin et al. (2011) between haplotypes included in this cluster. Four of the haplotypes (Rr2, Rr4, Rr6 and Rr7) have been identified for the first time, while the others (Rr1, Rr3 and Rr5) have been previously identified in populations from other geographic regions. The Rr1 haplotype is the most frequent and widely distributed both in the Canary Islands and in the rest of the world. This haplotype has been detected in Europe and in several countries in America, Africa and Oceania, its presence in the two last continents possibly originating from Cook’s expedition, as suggested by Tollenaere et al. (2010).

Table 1 List of haplotypes and genetic diversities results for each population of *R. rattus* and *R. norvegicus* analyzed in this study

Species	Population	Site	Haplotypes (N)	<i>h</i>	π
<i>R. rattus</i>	Canary Islands	La Palma	Rr1(6)	0.00	0.0000
		El Hierro	Rr1(2), Rr3(1), Rr4(2)	0.80	0.0014
		La Gomera	Rr1(1)	–	–
		Tenerife	Rr1(9), Rr2 (2)	0.33	0.0005
		Gran Canaria	Rr1(11)	0.00	0.0000
		Fuerteventura	Rr5(12)	0.00	0.0000
		Lanzarote	Rr1(2), Rr4(10), Rr5(3)	0.53	0.0012
	Spain (Iberian Peninsula)	Sevilla	Rr1(2), Rr5(1), Rr6(1)	0.83	0.0024
	France	Banyuls-sur-Mer	Rr1(3)	0.00	0.0000
	Tunisia	Chebba	Rr1(1), Rr7(1)	1.00	0.0014
	Senegal	Dakar (Lago Rosa)	Rr1(5)	0.00	0.0000
	Benim	Cotonou	Rr1(7)	0.00	0.0000
	<i>R. norvegicus</i>	Canary Islands	La Palma	Rn2(1)	–
Tenerife			Rn1(1)	–	–
Gran Canaria			Rn1(1)	–	–
Finland			Rn1(2)	–	–

h Haplotype diversity, π nucleotide diversity

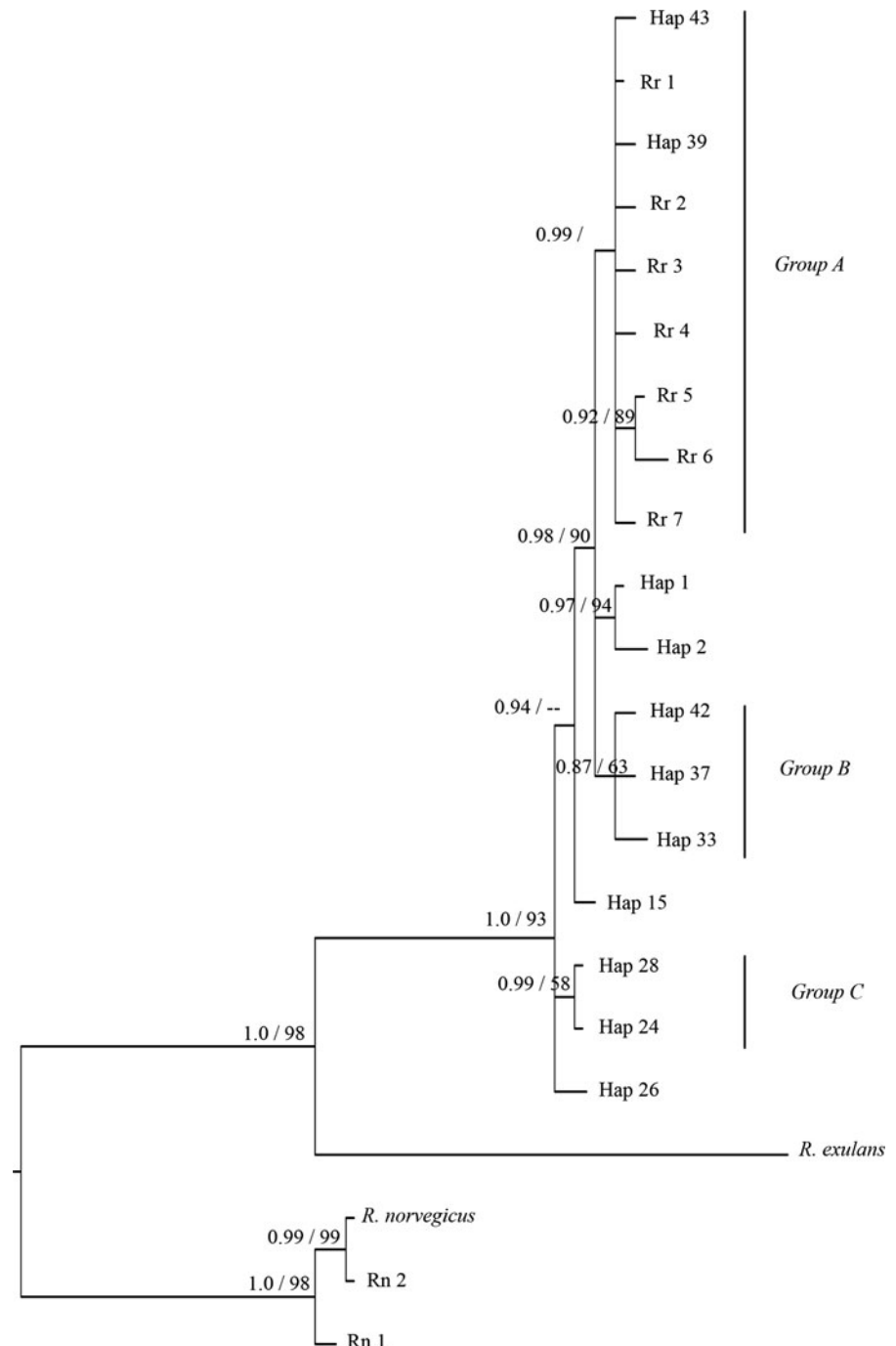
The haplotype distribution found in the Canaries (Fig. 3) was not homogeneous, the haplotypes Rr4 and Rr5 being almost exclusively confined to the easternmost islands Lanzarote and Fuerteventura. The Rr5 was the only haplotype found in Fuerteventura, and its presence in Lanzarote suggests a contact between these islands or an independent introduction to each island. In contrast, haplotype Rr4 was present exclusively in Lanzarote. For these two eastern islands only two specimens with Rr1 haplotype have been found, specifically in Lanzarote, in a coastal area close to the main seaport, pointing to a recent introduction. Whereas, the central and western islands showed a different haplotype distribution, with the Rr2, Rr3, and the most frequent Rr1 haplotypes as a hallmark for these islands. These findings suggest a different colonization pattern of the two groups of islands (eastern and central/western) (Fig. 3).

The introduction of the rats runs parallel with human colonization in the Canary Islands. Lanzarote and Fuerteventura were the first islands visited by Europeans, which probably caused the differences between haplotypes detected in the eastern islands with respect to the western ones. Moreover, there seems to be differences between the specimens from eastern islands, Fuerteventura and Lanzarote. In the last one, the most frequent haplotype Rr4, widely distributed on

the island, has not been detected in other populations. The fact that Lanzarote was in contact with European cultures before the conquest could explain the differences found between this two islands. The presence of Rr4 haplotype in the possible Mediterranean source populations, suggested by historical data, would support this hypothesis. In contrast, the Rr5 haplotype found in Fuerteventura has been detected in the Spain samples analyzed, suggesting a later colonization of this island. These findings agree with paleontological data obtained by Rando et al. (2011), who proposed that the introduction of black rat in Lanzarote occurred before 650 AD which is prior to European contact, probably by other Mediterranean cultures such as the Romans. On the contrary, in Fuerteventura these authors dated bones with an age coincident to the islands' conquest. The presence of the Rr4 haplotype (the most frequent in Lanzarote) in the westernmost El Hierro island seems to be in contradiction with the above hypothesis. However, there is historical evidence of the "Norman" conquest of El Hierro from the easternmost islands and mainly from Viera y Clavijo (1772), which could explain this result.

The hypothesis of a double colonization of the Canary Islands has been previously suggested by Foronda et al. (2011), who recently proposed similar pattern of colonization for the rodent parasite

Fig. 1 Bayesian tree for all the *R. rattus* (Rr1–Rr7) and *R. norvegicus* (Rn1–Rn2) haplotypes analyzed in this work, together with those of different lineages/groups found in other world regions and the closely related species *R. exulans* and *R. norvegicus* (Acc. Nos. EF186433 and EF186402, respectively). Nodal support values are indicated BPP/ML. Dash indicates nodes that had support values <50 for ML. [Hap1–Hap39 and groups name follow Tollenare et al. 2010; Hap42 and Hap43 published by Russell et al. (2011)]



Hymenolepis diminuta (Cestoda) in these islands. In that case, the haplotypes of the eastern islands were closely related and showed a high differentiation from the central/western islands.

Finally, the molecular characterization of *R. rattus* populations allowed us to detect a possible

role of the Canary Islands as a faunal link with the American and European continents. These Atlantic islands could have acted not only as a receiving population but also as a source one. The occurrence of haplotypes Rr3 in Guyana and Rr5 in Brazil and USA west coast (Aplin et al. 2011) support this

Fig. 2 Median-joining network of the 7 *R. rattus* cytochrome *b* haplotypes (706 bp). Node sizes are proportional to haplotype frequency in the whole dataset

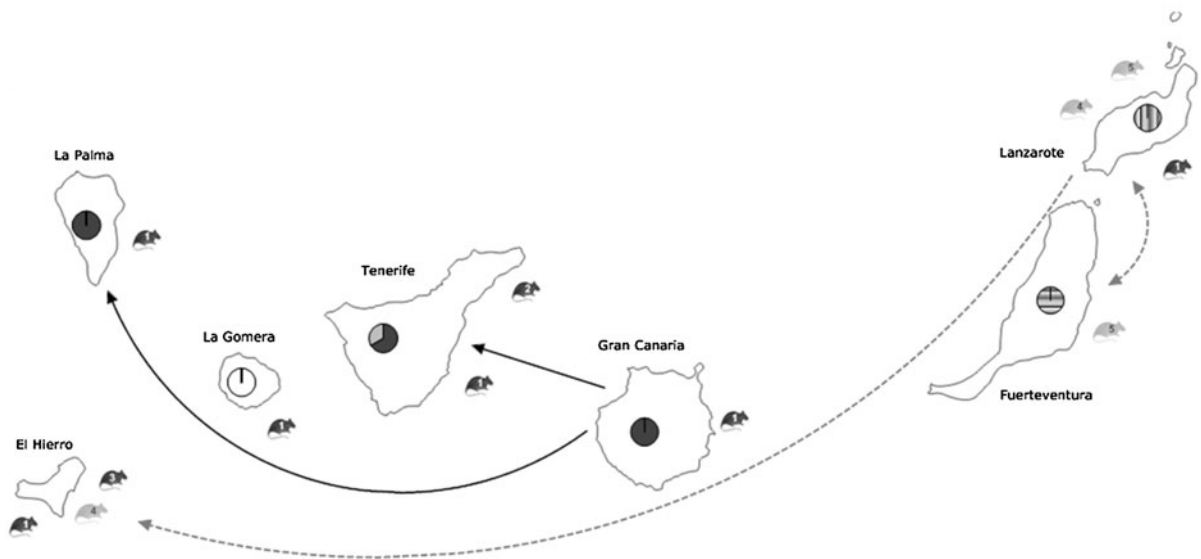
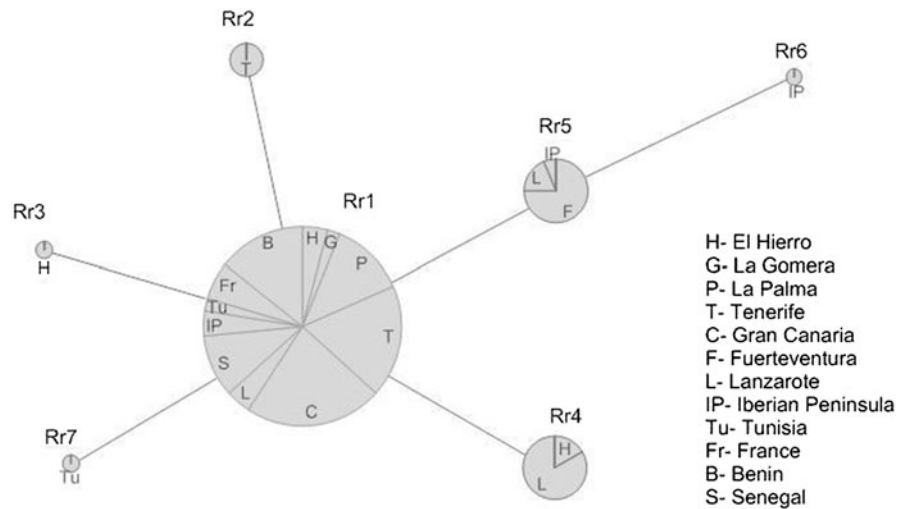


Fig. 3 Map of the Canary Islands showing the double colonization hypothesis for *Rattus rattus* and its relationship with the parasite *Hymenolepis diminuta*. The numbers in the rats indicate the *Rattus rattus* haplotype. Circles symbolize

Hymenolepis diminuta haplotypes (distribution and frequencies are based on Foronda et al. (2011)). Arrows indicate the colonization direction of *Rattus rattus*

suggestion. Their presence in America could be related to the colonization of these territories by Europeans since the sixteenth century (Bethell 1984) or to the illegal migration that took place from Canary Archipelago to Venezuela. Thus for example, during the mid-twentieth century, Guyana and Brazil acted as ports of call to this country (see González 2005). Interestingly, there are data on human transportation from the island of El Hierro to Cayenne, the capital of French Guyana (Acosta

1993). Because Rr3 is frequent in El Hierro, this relationship is highly probable. Regarding to the Rr5 haplotype, there are few records about clandestine shipments from the eastern islands that maybe link its presence in Brazil with the Canary migration.

This study provides the first molecular data on rats from Canary Islands and Spain, and adds information on their origin in the islands, from the northwestern Africa and part of Mediterranean Europe. However,

these preliminary results need exhaustive analysis, mainly of samples from the Mediterranean coast, to confirm our hypothesis.

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