

General palaeontology

Rodents and palaeogenetics: New perspectives

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Abstract

Rodents are the most diversified mammalian order (484 extant genera including 2277 species), and they have a worldwide distribution. Palaeontological, morphological and molecular data have greatly helped to resolve their systematics and evolutionary history. However, some discrepancies remain between palaeontologists and molecular biologists. New techniques in molecular biology, and especially in palaeogenetics, allow us to have direct access to the hereditary material of extinct organisms, and they can compensate for some morphological limits. Unfortunately, few studies are dealing with rodent palaeogenetics, despite the amount of museum and fossil material available. Here, we review the major research activities in rodent palaeogenetics (phylogeny, genetic diversity, migration), and we present the promising research perspectives in this field (phylochronology, palaeoparasitology).

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Résumé

Rongeurs et paléogénétique : nouvelles perspectives. Parmi les Mammifères, l'ordre des Rongeurs est de loin le mieux représenté (484 genres, comprenant 2277 espèces) à travers le monde. L'histoire évolutive et la systématique de ce groupe ont largement été étudiées par le biais de données paléontologiques, morphologiques et moléculaires. Cependant, il persiste certains points de désaccord entre paléontologistes et biologistes moléculaires. L'avancée des techniques de biologie moléculaire, et particulièrement en paléogénétique, permet désormais d'accéder au patrimoine génétique d'organismes disparus depuis plusieurs milliers d'années, mais aussi de pallier certaines limites de la morphologie. Malheureusement, peu de travaux se rapportant aux rongeurs intègrent des données paléogénétiques, malgré l'importante quantité de fossiles et de matériel conservés dans les musées. Nous présentons ici une synthèse des travaux effectués en paléogénétique (phylogénie, diversité génétique, migration) depuis l'origine de la discipline, ainsi que les champs disciplinaires prometteurs dans ce domaine (phylochronologie, paléoparasitologie).

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Mots clés : Rongeurs ; ADN ancien ; Phylogénie ; Diversité génétique ; Phylochronologie ; Paléoparasitologie

1. Introduction

Rodents (Rodentia, Mammalia) are the most diversified order of eutherian mammals, representing over 40% of all extant species (2277 species), and they have

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a worldwide distribution [13,73]. With their great fossil (743 extinct genera) [71] and extant (484 extant genera) [13] diversity, rodents are an excellent model group for evolutionary studies: most species are characterized by a short generation time and a fast evolving genome; some species are good ecological, climatological, and geographical indicators (Fig. 1A). The fossil record sup-

ports a rodent radiation 65–55 Myr ago (Palaeocene epoch) [42], whereas a Palaeocene or even a Late Cretaceous age is suggested by recent molecular estimates (75 Myr, [1]; 60 Myr [26]; 63.5–74.5 Myr [27]; 56 Myr [48]; 70–77 Myr [88]). The monophyly of Rodentia is strongly supported by morphological, palaeontological and molecular data [1,42,70,85], although it was seri-

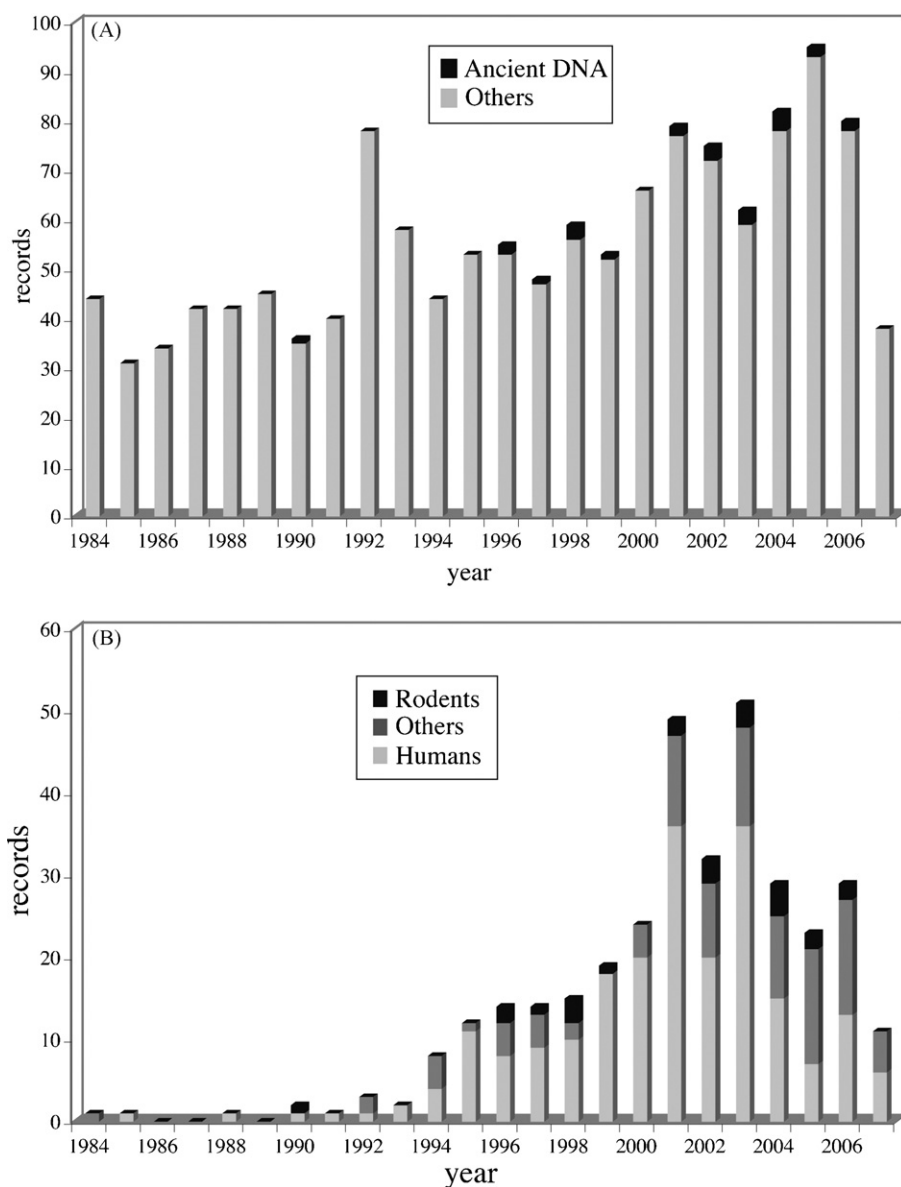


Fig. 1. Number of publications retrieved from public reference data banks (ISI Web of Sciences, PubMed) in July 2007: **A**, studies dealing with rodent evolution (e.g., palaeontology, archaeology, morphology, morphometrics, phylogeny, phylogeography, cytogenetics, population genetics, and palaeogenetics); **B**, studies based on ancient DNA sequences from mammals.

Fig. 1. Nombre de publications recueillies en juillet 2007 à partir de bases publiques de références bibliographiques (ISI Web of Sciences, PubMed) : **A**, études se rapportant à l'évolution des rongeurs (paléontologie, archéologie, morphologie, morphométrie, phylogénie, phylogéographie, cytogénétique, génétique des populations, paléogénétique, etc.) ; **B**, études intégrant des séquences d'ADN ancien de Mammifères.

Table 1

Published works dealing with rodent palaeogenetic studies. Nomenclature is based on Carleton and Musser [13]

Tableau 1

Liste des travaux publiés se rapportant aux études paléogénétiques de rongeurs. La nomenclature suit Carleton et Musser [13]

Taxa	DNA Source	Genetic Marker	Age	Origin	Authors
Cricetidae					
<i>Phyllotis limatus</i> Lima pericote	fecal pellets	cytochrome <i>b</i> (273 bp)	10,120 ± 150 yr	midden	[59]
<i>Microtus montanus</i> Montane vole	teeth (upper first molar)	cytochrome <i>b</i> (312 bp)	present to 2860 ± 70 yr	cave	[39]
Ctenomyidae					
<i>Ctenomys socialis</i> Social tuco-tuco	teeth	cytochrome <i>b</i> (150 to 253 bp)	present to ≈ 10,000 yr	barn owl roost	[16,38]
Echimyidae					
<i>Mesomys hispidus</i> Spiny tree rat	dried skin	cytochrome <i>b</i> (331 bp)	1817	museum	[74]
Geomyidae					
<i>Pappogeomys alcorni</i> Alcornis pocket gopher	dried skins	cytochrome <i>b</i> (402 bp)	1950 and 1966	museum	[22]
<i>Thomomys talpoides</i> Northern pocket gopher	teeth	cytochrome <i>b</i> (164 bp)	present to 2860 ± 70 yr	cave	[39,42]
Heteromyidae					
<i>Dipodomys panamintinus</i> Panamint kangaroo rat	dried skins	control region (225 bp)	1911, 1917 and 1937	museum	[93]
Muridae					
<i>Rattus exulans</i> Pacific rat	bones (femora, mandibles) and teeth (incisives)	control region (173 to 239 bp)	400 to 2000 yr	museum and archaeological sites	[65,66,67]
Various rodent species	taxidermized specimens		1941 to 1975	museum	[4]

ously challenged in the 1990s [12,23,33,62]. However, some disagreements remain within and among palaeontologists and molecular biologists about the divergence dates of major lineages (e.g., the *Mus/Rattus* split) [1,26,47,52,53,88], and the relationships among families [1,14,17,28,42,47,51,72,94].

Since the mid-1980s, palaeogenetics (i.e. studies of ancient DNA) has added another temporal dimension to evolutionary studies [44,77]. The first most common use of ancient DNA was systematics, whereas a wide variety of evolutionary issues (notably phylogeography, genetic diversity through time, population response to climate and environmental changes, domestication origin, past human migrations) are investigated today [80]. The majority of palaeogenetic studies have focused predominantly on mammal species (Fig. 1B) [45,80,98], but few of them are dealing with rodents, despite the amount of museum and fossil material available for evolutionary hypothesis testing. Here, we review the major research activities in rodent palaeogenetics, and we present the promising research perspectives in this field (see Table 1

for an overview). We deliberately refrain from dealing with technical aspects of ancient DNA works and the issue of authenticity, as it has been widely discussed in the literature [6,20,45,55,80,96,98].

2. Sources of ancient DNA

In the 1980s, the technical improvements in Polymerase Chain Reaction (PCR) have opened up the possibility of ancient DNA studies on museum specimens [19,56,78,81,97]. The first study on rodent ancient DNA was performed on dried skin tissue from museum specimens [93]. A 225-base pair (bp) fragment of the mitochondrial DNA (mtDNA) control region was amplified and sequenced. Later on, other studies used the same DNA source to address taxonomic questions [74] or to investigate phylogenetic relationships of species [22]. Likewise, owl pellets stored in museums or collected in the field are also considered as another good DNA source [91]. The DNA contained in that kind of samples could be protected from cross-contamination and degradation

inside pellets. However, dried skins and owl pellets are usually few decades old, and they do not allow phylogenetic and phylogeographic studies to be carried out on long time periods.

On the other hand, archaeological and palaeontological field collections can also provide fossil bone and tooth remains. The sampling is thus widely increased and it covers several thousand years (e.g., 2000 to 10,000 yr, [16]; 400 to 2000 yr, [67]). However, palaeogenetic analyses of fossil bones and rodent teeth are relatively rare. Post-excavation conditions and museum storage cause DNA degradation in fossil samples [84]. Consequently, bones and teeth used in most of ancient DNA studies of rodent remains should be freshly excavated [8,37,38,64–66]. Later on, fossil samples aimed at ancient DNA extractions should be kept at low temperatures shortly after excavation, and from the field to the lab [10].

Fossil bones and teeth of rodents are generally collected in caves (e.g., Lamar Cave, Wyoming, USA, [37,39]; Polynesian archaeological sites and caves; [8,64–67]; raptor roost, Argentina, [38]). Even if DNA preservation conditions are really different in terms of taphonomy (e.g., soil acidity, humidity, sediment porosity) inside each cave, this environment is less subject to temperature fluctuations (e.g., [31,32,40,45,46,83,84,87]). The low amplitude of temperature fluctuations as well as the cold to temperate temperatures is supposed to favour DNA preservation. Moreover, rodent skeletal remains are abundant there, essentially due to the accumulation of owl pellets [14,91].

Faecal material is also commonly found in caves frequented by animals [45,83], but also in much more unusual environments, such as rodent middens [59]. In this latter case, DNA was extracted from faecal pellets in order to identify the agent of an ancient midden located in the Atacama Desert (Salar de Atacama, Chile). To date, these remains are the oldest material used for ancient DNA studies on rodents (~11,700 yr BP) [59]. The reason of such an exceptional DNA preservation (in crystallized urine strongly cemented as adobe brick) is still questioned, but it suggests new perspectives for potential sources of ancient DNA retrieval.

Most palaeogenetic studies are dealing with North and South American rodents. Many European archaeological sites have yielded rodent remains (e.g., Gigny, Jura, France, [11], Pilisszántó, Hungary, [54], Bacho Kiro, Bulgaria, [58], Bedburg-Königshoven, Germany, [89], British Islands, [57,90]), but they remain understudied [82]. The main reason seems to us related to the difficulties encountered to extract ancient DNA from tiny

teeth and bones. Even in the best conditions of preservation, the recoverable quantity of DNA from one tooth or bone is small. In order to compensate for this drawback for phylogenetic studies, some authors propose to pool, during extraction and PCR analyses, several samples belonging to one species to get a consensus DNA sequence [24]. However, in these conditions, the results are completely unreliable, and they make the authentication of ancient DNA sequences nearly impossible. For phylogeographic studies, it is much more problematic: two (or more) samples from the same fossil site could have different haplotypes. Unfortunately, no paper related to rodent palaeogenetics discusses this fact.

3. Phylogenetics and phylogeography

Ancient DNA data are usually used in a systematic context (e.g., [5,35,44,75,84,86,100]). In fact, ancient DNA sequences provide an accurate vision of genetic differentiation through time to conduct phylogenetic and phylogeographic studies. Sometimes, species identification is difficult because of unidentifiable broken samples [65], or insufficient morphological features for species differentiation [22]. In such conditions, analyses of ancient DNA can be very helpful [61]. Ancient sequences are compared with those of the closest extant taxonomic species in order to confirm the identification of the specimen. In the case of rodent remains, this technique was first used by Matisoo-Smith et al. [65]. These authors validated the identification of the Pacific rat (*Rattus exulans*) fossil remains by both palaeogenetic and morphological methods.

Phylogenetic reconstructions based on ancient DNA sequences, associated with modern DNA analyses, can help to solve the phylogenetic relationships of extinct and extant species, and to improve the systematics significantly. For instance, the spiny rat (*Mesomys hispidus*) lacks adequate taxonomic definition, and the geographic origin of the holotype described in 1817 and stored in the 'Museum National d'Histoire Naturelle' (Paris, France) is unknown [74]. The mtDNA cytochrome *b* (cytb) gene sequence (331 bp) and morphometric analyses based on 22 cranial and dental measurements show a clear affinity of the holotype to extant specimens of *M. hispidus* from French Guiana [74]. Therefore, the holotype probably came from an area close to French Guiana, but its exact geographic origin is still unresolved. Likewise, the systematics of a rare pocket gopher (*Pappogeomys alcorni*) was investigated with cranial measurements and ancient DNA sequences [22]. The DNA was extracted from dried skins of specimens collected between 1950 and 1966. The monophyly of the genus *Pappogeomys* is strongly

supported (bootstrap value = 100%) by DNA sequences of *P. alcorni* and *P. bulleri*. From the tree topology, *P. alcorni* is included in the *bulleri* clade. From the morphometric analysis, there were no significant differences between *P. alcorni* and *P. bulleri* for each one of the 12 characters examined. Phylogenetic and morphometric analyses suggest that *P. alcorni* is a subspecies of *P. bulleri* rather than a distinct species [22].

Phylogeographic studies on rodents are mostly based on extant populations [18,21,43,49,50,76,99]. However, temporal changes are not explicitly taken into account in these studies. For this reason, sequence information from ancient DNA is also used to test the reliability of evolutionary hypotheses. Some fossil deposits hold the potential to carry out temporal and spatial studies of genetic diversity at the population level [16,37,39,64,79,96]. Genetic changes in rodent populations are tracked through time in order to point out migration events [37,38,39,64,66,67,93]. Compared with climatic changes, these events allow us to investigate evolutionary responses of rodent populations to environmental changes [16,37–39] (for details, see § *Phylochronology* below). Indeed, some migration routes and movements of past human populations can also be tracked back by studies of temporal and spatial genetic changes from some rodent populations. For instance, the Pacific rat (*Rattus exulans*), a commensal rat often transported as a food item in colonizing canoes, is an indirect valuable tool for tracing prehistoric human migration within Polynesia [8,64–67] (for details, see § *Rodents and human migrations* below).

4. Phylochronology

Phylogeography (i.e. the study of the processes governing the geographical distribution of genealogical lineages) has improved our understanding of the geographical distribution, phylogenetic relationships, and genetic diversity within and among animal and plant species [7]. This discipline offers the opportunity to interpret the effects of climatic and environmental changes on spatial distribution and population dynamics of living organisms. However, it gives a limited access to the past because the fossil record is not taken into account. Hadly et al. [39] have proposed a new approach, named phylochronology, to study populations in space and time using phylogenetic and population genetic methods. This approach uses both ancient and modern DNA data, and it integrates fossil abundance data, ecological parameters, as well as historical climate records to infer microevolutionary processes [39,95]. In this framework, analyses of ancient DNA provide

the opportunity to track the response of past populations, as well as to predict that of the extant biodiversity to environmental changes [16]. Phylochronology was elaborated from studies on North and South American rodent populations: the northern pocket gopher (*Thomomys talpoides*) and the montane vole (*Microtus montanus*) from the Lamar Cave (Yellowstone National Park, Wyoming, USA) [37,39], and the social tuco-tuco (*Ctenomys sociabilis*, Estancia Nahuel Huapi and Cueva Traful, Argentina) [16,38].

Previous palaeontological studies on Lamar Cave faunas (fossil sequence spanning the last 3000 yr) have shown the influence of climatic change on population dynamics and phenotypic response [36]. In addition, ancient DNA analyses indicate that some species (*T. talpoides*) exhibited lowered gene diversity with decreasing population size at the time of the Medieval Warm Period (470–1438 yr), whereas others (*M. montanus*) did not [39]. The opposite responses seem to be due to differences in demographic dispersal patterns of these species. *Microtus* migrations could occur more frequently in and between low-density populations, whereas *T. talpoides* could experience a long-term isolation [37,39]. In fact, these analyses have documented environmental change, population response, genetic diversity change, and the correlation between the three [39].

Likewise, the response of *C. sociabilis* to climate change was investigated on a period of 10,000 yr [16,38]. Modern populations share the same cytb haplotype (M), while eight haplotypes were identified for the Cueva Traful fossil samples: M and seven historical variants. Prior to ca. 3000 yr, *C. sociabilis* was characterized by a greater genetic diversity than the present specimens living in the Cueva Traful area. Moreover, based on the tooth abundance from several stratigraphic levels, the population density decreased between approximately 8200 and 3000 yr [39]. Several factors may have contributed to this population bottleneck: changes in vegetation, volcanic eruption, or competition with the Haig's tuco-tuco (*C. haigi*). The survival of *C. sociabilis*, despite low modern genetic variation, is probably due to its unusual social system, either as a cause or consequence of the bottleneck [15].

5. Rodents and human migrations

The dynamic reconstruction of the human past migrations is a real challenge. Palaeogenetic studies have the potential to shed light on these migrations. Unfortunately, they can encounter methodological difficulties due to contamination with modern DNA, insufficient

samples and ethical problems preventing sample destruction [45,66,67,80,98]. An alternate approach is to focus ancient DNA studies on the genetic variation of commensal or domesticated animals and plants (e.g., [2,29,41,60,66,69]).

Human settlement in the Pacific, and particularly in Polynesia, was a major event in world prehistory, and it is still cause for debate. It represents one of the last human population migrations. Works in various disciplines (archaeology, human skeletal biology, cultural anthropology, linguistics, and human genetics) have improved our understanding of this event, but questions remained in abeyance. Where was the starting point of the Polynesian populations? What was their dispersal pattern throughout Polynesia? What were the settlement process and population interactions? [66,69]. The Pacific rat (*Rattus exulans*) provided an ideal model for telling the story of human colonization through Near and Remote Oceania (respectively, western and eastern Pacific) and the Polynesian triangle (French Polynesia, New Zealand, Chatham and Kermadec islands, Rapa Nui, and so on) [8,63–69]. In fact, this rat, carried intentionally by ancestral Polynesians as a food item, cannot swim more than a few metres in the open ocean; it was thus dependent upon humans for its dispersal across waters. Moreover, it is characterized by a short generation time and a fast evolving genome.

The history of human colonization in the Pacific can be divided in two major phases: the colonization of Near Oceania approximately 40,000 yr BP ago and that of Remote Oceania around 3100 yr BP ago [66]. From modern, museum (1921–1963) and archaeological (400 to 2000 yr) specimens of *R. exulans*, mtDNA control region phylogenies provide an indication of the degree of interaction between the various Polynesian archipelagos: isolation after colonization of some islands (the Marquesas, Chatham islands, Rapa Nui) or multiple contacts with some islands (Hawaii and New Zealand) [8,64,68,69]. Colonization of the eastern Polynesian islands occurred in a broad central area of the Polynesian triangle, but there was no evidence from *R. exulans* molecular data for a dispersal centre restricted to any particular archipelago [69].

In fact, molecular studies of *R. exulans* on a larger scale (Southeast Asia in addition to Near and Remote Oceania, including Polynesian islands) allow us to identify three distinct haplogroups (I, II, and III) [66]. Haplogroup I clusters with some Southeast Asian populations, whereas haplogroup II consists of populations from Southeast Asia and Near Oceania, and haplogroup III includes most of Remote Oceanic populations. From these phylogeographic results, Southeast Asia appears

as the starting point of *R. exulans* populations toward Near Oceania. Likewise, the most likely origin for rat populations from Remote Oceania might be Halmahera (between the Philippines and New Guinea), since Halmahera rats are found in haplogroups II and III [66]. The clear distinction between these two latter haplogroups suggests that *R. exulans* was introduced at least twice into Oceania. Morphological studies of this species support the following hypothesis: one population (haplogroup II) could have been introduced from Southeast Asia through Melanesia into Near Oceania, and the other one from Southeast Asia through Micronesia into Remote Oceania [92]. The distribution and variation of haplogroup III confirms the rapid dispersal of *R. exulans* populations (and therefore of human populations) through Pacific islands (Mobile Founding Migrant models; [34,66]). These results are consistent with data from human language analysis, comparisons of human populations and cultures.

6. Palaeoparasitology

Rodents are known to convey indirectly pathogen agents such as the plague agent, *Yersinia pestis*, as well as internal and external parasites. Some of these parasites were found with coprolites or mummies of rodents in archaeological sites, mainly in South America. In coprolites, two kind of internal parasites were identified: (1) the nematode *Strongyloides ferreirai* from Brazilian archaeological sites (2000 to 8000 yr BP) [3]; (2) eggs from a potentially extinct species of the nematode *Trichuris* (8450 to 30,000 yr BP) [30]. On the other hand, well-preserved ectoparasites (lice, fleas, mites) were discovered in the fur of various guinea pig mummies (*Cavia aperea*) belonging to the Chiribaya Culture (900–1100 AD, Moquegua Valley, southern Peru) [24,25]. Guinea pig (159 individuals of *Cavia aperea*) and dog (17 individuals of *Canis familiaris*) mummies were found in Moquegua Valley sites. Over 1200 fleas of the genus *Pulex* were recovered from animal mummies. Phylogenetic reconstructions based on sequences from 300 fleas (taken from three dogs) did not allow us to identify clearly the *Pulex* species (*irritans* or *simulans*). However, two lines of research could benefit from this study: taxonomic and medical interests [25].

Pathogen agents and parasites are common in soil or animal hosts, raising the possibility of contamination of past samples with modern DNA [80,96]. For this reason, very few ancient DNA analyses were performed on rodent parasites. PCR was used to study the possibility of *Trypanosoma cruzi* kinetoplast DNA extraction from experimentally desiccated mouse tissue.

The results suggest that the application of this technique to the detection of *T. cruzi* in archaeological remains is feasible [9]. On the other hand, PCR was also applied to 39 taxidermized rodents collected between 1941 and 1975 (National Museum, Rio de Janeiro, Brazil) in an attempt to examine the role of rodents in the transmission cycle of leishmaniasis. Five of 39 rodents were found to be positive for *Leishmania* [4].

7. Conclusion

Rodent palaeogenetic research is just at its beginning. Few works focus on rodent palaeogenetics, despite the amount of material available in museums and fossil sites. Fossil and museum rodent specimens offer the opportunity: (1) to be more specific about the inter- and intra-specific phylogenetic relationships; (2) to test and validate evolutionary hypotheses elaborated from morphological, palaeontological/archaeological and/or modern molecular data; (3) to understand the response of rodent populations to past climatic and ecological changes. In fact, phylochronological studies provide a unique insight into the past and the potential ability to separate the cause from the effect [39].

Another promising field of research is palaeoparasitology, because of the ability of rodents to carry and vector indirectly diseases (plague, Lassa fever, leptospirosis). Experimental analyses on sub-recent (last 200 yr) rodent pathogens show that ancient DNA techniques can be applied to epidemiological studies of the past [4]. The contribution of palaeoparasitology is a major stake to study and understand the role of rodents in the transmission cycle of pathogens and parasites. Diseases of rodents or the transmission way to humans could be like this elucidated.

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