Research Article

Population genetic structure of black rats in an urban environment: a case study in Cotonou, Benin

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Abstract

The cosmopolitan black rat, *Rattus rattus*, has invaded many cities throughout the world. Although the species is responsible for major damages to food stocks and crops and is involved in the maintenance, circulation and transmission of many zoonotic pathogens to humans and animals, a lot remains to be known about its eco-evolutionary characteristics, especially in highly modified environments like the urban habitat. In particular, very few studies were conducted on the genetic structure of urban black rat populations, which is yet a prerequisite for defining effective management units. Here, we focus on the population genetics of *Rattus rattus* in Cotonou, Benin. Forty different localities were sampled throughout the city and 457 individuals were genotyped using 18 microsatellite markers. Our results reveal two poorly distinguishable but significant genetic clusters, one of each side of Cotonou channel, that tend to homogenize into a single group due to probable gene flow between the two shores. Implications in terms of management units and rodent control are discussed.

Introduction

Africa is expected to show the largest urban growth and sprawl in the coming decades, with West Africa displaying the fastest urbanization rate (United Nations, 2014). When such a rapid urbanization exceeds the capacity of states and local authorities to deliver basic services, it may lead to the development of wide and poorly equipped areas that are characterized by degraded roads, inadequate and unsafe housing, limited water and electricity supplies, lack of garbage disposal as well as poor or non-existent sanitation systems. As a result, 190 millions of African urban dwellers currently reside in slums (UN DESA, 2019). This paucity of urban management translates into chronically degraded socio-environments which, in turn, provide shelter and food for anthropophilic rodents and favors their year-long reproduction and proliferation (e.g., Promkerd et al., 2008; Feng and Himsworth, 2014; Vadell et al., 2014; Garba et al., 2014; Panti-May et al., 2017). As a consequence, many city inhabitants face rodent-associated issues on a regular basis, such as infection by zoonotic pathogens (e.g., de Faria et al., 2008; Firth et al., 2014 reviewed in Meerburg et al., 2009) and destruction of food stocks and infrastructures (e.g., Garba et al., 2014; Dossou et al., 2015, 2020). In addition, cities are sea, air, river and/or road transport hubs. As such, they constitute privileged sites for import and export of anthropophilic rodents like mice and rats (e.g., Berthier et al., 2016; Combs et al., 2018a; Stragier et al., 2019) which, as invasive species,

are generalist, opportunistic and highly adaptable, including to urban ecosystems (Aplin et al., 2003).

Despite their deep impact on human welfare, health and infrastructures, and although they are the subject of a clear revival of interest (e.g. special issue on urban rodents in Frontiers in Ecology and Evolution, 2020), especially as models in the growing field of urban evolutionary ecology (e.g., Lawrence, 1999; Alberti, 2015; Johnson and Munshi-South, 2017; Rivkin et al., 2019; Parsons et al., 2020), urban rodent biology remains surprisingly under-documented (Parsons et al., 2017). For instance, the few studies available on the genetic diversity and structure of rodent populations within cities have been conducted mainly on species that are in fact dependent on urban parks (e.g., Chiappero-Martinetti and Sabadash, 2010; Munshi-South and Kharchenko, 2010; Munshi-South and Nagy, 2014) and, more rarely, on rodents that are truly anthropophilic (see below).

Population genetic studies may help to define meaningful spatial units for rodent control (Combs et al., 2018a; Kajdacsi et al., 2013; Richardson et al., 2017). Although brown rats (*Rattus norvegicus*) appear as the dominant rodent species in many European and American cities, they were genetically investigated in only four American towns (i.e., Salvador, Brazil: Kajdacsi et al., 2013; Richardson et al., 2017; Baltimore, USA: Gardner-Santana et al., 2009; New York, USA: Combs et al., 2018a; the same three cities plus Vancouver, Canada: Combs et al., 2018b). In African towns, the house mouse (*Mus musculus*) and the black rat (*Rattus rattus*) are often dominant, but were studied from the genetic perspective only in Dakar for the house mouse (Senegal; Stragier et al., 2019), and in Niamey (Niger; Berthier et al.,

2016) and Franceville (Gabon; Mangombi et al., 2016) for the black rat. In such a context, the present work aims at investigating the genetic diversity and structure of black rats in a third African city, namely Cotonou, Benin.

The black rat (also called ship or roof rat; *R. rattus* lineage 1 *sensu* Aplin et al., 2011) is a major pest and is considered as one of the most widespread invasive animal species worldwide (Drake and Hunt, 2009). A recent cytochrome b gene-based phylogeographical study suggests that it was probably imported into Dahomey (actual Benin) during the spice trade between the 15th and the 17th centuries through the Indies Road connecting Europe to Asia, as well as more recently following the intensification of globalized trade from the eighteenth century (Etougbétché et al., 2020). Today, it is the most widespread domestic and peri-domestic species in southern Benin, including Cotonou (Houéménou et al., 2018, 2019)(where it carries several pathogens such as *Leptospira* spp. (Dobigny et al., 2018; Houéménou et al., 2019), *Trypanosoma lewisi* (Dobigny et al., 2019) and *Bartonella* spp. (Martin-Alonso et al., 2016). Our study aims at providing a better knowledge of the population genetic structure of this widespread species, which is still poorly documented in the African urban environment, in order to contribute to the production of biological elements that may guide future pest control policies within Cotonou. More specifically, we wanted to evaluate whether the population genetic structure of *R. rattus* in Cotonou was explained by some characteristics of this urban landscape.

Materials and methods

Study area

Benin is located on the West African coast of the Gulf of Guinea. The city of Cotonou was created by French settlers in the middle of the 19th century. It is currently the largest city and economic capital of Benin. It lies at the extreme south of the country, between the Atlantic Ocean and the Lake Nokoué. It is characterized by a sub-equatorial climate with two rainy seasons (April-July and September-October). The expansion of the city first occurred slowly, but from the late 1960s onwards, it sprawled very rapidly onto the surrounding mangroves and plantations, thus encompassing the few rare isolated villages lying along

the shores of Lake Nokoué (Choplin and Ciavolella, 2008). Thanks to its international seaport, Cotonou is at the heart of intercontinental trade that generates intense commercial, handling and logistics activities (http://www.pac.bj; Janin, 1964). The town is crossed by the so-called "Cotonou channel" that connects the Atlantic Ocean to the Lake Nokoué. Dug in 1855, it is 4.5 km long and 300 m wide, with an average depth of 5 to 6 m. (Badahoui et al., 2009).

Sampling

Rodents were collected during 2009 and 2010 (with some extra samples being collected in the neighborhood of Fifadji (see FIF on Fig. 1) in 2015 and 2016) in 40 neighborhoods throughout Cotonou and on both shores of the Cotonou channel, in order to maximize the geographical coverage of the city (Fig. 1). In each neighborhood, 8–12 households about 50 m distant from each other were prospected for three consecutive nights using locally made wire-mesh traps (30×10×10 cm) that were placed inside bedrooms, kitchens and/or in courtyards. Wheat flour-sardine mixture or smoked fish was used as bait. Traps were checked every morning: empty traps were rebaited while those with rodents were brought to the lab for processing usually within the same day. Exact geographic location at each investigated household was not registered; however, GPS coordinates were systematically noted for the neighborhood as a whole at the approximate middle of the sampled houses.

Ethical aspect and data sharing

Fieldwork in Benin was conducted under the research agreement between the Republic of Benin and the French National Institute for Sustainable Development (IRD) that was reapproved on the 6th April 2017 (available on demand). Black rat has no protected status (see IUCN and CITES lists). All trapping sessions were conducted under explicit agreement from local, traditional (i.e., family and household heads) and administrative (i.e., urban districts chiefs) authorities. Rodents were captured and brought alive to the lab where they were treated in a respectful manner in accordance with the guidelines of the American Society of Mammalogists (Sikes and Gannon, 2011) before being anesthetized using diethyl-ether and then sacrificed by
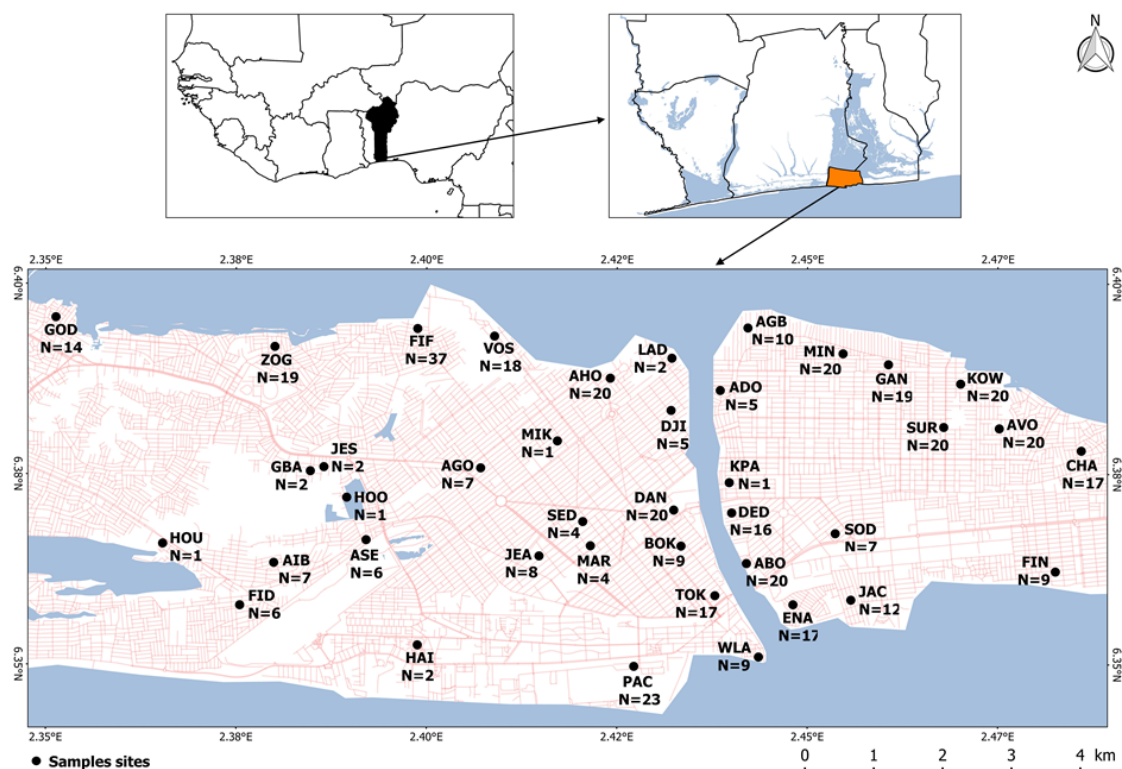

Figure 1 – Representation of the different sampling sites with the captures sizes.

cervical dislocation as recommended by Mills et al. (1995). Access to and benefit-sharing of genetic resources produced during the course of the present study was authorized by the Benin national authorities following the Nagoya international protocol; permit 608/DGEFC/DCPRN/PF-APA/SA). Samples and associated data were deposited in the Small Mammal Collection at the IRD/CBGP (https://doi.org/10.15454/WWNUPO) as well as at URIB/LARBA/EPAC; they are available upon request.

DNA extraction, amplification and genotyping

Genomic DNA was extracted from ethanol-preserved tissues (kidney, spleen or liver for animals captured alive, and toes for individuals that were found dead in the traps) with the Qiagen DNeasy Blood and Tissues kit as recommended by the supplier. Amplification was carried out in multiplex using a panel of 18 microsatellite markers, eight of which (D10Rat20, D11Mgh5, D11Rat56, D16Rat81, D2Mgh14, D5Rat83, D7Rat13 and D18Rat75) being originally developed for *R. norvegicus* (Jacob et al., 1995) and ten (Rr14, Rr17, Rr21, Rr22, Rr54, Rr67, Rr68, Rr93, Rr107 and Rr114) specifically for *R. rattus* (Loiseau et al., 2008). Polymerase Chain Reactions (PCR) and genotyping were conducted according to previously described procedures (Konečný et al., 2013; Mangombi et al., 2016; Berthier et al., 2016). Genotyping was performed at Amersham Biosciences with 2 µL of diluted PCR product to which a 15 µL mix of formamide, and the GS 500 LIZ size marker (ABI 3100 model) was added. Finally, microsatellite profiles were read independently by two persons using GeneMapper v.4.0. In case of ambiguous reading at a given microsatellite, the individual was genotyped de novo and analyzed for that microsatellite until a consensus was reached.

Data analyses

Analyses were performed using three different datasets. A first dataset includes all black rats genotyped from all 40 sampling sites. However, some localities were represented by very low sample size (sometimes only one single individual). In order to conduct population-based analyses while keeping as many sites as possible, a second dataset was also investigated that corresponds to sites where at least 16 individuals genotyped. Relatedness was estimated using the kinship coefficient (ρ) of Loiselle et al. (1995) between all pairs of individuals at each site, with SPAGeDI v.1.4 (Hardy et al., 2002), using genotype data for each site as the reference for allelic frequencies. The effect of relatedness on our results was evaluated by performing the same analyses on a third dataset, from which 60 individuals involved in the pairs associated with the highest relatedness (>0.3) were removed.

The first complete dataset was used to investigate private alleles at each site. The other analyses were conducted on the second and third datasets. Results were very similar, and only those retrieved from dataset 2 (see Supplemental Material S1-S4 for a summary of results obtained with dataset 3).

Genetic diversity

Linkage disequilibrium between each pair of the 18 loci at each site was tested in Genepop v.4.6 (Rousset, 2008). Deviation from Hardy-Weinberg (HW) equilibrium was also investigated at each site and each locus using Genepop. Microchecker v.2.2.3 (Van Oosterhout et al., 2004) was used to test for the presence of null alleles. At each site, the expected (H_E) and observed (H_O) heterozygosities were estimated using Geneclass2 (Piry et al., 2004), the allelic richness (A) using FSTAT version 2.9.3 (Goudet, 2001) and F_{IS} (Weir and Cockerham, 1984) using Genepop. Finally, the presence of private alleles at each site was explored using GeneClass2.

Population genetic structure

Genetic differentiation between sites was investigated by pairwise F_{ST} estimates (Weir and Cockerham, 1984) using FSTAT with all sites and without those just next to the bridges above the Cotonou channel (DED and ENA). Pairwise differentiation between sites was tested with 10000 permutations using FSTAT. A 95% confidence interval (CI) for mean

F_{ST} was generated by bootstrap resampling across loci. An analysis of molecular variance (AMOVA) was performed using Arlequin v.3.5 (Excoffier and Lischer, 2010) in order to test specifically the influence of the Cotonou channel as a possible geographical barrier to gene flow, taking all populations on the one hand but also removing the populations (DED and ENA) just next to the bridges on the other hand.

Isolation by distance (IBD; Wright, 1943) was estimated using a Mantel test performed between Euclidean geographical distances and genetic differentiation (estimated as $\frac{F_{ST}}{(1-F_{ST})}$) calculated between sites, using Genepop (10000 permutations). IBD was tested among all sites on the one hand, as well as on each shore of Cotonou channel independently on the other hand.

Population genetic structure was then explored using the clustering approach implemented in STRUCTURE V.2.3.4 (Pritchard et al., 2000) in order to estimate the number of homogeneous genetic groups (K) in the dataset. The analyses were performed with a model including admixture and correlated allele frequencies, for K ranging from 1 to 10. Each run included a burn-in phase of 200000 iterations followed by 600000 iterations. We performed 10 independent analyses for each K value. The number of genetic groups was inferred by the delta-K method applied to the log probabilities of data (Evanno et al., 2005).

Population genetic structure was finally also investigated through Discriminant Principal Component Analysis (DAPC) which can handle the absence of HW equilibrium (Jombart et al., 2010). Analyses were conducted under the R software using the `adegenet` and `devtools` packages. The most likely number of genetic groups (K) was determined using the Bayesian Information Criterion (BIC; Lebarbier and Mary-Huard, 2006) using the empirical criterion of a delta-BIC less than 6 (Kass and Raftery, 1995).

Results

The total number of genotyped black rat individuals reached 457, with 1 to 37 individuals in each site (see Supplemental Material S5). Relatedness was rather limited between individuals at each site (see Supplementary Material S3), and the removal of the 60 individuals that were involved in the most-related pairs (ρ>0.35) did not affect the results (data not shown). This is the reason why we here below present only the results obtained using dataset 2 that contains 323 individuals from 16 sites, 169 individuals of which being from 9 sites located on the eastern shore of the Cotonou channel, and 154 from 7 sites on the western one (Tab. 1).

Genetic diversity

Only one significant linkage disequilibrium association was found after correction for multiple testing out of the 2379 tests performed. Consequently, loci were considered as genetically independent.

All loci were found at HW equilibrium after correction for multiple testing, except locus D18Rat75. An analysis using Micro-checker showed that the imbalance found at this locus could be explained by the presence of null alleles. However, their estimated mean frequency was very low (f=0.05), suggesting that null alleles at this locus should have a minor effect on genetic diversity and differentiation estimators. As a consequence, all eighteen microsatellites were retained for further analyses.

Mean allelic richness was 4.4±0.2, with the lowest and highest values obtained in TOK (3.80±1.5) and MIN (5.37±1.7), respectively. The mean observed heterozygosity varied from 0.46±0.1 (PAC) to 0.66±0.1 (AHO) with a mean value of 0.55±0.05 (Tab. 2). Mean F_{IS} values ranged from -0.104 in AHO to 0.210 in PAC.

The examination of allelic frequencies over all genotyping data showed the occurrence of 27 private alleles from 15 different microsatellites in 13 different sites. Chankpamè (CHA), Dédokpo (DED), Fifadji (FIF) and the seaport (PAC) showed the highest number of private alleles, i.e. four each (Tab. 1).

Table 1 – Genetic estimates of key statistics within sampling sites with more than 16 genotyped individuals of *Rattus rattus* in Cotonou. Number of genotyped individuals (N), allelic richness (A), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), private allele number (NPA) on dataset 1 (here are shown the results only for the sites with at least 16 individuals captured).

Site	N	A	H_O	H_E	F_{IS}	NPA
East shore						
Abokicodji (ABO)	20	4.72	0.580	0.602	0.052	0
Avotrou (AVO)	20	4.38	0.570	0.595	0.043	1
Chankpamè (CHA)	17	4.97	0.570	0.603	0.083	4
Dédokpo (DED)	16	4.61	0.537	0.567	0.038	4
Enagnon (ENA)	17	3.99	0.504	0.560	0.119	1
Gankpodo (GAN)	19	3.88	0.526	0.554	0.018	1
Kowégbo (KOW)	20	4.16	0.517	0.576	0.102	1
Minontchou (MIN)	20	5.37	0.571	0.592	0.056	0
Suru-Léré (SUR)	20	4.88	0.509	0.618	0.176	0
total	169	4.55	0.543	0.585	0.076	
West shore						
Ahouansori-Agué (AHO)	20	3.90	0.667	0.594	−0.104	2
Fifadji (FIF)	37	4.64	0.538	0.612	0.164	4
marché Dantokpa (DAN)	20	4.06	0.567	0.567	0.045	1
Port Autonome de Cotonou (PAC)	23	4.70	0.500	0.631	0.210	4
Tokpa-Hoho (TOK)	17	3.80	0.522	0.533	0.010	1
Vossa-kpodji (VOS)	18	4.27	0.611	0.606	−0.017	2
Zogbohouè (ZOG)	19	4.45	0.525	0.559	0.080	1
total	154	4.26	0.561	0.586	0.055	
Total / Global mean	323	4.41	0.552	0.585	0.066	

Population genetic structure

Pairwise F_{ST} values (Tab. 2) ranged between 0.01 (between the two closely located sites of SUR and AVO) and 0.21 (between GAN on the eastern shore, and DAN on the western shore), with a global mean F_{ST} value of 0.107 (95% CI=0.089–0.111) (see Fig. 1 and Tab. 2). When the DED and ENA populations that are next to the bridges above the Cotonou channel are excluded from the analysis, the global mean F_{ST} remains similar (0.109). Differentiation was significant for all pairs of sites ($p<0.0004$) except between the three pairs of closely located sites of SUR and AVO, SUR and CHA as well as VOS and FIF.

Molecular analysis of variance (AMOVA) showed that differentiation between the two shores of the channel was very weak (only 1.36% of variance between groups, vs. 9.43% and 89.20% of variance between sites of the same group and within sites, respectively) but significant ($p<0.001$). When the analysis is done without DED and ENA, the variance between groups increases a little bit (1.84%) and was still significant ($p<0.001$).

Isolation by distance was not significant, neither when considering the whole dataset ($p=0.15$) or when considering each shore separately (East: $p=0.16$; West: $p=0.08$).

The program STRUCTURE was used to investigate whether rat populations were spatially structured in Cotonou. The highest delta-*K* value was rather low, and was obtained for $K=2$ (delta-$K=141.38$ for $K=2$, and below 52.20 otherwise), thus pointing towards the coexistence of two genetic groups (Fig. 2A). Overall, individuals from the western shore (AHO, FIF, DAN, PAC, TOK, VOS, ZOG) were mainly assigned to a first group, while those from the East shore (ABO, AVO, CHA, GAN, KOW, MIN and SUR) were largely assigned to a second one (Fig. 2). Individuals of the two populations (DED and ENA), located on the East shore but both very close to the bridges above the Cotonou channel were also mainly assigned to the first (western) group. Nevertheless, genetic segregation is far from complete with all populations showing admixed individuals or individuals assigned to the other group.

In the DAPC analysis, the highest delta-BIC value (8.57) was also recovered for $K=2$. However, the pattern observed was not geographically coherent and mainly related to one locus (Rr54): when this locus was removed from the dataset, the pattern observed was not reliable among runs.

Discussion

In this study, we aimed to investigate the population genetic structure of *Rattus rattus* in the city of Cotonou. We wanted to characterize the genetic structure of this synanthropic species, and evaluate its relationships with specific landscape features such as the Cotonou channel, which separates the city in two parts. Our results reveal two poorly distinguishable but significant genetic clusters.

It is unlikely that the two genetic clusters evidenced in Cotonou would reflect *R. rattus* lineages introduced in Benin at different periods and/or from different origins. A recent cytochrome b-based phylogeographic analysis suggested that the invasion of *R. rattus* in Benin has occurred through at least two independent introduction events (Etougbétché et al., 2020). However, some individuals of this recent study were also included in the present study and were found in both genetic clusters detected by our microsatellite-based analyses (data not shown). Therefore, the genetic structure identified by microsatellite markers in Cotonou does not seem to reflect the introduction history of the species in Benin.

Alternatively, we would expect the Cotonou channel (300 meters wide on average) to be a major barrier to rat dissemination across the city. This was consistent with the two weakly but significantly genetic clusters retrieved by our population-based analyses (i.e. Structure and AMOVA). However, the existence of these two distinct clusters was not supported by our individual-based analyses (i.e. DAPC), thus showing that the channel may present an incomplete barrier to gene flow. This is congruent with *R. rattus* being sometimes reported to be an excellent swimmer (up to 400 m; Russell et al., 2005; Abdelkrim et al., 2005). Accordingly, they were observed in large numbers and able to swim in the lacustrian and semi-lacustrian villages of Lake Nokoué, located immediately north of Cotonou (Houéménou et al., 2019; Savassi et al., 2021), thus demonstrating their ability to move across open waters of Southern Benin. Interestingly, two populations located close to the three bridges that cross the channel (DED and ENA) appeared as a genetic admixture between the east and west genetic pools in STRUC-

Table 2 – Genetic differentiation between sampled sites with at least than 16 genotyped individuals of *Rattus rattus* in Cotonou. F_{ST} were calculated with the 18 microsatellites markers. Underlined localities correspond to the sites from the west shore of Cotonou channel. As a consequence, values in bold correspond to west-west F_{ST} values, while values in italics are east-east F_{ST} values.

Site	AHO	FIF	DAN	PAC	TOK	VOS	ZOG	DED	ENA	ABO	AVO	CHA	GAN	KOW	MIN
FIF	**0.06**														
DAN	**0.14**	**0.11**													
PAC	**0.11**	**0.10**	**0.14**												
TOK	**0.13**	**0.10**	**0.13**	**0.17**											
VOS	**0.07**	**0.02**	**0.12**	**0.11**	**0.11**										
ZOG	**0.08**	**0.07**	**0.14**	**0.09**	**0.10**	**0.09**									
DED	0.08	0.08	0.11	0.12	0.11	0.11	*0.10*								
ENA	0.12	0.09	0.13	0.12	0.09	0.11	*0.12*	0.10							
ABO	0.09	0.08	0.12	0.11	0.10	0.09	*0.08*	0.07	0.10						
AVO	0.08	0.06	0.13	0.11	0.09	0.08	*0.07*	0.08	0.06	0.07					
CHA	0.07	0.08	0.13	0.09	0.13	0.10	*0.09*	0.08	0.09	0.09	0.04				
GAN	0.13	0.13	0.21	0.17	0.15	0.14	*0.14*	0.14	0.14	0.10	0.09	0.10			
KOW	0.14	0.09	0.18	0.19	0.17	0.11	*0.17*	0.16	0.16	0.15	0.09	0.11	0.17		
MIN	0.06	0.06	0.15	0.09	0.13	0.08	*0.06*	0.08	0.10	0.05	0.04	0.05	0.05	0.12	
SUR	0.07	0.06	0.11	0.09	0.10	0.07	*0.07*	0.07	0.08	0.07	0.01	0.02	0.10	0.08	*0.057*

TURE analyses, suggesting that these bridges may also favor rat dissemination from one shore to the other. They were built in 1930, around the 1980s and in 2004, respectively, thus having provided opportunities for rat exchanges between the two sides of Cotonou channel for several decades. These infrastructures allow for an intensive daily traffic of goods via a very large number of vehicles that constantly transit between the two parts of the city. In addition, many pirogues also connect the two shores (Badahoui et al., 2009), thus representing another probably more ancient but still extensive type of transports of goods and people. These types of river and road transports have already been shown to facilitate long-distance dispersal of commensal black rats (Duplantier et al., 1991; Lack et al., 2013; Berthier et al., 2016; Hima et al., 2019); it would not be surprising that they could also act on shorter distances, as here within a city.

Even though permeable due to rats swimming abilities, pirogue- and/or road-mediated transport, the role of the Cotonou channel as a

possible intra-urban geographic barrier, echoes some of the results obtained in Norway rats from American cities where large urban landscape elements were also identified as barriers to gene flow, too (e.g., valleys in Salvador, Brazil; low human density areas in New York, USA; major shipping lanes in New Orleans, USA; large roads in Vancouver, Canada, and Salvador, Brazil; Richardson et al., 2017; Combs et al., 2018a,b).

Besides the weak impact of the Cotonou Channel on the population genetic structure of the black rat in the city, it is interesting to notice some differences among sites in terms of genetic diversity levels and private allele numbers. Indeed, CHA, DED and FIF, which all show relatively high allelic richness and numbers of private alleles ($A \geqslant 4$ and Al=4) are sites that lye on the shores of Lake Nokoué. As such, they may constitute berth zones by pirogues that constantly connect Cotonou with the surrounding cities (e.g. Porto-Novo) and, beyond, with Nigeria. This may potentially lead to the contribution of allochtonous alleles in the genetic diversity of these localities. In the same manner, the international seaport of Cotonou (PAC) also shows rather elevated values (A=4.7 and Al=4), maybe following long-distance maritime exchanges and subsequent introduction of new individuals and alleles. Such a long-distance contribution to allelic novelty in already established genetic pools, though intuitive, is not trivial and would deserve to be further investigated.

The four studies of black rat urban population genetics were all performed in West African cities (Dakar, Senegal, Konečný et al., 2013; Niamey, Niger, Berthier et al., 2016; Franceville, Gabon, Mangombi et al., 2016; Cotonou, Benin, this study) and using the same microsatellite markers, thus making them "quite comparable". In Cotonou, allelic richness (mean A=4.41±0.2) is between mean values obtained in Niamey (A=3.42) and Dakar (A=3.99) on the one hand, and Franceville (A=5) on the other hand. In the same manner, mean expected heterozygosity (mean H_E=0.58±0.03) is between values obtained in Niamey (H_E=0.53) on one hand, and Franceville (H_E=0.66) and Dakar (H_E=0.67) on the other hand. It is highly probable that the colonization of these four agglomerations by black rats occurred independently, most probably following their own evolutionary trajectories that were influenced by different historical events as well as different socio-environmental contexts and dynamics. Black rat populations in Niamey probably correspond to very recent and multiple introduction events (Berthier et al., 2016), as suggested by lower genetic diversity levels and by higher genetic differentiation (mean F_{ST}=0.25). Well-connected populations that show low to no genetic differentiation were already observed for the black rat in Franceville (Mangombi et al., 2016). Like Dakar and Franceville, Cotonou has a mid-19th century origin, and has experienced a particularly rapid demographic expansion during the last 50 years, which may explain similarities in the genetic structure of the black rat populations in the three cities.
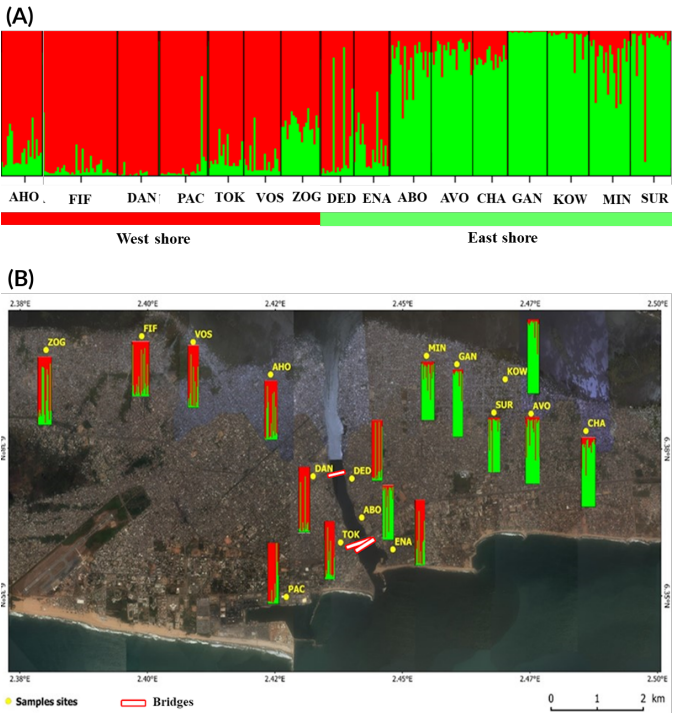

Figure 2 – Spatial genetic structure of *Rattus rattus* in Cotonou using STRUCTURE. A) STRUCTURE analysis results are presented for K=2 with barplots indicating individual ancestry estimates (each vertical line represents an individual) in each site with at least 16 genotyped individuals of *Rattus rattus* in Cotonou.

Population genetics may be useful to define management/eradication units and to guide control strategies (Combs et al., 2018b). It may be of particular interest for rodent control in insular contexts where it helps to identify the probable routes of new invasions or post-eradication recolonization (e.g., Gatto-Almeida et al., 2020; Iannucci et al., 2018; Sjodin et al., 2020. However, islands are close terrestrial ecosystems which may reduce the range of colonization routes. By essence, cities display many interfaces with surrounding landscape matrixes that may serve as permanent rodent sources. In addition, cities are highly connected, by road, maritime and/or fluvial trade, thus providing opportunities for potentially long-distance imports of rodents. This implies that many introduction hotspots (e.g. seaport, truck stations and transbording platforms) need to be surveyed, thus making surveillance and avoidance of post-control recolonization events quite difficult. The identification of barriers to gene flow within the city theoretically make it possible to adjust control campaigns to the urban landscape elements that act as obstacles to re-infestation by neighboring colonies (propagules) in order to guide more sustainable rat control (Combs et al., 2018b). In practical terms, this is expected to be useful for the tailoring of anti-rodent actions at a reasonably manageable spatial scale. Unfortunately, in the case of black rats in Cotonou, the widespread and highly connected populations (which, in fact, should be considered as one, at best two populations) appear as a major obstacle to such localized control campaigns, especially when using rat-killing strategies (i.e. poisons, traps, etc) since post-control recolonization through short-distance mobility and local recruitment would probably be extremely rapid. We believe such densely and obviously well adapted populations of pest rodents can only be controlled through deep environmental modifications, i.e. wide-scale, publicly led and determined urban planning policies. Keeping in mind the huge amount of human resources that is needed to achieve such a goal, this has to rely on community-based approaches, thus requiring dialogue and collaboration between public services involved in pest control and local NGOs, associations and communities (Parsons et al., 2017). In Benin, this must go through an increased awareness of rodent-associated issues by national as well as local representative authorities and through a transfer of knowledge towards inhabitants who should be fully involved in the design of sustainable modifications of their own environment. Relying of the quite dense and efficient network of community workers could constitute a first step towards such community-based mitigation of rodent-associated issues. ✍

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Supplemental information

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

Table S1 Number of genotyped individuals. allelic richness, observed and expected heterozygosity and inbreeding coefficient from dataset 3.

Table S2 F_{ST} per population pairs as calculated with the 18 microsatellites markes.

Figure S3 Genetic structure of *Rattus rattus* populations in Cotonou at K=2.

Figure S4 Graphical representation of the individuals genetic affiliation.

Table S5 Number of genotyped individuals. allelic richness, observed and expected heterozygosity and inbreeding coefficient from dataset 1.

Figure S6 Summary of Loiselle coefficients in each dataset.