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Faculty of Science
Study programme: Zoology

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Allopatric evolution in rousettine fruit bats: from population and landscape genetics to phylogeography

Alopatrická evoluce u kaloňů rodu *Rousettus*: od populační a krajinné genetiky k fylogeografii

Doctoral Thesis

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Praha, 2018

Prohlášení:

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V Praze, 16.7.2018

Acknowledgements:

I would like to thank the following foundations:

„Nadání Josefa, Marie a Zdeňky Hlávkových“ for the financial contribution which helped to cover the costs of a travel to South Africa to attend the 17th International Bat Research Conference in 2016

“Fond mobility UK” which financially supported a scientific field research in South Africa.

I am particularly grateful for the assistance given by my supervisor Pavel Hulva.

I also wish to acknowledge the help provided by all my co-authors of the attached manuscripts. Detailed acknowledgements related to specific studies are included in particular manuscripts.

Finally, I would like to express my greatest appreciation to my beloved husband for his love, patience and all-embracing support.

Abstrakt

V západní části areálu výskytu kaloňů rodu *Rousettus* byla studována jeho biogeografie, populační struktura a fylogenetické vztahy. Současné populační vzorce tohoto rodu kaloňů na území Starého světa jsou ovlivňovány různými vlastnostmi prostředí souvisejícími s topografií, klimatem a krajinným pokryvem. Tyto proměnné se odráží v plesiomorfiích kaloňů spojených s ekologickou nikou tropických létajících plodožravců, stejně jako v apomorfiích rodu *Rousettus*, které zahrnují schopnost echolokace, osídlování jeskyní a schopnost šířit se i v nezalesněné krajině. Fylogenetické vztahy mezi druhy a poddruhy rodu jsou naznačeny a konfrontovány s dosavadními scénáři kolonizace. Ostrovní populace (včetně biotopů v pouštních oázách) vykazují častou genetickou diferenciaci od svých příbuzných na pevnině, což naznačuje úspěšné zakládání kolonií po překonání úseků nevhodných stanovišť. Genetická odlišnost kaloňů vyvíjející se na méně vzdálených ostrovechnaznačuje zapojení behaviorálních mechanismů, které udržují soudržnost izolovaných oblastí, jako jsou filopatrie a upřednostňování natálního habitatu. Na obrovské ploše sub-saharské pevninské Afriky, která sahá od jižní hranice Sahary až ke Kapskému poloostrovu, sdílí kaloň egyptský homogenní jaderný fond, avšak tvoří dvě mitochondriální haploskupiny, které se částečně vyskytují v sympatrii. Pozorovaný jev mohl vzniknout vlivem klimatických výkyvů v Plio- / Pleistocénu a diferenciací v rámci hypotetických lesních refugií v Konžské pánvi a / nebo Horní Guineji a ve východní Africe. Současné rozšíření obou haploskupin naznačuje různé způsoby rozšiřování z těchto refugií a možnost existence adaptivních vlastností spojených s mitochondriální DNA. Rozdílná situace byla odhalena na severní hranici rozšíření rodu na Středním východě. Složitá geomorfologie regionu spolu s efekty zakladatele a pozdější kolonizací způsobila mělkou diverzifikaci populací, kde genetické vzdálenosti odráží fenomén *„isolation by distance“* a časté ostrovní efekty. Pozorované patrnosti byly zjištěny pomocí mitochondriálních a rychle se vyvíjejících nukleárních markerů. Podrobná studie kontaktní zóny mezi středomořskými a pouštními oblastmi Levanty potvrdila prudký gradient jak genetických, tak morfologických rysů místních kaloňů, shodující se se vzorcem *„isolation by environment“* a *„isolation by adaptation“* a ukazující na přítomnost dvou ekotypů. Lidská aktivita zahrnující pěstování stromů v této oblasti zvýšila množství vhodných stanovišť pro úkryt a potravu kaloňů a tím ovlivnila ekologii obou ekotypů, avšak vliv lidské činnosti na jejich hybridizaci nebyl prokázán. Vzhledem k tomu, že kaloni hrají zásadní roli při opylování a disperzi semen velkého počtu rostlin, představují klíčové druhy pro dané lesní ekosystémy. Naneštěstí, především malé populace kaloňů jsou ohroženy zvyšujícím se využíváním půdy, zemědělstvím, jejich statusem škůdců, lovem pro maso a jejich kontroverzní rolí v přenosu virů (Ebola, Marburg, atd.). Z těchto důvodů a vzhledem k významu kaloňů rodu *Rousettus* pro obnovu lesa a jejich schopnosti nově kolonizovat i suchá a vzdálená místa, jsou vhodným předmětem studia ochranářské biologie a ekologie obnovy.

Abstract

Population structure, biogeography and phylogenetic relationships of the fruit bat genus *Rousettus* have been studied in Africa and adjacent regions. The current population patterns of rousettine fruit bats in the Old World are influenced by several environmental attributes, namely the topography, climate and land cover. These variables are mirrored in fruit bat plesiomorphies related to the ecological niche of tropical flying frugivore, as well as apomorphies of rousettines including echolocation ability, roosting in caves and dispersal capacity in open landscapes with discontinuous tree cover. Phylogenetic relationships among species and subspecies of the genus have been indicated and confronted with the existing colonization scenarios. Insular populations (including habitat islands within desert oases) show frequent genetic differentiation from their mainland relatives suggesting successful founder events after traversing stretches of unsuitable habitats. Genetic differentiation evolving in less distant islands suggests involving behavioural mechanisms maintaining cohesion of isolated demes as site fidelity and natal habitat-biased dispersal. In sub-Saharan mainland Africa within the large range reaching from the southern border of Sahara to Cape Peninsula, *Rousettus* populations share a homogeneous nuclear pool but form two mitochondrial haplogroups that occur partly in sympatry. The observed pattern is attributed to the Plio- / Pleistocene climatic oscillations and differentiation within hypothetic forest refugia in the Congo basin and/or Upper Guinea and in eastern Africa. The current distribution of both haplogroups implies different modes of dispersal from particular refugia and the possibility of adaptive traits associated with mitochondrial DNA. A contrasting situation was uncovered on the northern border of the genus' distribution, in the Middle East. A complex geomorphology of the region together with founder effects and a later colonization have caused fine-scale population diversification, isolation by distance and frequent insular effects. The observed patterns have been discovered by means of mitochondrial and fast evolving nuclear markers. A detailed study of a contact zone between Mediterranean and desert habitats in the Levantine region confirmed a steep gradient in both genetic and morphological traits of the local fruit bats, concordant with isolation by environment and isolation by adaptation and indicating the presence of ecotypic variation. Human activities including horticulture have increased the amount of roosting and foraging sites, thus affecting movement ecology of both ecotypes, however, the impact of anthropogenic changes in the environment on the admixture was not proved. Since fruit bats play a crucial role in pollination and seed dispersal of a large number of plants and trees, they represent a keystone species of respective forest ecosystems. Unfortunately, especially small populations of fruit bats are threatened by increasing land-use, agriculture, their conflicting status as pests, their hunting for bushmeat and controversial role as vectors of viruses (Ebola, Marburg, etc.). For these reasons and due to the importance of rousettine bats for forest recovery and their ability to pioneer dry and distant habitats, they are proper object in the fields of conservation biology and restoration ecology.

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Annex II.

Hulva, P., Marešová, T., Dundarova, H., Bilgin, R., Benda, P., Bartonička, T., & Horáček, I. (2012). Environmental margin and island evolution in Middle Eastern populations of the Egyptian fruit bat. *Molecular Ecology*

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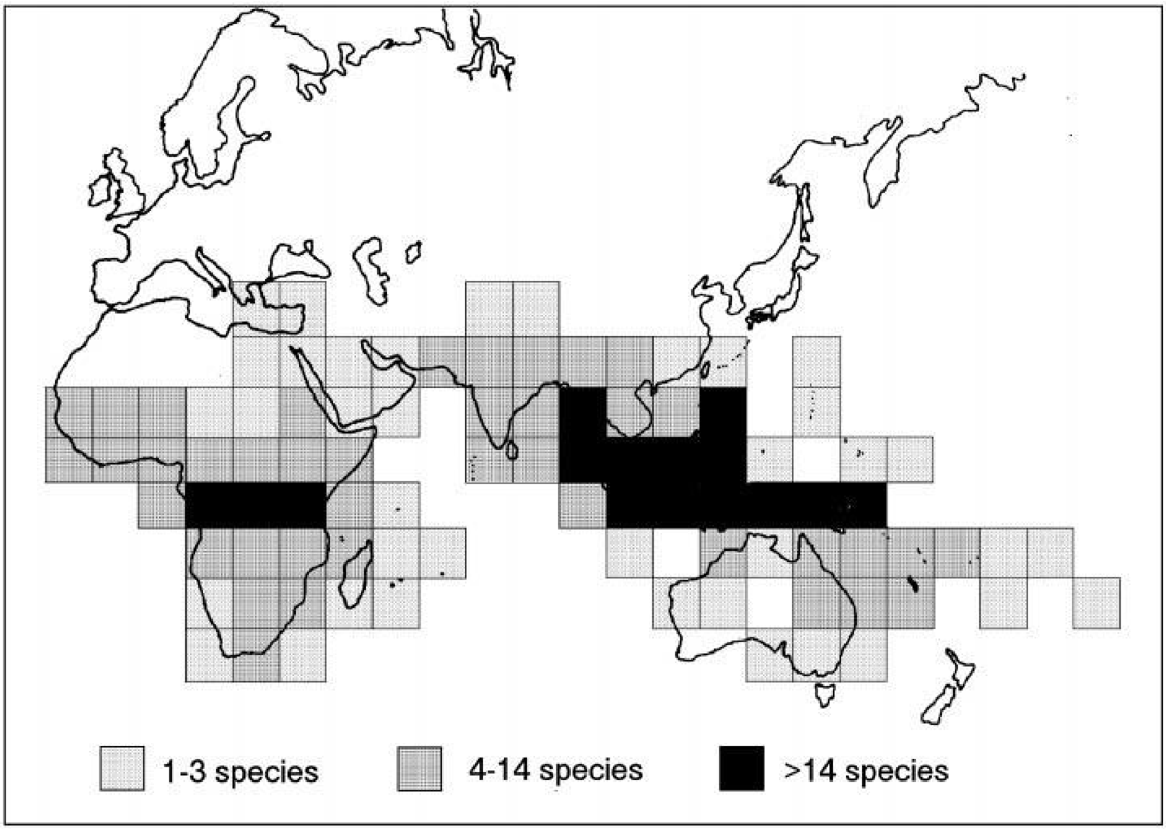
Centeno-Cuadros, A., Hulva, P., Romportl, D., Santoro, S., Stříbná, T, Shohami, D., … Nathan, R. (2017). Habitat use, but not gene flow, is influenced by human activities in two ecotypes of Egyptian fruit bat (*Rousettus aegyptiacus*). *Molecular Ecology*, 22, 6224-6237.

Introduction

The Old World fruit bats, Pteropodidae, represent a monophyletic taxon in the suborder Yinpterochiroptera. These flying mammals with many unique adaptations, phytophagy and an extraordinary ecological and economic importance probably originate in Asia and their main habitat lays in the tropics and subtropics of the Old World (Fig. 1). Various bats from average size to the largest in the world pertain to this family of 42 genera and about 186 species and many of them play an essential role as forest pollinators and seed dispersers. The adaptations for a diet of fruit, flowers, nectar and pollen encompass either heavy jaws with big canines to penetrate tough fruit and spacious grinding surfaces of molars and premolars or elongated narrower jaws with smaller teeth for reaching into blossoms bottom. The orientation for a stationary, colourful food made the vision and olfaction most important senses that are supported by large eyes and dog-like muzzles. They often forage in large groups and some migrate seasonally in relations with fruit phenology. Fruit bats roost mostly in trees or caves - a single individual or a group of even hundreds of thousands can be present at one site.

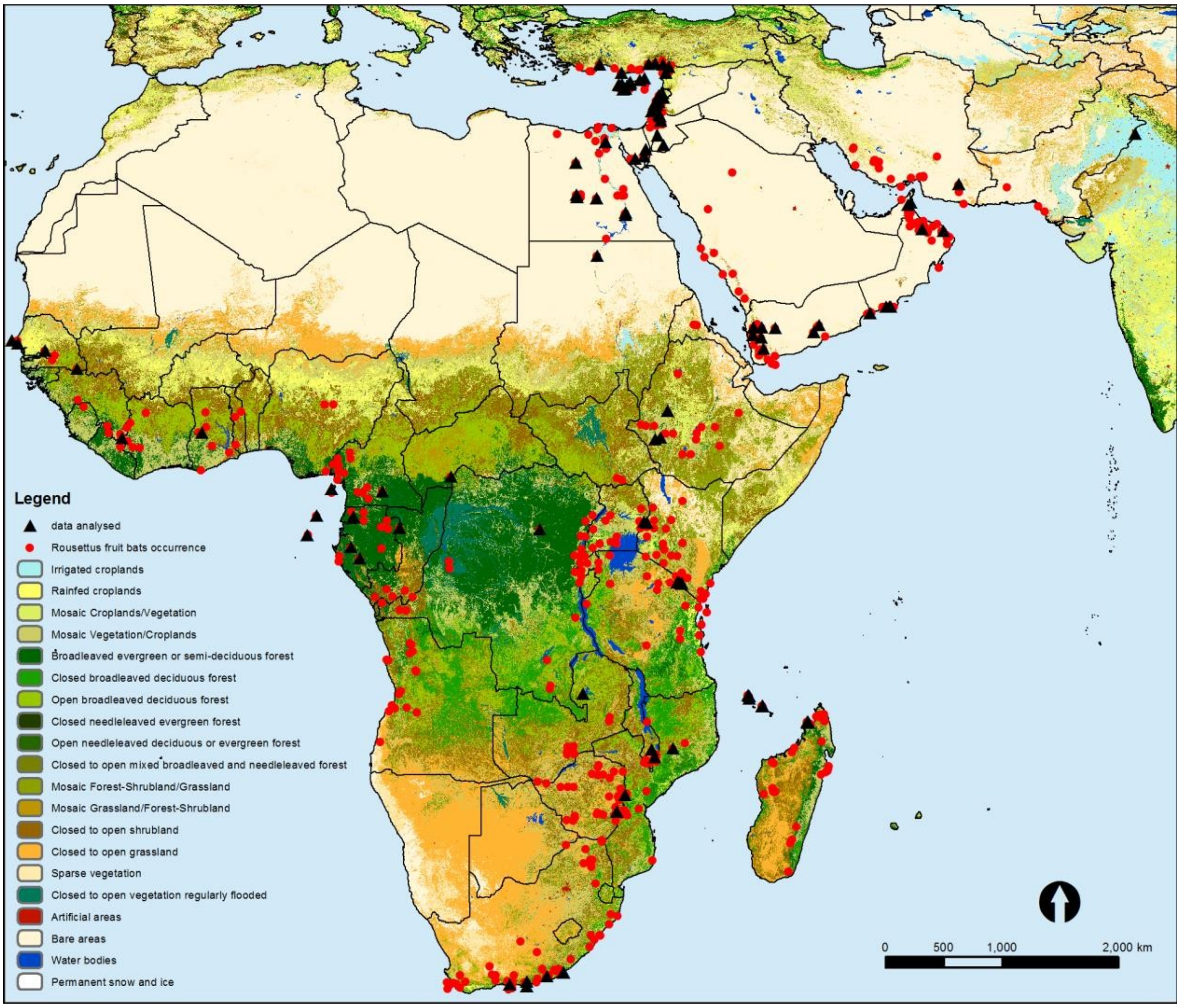
Fig. 1 Pattern of species richness distribution of Old World fruitbats in a 10 3 10-degree frame according to Corbet and Hill (1992), Mickleburgh et al. (1992), Bergmans (1994, 1997), and Bates and Harrison (1997), taken from Juste et al., 1999.



The majority and especially the singly living species tend to be brownish and inconspicuous but some males possess colourful long hair on their heads and shoulders emphasizing their scent glands. These traits are coupled with the sometimes very loud and elaborate courtships (Altringham, 2011).

Rousettus is a fruit bat genus with the broadest distribution partly thanks to the ability to echolocate (unique in fruit bats), which allows hiding in the darkness of caves during the day, and partly due to the exceptional dispersal capacity of these animals. Their distribution range covers the Oriental, the Afrotropical and the Saharo-Arabian biogeographical realms (Juste et al., 1999). As historical biogeographical patterns can be retrieved from a comparison of phylogenies of many different organisms and by an identification of biotic areas, full phylogenies are needed for the recognition of migration paths in a region (Hewitt, 2004). The impact of Plio-Pleistocene climatic oscillations in the temperate zone of the northern hemisphere is seemingly well studied and specific refugia were identified based on the phylogeographic patterns of different organisms (Hewitt, 1999). Such a clear outcome is lacking for the Afrotropical region and hypotheses concerning this topic vary. By exploring the current phylogeographic state, we are obtaining information on the natural history of the area and of the respective taxon. My dissertation focuses on biogeography and inner relationships of the genus *Rousettus* with an emphasis on the historical evolutionary scenarios that have led to the current population structure in the African and Middle Eastern parts of the genus' range (Fig. 2).

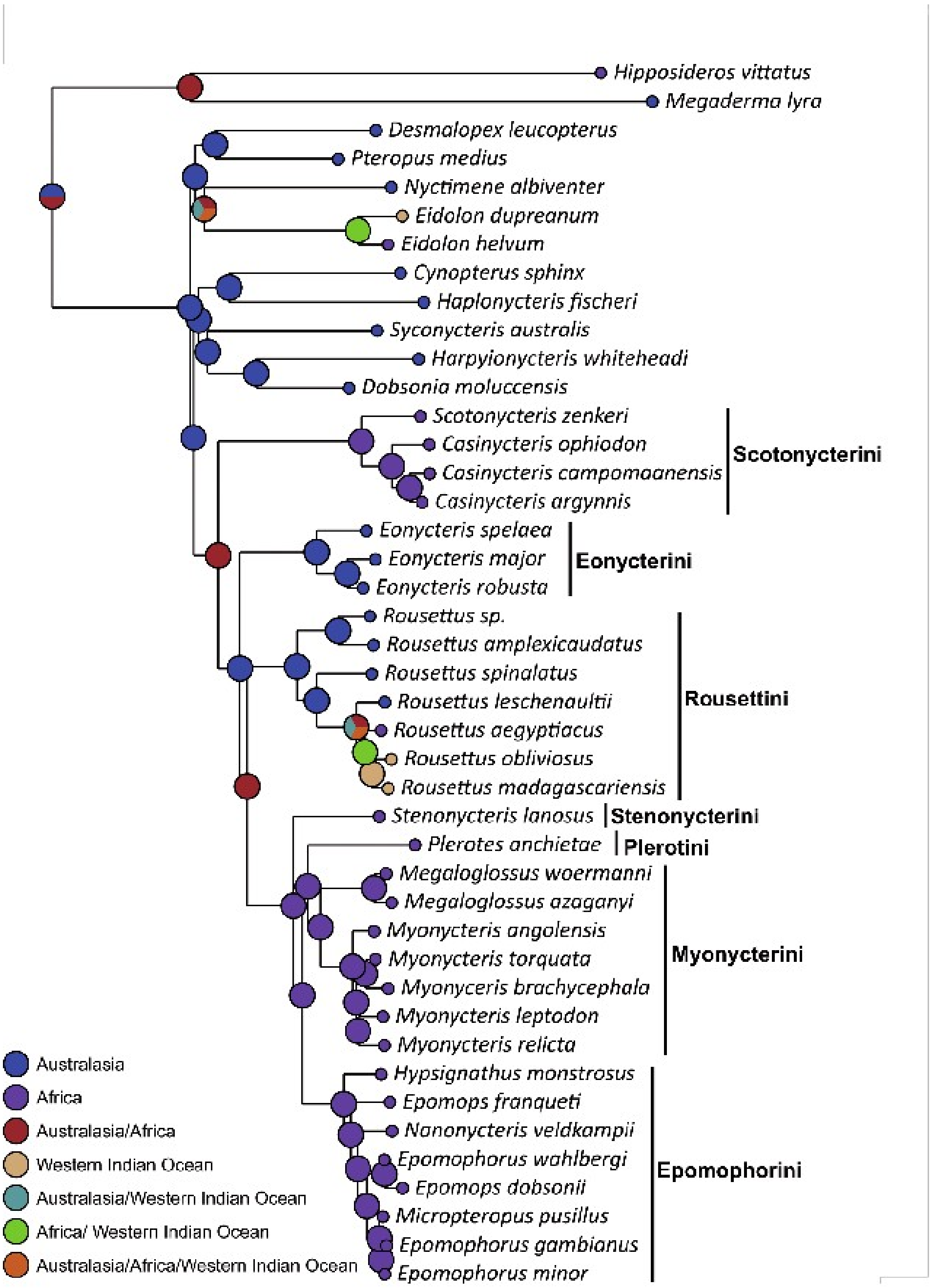
Fig. 2 Distribution range of the genus *Rousettus* in the studied areaon a background based on land cover data (ESA Climate Change Initiative). Black triangles indicate the localities with genetic data analysed (generated in the present study, Hulva et al. 2012 and Hassanin et al., 2016) and red dots indicate the localities from other studies (Bergmans, 1994; Monadjem et al., 2010; Goodman et al., 2010, Benda et al., 2012).



Phylogeography and biogeography

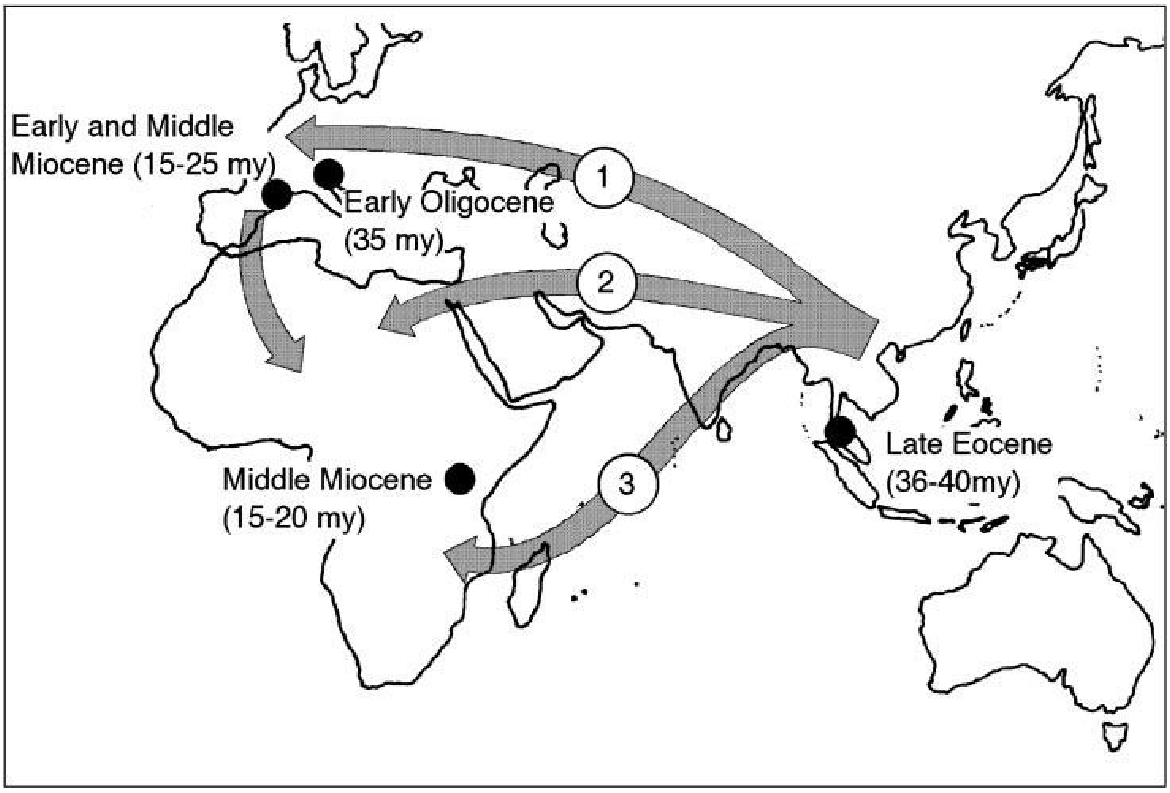
Eight species fall into the genus*Rousettus* (*R. amplexicaudatus, R. celebensis, R. linduensis, R. leschenaultii, R. spinalatus, R. madagascariensis, R. obliviosus* and *R. aegyptiacus*) four of which are endemic to islands in southeast Asia and western Indian ocean. *R. aegyptiacus* has been divided into six sub-species inhabiting discrete geographic areas by Bergmans in 1994; however, in some cases, we questioned the former subdivision by means of molecular methods and increased sampling of the species (Hulva et al., 2012; Stříbná et al., under review). All species of the genus *Rousettus* form the Rousettini clade, which constitutes one of four branches that encompass lineages present in Africa. It has been placed as a sister to the entirely African Epomophorinae + Stenonycterini + Plerotini + Myonycterini clade (Fig. 3, Giannini & Simmons, 2005; Almeida et al., 2011 & 2016) and was estimated to diverged from their common ancestor in Middle Miocene.

Fig. 3 Biogeographic reconstruction of Pteropodidae obtained from DIVA (Almeida et al., 2016).



From the Asian ancestor lineages *Rousettus* is believed to colonize Africa only once in the late Pliocene/early Pleistocene, the estimations of time period of this event laying between 2.6 (according to Almeida et al., 2016) and 1.7 Mya (according to Hassanin et al., 2016). Which route was followed to reach the current distribution is not exactly clear although authors of the newest phylogenies suggested that the route through Arabia was utilized (route 2, Fig. 4, Almeida et al., 2016). However, the extant Middle Eastern populations do not appear to be ancestors of the African ones (Hulva et al., 2012). Thus, the paleontological records shown in Fig. 4 indicate a larger Neogene range of the genus, but do not clarify the origin of current lineages as the fossils found must have pertained to an extinct population. For the settlement on islands in the Indian Ocean the route via stepping stone islands (route 3, Fig. 4) was also possible.

Fig. 4 Alternative colonization routes of Africa. Route 1: across Europe and stepping-stone islands and/or the Gibraltar bridge during the Late Miocene (Steininger et al., 1985). Route 2: through corridors along the Arabian Peninsula, which permitted several mammalian dispersal phases in the Neogene (Thomas 1985). Route 3: across the Indian Ocean via stepping-stone islands. Locations of the fossil records of fruit bats (Butler, 1984; Aguilar et al., 1986; Sige´ and Aguilar, 1987; Ducrocq et al., 1993) from Juste et al., 1999.



Phylogenetic relationships among species and sub-species of*Rousettus* have been resolved genetically by several authors with differing number of species, sub-species, individuals and markers used (Almeida et al., 2016; Goodman et al., 2010; Giannini & Simmons, 2005). Our assemblage of samples covered all of the species and sub-species living in the studied area and two Asian species – a unique representation analysed to date. Our findings show the tree base branching is in accordance with the works published to date and more detailed relationships can be seen on sub-species level, although not always with very high support (Fig.4 in Annex I.).

Based on broadly accepted taxonomic literature, in sub-Saharan Africa, two allopatric sub-species of *R. aegyptiacus* should be present on the western coast (*R. a. unicolor*) and in southern and most of eastern Africa (*R. a. leachii*). We found two mitochondrial lineages that partly correspond to the specified regions but coexist in sympatry in the east and northwest of the region.

In the northern part of the species range, morphologically distinct *R. a. egyptiacus* and *R. a. arabicus* were formerly recognised with a border delimiting their occurrence running roughly along the eastern borders of Jordan and Syria, separating colonies in the Levant, Cyprus, Turkey and Egypt from those in Arabian Peninsula, Iran and Pakistan (Bergmans, 1994). In this region, we found a mosaic of subpopulations based on nuclear microsatellite data, while the mitochondrial marker showed to some extent geographically localized haplotypes that indeed did not correlate with the two subgroups (Hulva et al., 2012; Stříbná et al., under review). However, with a closer look at the contact zone where the two potential sub-species meet, we found a steep gradient in environmental conditions reflected in ecology, morphology and genetics of the animals and suggested their variation as ecotypic (Centeno-Cuadros et al., 2017).

Feeding ecology

As their English name suggests, fruit bats feed primarily on various fruits of the Old World flora. Observations have shown that the preferential colour of the fruit is light inconspicuous tone of green to yellow or red to brown. This appearance is probably linked to the nocturnal foraging and adapted vison in Pteropodidae. There is a significant relationship between the moon light visibility of the rainforest fruits and the food selection of an Australian fruit bat (Richards, 1990; Hodgkinson et al., 2006). In addition to fruits, which are low in protein and thus insufficient for complete nutrition, fruit bats occasionally feed on flowers, leaves, pollen or insects. These complements form a small but nutritionally important part of the diet. Other mechanisms of digestion have been adapted to rise the income of proteins, which is a limiting factor. Rapid gut passage rate, longer intestine than in insectivorous bats or improved disintegration of pollen are just some examples (Courts, 1998; Funakoshi et al., 1993).

The specific diet of Pteropodidae, some of which feed on more than 40 species of plants, plays an important role in the forest ecosystems. As these bats forage on sites distant from their roosts and fly easily over other habitats, the incidental seed dispersal helps bridging of isolated forest fragments (Thomas, 1988). The species from genus *Pteropus*, characterized by largest body size within the family, feed up to 50km from their roost. Nevertheless, also smaller species (e.g. *Rousettus madagascariensis*) can travel daily more than 8 km to their foraging site making a round trip of up to 27km (Andrianaivoarivelo et al., 2011; Bollen & Elsacker, 2002; Picot, 2007).

Since fruit bats do not hibernate, their diet determines the distributional limits as an all-year fruit availability is crucial. Regarding latitude, *Rousettus aegyptiacus* occupying the Mediterranean is the furthest living fruit bat from the equator, who benefits from the regional agriculture. It feeds on native, introduced or cultivated fruit plants (Korine et al., 1999). An elaborate study based on nitrogen isotope analysis has shown that the intraspecific variation in protein sources is significantly higher in spring than in summer or winter in this area. As predicted, the elevated number of fruiting plants can offer wider choice of resources during spring in a seasonally changing region (Herrera et al., 2008). Differences in food choice between males and females were visible in summer when resources are scarce, and therefore females opt for more protein rich plants in the expectation of upcoming reproduction period (Lučan et al., 2016). In addition, the fruiting or flowering is more inconsistent in space and time in drier habitats than in forest ecosystems (van Schaik et al., 1993; Fleming & Eby, 2003) and local availability of food promotes annual migratory behaviour and gene flow in fruit bats (Thomas 1983; Palmer & Woinarski, 1999).

Social structure, movement ecology and roosting. The implications for population structure

Genetic population structure of any organism is influenced by many factors such as historical events, geographical, ecological and behavioural characteristics, capacity to disperse and others. Unlike terrestrial mammals of comparable size, bats are overcoming various geomorphological barriers with relative ease and are often able to fly long distances at once (Shilton et al., 1999; Heaney et al., 2005). Although it could be presumed that such vagility leads to large unstructured populations in bat species, it is not often the case. For example, among four co-distributed fruit bats of the genus *Cynopterus* in southeast Asia, substantial differences have been found between pairs of species that shared the same history and/or ecology, and thus interactions between multiple factors must have influenced the development of heterogeneous genetic patterns (Campbell et al., 2006). In Southeast and East Asia, where *Cynopterus sphinx* and *Rousettus leschenaultii* are abundant and widely co-distributed, contrasting genetic structure has been uncovered despite the same demographic history of both taxa. In this case, the roosting ecology of the two species has been suggested to be the cause as *Cynopterus* is a tree dwelling species while *Rousettus* dwells in caves. Also, the number of individuals sharing the same shelter is believed to influence population structure in such a manner that huge irregularly situated cave colonies tend to search for food resources over a larger space whereas smaller tree cavity/foliage roosting groups scattered all over a forest do not. Obviously, the levels of food sources competition are very different (Chen et al., 2010). To a concordant conclusion came authors of a sophisticated study of seven bat species co-distributed in an unmodified habitat of a continuous intact forest. The site of a Malaysian lowland rainforest was carefully chosen, so that the taxa experienced the same climatic and historical processes, and thus their genetic structure should be purely a result of their behaviour, ecology and social structure. Low vagility and high positive genetic structure was found in species that roost singly or in small groups in the trees, and the opposite was found for the species living in larger colonies of caves (Rossiter et al., 2012).

Rousettus aegyptiacus, which is widespread across most of the Sub-Saharan African mainland, shows a homogeneous nuclear gene pool in the whole area – a pattern consistent with the abovementioned findings for cave dwelling bats. However, at the same time two mitochondrial lineages are distinguishable within the areal. Historical climatic changes of the Quaternary are suggested to cause this situation as extreme conditions could have separated them for a period of time and the subsequent post-glacial climate stabilization could have allowed them to reunite (Stříbná et al., under review). The glacial cycles imposed repeated shifts of suitable habitat that has led to recurrent allopatry promoting differentiation and speciation events for many species (Hewitt, 1999, 2000 & 2004). The formation of evolutionary centres together with isolation of central and western forest blocks from the eastern forested mountains by increased aridification could have separated the originally cohesive population of *R. aegyptiacus*. One of the haplogroups is generally present at higher altitudes that may signify an association of adaptive traits coded in mitochondrial DNA with this environmental factor. Several authors have recently described such relationship (Zhang et al., 2017; Ma et al., 2015; Gu et al., 2012; Luo et al., 2012).

In contrast, *R. aegyptiacus* inhabiting the Middle East shows a structured, geographically localized pattern in nuclear genes indicating more recent formation of subpopulations. Such differentiation may be explained by the complex geomorphology of the region where stretches of unsuitable habitat represent barriers to gene flow and promote insular effects.

Spatial activity, navigational skills and foraging strategies have been thoroughly studied on *Rousettus aegyptiacus*. GPS or radiotelemetry tracking was employed to gain data on behaviour of this species and to make comparisons in relation to different seasons, sexes or experiments. The distance they travel from their roost to a feeding site was found similar in Cyprus, Israel and the north of South Africa (11–27 km), while smaller was observed in the southern tip of South Africa (1.5–6.9 km) (Andrianaivoarivelo et al., 2011, Centeno-Cuadros et al., 2017, Lučan et al., 2016; Tsoar et al., 2011; Barclays & Jacobs, 2011; Jacobsen et al., 1986). On Cyprus, in the almost northernmost population, differences in feeding habits were noticed between summer and winter seasons, pointing out the lack of food resources and plant diversity in summer leading to increased spatial activity and worse body condition in this period of the year (Lučan et al., 2016). Travels connected with food search also vary according to the surrounding setting. Neural activity differences were found in relation to whether Egyptian fruit bats lived in natural habitats or in human settlements (Dashevsky et al., 2017). Extraordinary navigational skills were discovered under experimental conditions that proved these mammals orientate by a visual-based navigation with multiple distant landmarks and are able to find their way from unfamiliar places (Tsoar et al., 2011). All these findings may help to explain the background of the geographical range of the species and namely its colonization of insular habitats.

Island biogeography

From the ongoing and increasingly precise studies of the animals inhabiting islands around the world, several principles have emerged for the insular taxa. Firstly, both dispersal and vicariance processes play their role in the settlement on oceanic islands despite their, often long, distance from the continents. Secondly, the genetic divergence linked to the geographical structure of an archipelago varies largely even among similar organisms like bats (Heaney et al., 2005; Hisheh et al., 1998). Thirdly, lineages on sets of islands frequently diversify, which leads to high endemism and species richness. Fourthly, the age of an island may be used to predict the phylogeny of its residents, as young islands often inhabit younger species than geographically closer but older islands. Fifthly, island biota is typified by persistence at least as much as extinction. And sixthly, the colonization direction does not always go towards an island; also continents are recolonized in several documented cases (Heaney, 2007).

Fruit bats of the genus *Rousettus* inhabit many islands within the tropics and subtropics of the Old World. Although not all these isolated populations are considered as independent taxa, insular rousettine bats very often differ from their mainland relatives to certain level. The population inhabiting Cyprus shows a decline in the genetic variation compared to its coastal relatives and the same applies to Saharan oases surrounded by desert, thus the oases are acting as inland islands (Hulva et al., 2012). The whole Middle Eastern region is characterised by shallow divergences supporting its recent colonization from African lineages (Benda et al., 2012; Stříbná et al., under review). In the Gulf of Guinea two islands are populated by *R. aegyptiacus* that are considered two separate sister subspecies *R. a. tomensis* (São Tomé) and *R. a. princeps* (Príncipe). The two taxa originate from mainland African population of *R. aegyptiacus* (Juste et al., 1996; Stříbná et al., under review). Deeper phylogenetic divergence lays between African and Indian ocean Rousettes as separate sister species have been recognised on Madagascar and Comoro Islands (*R. madagascariensis, R. obliviosus* respectively). Alternatives of colonization routes of the islands were discussed without definite conclusion in several papers (Almeida et al., 2016; Goodman et al., 2010). In southeast Asia, two endemic species inhabit Sulawesi (*R. linduensis*, *R. celebensis*), an island with high level of endemism. Another species lives in the Philippines, which, comprising over 700 islands, is a biodiversity hotspot. On these tropical islands, the origin of the genus may be placed since *R. amplexicaudatus* from the Philippines occupies the most basal branch in all phylogenies published to date (Almeida et al., 2016; Giannini & Simmons, 2005; Juste et al., 1999; Stříbná et al., under review). Unfortunately, no comparable genetic data are available for the two aforementioned species. On Malaysian and Indonesian islands lives *Rousettus spinalatus*, a vulnerable species with declining population trend as cave disturbance and forest loss takes place in the vicinity of its home area (Francis et al., 2008). Island endemic bats are significantly more threatened than broadly distributed species especially due to higher vulnerability towards anthropogenic threats, such as invasive species, land-use intensification, climate change, and overharvesting (Conenna et al., 2017; McCreless et al., 2016). From the four *Rousettus* species two are considered vulnerable, one near threatened and one has an unknown status due to data deficiency and their population trend is decreasing or unknown according to the IUCN Red List of Threatened Species (http://www.iucnredlist.org/).

In addition, the Cypriot population of *R. aegyptiacus* has a severely declining abundance for reasons mainly attributed to climatic extremes and negative effects of human activity (Lučan et al., 2016; Lučan et al., in preparation). In contrast with their insular relatives, widely distributed *Rousettus leschenaultii* and *Rousettus aegyptiacus*, the former found in South Asia, southern China and Southeast Asia and the latter from southern Turkey to South Africa, maintain a stable population trend.

Conservation status

Since fruit bats play a key role in pollination and seed dispersal, they provide irreplaceable ecological services to their environment and thus persistent bat populations are desirable (Shilton et al., 1999; Jenkins & Racey, 2008; Andrianaivoarivelo et al., 2011). Although for experts the need for their conservation is well known and obvious, for local communities living in proximity of bat roosts or foraging sites the immediate nutritional and economic potential of these animals may be much more important. Fruit bats are frequently hunted for bushmeat (Fig. 5) and served for example in restaurants in Madagascar, where hunting seasons are determined, but widely ignored and an unsustainable hunting practice, high offtake and the use of firearms becomes a serious threat (Jenkins & Racey, 2008).

Fig. 5 The results of a daily hunting trip to a cave roost of *Rousettus madagascariensis* on Nosy Boraha, Madagascar (Jenkins & Racey, 2008, Photo: F. H. Randrianandrianina).

Another explanation for the eradication of fruit bat colonies is the crop protection efforts of local people, which also occurs in other parts of the world (Andrianaivoarivelo et al., 2007; Albayrak et al., 2008). A further markedly discussed topic is the recognition of bats as hosts for pathogens that affect humans and livestock. Researchers have shown that bats carry potentially zoonotic viruses including lyssa-, corona-, henipa-, and filoviruses. Conservation strategies for these mammals thus need to consider and unite several contradictory factors. Deliberate human-bat interactions may be decreased with the help of community-level education respectful of ecological as well as local spiritual and traditional issues. Where hunting is allowed, cooperation among local people, government and scientists is needed and the protection of roost sites together with the prohibition of firearms may lead to success (Anti et al., 2015; Leroy et al., 2005; Pourrut et al., 2009; Hassanin et al., 2016). In the Mediterranean region, the revaluation of introduced plants as alien at least in the vicinity of fruit bats was suggested together with the proposal of artificial water sources construction (Lučan et al., 2016).

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Annex I.

Journal of Biogeography

PAN-AFRICAN PHYLOGEOGRAPHY OF ROUSETTINE FRUIT BATS: PLEISTOCENE REFUGIA, HOLOCENE PANMIXIA, AND ISLAND EVOLUTION

Journal:	*Journal of Biogeography*
Manuscript ID	Draft
Manuscript Type:	Research Paper
Date Submitted by the Author:	n/a
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Key Words:	Rousettus, Pleistocene, forest refugia, island evolution, fruit bats, mitochondrial DNA, nuclear microsatellites

PAN-AFRICAN PHYLOGEOGRAPHY OF ROUSETTINE FRUIT BATS: PLEISTOCENE REFUGIA, HOLOCENE PANMIXIA, AND ISLAND EVOLUTION

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Abstract

Aim: The impact of Pleistocene climate oscillations on tropical biomes is associated with changes to the extent of available forest cover. Fruit bats, which are generally forest;dependent, represent keystone taxa of Old World tropical forests, thus they presumably participated in forest expansion during interglacial periods through their roles in pollination and seed dispersal. To contribute to an understanding of African biogeography, we aim to assess the population structure of the genus *Rousettus* and infer the role of historical versus environmental factors in explaining current phylogenetic and phylogeographical patterns.

Location: Afrotropical, Madagascan and Saharo-Arabian biogeographic realms

Taxon: Genus *Rousettus* (Pteropodidae)

Methods: Twenty nuclear microsatellite loci were genotyped and two mitochondrial regions were sequenced. The data were processed using modern approaches within population genetics and phylogeography.

Results: The Asian origin of the genus *Rousettus* was confirmed and the importance of the western Indian Ocean region for the origin of African radiation was indicated. We found a homogenous nuclear gene pool across the African mainland but with two mitochondrial haplogroups having different demographies. Haplogroup I is widespread in all regions with tree cover and represents the ancestral mitochondrial lineage for populations in the Mediterranean region and on islands in the Gulf of Guinea. Haplogroup II is found only in eastern and southern Africa and Senegal, distributed in deciduous forests.

Main Conclusion: The observed patterns indicate periods of Pleistocene allopatry, probably in glacial refugia in the Congo basin and/or Upper Guinea and in eastern Africa. The group’s high dispersal abilities led to interglacial panmixia on the continent; however, this was likely limited by female philopatry. The patterns observed in the Middle East are consistent with a colonization event during a pluvial climate window and the Sahara pump hypothesis. Data at different scales shows an important role of insular effects. Due to the importance of rousettine bats for forest recovery and their ability to pioneer dry and distant habitats, they are proper object of the fields of conservation biology and restoration ecology.

Key words: *Rousettus*, Pleistocene, forest refugia, island evolution, fruit bats, mitochondrial DNA, nuclear microsatellites.

1. INTRODUCTION

While the phylogeographic patterns of different organisms of the northern hemisphere temperate zone are well studied, less is known about the impact of Plio-Pleistocene climatic oscillations at lower latitudes. In the north, glaciation and cyclic changes to the proportions of forested and open habitats, which resulted in alternation of allopatric and sympatric periods promoting speciation, are considered the main drivers of different proposed phylogeographic scenarios (Hewitt, 2000, 2004), but the effects of Pleistocene climate oscillations in the tropics are still rather puzzling. The idea of the retreat and fragmentation of tropical rainforest because of a drier climate during glacial periods, which resulted in the periodic formation of forest refugia separated by xeric habitats, was proposed as a possible mechanism for speciation in lowland species (Haffer, 1969). This concept became the object of considerable debate as empirical biogeographic patterns observed in the tropics are complex, the ecological characteristics of particular species are important, and many other factors could also potentially play a role (e.g., Nores, 1999). For example, the altitudinal range shifts in high mountain systems may result in periodic range expansion and the contraction of mountain species or ecotypes serving as a “species pump” for lowland areas (Fjeldså, 1994). With regard to the Pleistocene forest dynamics and refugia hypothesis, the Amazonian forest has attracted most attention (e.g., Lessa, Cook, & Patton, 2003). In the Old World, Africa, with a large forest mass situated on the equator, represents the most important continental generator of evolutionary novelty such as radiations of large mammals and humans. The alternation of large mammal communities dominated by grazers during glacial periods versus browsers and mixed feeders during interglacial periods (Klein, 1980) is one source of evidence for alteration in the proportion of forest versus open habitats during the Pleistocene. More recently, molecular tools have identified glacial forest refugia in the Afrotropics for some taxa such as rainforest trees or rainforest dwelling vertebrates (e.g., Anthony et al., 2007, Born et al., 2011).

With regard to the illumination of phylogeographic scenarios in Africa, species that have evolved in co; evolution with tropical trees, and thus play a keystone role in forest ecosystems and actively impact the forest dynamics, are especially interesting. One such group is fruit bats; represented in the Old World tropics by the family Pteropodidae. These animals are largely fruit eating (in some cases nectar, pollen, flowers and leaves), are capable of flying considerable distances and they possess apomorphies associated with pollination (chiropterophily) and seed dispersal (chiropterochory) for an appreciable number of plant species (e.g., Shilton, Altringham, Compton, & Whittaker, 1999; Raghuram et al., 2011).

The biogeography of Pteropodidae has been addressed in the last few decades by many authors (e.g.: Hollar, & Springer, 1997; Bergmans, 1997; Juste et al., 1999; Romagnoli, & Springer, 2000; Giannini, & Simmons, 2003, & 2005; Almeida, Giannini, DeSalle, & Simmons, 2011; Almeida, Giannini, & Simmons, 2016; Hassanin et al., 2016). The African fruit bats form four separate branches within the Pteropodidae tree, pointing to successive colonization events of Africa from the Asian ancestor lineages (Almeida et al., 2016). The genus *Rousettus* has characteristics of a unique evolutionary history, placed as a sister to Epomophorinae + Stenonycterini + Plerotini clade as the sole member of the tribus Rousettini that diverged from their common ancestor in the Middle Miocene (Giannini, & Simmons, 2005; Almeida et al., 2011, & 2016; Hassanin et al., 2016).

This group has exceptional dispersal ability and overcomes with relative ease different geographical barriers such as mountains, deserts, climatic clines or open water. Their ability to echolocate, largely unique in fruit bats, allows them to use caves for day;roost sites rather than having to use the foliage of trees. In comparison to other fruit bats, rousettines are hence not strictly forest;dwelling and readily cross and colonize areas with non-continuous canopy cover. As a consequence, *Rousettus* is the fruit bat genus with the broadest distribution, comprising the Oriental, the Afrotropical and the Saharo-Arabian biogeographical realms (Juste et al., 1999). The colonization of Africa by *Rousettus* is believed to have occurred only once in the late Pliocene/early Pleistocene and the specific period of this event being between 2.6 and 1.7 Mya, depending on the analyses from different studies (Almeida et al., 2016; Hassanin et al., 2016). Repeated paleontological records of a pteropodid morphologically similar to extant *Rousettus* in the Early and Middle Miocene cave deposits in southern France (see e.g. Aguilar et al., 1986) support a hypothesis that the Neogene range of the genus included also most of the Mediterranean region and the Middle East. The effects of the Quaternary climatic deterioration probably led that population to extinction as genetic data on the extant Mediterranean population show no sign of survival of ancient clades in the region (Hulva et al. 2012).

According to the latest taxonomic studies, the genus encompasses eight species (*R. amplexicaudatus, R. celebensis, R. linduensis, R. leschenaultii, R. spinalatus, R. madagascariensis, R. obliviosus* and *R. aegyptiacus*), four of which are endemic to islands in Indonesia (*R. linduensis, R. celebensis*) and the western Indian Ocean (*R. madagascariensis, R. obliviosus*). Two subspecies of *R. aegyptiacus* have been identified on islands in the Gulf of Guinea (*R. a. tomensis* and *R. a. princeps*) and four subspecies have been described in Africa and the Middle East (*R. a. aegyptiacus, R. a. arabicus, R. a. unicolor, R. a. leachii*). The former subspecific classification within the Middle East was later rejected in the original sense (Hulva et al., 2012). Both *R. bidens* and *R. lanosus*, previously included in the group (as summarised in Bergmans, 1994; Simmons, 2005), were later reclassified as two unrelated genera on the basis of molecular analyses (Giannini, Almeida, & Simmons, 2009; Nesi et al., 2013).

The internal nodes of the genus have been assessed in many studies with differing representation of species and subspecies (e.g.: Giannini, & Simmons, 2003, & 2005; Juste, Machordom, & Ibáñez, 1996; Juste et al., 1999; Juste, López;González, & Strauss, 2001; Guan et al., 2006; Goodman, Chan, Nowak, & Yoder, 2010; Hassanin et al., 2016; Almeida et al., 2011 & 2016). Generally, Asian species have been recognized at the base, with *R. amplexicaudatus* occupying the most basal branch, *R. spinalatus* the second and with *R. leschenaultii* following as a sister species to the African and Indian Ocean species. To date, the internal relationships within the latter group remain unresolved as there is little support for the constructed phylogenetic trees. There has been no reported study conducted on the phylogenetic position of the remaining two Indonesian species.

Using fast evolving molecular markers, we aim to describe the population structure of rousettine fruit bats in the Afrotropical, Madagascan and Saharo-Arabian realms, resolve the relationships of recognized species, provide the biogeographical context of their evolution, and describe the phylogeographic scenario associated with their Pleistocene history.

2. MATERIAL AND METHODS

Sampling and DNA assays

Samples of 388 individual *Rousettus* were assembled and analysed from 120 localities across the range of this genus (Fig. 1). Biopsies and cells from buccal swabs were preserved in a 96% ethanol or EDTA solution. The purification of total genomic DNA was performed in accordance with standard protocols with QIAGEN DNeasy Blood & Tissue Kit (QIAGEN Ltd) or Macherey Nagel NucleoSpin® Tissue Kit (MACHEREY;NAGEL GmbH & Co.). Twenty microsatellite loci were genotyped using the primers and procedures detailed in Hulva et al. (2012). Allele calling was executed manually using the program GENEMARKER v.1.85 (www.softgenetics.com). Allele binning was performed by the FLEXIBIN program (Amos et al., 2006) to avoid errors in identifying indistinct allele class boundaries. Primers and conditions specified in Table S1.1 (Appendix S1 in Supporting Information) were used for the amplification of mitochondrial DNA fragments of cytochrome *b* gene (cyt *b*; 964 bp) and hypervariable domain II of control region (CR; 307 bp). The amplicons were purified using the QIAquick Gel Extraction Kit (QIAGEN Ltd.) and sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit and capillary electrophoresis on a 3130 Genetic Analyzer (Applied Biosystems). Sequences were assembled and edited using GENEIOUS 9.0.5. (http://www.geneious.com, Kearse, et al., 2012) and deposited in GenBank (see Data accessibility). Data were combined with sequences from GenBank and the final alignment was made using the G;INS;i method in MAFFT version 7 (Katoh & Standley, 2013).

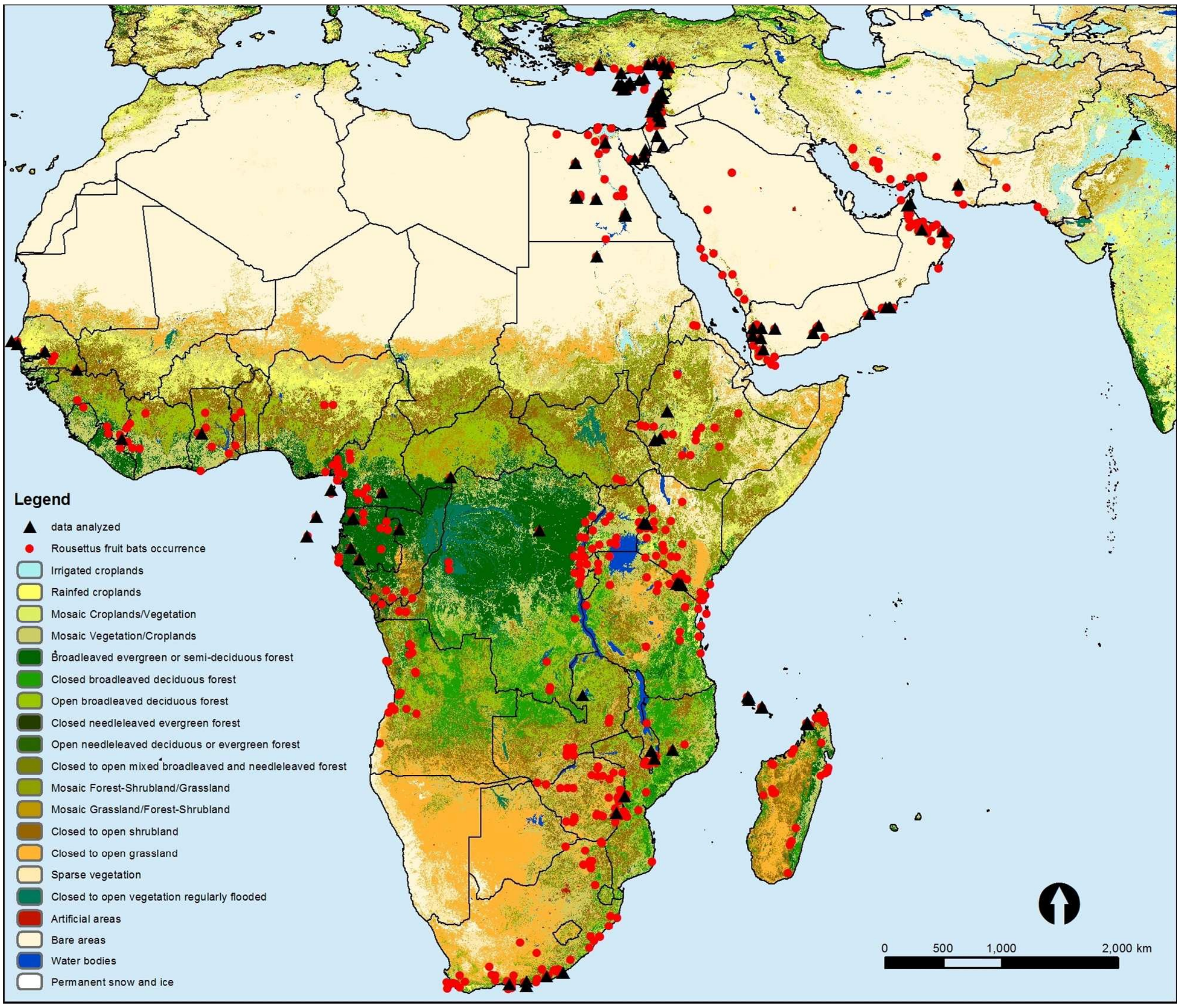

Fig. 1 Map displaying the distribution of *Rousettus* bats in the Afrotropical, Madagascan and Saharo-Arabian realms on a background based on land cover data (ESA Climate Change Initiative). Black triangles indicate the localities with genetic data analysed (generated in the present study, Hulva et al. 2012 and Hassanin et al., 2016) and red dots indicate the localities from other studies (Bergmans, 1994; Monadjem, Taylor, Cotterill, & Schoeman, 2010; Goodman et al., 2010, Benda et al., 2012).

Data analyses

Microsatellites

The occurrence of any genotyping errors was tested for using a Monte Carlo simulation of expected allele-size differences by MICRO;CHECKER 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). The data were scanned in CERVUS 3.0 (Kalinowski, Taper, & Marshall, 2007) for possible matching genotypes (identity analysis). Individual based Bayesian clustering analyses were performed for a dataset of 281 individuals from across the range of *Rousettus* and subsequently were limited to continental Africa. Each analysis was run 10 times for each number of population K (K values from 1 to 13) for 1,000,000 iterations with 100,000 burn;in steps using the admixture model with correlated allele frequencies in STRUCTURE v2.3.2 (Pritchard, Stephens, & Donnelly, 2000; Falush, Stephens, & Pritchard, 2007). The resulting membership coefficients (q values) from each run were averaged using the Greedy algorithm of CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007) and were graphically visualised in software DISTRUCT v1.1 (Rosenberg, 2004). The allelic patterns expressed by observed heterozygosity, expected heterozygosity, inbreeding coefficient, gene and allelic diversity, and allelic richness across populations retrieved from the Structure program analysis were calculated by GENEALEX 6.503 (Peakall & Smouse, 2006 & 2012). Genetic differentiation between particular clusters, measured using pairwise F_{ST} according to Weir & Cockerham, was calculated in FSTAT 2.9.3.2 (Goudet, 1995). The allelic patterns across large population samples (more than nine individuals) were compared in GENEALEX 6.503 (Peakall & Smouse, 2006 & 2012).

Sequences of mitochondrial DNA

To visualize the phylogenetic patterns, relationships among haplotypes based on 1058 bp long concatenated sequences were displayed using the Median-Joining network (Bandelt, Forster, & Röhl, 1999) in NETWORK 5.0.0.1 (www.fluxus;engineering.com). To reconstruct deep nodes among studied taxa, Bayesian inference in the MRBAYES v3.2.6 program (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) was used. The dataset involved cyt *b* data to avoid the occurrence of homoplasies in the fast evolving control region. The SYM+G model of evolution was selected by Akaike criterion in the MRMODELTEST 2.3 program (Nylander, 2004). The program was run for 82.2x10^6 generations until the standard deviation of split frequencies was below 0.01, sampled every 100000 generations with burn-in of 25%.

The descriptive parameters of cyt *b* gene sequence polymorphism of clusters retrieved from the Network analysis (the number of haplotypes Nh, haplotype diversity h, Tajimas D, Fu and Li's D*, Fu and Li's F*, and Fu's Fs statistic) were computed in the DNASP 5.10.0.1 software (Librado & Rozas, 2009). Distance analysis as one of the primary approaches to analysing sequences was applied using p;distances of aligned cyt *b* gene sequences in MEGA7 (Kumar et al., 2016).

Range mapping

The distributional data of the *Rousettus* fruit bats were compiled from our genetic sampling and published studies (Benda, Vallo, Hulva & Horáček, 2012; Bergmans, 1994; Monadjem et al., 2010; Goodman et al., 2010; Hassanin et al., 2016). Current land cover data derived from the ESA Climate Change Initiative ; Land Cover (2017) were generalized into 16 classes to express a given species affinity to particular habitats. Moreover, the concept of anthropogenic biomes (Ellis & Ramankutty 2008) was used in order to evaluate the potential human impact on habitats inhabited by the *Rousettus* spp.

3. RESULTS

Analyses of nuclear microsatellite loci

The nuclear dataset contained 387 genotypes (including 194 from Hulva et al., 2012) and our microsatellite loci exhibited between 15 and 39 alleles. One to three loci showed homozygote excess. Results from Bayesian genotype clustering for K=2 to K=11 are presented in Fig. 2. The dataset bifurcated into clusters including Mediterranean and Middle Eastern *R. aegyptiacus* individuals and the rest of the genotypes at K=2. Between K=3 and K=11, *R. obliviosus*, *R. amplexicaudatus*, *R. leschenaultii*, *R. madagascariensis* and insular sub;species of *R. aegyptiacus* (*R. a. tomensis*, *R. a. princeps*) populations gradually became separated. Samples including sub;Saharan continental African localities and Bioko Island formed one cluster when analysed both as part of the whole dataset and separately (Fig. S1.1).

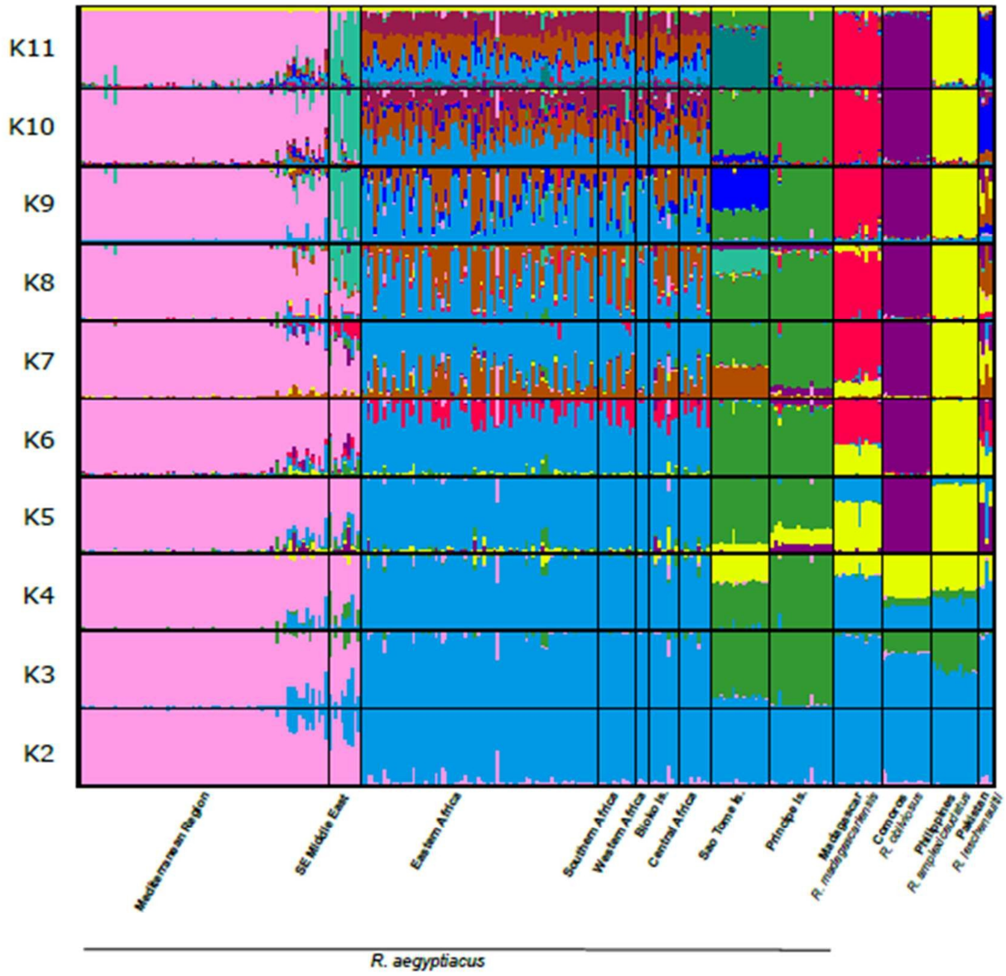

Fig. 2 The population structure of *Rousettus* fruit bats from Africa and adjacent regions estimated from Bayesian clustering. Estimates for K=1 – 11 are based on data from 20 microsatellite loci for 281 individuals. Cluster membership coefficients (*q*) creating each individual's line are distinguished by colours.

Population genetic descriptive characteristics of distinct clusters are presented in Table 1, allelic patterns across large populations (more than nine individuals) are plotted in Appendix S1, Fig. S1.2, and pairwise F_{ST} values are in Table S1.2. Populations from the Philippines and Madagascar show similar gene and allelic diversity and allelic richness to one another as do the sub;Saharan continental and the Bioko Island populations (Gulf of Guinea). Populations from isolated islands and the Middle East show the lowest indices. Allelic patterns are similarly lower for the Middle Eastern populations when compared to island or sub-Saharan continental African populations.

Table 1 The descriptive characteristics of mitochondrial and nuclear diversity of *Rousettus* bats. The main clusters retrieved from Median;Joining Network and Structure analysis are present. Ng – number of individuals genotyped; h – gene diversity; A – allelic diversity; AR – allelic richness; HO – observed heterozygosity; He – expected heterozygosity; F_{IS} – inbreeding coefficient; Ns – number of individuals sequenced; Hd – haplotype diversity; Nh – number of haplotypes.

	Nuclear DNA (microsatellite) diversity							**Mitochondrial DNA (cyt *b*) diversity**						
Region	Ng	h	A	AR	Ho	He	F_{IS}	Ns	Hd	Nh	Tajimas D	Fu and Li's D*	Fu and Li's F*	Fu's Fs statistic
The Mediterranean (*R. aegyptiacus*)	76	0.703	9.8	1.7026	0.65	0.7	0.08	10	0.778	4	0.14316	;0.33833	;0.24721	;0.077
Southeastern Middle East (*R. aegyptiacus*)	10	0.6924	5.65	1.6867	0.59	0.65	0.15							
Africa – *haplogroup I* (*R. aegyptiacus*)	108	0.8831	16.6	1.8826	0.78	0.88	0.12	34	0.986	28	;1.81124*	;1.43177	;1.84667	;21.455
Africa – *haplogroup II* (*R. aegyptiacus*)								10	0,978	9	;0,10381	;0,51176	;0,46262	;1,987
São Tomé Island (*R. a. tomensis*)	18	0.7751	6.85	1.76	0.66	0.73	0.15	16	0.714	6	;1.75814	;1.73679	;1.99708	;2.05
Principe Island (*R. a. princeps*)	20	0.6959	6.9	1.6623	0.58	0.63	0.12	19	0.865	8	;0.64796	;0.54628	;0.66359	;3.135
Madagascar (*R. madagascariensis*)	15	0.89	10.2	1.8877	0.83	0.86	0.07	14	0.978	12	;1.49578	;1.86275	;2.02209	;5.908
The Comoros (*R. obliviosus*)	15	0.7018	6.6	1.7007	0.67	0.68	0.05	13	0.923	8	;0.77313	;0.66416	;0.79179	;0.861
The Philippines (*R. amplexicaudatus*)	15	0.8804	8.35	1.8802	0.6	0.7	0.21	10	0.978	9	;0.92892	;1.10655	;1.19847	;2.294
Pakistan (*R. leschenaultii*)	4	0.7959	4.4	1.7567	0.59	0.66	0.26	4	1.000	4	;0.1297	;0.1297	;0.13174	;0.095

Analyses of mitochondrial DNA sequences

Sequence data were obtained from 130 individuals. A Median;Joining haplotype network and a Bayesian tree (Fig. 3, Fig. 4) show a similar subdivision to the nuclear markers, with one major exception: the animals from sub;Saharan Africa, which form a homogenous nuclear cluster, are separated into two haplogroups. One individual from the São Tomé Island in the Gulf of Guinea clusters closer to continental haplogroups, while bats from other islands (Príncipe, Madagascar, the Comoros, the Philippines) form separate and distinct clusters (see Fig. 3). The geographic placement of the haplogroups is mapped in Fig. 5.

The descriptive characteristics of haplogroups retrieved from the haplotype network are summarized in Table 1. No haplotypes are shared among individuals from sub;Saharan continental Africa and Bioko Island, whereas one to five haplotypes are shared on islands surrounding Africa. Intra and inter;population p;values ranges based on cyt *b* sequences are summarized in Tab. S4.

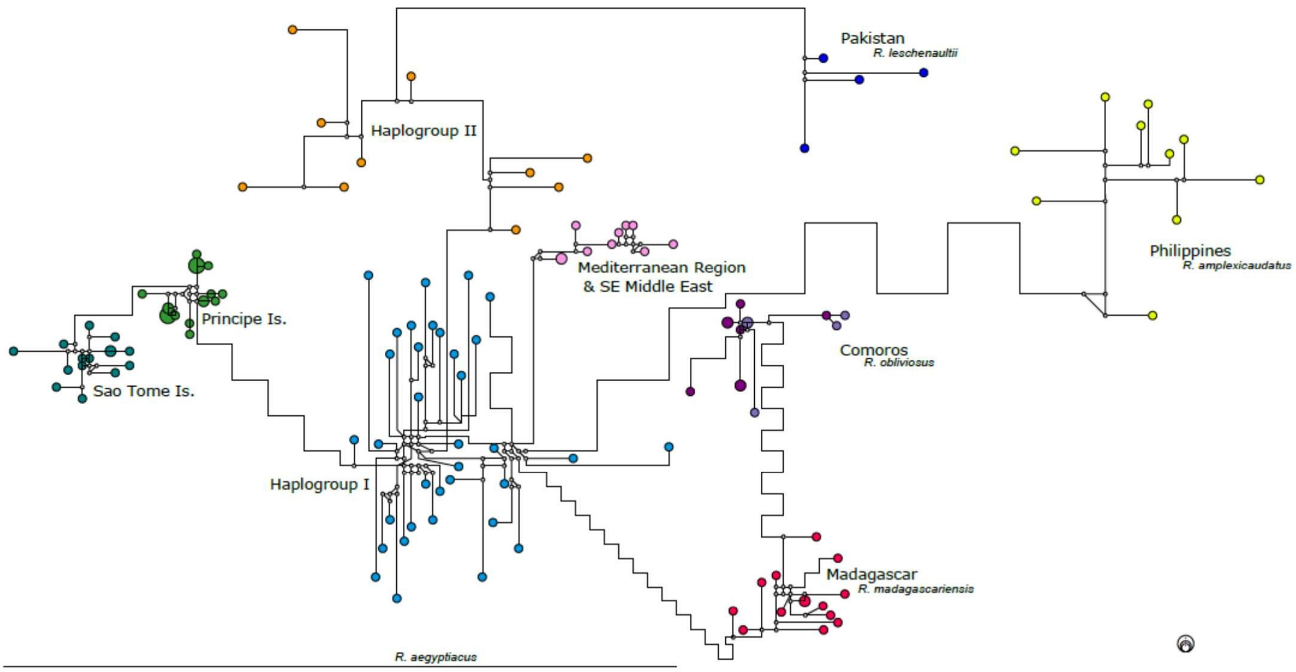

Fig. 3 The Median;Joining haplotype network of the mitochondrial sequences of *Rousettus* fruit bats. Lines between haplotypes are proportional to the number of mutations between them, colour codes denote particular haplogroups. Two colours in the haplogroup from the Comoros distinguish two islands of the archipelago (dark violet – Grande Comore, pale violet – Anjouan).

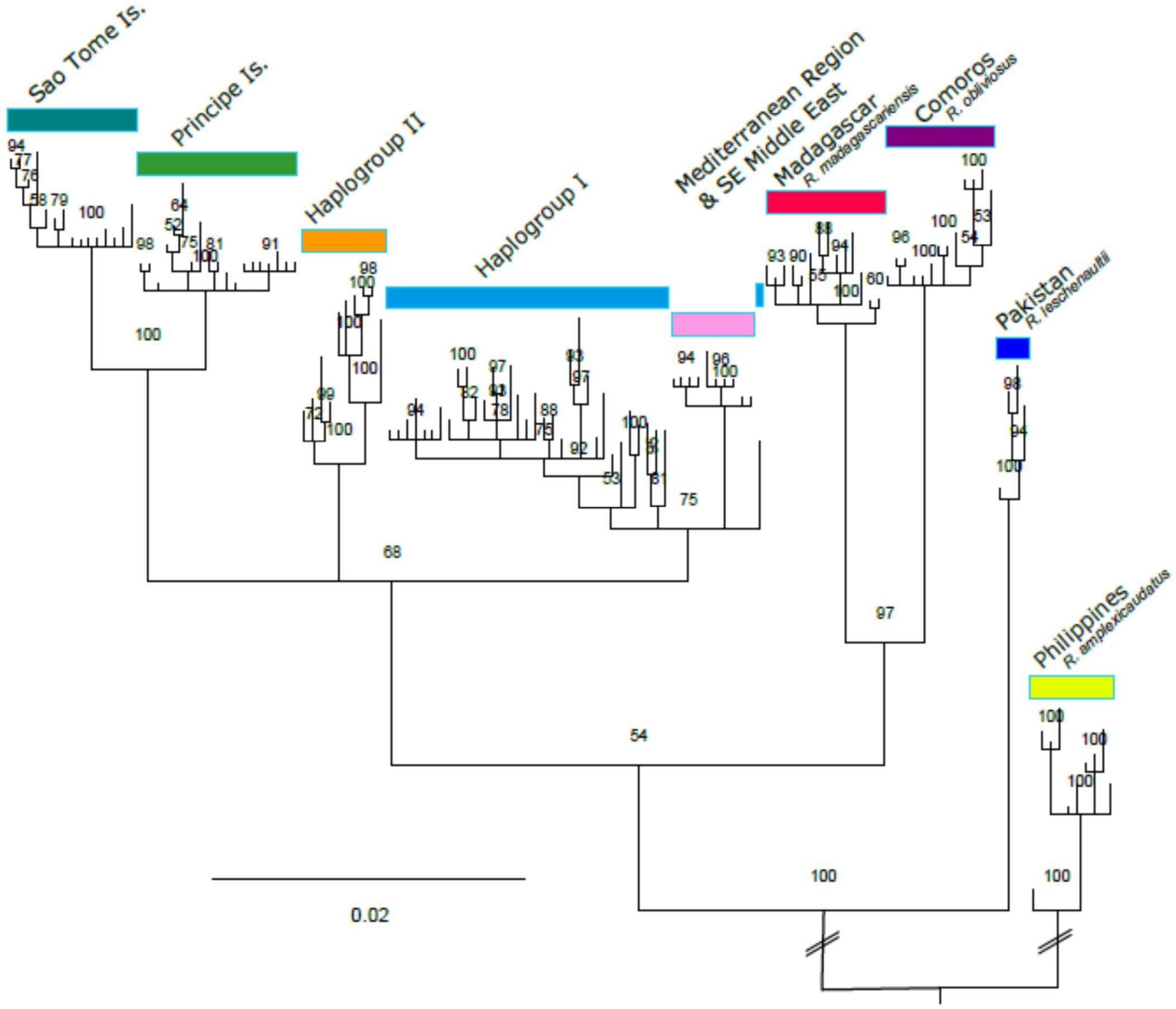

Fig. 4 A phylogenetic tree of rousettine bats obtained by Bayesian inference.

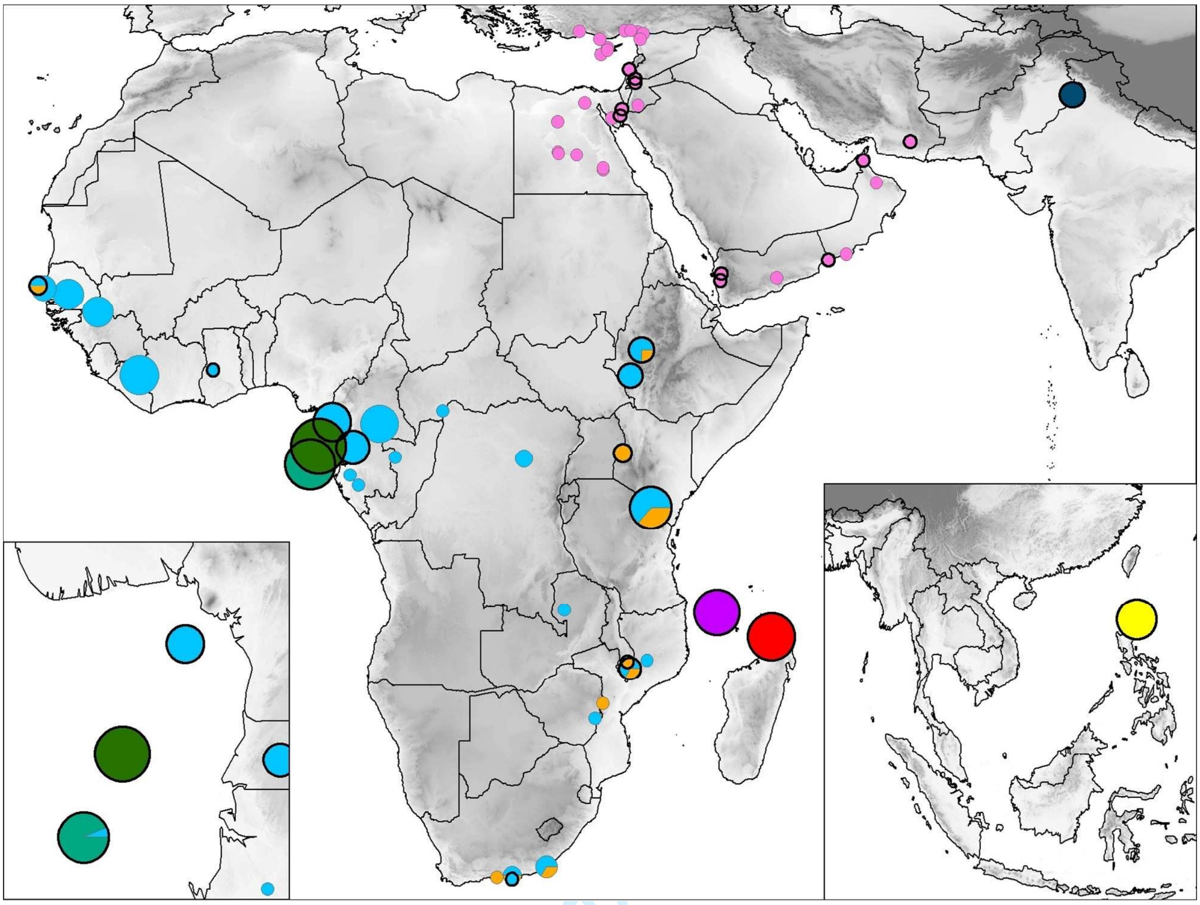

Fig. 5 The diversity of *Rousettus* fruit bats mtDNA displayed on a map with altitude shading. The pie chart colour codes correspond to haplogroups from Fig. 3. The number of individuals from each locality is reflected in the chart size. Those charts enclosed by dark circles show data from both cyt b and CR sequences, and those charts without enclosed circles show only cyt b (Hassanin et al., 2016) or CR sequences (present study and Hulva et al., 2012).

4. DISCUSSION

Phylogenetic relationships and Pleistocene differentiation

The present results indicate the distant position of Asian *R. amplexicaudatus* and *R. leschenaultii* from the African rousettine lineages, which confirms previous studies (Juste et al., 1999, Almeida et al., 2016) and indicates the Asian origin of the genus *Rousettus*. The basal position of the western Indian Ocean *Rousettus* (*R. madagascariensis* and *R. obliviosus*) within the African radiation suggests this region's role in the biogeography of the group. The mainland populations confirm the signature of a split into two haplogroups (Benda et al., 2012, Hassanin et al., 2016). Haplogroup I has a broad distribution across sub;Saharan Africa over diverse biomes, and is the only variant occurring in broadleaf evergreen rainforests. It is also a probable source for the colonization of the Mediterranean region and the islands in the Gulf of Guinea (Fig. 3 and Fig. 4). The distribution of haplogroup II covers eastern and southern Africa and probably also the belt south of Sahel (explaining the occurrence in Senegal), which corresponds with the preference for broadleaf deciduous open forests.

The fact that this pattern is not mirrored by fast;evolving microsatellite markers indicates the complex demographic history of the species. When considering the p;distance of 2;4% in cytochrome *b* and the distribution of the two lineages, we have hypothesized that they evolved in allopatry in different forest refugia during cold periods of the Pleistocene followed by the expansion and merging of both lineages during warm periods, leading to the admixture of nuclear gene pools and partial overlap of ranges of the mitochondrial haplogroups. The occurrence of two variants of mtDNA overlaid on a homogenous nuclear background has been observed in a variety of organisms, including bats (Hulva et al., 2010; Navajas, Lagnel, Gutierrez, & Boursot, 1998; Melnick & Hoelzer, 1991; Brown Gladden, Ferguson, Friesen, & Clayton, 1999). The present spatiotemporal distribution of both *R. aegyptiacus* haplogroups is consistent with the observation that Pleistocene lineages often evolve due to peripatric evolution on the edges of main rainforest blocks or on adjacent islands, in contrast with sister lineages in lowland rainforest that are frequently of pre;Pleistocene age (Fjeldså & Lovett, 1997), and corroborates the hypothesis of glacial allopatry between forest refugia in the Congo basin and/or Upper Guinea (haplogroup I) and in eastern Africa (haplogroup II) (Maley, 1996). Similar observations have been made in Peter's dwarf epauletted fruit bat (*Micropteropus pusillus*; Hassanin et al., 2016). The topographic complexity of eastern Africa, in association with the respective eco;climatic properties, supported the role of biodiversity refuge and speciation hotspot of many organisms, including humans (Liu, Prugnolle, Manica, & Balloux, 2006). For example, orographic rain and mist may have been key factors and could have enabled the persistence of forests, as water is a critical limiting factor during dry glacial periods (Elias, 2013). High altitudinal range may also have facilitated the altitudinal shifts of the respective ecosystems during climate oscillations (Schmitt, 2009). The range of haplogroup II is typical for many African mammals occurring in habitats with partially open land cover, including oribi (*Ourebia ourebi*), bohor reedbuck (*Redunca redunca*) or common waterbuck (*Kobus ellipsiprymnus*) among large herbivores, or the yellow;winged bat (*Lavia frons*), the Egyptian slit;faced bat (*Nycteris thebaica*), the Midas free;tailed bat (*Mops midas*), Schlieffen's bat (*Nycticeinops schlieffenii*) or the rusty pipistrelle (*Pipistrellus rusticus*) among bats (Kingdon et al., 2013). Such an ecological valence indicates that during some stages of dry periods the extent of the open landscapes (and thus the range of their inhabitants including rousettes of haplogroup II) could have been larger; this is consistent with the recent glacial relict type of distribution and species pump hypothesis.

Holocene panmixia on the African mainland

The uniformity of nuclear variation and the broad distribution and partial sympatry of mitochondrial haplogroups characterize continent;wide panmixia in *Rousettus aegyptiacus* (Peel et al., 2013); this is likely consistent with seasonal movements and a dispersal capacity connected with a cave roosting strategy (Chen et al., 2010). For example, physical barriers such as the Cameroon volcanic line or the Niger Delta have probably resulted in population differentiation in several fruit bat species (Nesi, et al., 2013) but do not affect the population structure in *R. aegyptiacus*. A homozygote excess observed in one to three loci is probably related to a weak Wahlund effect, namely the presence of related individuals obtained during sampling at roosting sites and inbreeding (reflected also by positive values of F_{IS}; Table 1). Considering the large extent of the study area, we have presumed the role of these factors rather than this being an observation bias caused by null alleles (cf. Pilot, et al., 2006). A prerequisite for the spatial restriction of the haplogroup II range when compared to the presumed panmixia of nuclear loci is the female philopatry in this species, which is a pattern observed in many mammals including bats (e.g., Hulva et al., 2010). Several other factors could have contributed to this. An excess of low frequency polymorphisms in haplogroup I, indicated by negative and significant values of Tajima's D, and strongly negative Fs value (Table 1) point to a recent population expansion. Such asymmetry in the demographic trends of the haplogroups could hypothetically be responsible for the differences in their distribution. A conceivably higher dispersal resistance (McRae, 2006) of a rainforest environment when compared to open habitats could also play a role. Since there is usually a negative relationship between resource availability and displacement distances (Mueller et al., 2011; Tucker et al., 2018), smaller home ranges and thus higher dispersal resistance may be expected in a highly productive rainforest biome with regular fruit production.

Ecotypic variation was referred to in the population inhabiting contrasting biomes in the Middle Eastern part of this species' range (Centeno;Cuadros et al., 2017) and an adaptive reaction cannot be excluded for continental Africa. An association of adaptive traits coded in mitochondrial DNA with environmental factors, particularly altitude, has recently been described (Zhang et al., 2017; Ma, Kang, Chen, Zhou, & He, 2015; Gu et al., 2012; Luo, Chen, Liu, & Yuqi, 2012), and the localities of haplogroup II are generally at higher altitudes in the east African section of its current range. The sub;Saharan east;west differentiation in *R. aegyptiacus* is reflected in traditional sub;specific taxonomy, with the recognition of western *R. a. unicolor* (Gray, 1870) and eastern and southern *R. a. leachi* (Smith 1829) being separated by the 17°E meridian (Bergmans, 1994); however, the current study does not fully confirm this classification. Further studies on genomic, phenotypic and ecological traits are needed in order to reveal details about this divergence and a potential isolation by adaptation (Orsini, Vanoverbeke, Swillen, Mergeay, & De Meester, 2013) in particular populations.

Out of the tropics

The range of *R. aegyptiacus* includes areas with tree cover and generally excludes open biomes such as deserts, grasslands and dry savannahs. Thus, the sub;Saharan range of the species includes a distributional hiatus associated with stretches of savannah in the Dahomey Gap, in southwestern Africa and in the Horn of Africa. However, these distributional gaps do not substantially affect the population genetic structure of the species, which indicates the ability of effective dispersal over open habitats. A consequence of this dispersal capacity includes the colonization of environments with desert and Mediterranean climates. The Middle Eastern populations show a combination of a mitochondrial lineage descendant from African haplogroup I (p;distance of 1.4;3.7%) and a substantial differentiation of microsatellite markers, displaying a separation from the rest of the range already at K=2. This pattern indicates a Pleistocene connectivity between the northern territories and sub;Saharan Africa, possibly during pluvial periods which were characterized by high precipitation and more mesic conditions in what is today the Sahara.

We propose the scenario that the pronounced allopatry evolved after the southward retreat of monsoons, mirrored by marked differentiation of microsatellite markers, consistent with the Sahara pump hypothesis (Van Zinderen;Barker, 1962). The current diet of *R. aegyptiacus* in the xeric biomes of the Middle East relies largely on introduced and agricultural plants (Korine, Izhaki, & Arad, 1999; Centeno; Cuadros et al., 2017). Thus, anthropogenic factors including roosting opportunities in buildings or ruins may substantially modulate the recent distributional patterns of *Rousettus*. The impact of highly populated areas (Fig. S1.2) may potentially also alter the population connectivity in drier regions of Africa; however, further data are needed.

Island evolution

The important impact of insular effects represent a general trend within the genus *Rousettus*, as nested patterns of insular differentiation occur at different levels. For example, Hulva et al. (2012) showed a decline in the genetic variation in *R. aegyptiacus* at a population level from Cyprus and Saharan oases in a study that focused on the Middle Eastern part of their range. The present study shows a differentiation of insular populations on a larger geographic scale indicated by the values of F_{ST} distances and a reduction in genetic variation, indicating the role of insular effects as founder events and genetic drift.

The results from Madagascar (the Gondwana fragment that has been isolated from Africa and India since the Mesozoic Era and which is more recently 400 km from the African mainland) and the Comoros (*in situ* volcanic islands situated 300 km from the African mainland) confirm the pronounced genetic differentiation of resident populations from the continental groups in both mitochondrial (p;distance of 4.7; 8.0% and 6.4;8.2%) and nuclear markers (4% and 10%), corresponding to two separate species (*R. madagascariensis* and *R. obliviosus)*(Goodman et al., 2010).

In a similar manner, the populations from islands formed by the Cameroon volcanic line in the Gulf of Guinea show genetic differentiation. While the population from Bioko Island (32 km from the African mainland, formerly attached) shows no differences from the continental lineage, populations from Príncipe and São Tomé (220 and 280 km from the African mainland, respectively) are differentiated in both types of markers (2.3;5.3% and 15%). This finding is in accordance with previous studies based on allozymes of *R. aegyptiacus* (Juste et al., 1996), as well as with sequencing studies in other fruit bat (Peel et al., 2013) and bird (Melo, Warren, & Jones, 2011) species. However, a single individual was found on São Tomé with a continental haplotype but with a nuclear genotype belonging to an insular cluster. This indicates introgression of continental mitochondrion and that the respective population maintains genetic distinctness despite some level of historical gene flow and admixture with mainland populations.

In general, the present study illustrates that rousettine bats have a relatively simple phylogenetic structure on the African continent with several isolated island lineages. This finding is consistent with the frequent reduction of migratory behaviour in flying vertebrates on islands (Voelker & Light, 2011). An explanation can be seen in species population biology: a combination of an ability for long distance dispersals and a social way of living resulting in frequent successful colonization events on remote islands and trends in the evolution of social tradition and site fidelity on the islands. The situation on Anjouan and the Grande Comore Islands of the Comoros archipelago that show private mitochondrial haplotypes but with an admixture in nuclear loci supports male;biased gene flow. During dry glacial periods, the extent of the island areas was larger when compared to the extent more recently due to the drop in sea levels (Voris, 2000; Sathiamurthy & Voris, 2006) and many islands likely represented forest refugia as a consequence of their more humid oceanic climates in comparison to the climates on large continents like Africa (Fernandez;

Palacios et al., 2011). The above;mentioned factors demonstrate an important role of islands as generators of evolutionary novelty combined with a refugial role which preserves ancestral polymorphisms within the whole family Pteropodidae, as illustrated by centres of endemism often situated on islands and archipelagos (Goodman et al., 2010; Almeida et al., 2016).

5. CONCLUSIONS

The observed phylogeographic patterns in *Rousettus* fruit bats with shallow divergences and incongruencies between mitochondrial and microsatellite markers suggest complex demographic history with periods of allopatry and a high level of gene flow in males. In considering the co;evolution of fruit bats and many species of trees, fruit bats represent an interesting model organisms that enables the tracing of forest refugia but are also a keystone species that may have actively contributed to the expansion of tree cover during interglacial periods as well as to the recovery of deforested regions in the Anthropocene. This is especially relevant to rousettine bats with their ability to spread to dry habitats and so in this respect can be considered a pioneer taxon.

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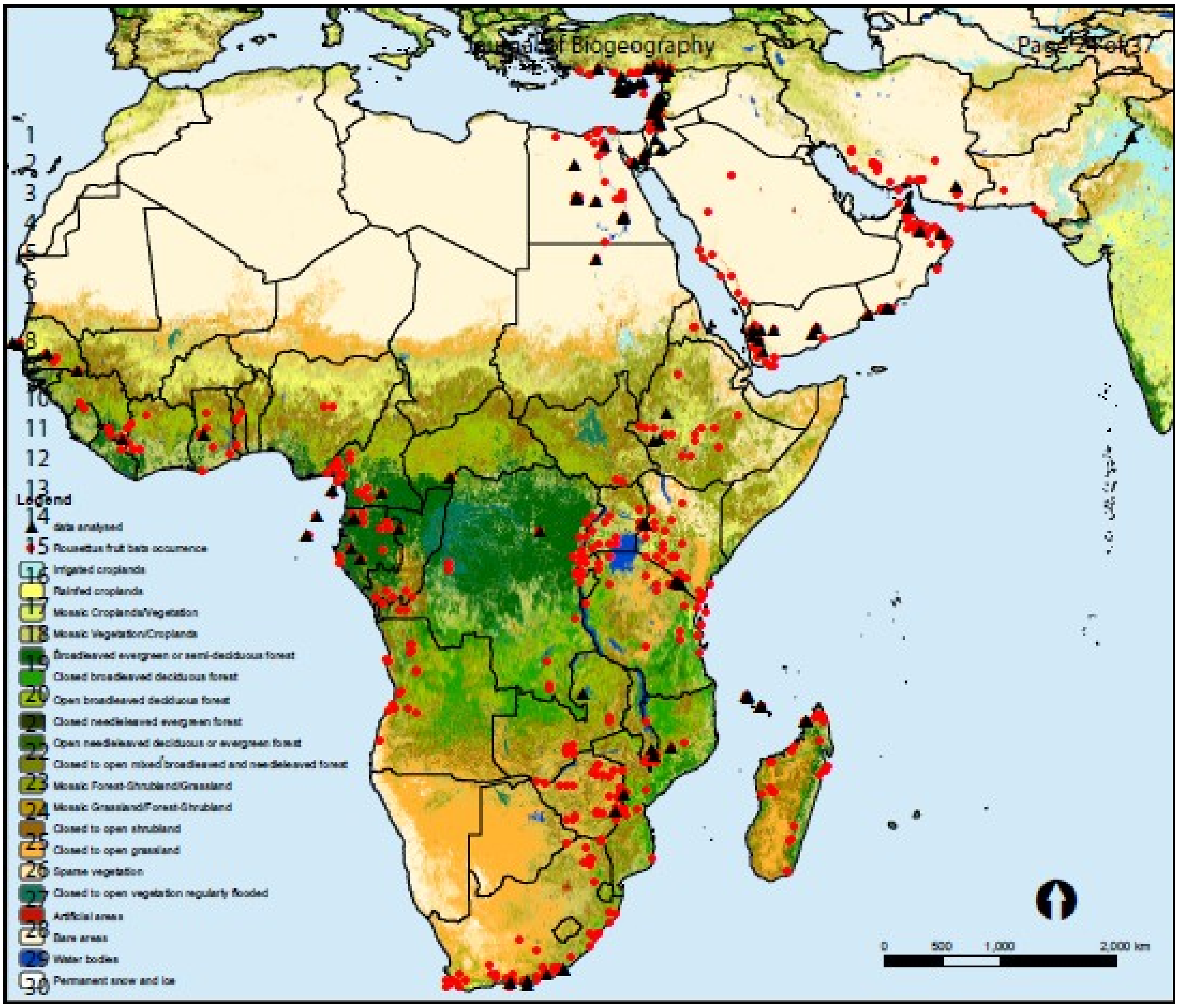
Acknowledgements

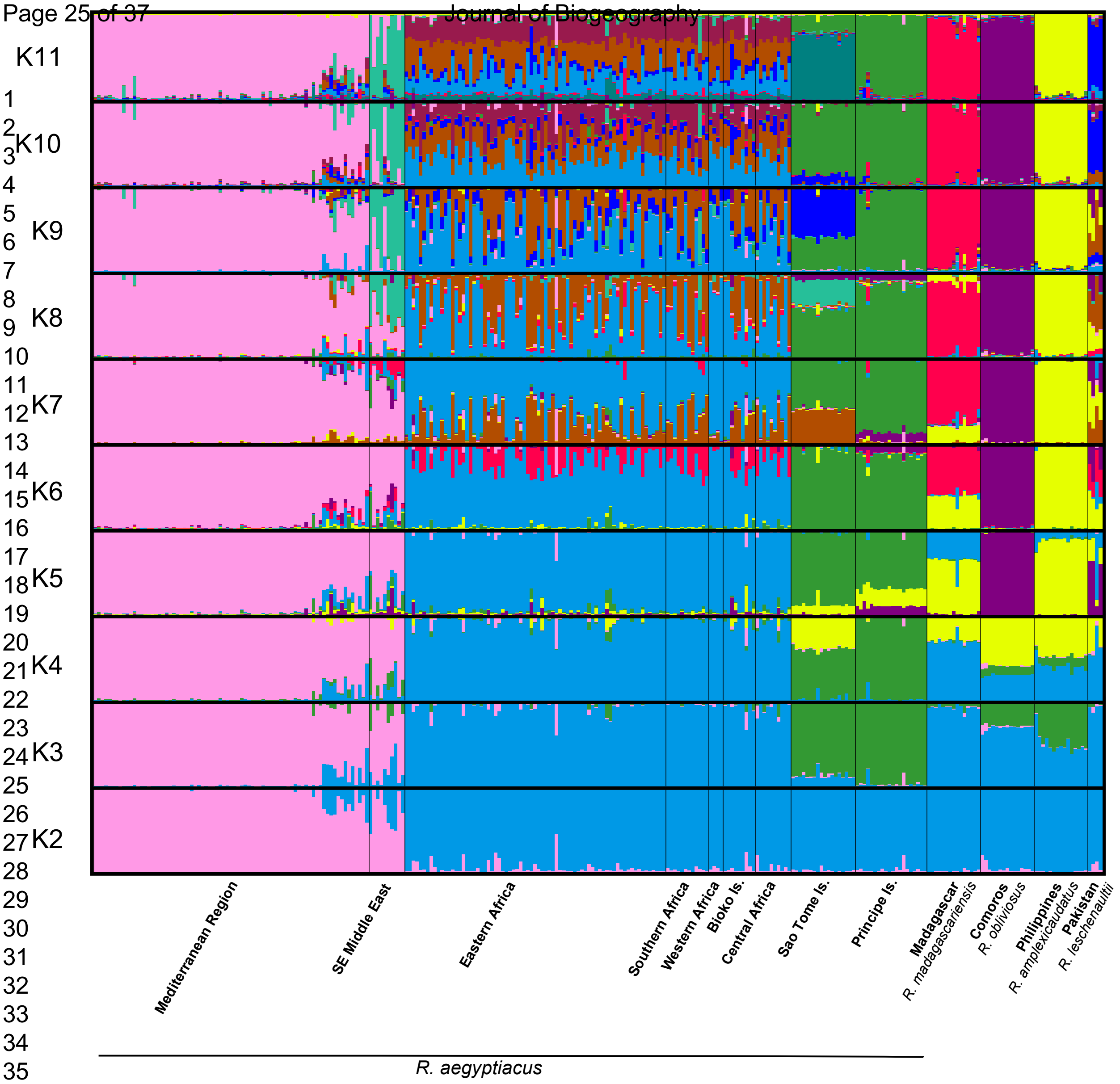
We are grateful to Dr. Arshad Javid and Peter Vallo for providing us with valuable samples. Furthermore, we would like to thank certain museums for loaning material, in particular, The Amathole Museum and The Durban Natural Science Museum, South Africa; Field Museum of Natural History, Chicago, USA; National Museum (Natural History), Prague, Czech Republic; and Doñana Biological Station, Seville, Spain.

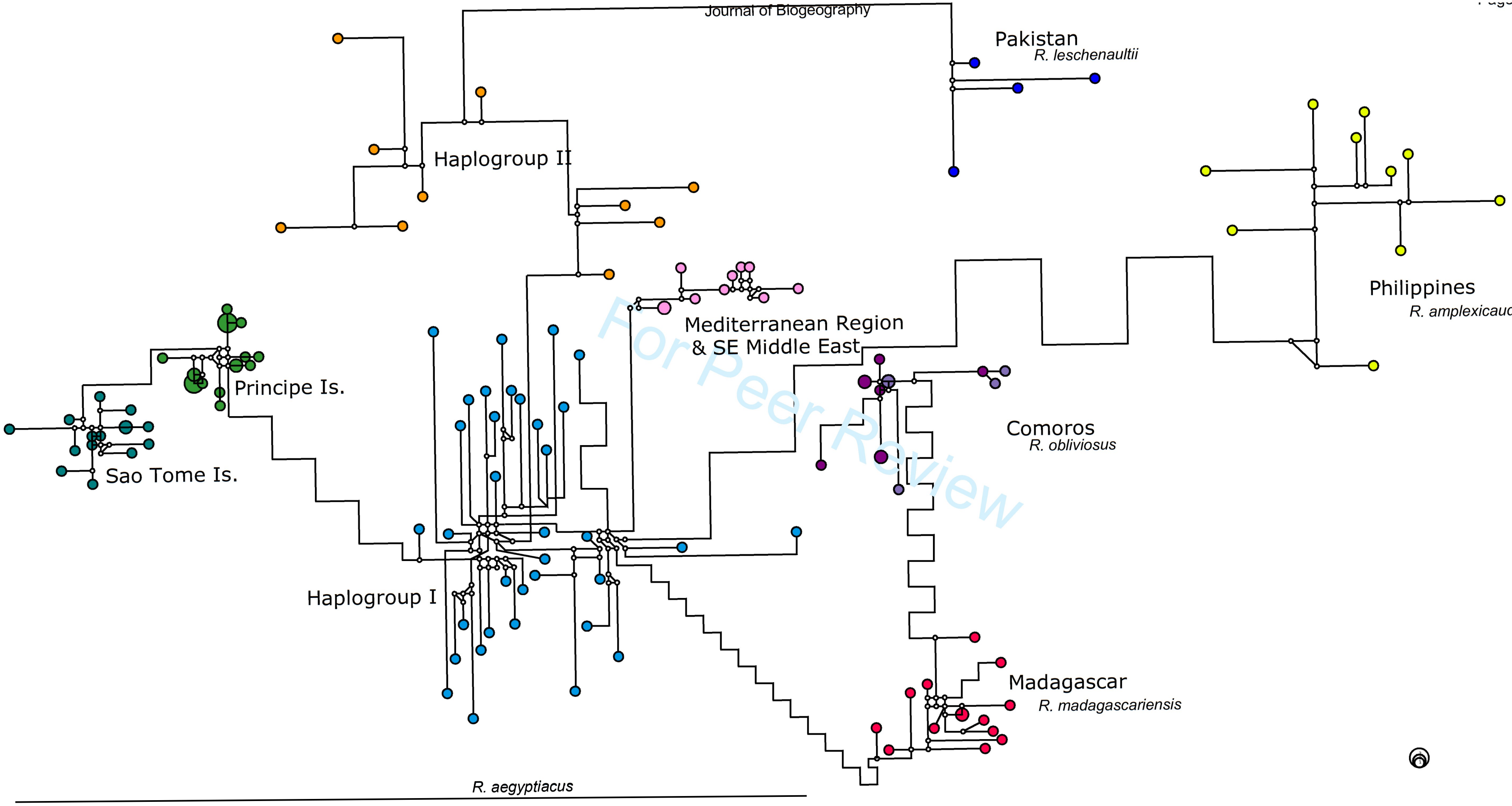
Data accessibility: Genotypes and accession numbers of sequence data used for this study will be deposited in Dryad.

Biosketch: Tereza Stříbná is a PhD student at the Zoology department, Faculty of Science, Charles University in Prague. This paper forms part of her dissertation thesis, which is focused on the biogeography of fruit bats of the genus *Rousettus*.

Author contributions: T.S. and P.H. initiated the study and conceived the ideas; T.S. generated and analysed the data; P.H. supervised the whole project and led the writing; D.R. prepared the maps; P.B., S.M.G., I.H., J.J., M.T. and A.V. provided their collections of samples. All authors have read and approved the manuscript.


Legend
data analysed
Rousettus fruit bats occurrence
Irrigated croplands
Rainfed croplands
Mosaic Croplands/Vegetation
Mosaic Vegetation/Croplands
Broadleaved evergreen or semi-deciduous forest
Closed broadleaved deciduous forest
Open broadleaved deciduous forest
Closed needleleaved evergreen forest
Open needleleaved deciduous or evergreen forest
Closed to open mixed broadleaved and needleleaved forest
Mosaic Forest-Shrubland/Grassland
Mosaic Grassland/Forest-Shrubland
Closed to open shrubland
Closed to open grassland
Sparse vegetation
Closed to open vegetation regularly flooded
Artificial areas
Bare areas
Water bodies
Permanent snow and ice
0
500
1,000
2,000 km


K11
K10
K9
K8
K7
K6
K5
K4
K3
K2
Mediterranean Region
SE Middle East
Eastern Africa
Southern Africa
Western Africa
Bioko Is.
Central Africa
Sao Tome Is.
Principe Is.
Madagascar
R. madagascariensis
Comoros
R. obliviosus
Philippines
R. amplexicaudatus
Pakistan
R. leschenaultii
R. aegyptiacus


Pakistan
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Haplogroup II
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R. amplexicaudatus
Mediterranean Region
& SE Middle East
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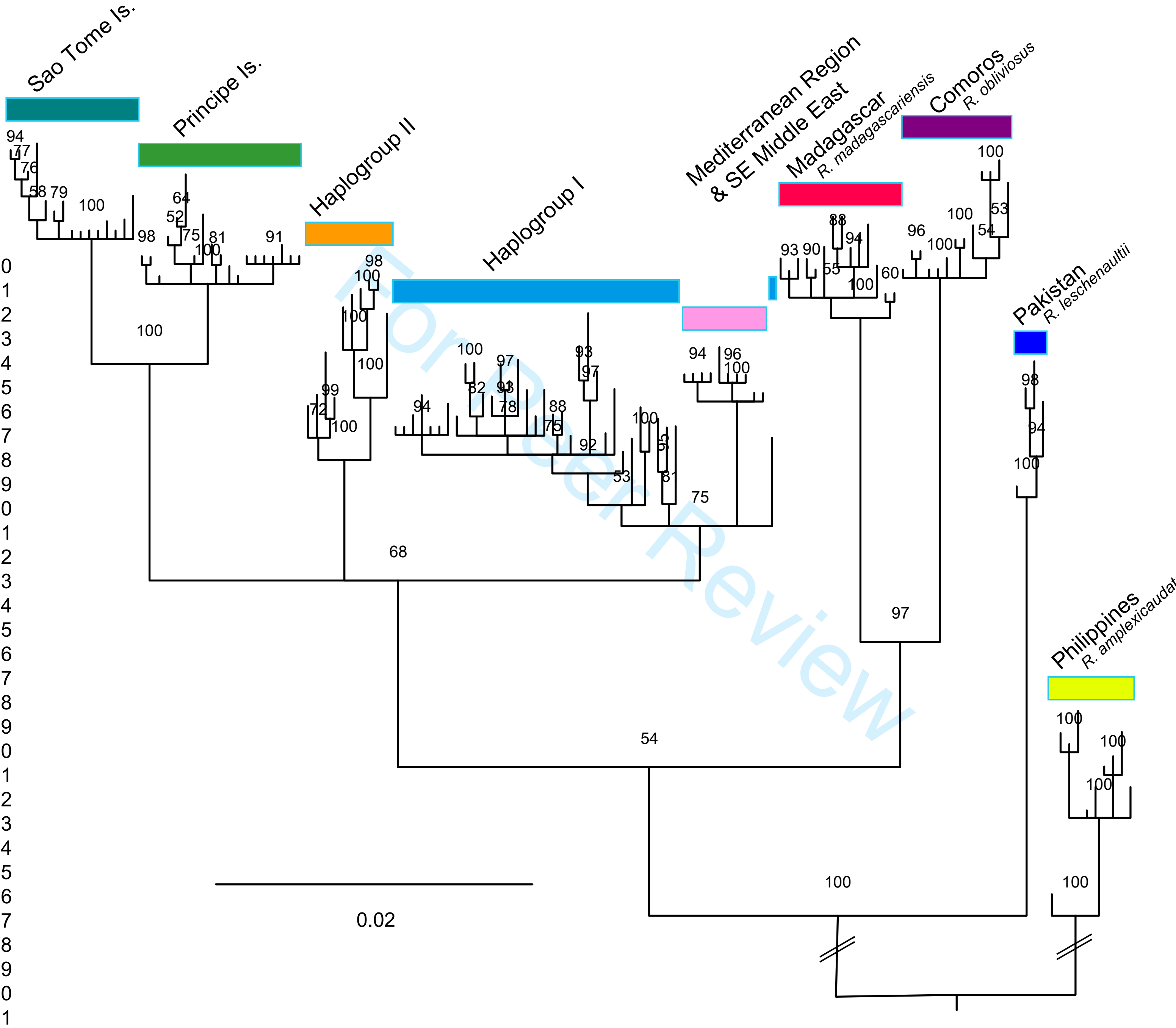
R. aegyptiacus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& SE Middle East
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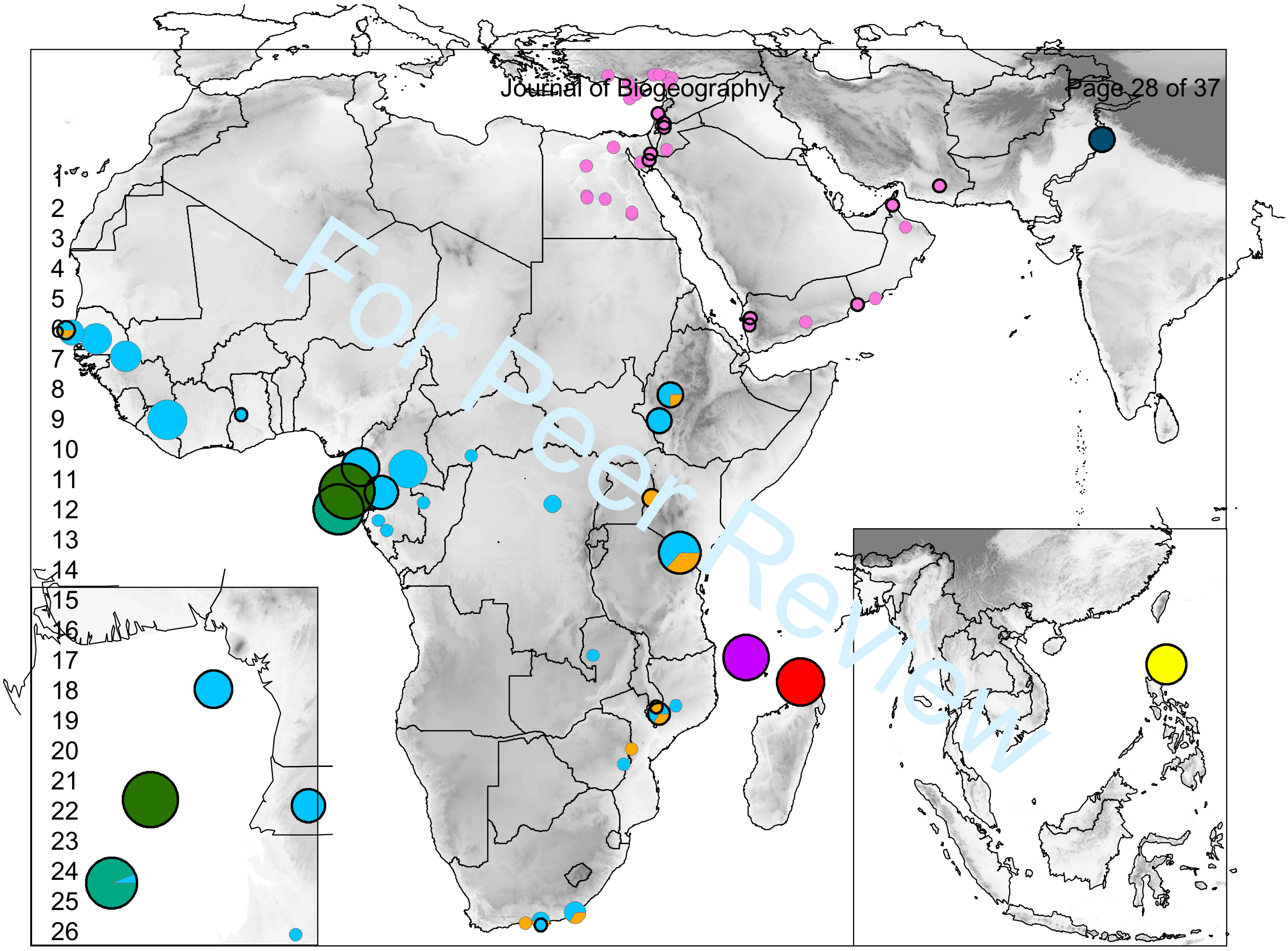

PAN-AFRICAN PHYLOGEOGRAPHY OF ROUSETTINE FRUIT BATS: PLEISTOCENE REFUGIA, HOLOCENE PANMIXIA AND ISLAND EVOLUTION

Appendix S1

Primers:

Cyt*b* gene:	Fw: L14724 (5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′) (Irwin, Kocher & Wilson, 1991)
	Rv: H15748 (5′- GGCTGTCCGCCAATTCATGT-3′) (modified, based on Lopez-Oceja, Gamarra, Borragan, Jiménez-Moreno & de Pancorbo, 2016)
CR:	Fw: L16517r (5′-GTATGTCCTGCTACCATTAACTG-3′)
	Rv1: LC2r (5′-GTATGTCCTGCTACCATTAACTG)
	Rv2: HSCr (5′-CTGTTTTTGGGGTTTGGCGGGA-3′) (all modified, based on Fumagalli, Taberlet, Favre & Hausser, 1996)

Table S1.1 Polymerase chain reaction conditions

PCR setting			
Step	cytb	CR-RV1	CR-RV2
Pre-denaturation	94°C, 5 min	95°C, 5 min	95°C, 5 min
Denaturation*	94°C, 30 sec	95°C, 45 sec	95°C, 45 sec
Annealing*	58°C, 30 sec	53°C, 45 sec	63°C, 45 sec
Extension*	72°C, 90 sec	72°C, 1 min	72°C, 1 min
Final elongation	72°C, 8 min	72°C, 7 min	72°C, 7 min
Preservation	12°C, for ever	12°C, for ever	12°C, for ever
* steps repeated 35 times			

Reagents	Concentrations
DNPs	0.25 mM each
primer	0.25 μM each
DNA	10-50 ng
MgCl2	2.5 mM
Taq Polymerase	1 U
5x reaction buffer	1/5 of final volume

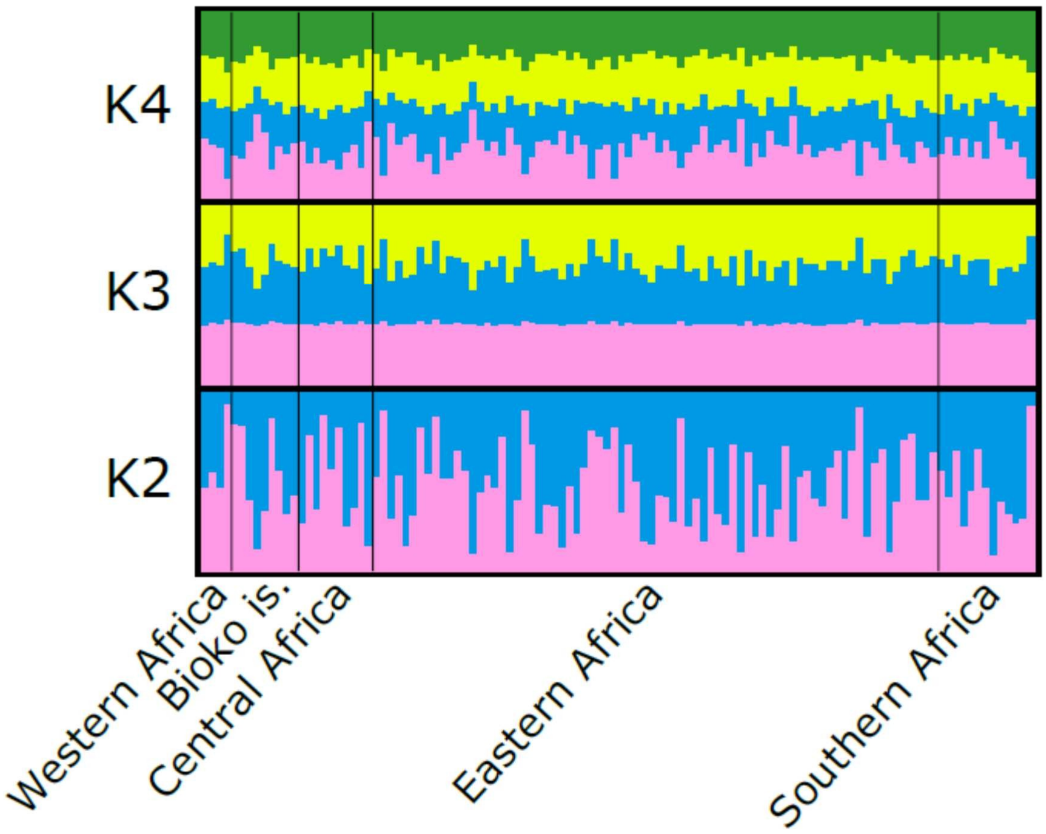

Fig. S1.1 Bayesian clustering of Rousettine fruit bats from sub-Saharan African locations.

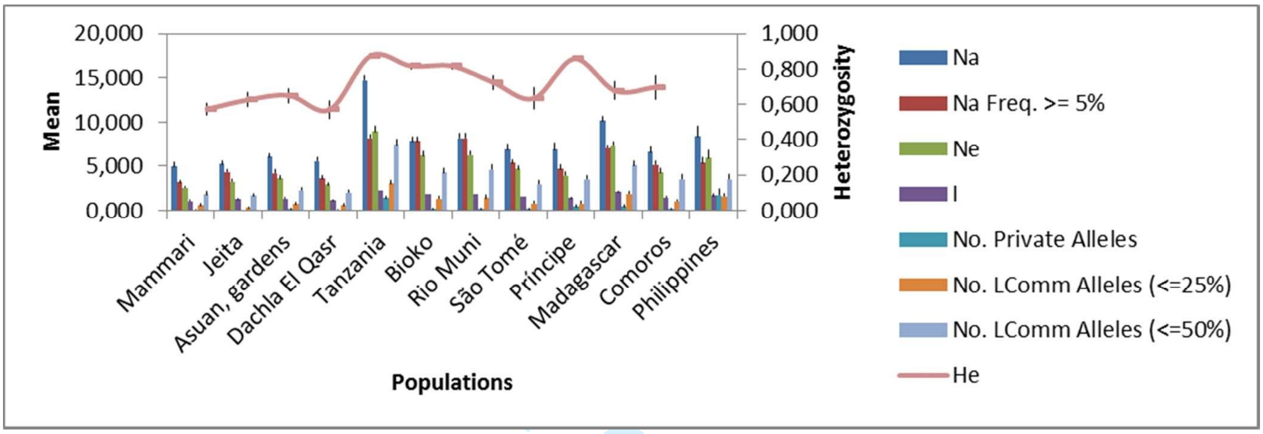

Fig. S1.2 Allelic patterns across *Rousettus* populations with more than nine genotyped individuals.

Table S1.2 Pairwise F_{ST} values among *Rousettus* populations in Afrotropical, Saharo-Arabian and Madagasacar biogeographic realms.

	Mediterranean Region	SE Middle East	Sub-Saharan Africa + Bioko Is.	São Tomé Is.	Príncipe Is.	Madagascar	Comoros	Philippines	Pakistan
Mediterranean Region	**0.0000**								
SE Middle East	0.0742	**0.0000**							
Sub-Saharan Africa + Bioko Is.	0.0940	0.0816	**0.0000**						
São Tomé Is.	0.1706	0.1677	0.0734	**0.0000**					
Príncipe Is.	0.1776	0.1666	0.0874	0.1463	**0.0000**				
Madagascar	0.1458	0.1294	0.0385	0.0991	0.1342	**0.0000**			
Comoros	0.1857	0.1603	0.1023	0.1949	0.1943	0.1354	**0.0000**		
Philippines	0.1576	0.1691	0.0651	0.1291	0.1641	0.0692	0.1786	**0.0000**	
Pakistan	0.1691	0.1498	0.0727	0.1702	0.2003	0.1017	0.1368	0.0954	**0.0000**

Table S1.3 Inter- and intra-population p-distances based on cyt *b* sequences of *Rousettus* bats. Clusters are based on Median-Joining network analysis results.

	Príncipe Is.	São Tomé Is.	Mediterranean Region + SE Middle East	Haplogroup I	Haplogroup II	Madagascar	Comoros	Pakistan	Philippines
Príncipe Is.	**0-0.006**								
São Tomé Is.	0.016-0.023	**0.000-0.004**							
Mediterranean Region + SE Middle East	0.035-0.041	0.033-0.039	**0.000-0.004**						
Haplogroup I	0.025-0.051	0.023-0.053	0.014-0.037	**0.000-0.029**					
Haplogroup II	0.043-0.053	0.045-0.053	0.027-0.035	0.021-0.043	**0.000-0.018**				
Madagascar	0.070-0.082	0.062-0.074	0.060-0.072	0.049-0.078	0.066-0.082	**0.000-0.012**			
Comoros	0.074-0.084	0.074-0.084	0.068-0.076	0.064-0.080	0.066-0.082	0.055-0.072	**0.000-0.010**		
Pakistan	0.080-0.086	0.078-0.086	0.064-0.070	0.062-0.078	0.060-0.074	0.072-0.082	0.070-0.080	**0.006-0.010**	
Philippines	0.111-0.125	0.117-0.127	0.113-0.123	0.105-0.127	0.115-0.129	0.107-0.127	0.119-0.131	0.117-0.129	**0.000-0.021**

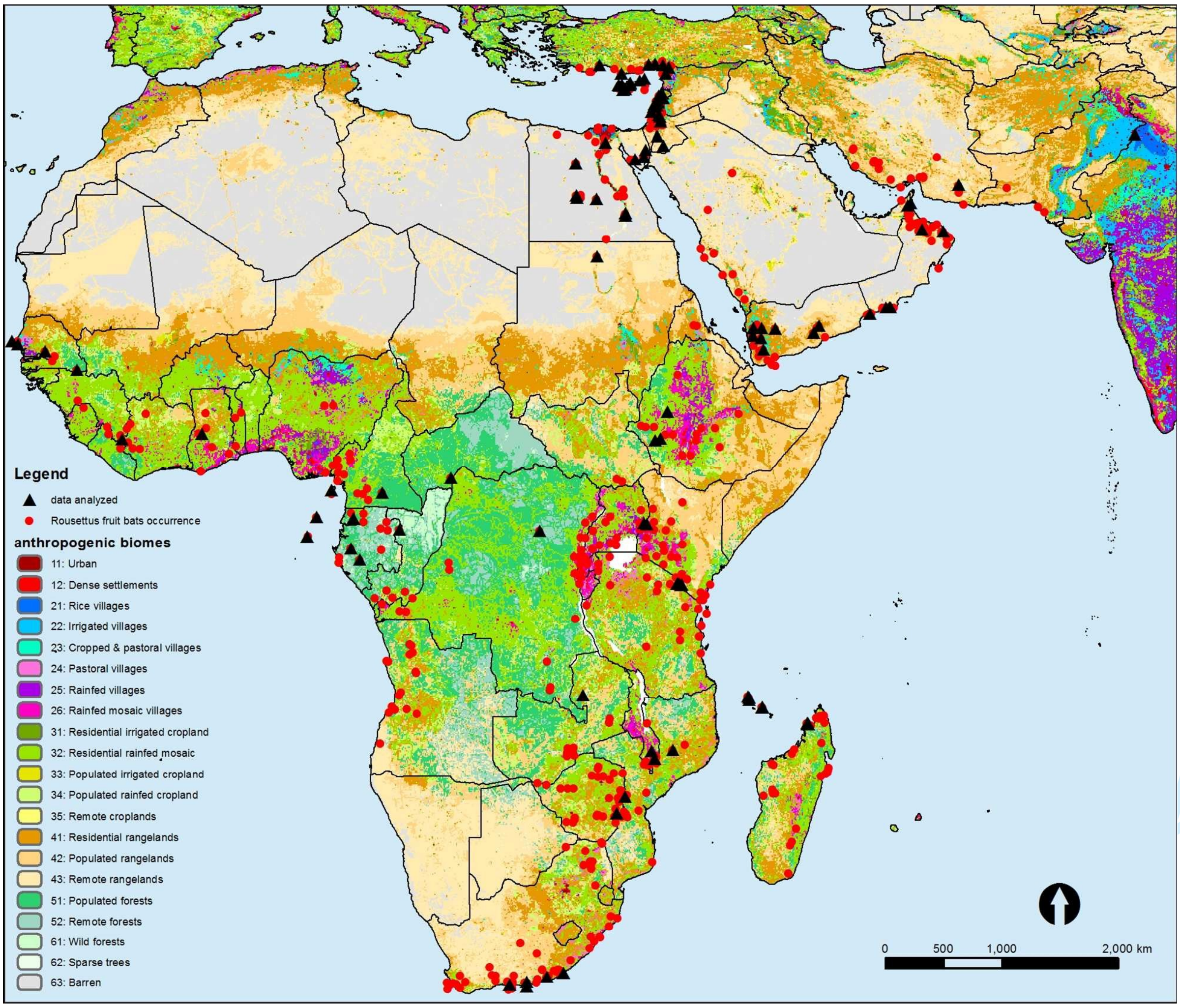

Fig. S1.3 Map of distribution of the genus *Rousettus*. The concept of anthropogenic biomes was applied to the map in the background. Black triangles denote analysed in this study, red dots show localities from previous studies (Bergmans, 1994; Monadjem, Taylor, Cotterill, & Schoeman, 2010; Goodman et al., 2010; Hassanin et al., 2016; Benda et al., 2012).

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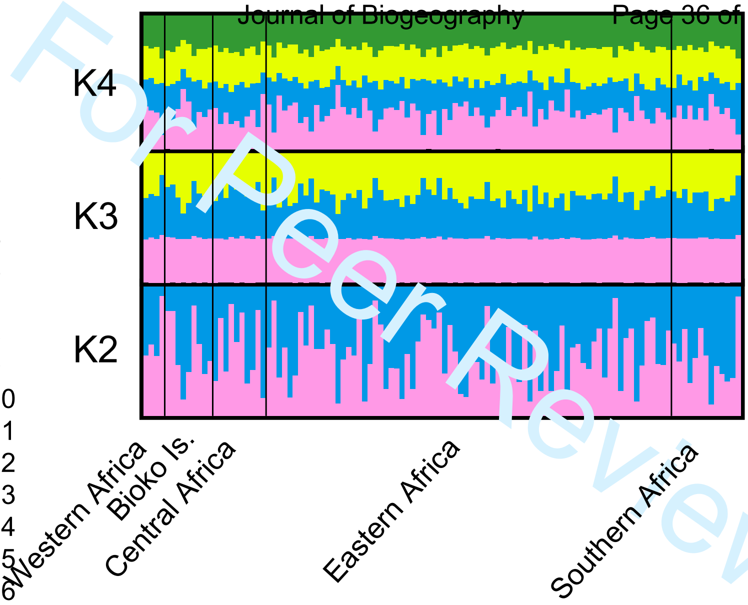
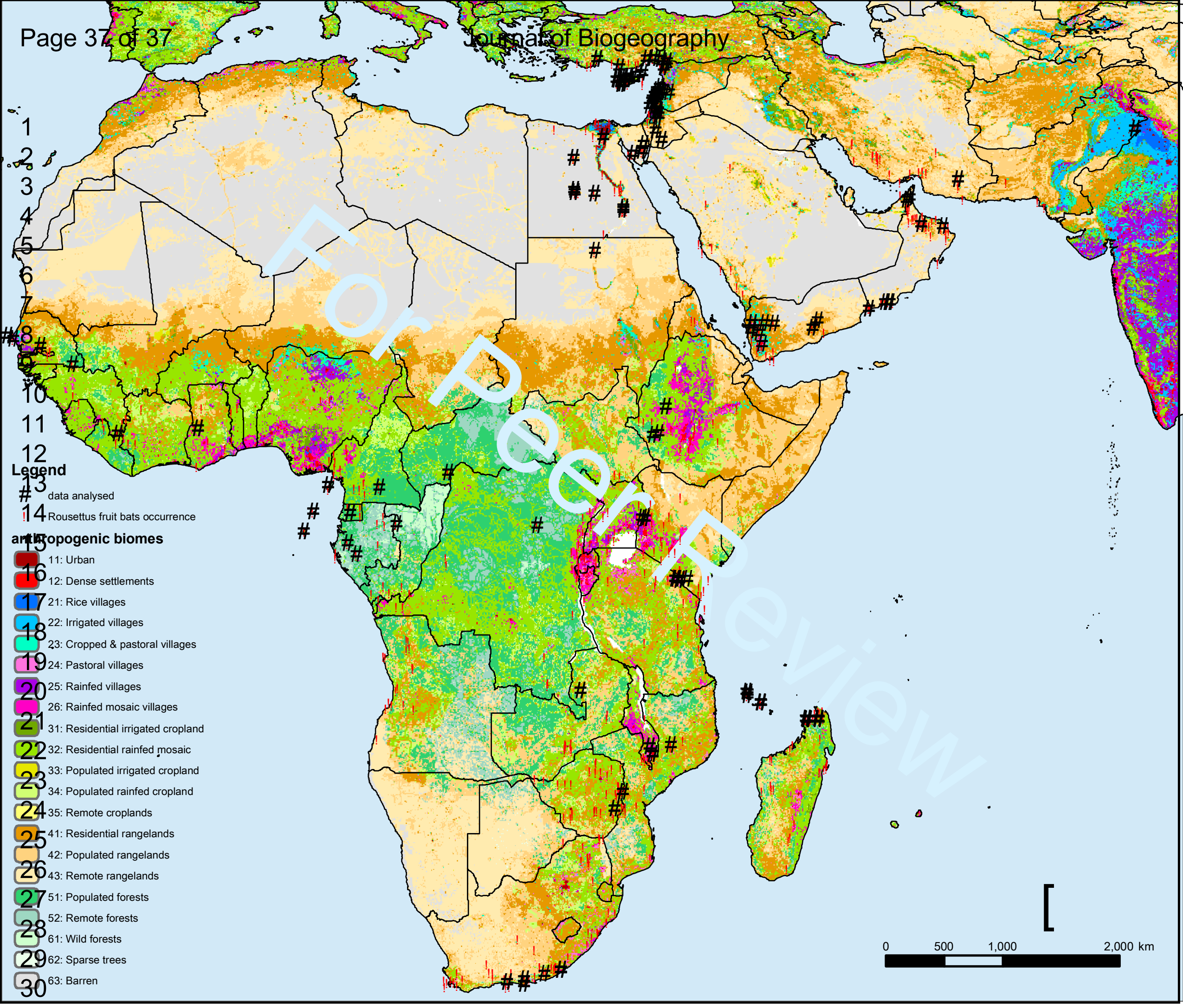

K4
K3
K2
Western Africa
Bioko Is.
Central Africa
Eastern Africa
Southern Africa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data analysed
! Rousettus fruit bats occurrence
anthropogenic biomes
11: Urban
12: Dense settlements
21: Rice villages
22: Irrigated villages
23: Cropped & pastoral villages
24: Pastoral villages
25: Rainfed villages
26: Rainfed mosaic villages
31: Residential irrigated cropland
32: Residential rainfed mosaic
33: Populated irrigated cropland
34: Populated rainfed cropland
35: Remote croplands
41: Residential rangelands
42: Populated rangelands
43: Remote rangelands
51: Populated forests
52: Remote forests
61: Wild forests
62: Sparse trees
63: Barren
0
500
1,000
2,000 km

Annex II.

MOLECULAR ECOLOGY

Molecular Ecology (2012) 21, 6104–6116 doi: 10.1111/mec.12078

Environmental margin and island evolution in Middle Eastern populations of the Egyptian fruit bat

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Abstract

Here, we present a study of the population genetic architecture and microevolution of the Egyptian fruit bat (Rousettus aegyptiacus) at the environmental margins in the Middle East using mitochondrial sequences and nuclear microsatellites. In contrast to the rather homogenous population structure typical of cave-dwelling bats in climax tropical ecosystems, a relatively pronounced isolation by distance and population diversification was observed. The evolution of this pattern could be ascribed to the complicated demographic history at higher latitudes related to the range margin fragmentation and complex geomorphology of the studied area. Lineages from East Africa and Arabia show divergent positions. Within the northwestern unit, the most marked pattern of the microsatellite data set is connected with insularity, as demonstrated by the separate status of populations from Saharan oases and Cyprus. These demes also exhibit a reduction in genetic variability, which is presumably connected with founder effects, drift and other potential factors related to island evolution as site-specific selection. Genetic clustering indicates a semipermeability of the desert barriers in the Sahara and Arabian Peninsula and a corridor role of the Nile Valley. The results emphasize the role of the island environment in restricting the gene flow in megabats, which is also corroborated by biogeographic patterns within the family, and suggests the possibility of nascent island speciation on Cyprus. Demographic analyses suggest that the colonization of the region was connected to the spread of agricultural plants; therefore, the peripatric processes described above might be because of or strengthened by anthropogenic changes in the environment.

Keywords: Cyprus, Egyptian fruit bat, founder effect, island speciation, microsatellites, Middle East, mitochondrial DNA, range margin

Received 29 June 2012; revision received 7 September 2012; accepted 10 September 2012

Introduction

The factors causing the restriction of species ranges remain only partially explored. At environmental margins, large populations in evolutionary stasis often reach the limits of their ecological valence with several mechanisms preventing the evolution of adaptations to novel environments beyond the edge of the range (Bridle & Vines 2006). In addition to historical constraints, the depletion of genetic variance could play a role in decreasing the adaptive potential of peripheral populations. This may be caused by spatial restriction of the mutational input, and the establishment of viable populations may also be complicated by the Allee effect. If the gene flow between the margin and centre remains intense, the evolution of novelty in the periphery may be prevented by the input of locally deleterious alleles

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from the main population. If the populations at the range margins remain fragmented (e.g. because of the patchiness of suitable habitats), genetic drift in the isolates and, according to the metapopulation model, founder effects in newly established demes can contribute to the loss of genetic variability, particularly in populations that result from expansion compared with the rear edge (Diekmann & Serrão 2012).

However, small peripheral populations can possess some degree of enhanced evolutionary potential and shift to new adaptive peaks, for example, as described in the shifted balance theory (Wright 1932) and models connected with the founder effect, that is, genetic revolutions model (Mayr 1954), founder flush model (Carson 1968) or transilience model (Templeton 1980). Potential admixture among different peripheral demes may further facilitate adaptive evolution by outbreeding, combinations of site-specific advantageous mutations and heterosis, the formation of transgressive phenotypes and other mechanisms connected to hybridization (Dowling & Secor 1997). Within the biogeographic perspective of the island model of environmental margin, populations may be influenced by other factors of island evolution, such as the relaxation of competitive, predatory or parasitic pressure, divergence in life-history traits and the occupation of novel niche. The resultant situation depends on the interference of the above-described factors and may culminate in peripatric speciation in some cases. In this study, we aimed to inspect the processes that occurred at the environmental margin by studying the population genetic architecture of the Egyptian fruit bat (Rousettus aegyptiacus) in the Middle East, which represents the northern distributional limits of megabats. The study area represents a zone of admixture of Palearctic, Afrotropic and Oriental biotas, and the region has an intricate paleogeology, geomorphology and diverse refugial histories of particular taxa with fragmentary knowledge of general phylogeographic patterns, particularly in mammals and bats (Hulva et al. 2007; Furman et al. 2009).

Megabats, forming the family Pteropodidae, represent the only frugivorous bats of the Old World. Because of their nutrient dependency on fruit, nectar and pollen, the patterns of distribution are primarily the tropical and subtropical evergreen forest habitats of Africa and Indo-Australasia. According to phylogenetic and paleontological studies, the origin of the family is connected with a Malay-Indonesian biodiversity hotspot (Juste et al. 1999). The dispersal to the western part of the range and the origin of hotspot in the Congo basin was presumably connected to three waves of colonization. The ancestors of epomophorines and myonycterines likely occurred in Africa via forested biotopes connecting Asia and Africa in Europe and/or Arabia during the Neogene mammalian dispersal phases (Thomas 1985) in the Miocene, similar to other rainforest-associated groups (e.g. the bat family Nycteridae; Griffiths 1997). The genus Eidolon, which is phylogenetically separate from other African fruit bats, likely colonized Africa in a separate dispersal event. Considering its capacity to fly for long distances, this may have occurred via stepping-stone islands in the Indian Ocean. The genus Rousettus Gray, 1821 likely arrived in Africa more recently than other fruit bats (Kirsch et al. 1995). The echolocation capacity sensu stricto, unique among fruit bats, enabled roosting in caves and spreading after the disappearance of forest blocks between the two continents. However, regardless of the fact that the most basal species of the genus, Rousettus amplexicaudatus, has an Asian distribution, the biogeographic analysis by Almeida et al. (2011) supported Africa as the ancestral area of the genus. The ecological characteristics of rousettes resulted to a unique distribution pattern among fruit bats, including both Asia and Africa, and also allowed R. aegyptiacus and R. leschenaulti to spread to relatively high latitudes, characterized by comparatively dry, cold and seasonal climates, which represent conditions close to the limits of the abiotic selective gradient of the group. Recently, R. aegyptiacus has an extensive disjunct range and is considered polytypic (Bergmans 1994) with four continental subspecies: R. a. egyptiacus (Geoffroy, 1810) distributed in Cyprus, Turkey, the Levant and Egypt; R. a. arabicus Anderson, 1902 in the Arabian Peninsula, Iran and Pakistan; R. a. leachi (Smith, 1829) in East Africa; and R. a. unicolor (Gray, 1870) in West Africa and two endemic insular subspecies from the Gulf of Guinea, including R. a. princeps Juste & Ibáñez, 1993 from Principe island and R.a. tomensis Juste & Ibáñez, 1993 from São Tomé Island.

The cave-dwelling strategy has also affected the population structure of the Rousettus species. Tight connections of rousettes with this unequally distributed microhabitat have led to the evolution of large colony sizes and a tendency towards long distance movements among particular cave roosts. This behaviour generates a high level of gene flow and resulted in substantial population genetic homogeneity compared with tree-roosting species with smaller social units and higher site fidelity. This tendency was revealed by molecular ecology studies in the rainforest biome, which is characterized by long-term climatic stability. Therefore, the genetic structure reflects mainly ecological, not historical, factors. These studies include a comparison of the genetic structure in codistributed fulvous fruit bat (Rousettus leschenaulti) and the greater short-nosed fruit bat (Cynopterus sphinx) in Southeast and East Asia (Chen et al. 2010) and comparison of cave vs. tree-dwelling species from microchiropteran genera Kerivoula and Rhinolophus in Malaysia (Rossiter et al. 2012). The gene flow may also be linked to the annual migratory behaviour, influenced by seasonal changes in the availability of ripe fruits or flowering trees, which is observed in many fruit bat species (Thomas 1983; Palmer & Woinarski 1999). For example, the phenology of plant communities in dry habitats may result in the formation of local patches of food resources that are richer than those in forest ecosystems (van Schaik, Terborgh & Wright 1993; Fleming & Eby 2003). In agricultural landscapes, farming could also play a role in fruit availability.

However, at the edge of the range, the population structure may be complicated by the abovementioned historical factors connected to demography. Additional factors could play a role in the case of the northern populations of R. aegyptiacus, such as range pulses raised by climatic oscillations, seasonality and effects caused by population fragmentation and insular evolution. We hypothesize that these phenomena limit the genetic exchange between particular patches, prevent the evolution of population homogeneity on a large geographic scale and lead to peripatric effects. Using detailed sampling of Middle Eastern populations and fast-evolving genetic markers (partial sequence of a mitochondrial control region and 20 nuclear microsatellites), we aim to achieve the following: (i) inspect the genetic variability in the northwestern section of the range, depth of the respective divergences and character of the genealogical patterns; (ii) describe the geographic distribution of genetic variability and identify the potential substructures, respective boundaries and possible barriers to gene flow using a landscape genetics approach; (iii) assess the effect of an island environment on population genetic architecture and (iv) infer the demography of the respective populations using a coalescent approach. We discuss these results together with other data concerning the life history, trophic niche, spatial behaviour, social organization and other traits to achieve a better comprehension of the population structure and microevolution of this unusual element in the eastern Mediterranean.

Materials and methods

Sampling design

The samples were collected during expeditions to the Middle East and East Africa from 2005 to 2011. The populations were sampled mainly in proximity to particular colony roosts (cave entrances or abandoned buildings), and individuals were also captured near feeding sites (e.g. in proximity of fruiting trees), small day roosts (rock crevices) and at flying corridors (above brooks) using mist netting. Each locality was georeferenced. We obtained genetic material from 568 bats from 70 sites, which representatively covered the distribution of the species in the region (Fig. 1, Table S1, Supporting Information, Dryad entry: doi:10.5061/dryad.k68k8). We used wing punch biopsies (Worthington Wilmer & Barratt 1996) or buccal swabs to obtain cellular material. The samples were fixed with pure ethanol and stored at 20 °C after transportation to the laboratory.

Sequencing and genotyping

Genomic DNA was isolated using the DNA Blood and Tissue Kit (Qiagen) or NucleoSpin Tissue Kit (Macherey-Nagel). Part of a mitochondrial control region (portion of hypervariable region II and of the central conserved domain) was sequenced. The studied segment was amplified using the forward primers L16517r (5′ CAT CTG GTT CTT TCT TCA GG 3′; modified according to Fumagalli et al. 1996) or 1211925r (5′ACG CAG ATC ACC ACC AAT GG 3′; modified according to Sun et al. 2009), located in the central conserved region, and the reverse primer HSCr (5′ CTG TTT TTG GGG TTT GGC GGG A 3′; modified according to Fumagalli et al. 1996), located in the Phe-tRNA region. PCRs were performed in 25 lL volumes containing 1 9 Taq buffer, 2.5 mM $MgCl_2$, 200 lM dNTPs, 0.5 lM primers, 1 U Taq polymerase (Promega) and 100 ng template DNA. The temperature profile on the iCycler Thermal Cycler (Bio-Rad) consisted of predenaturation (94 °C, 3 min), followed by 10 touchdown cycles of denaturation

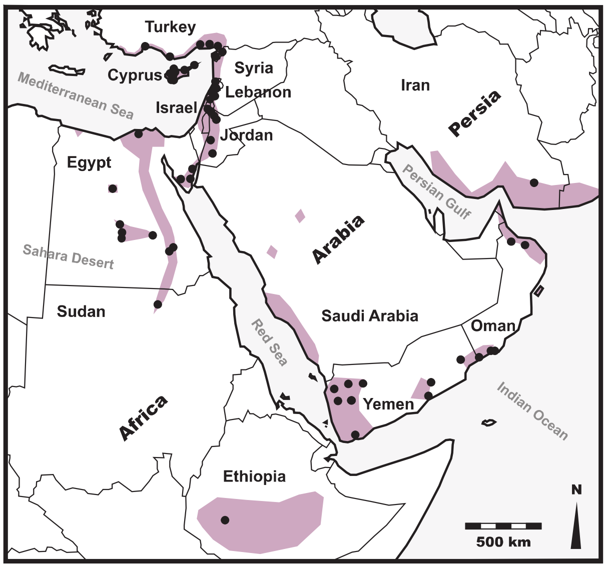

Fig. 1 A map of the Middle East and the sampling design. The extant range of the species is indicated in violet (modified from Kwiecinski & Griffiths 1999; Benda et al. 2012 and www.iucnredlist.org) and the sampling localities as black dots. The East African sample is only partially displayed because of space limitations.

(94 °C, 1 min), annealing (68 °C, decreasing by 0.5 °C in each step, 1 min) and extension (72 °C, 1 min), 25 cycles with constant annealing temperature (63 °C, 1 min) and a final extension (72 °C, 4 min). The amplicons were separated on 1% agarose gels, excised and purified using the QIAquick Gel Extraction Kit (Qiagen) and sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit and capillary electrophoresis on a 3130 Genetic Analyzer (Applied Biosystems). The chromatograms were compiled in SeqMan 5.05 (Swindell & Plasterer 1996). The sequences were aligned in MAFFT version 6 (Katoh et al. 2009) using the G-INS-i algorithm. The haplotype data were submitted to GenBank (accession numbers JX198427–JX198505; Dryad entry: doi:10.5061/dryad.k68k8).

For the amplification of the 20 nuclear microsatellite loci, the primers M3 (1, 6, 8, 120, 121), published in the study by Hua et al. (2006), and 66HDZ (20, 80, 82, 105, 106, 110, 117, 304, 327, 334, 340, 341, 343, 407, 413), from the study by Andrianaivoarivelo et al. (2008), were used (Table S2, Supporting Information). The fragments were amplified using fluorescently labelled forward primers in four 10-IL multiplex reactions with the Multiplex PCR Kit (Qiagen) according to the manufacturer's protocols in an iCycler Thermal Cycler (Bio-Rad). The products were separated on an ABI Prism 3100 Avant Genetic Analyzer using Gene ScanTM 500 LIZ® Size Standard (Applied Biosystems). Allele calling was performed manually using the program GeneMarker v.1.85 (www.softgenetics.com) and taking into account the amplification and electrophoresis artefacts. The binned values of allele sizes were constructed considering discrete breaks in the periodic size classes and off-ladder microvariants using Autobin (Guichoux et al. 2011). Frequency plots of the fragment size distributions were visually compared for the raw and transformed data. The genotypes of all individuals are available in the Dryad repository (doi:10.5061/dryad.k68k8).

Genetic variability

The descriptive parameters of sequence polymorphism (the number of haplotypes Nh and haplotype diversity h) were computed using DnaSP 5.10 (Rozas et al 2003). The haplotype relationships were displayed using the Median-Joining network (Bandelt et al. 1999) in Network 4.6.0.0 (www.fluxus-engineering.com). This method allows for displaying alternative phylogenetic hypotheses and is useful for the visualization of shallow genealogies. Because the East African haplotypes were recognized as distant, further analyses were performed solely on the Middle Eastern populations. The sequence evolution model was inferred using jModelTest 0.1.1 (Posada 2008). The model of sequence evolution was determined using Akaike (AIC) and Bayesian (BIC) information criteria.

To analyse the demographic history of the Middle Eastern populations in detail, we used Bayesian skyline plots (BSPs), which is a method based on coalescent theory independent of a priori defined models and enables the detangling of complex population dynamics scenarios (Drummond et al. 2005). The analysis was performed for the Middle Eastern populations using the BEAST 1.6.2 program (Drummond & Rambaut 2007). The Markov chain Monte Carlo (MCMC) was run three times with 3 9 10^{7} iterations, a piecewise linear demographic function, the model of sequence evolution compatible with the results of the jModelTest and the options available in BEAST (GTR) and a strict molecular clock. The genealogy and model parameters were sampled every 1000 iterations. After discarding 10^{7} iterations of each particular run as burn-in, the resulting log files were combined using LogCombiner and summarized as BSPs using Tracer v1.5.

The presence of null alleles or scoring errors, because of stuttering and large allele drop-out, in the microsatellite data set was assessed for each population using a Monte Carlo simulation of the expected allele size differences in MicroChecker (Van Oosterhout et al. 2004). The potential occurrence of recurrent sampling of a particular individual was checked by identity analysis in Cervus 3.0 (Kalinowski et al. 2007). The observed heterozygosity (H_O) and expected heterozygosity (H_E) were computed using the identical software. The allelic diversity (A), mean allelic richness corrected per sample size (AR) and the inbreeding coefficient (F_{IS}) were analysed using FSTAT 2.9.3 (Goudet 1995).

The population architecture was explored using model-based Bayesian clustering of individual genotypes (Pritchard et al. 2000; Falush et al. 2003) implemented using Structure 2.3.3. We performed 10^{6} MCMC reps after a 10^{5} burn-in period 10 times for each K, which varied from 1 to 10 with the admixture model and allele frequencies independent of the populations. The statistical support for the results of each K was then analysed using the method described by Evanno et al. (2005) with the aid of Structure Harvester v 0.6.8 (Earl & vonHoldt 2011).

Geographic structure

The occurrence of isolation by distance (sensu Wright 1943) was analysed in a relatively continuous part of the range in the Mediterranean basin and for the entire Middle Eastern region. The matrix of geographic distances was calculated using Geographic Distance Matrix Generator 1.2.3. (Ersts 2011). The genetic distances were measured as (a) Φ_{ST} or Rousett's distance $\Phi_{ST}/(1 \quad \Phi_{ST})$ for populations with $n > 1$ for the mitochondrial sequences and (b) F_{ST} or Rousett's distance $F_{ST}/(1 \quad F_{ST})$ for populations with $n > 4$ for the nuclear microsatellite genotypes. Isolation by distance (IBD) has been illustrated by plotting the genetic distances compared with the geographic distances in km. The correlation between the two matrices was tested using the Mantel test with 1000 randomizations, and a regression line was calculated using the RMA (Reduced Major Axis) across Isolation By Distance Web Service v 3.16 (Jensen et al. 2005).

We used a landscape genetics approach implemented in the R package of Geneland (Guillot et al. 2005) to analyse the spatial distribution of genetic variability within Eastern Mediterranean. Initially, the number of clusters was determined by running the program five times with 10^6 MCMC iterations, a thinning of 100, the number of populations varying from K = 1 to K = 10, an uncorrelated allele frequency model and noise blurring of the coordinates set to 5 km. A model assuming a multinomial distribution of the genotypes conditionally on the haplotype frequencies/subpopulation in Hardy–Weinberg equilibrium was used in the case of the haploid/diploid markers. Finally, the model was re-run with 10^7 iterations, a burn-in of 10^4, the number of subpopulations fixed to K inferred from the initial screening and the remaining parameters were identical to those outlined previously. The inferred Voronoi tessellations were displayed as maps of the posterior probabilities of population memberships. The genetic discontinuities uncovered by the procedure were linked to potential geographic barriers by a visual comparison with maps of the region.

Results

Genetic clustering

The 239 bp alignment of the mitochondrial sequences contained 304 individuals. The visualization of the relationships among the mitochondrial haplotypes revealed a relatively deep separation between the Middle Eastern and East African branches (p = 9.7–18%), large genetic distances within the East African haplogroup (9.2–17%) and a moderately diversified structure in the northern cluster (Fig. 2). The basal position in the northern haplogroup is occupied by Sinaic and Jordan individuals. Within the Mediterranean, widespread dominant haplotypes tend to be partially geographically localized and are often connected to site-specific rare variants. The haplotypes from the Persian and Arabian range patches are localized at the tips; however, they are not reciprocally monophyletic to the rest of the network. The TIM1 + G model was inferred using the Akaike, and TrNef + G model was inferred using the Bayesian information criterion. The Bayesian skyline plot suggested that the population size was approximately constant in the past and indicated a recent pulse in population growth (Fig. 3). The descriptive population genetics parameters for particular colonies are displayed in Table 1.

The identity analysis revealed that no individuals were resampled. Bayesian clustering of 553 microsatellite genotypes (Fig. 4) captured the isolated position of the East African and Cypriot island populations for K = 2. This subdivision generated the highest value of DK (Fig. 5). The patterns obtained for the increasing values of K showed further substructuring. The East African genotypes were sequentially subdivided into a northern Sudan population (which exhibited similarity to the Egyptian cluster) and a sub-Saharan group. The colonies from southern Jordan, Sinai, the southern Arabian Peninsula and Iran tended to cluster together from K = 3, with the emergence of a separate group that included eastern Oman and Iran at K = 7. The Egyptian and northern Sudanese individuals formed a unique cluster from K = 4. Animals from the Nile basin and delta exhibited similarity with the Levantine populations, whereas isolated demes from the Saharan oases west of the Nile

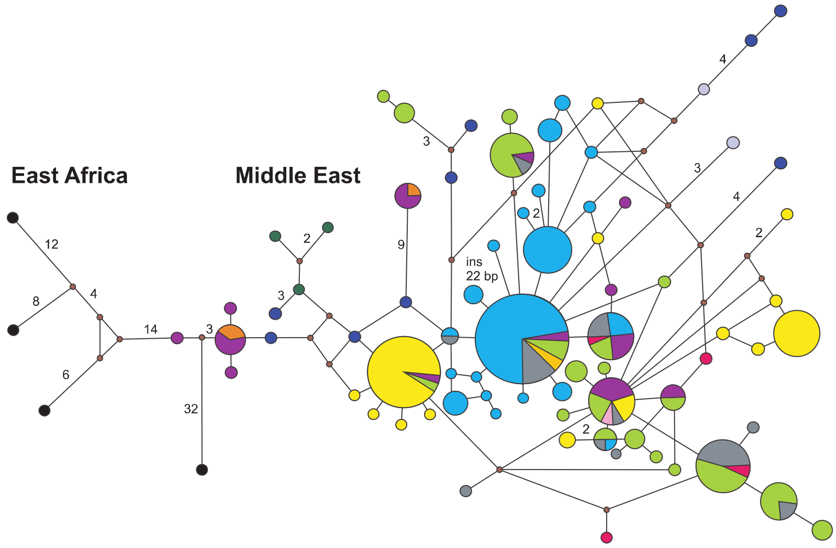

Fig. 2 The Median-joining network for the East African and Middle Eastern samples. The haplotypes are marked as circles, and their size is proportional to the number of individuals with the particular haplotype. The missing haplotypes are denoted as small brown dots. The numbers at the branches represent the number of mutation steps (displayed for n > 1). Colour codes: black-East Africa, violet-Jordan, orange-Sinai, dark blue-Yemen, dark green-Iran, yellow-Egypt, bright blue-Cyprus, bright green-Lebanon, dark grey-Turkey, red-Syria, pink-Israel and bright grey-Oman.

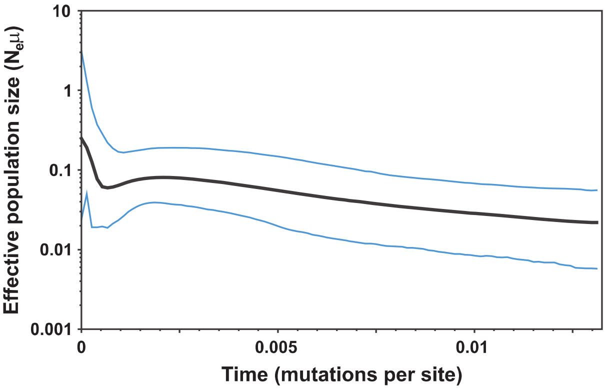

Fig. 3 The Bayesian skyline plot of the Middle Eastern populations showing changes in the effective population size estimate (N_e) over time and measured in mutations per site. The black line indicates the median estimate, and the blue lines delimit the highest 95% posterior density interval.

exhibited an exclusive status. Animals from the Levant (northern Jordan, Israel, Lebanon and Syria) and Turkey formed a relatively homogenous group.

Isolation by distance and landscape genetics

Positive correlations between the genetic and geographic distances were reflected using Mantel tests in all of the inspected cases. The results for the Mediterranean basin are displayed in Fig. S1 (Supporting information) ($Z = 129348.3845$, $r = 0.3856$, $P < 0.0001$ for mitochondrial sequences;$Z = 23581104.9530$,$r = 0.2968$, $P < 0.001$ for nuclear microsatellites). For the nuclear data, the F_{ST} of the population pairs, including Cyprus, showed relatively high values of up to $F_{ST} = 0.181$.

The analysis using Geneland generated similar results for the mitochondrial and nuclear data (Fig. 6). Both types of markers resulted in distinguishing the spatial

Table 1 The descriptive genetic parameters for particular localities with at least five individuals analysed for particular data sets. Only the data for sites with at least five individuals genotyped for microsatellites are displayed

Country	Locality	N_s	N_h	h	N_g	A	AR	H_E	H_O	F_{IS}
Cyprus	Ahanas. Androlika	13	5	0.756	16	4.3	1.574	0.574	0.574	0.004
Cyprus	Pissouri				12	3.7	1.592	0.592	0.473	0.210
Cyprus	Smigies	8	4	0.786	9	3.5	1.554	0.554	0.530	0.048
Cyprus	Ergates	14	7	0.813	14	3.9	1.574	0.574	0.539	0.040
Cyprus	Gerolakkos-Alaykoy				8	3.4	1.579	0.579	0.461	0.204
Cyprus	Mammari	16	5	0.675	25	4.7	1.582	0.582	0.505	0.147
Cyprus	Afendrika	8	5	0.786	8	3.8	1.599	0.599	0.586	0.025
Cyprus	Yedikonuk	12	5	0.727	13	4.2	1.600	0.600	0.563	0.064
Turkey	Antalya	7	3	0.667	16	5.1	1.674	0.674	0.672	0.006
Turkey	Adana				17	5.2	1.642	0.642	0.640	0.003
Turkey	Sayköy	8	5	0.786	19	5.6	1.671	0.671	0.656	0.012
Turkey	Cevlik				9	4.8	1.666	0.666	0.674	0.002
Turkey	Demrek. Dipsiz cave	5	4	0.900	16	5.2	1.647	0.647	0.687	0.073
Turkey	Harbiye	5	4	0.900	15	5.2	1.659	0.659	0.636	0.022
Lebanon	Adloun				8	4.3	1.636	0.636	0.645	0.025
Lebanon	Berquayl cave				10	3.5	1.622	0.622	0.425	0.283
Lebanon	Jeita	14	10	0.934	20	5.2	1.648	0.648	0.616	0.071
Lebanon	Jezzine. Pont Al Khalass	7	3	0.762	6	3.9	1.654	0.654	0.563	0.132
Lebanon	Mtal al Azraq	15	8	0.876	24	5.5	1.649	0.649	0.646	0.009
Lebanon	Ouataouite cave				15	5.5	1.661	0.622	0.584	0.044
Lebanon	Aamchit. Saleh cave	10	9	0.978	11	5.0	1.662	0.622	0.584	0.016
Lebanon	Antelias. Kanaan cave	8	8	1	9	4.3	1.644	0.625	0.584	0.096
Jordan	Kufranja. Iraq Al Wahaj cave	5	3	0.800	12	5.2	1.674	0.630	0.593	0.024
Jordan	Iraq al Amir. Wadi As Sir	14	7	0.824	27	7.0	1.682	0.633	0.596	0.027
Jordan	Wadi Dhana	9	4	0.639	12	6.3	1.742	0.636	0.603	0.024
Egypt	El Qahirah				6	3.8	1.623	0.639	0.608	0.072
Egypt	Aswan	16	8	0.858	22	5.9	1.656	0.641	0.609	0.070
Egypt	El A’aqab				17	5.2	1.648	0.628	0.592	0.021
Egypt	Dakhla. El Qasr	28	8	0.646	45	5.5	1.571	0.642	0.611	0.071
Egypt	Dakhla. Mut				10	4.0	1.594	0.641	0.608	0.018
Egypt	El Kharga	13	2	0.154	17	4.7	1.607	0.640	0.606	0.081
Oman	Ain Tabruq				7	5.5	1.696	0.639	0.604	0.027
Oman	Taiq cave				9	6.2	1.735	0.638	0.600	0.083
Oman	Al Nakhar				6	3.9	1.637	0.637	0.596	0.081

Number of individuals sequenced (N_s), number of haplotypes (N_h), haplotype diversity (h), number of individuals genotyped (N_g), allelic diversity (A), mean allelic richness corrected by sample size (AR), expected heterozygosity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}).

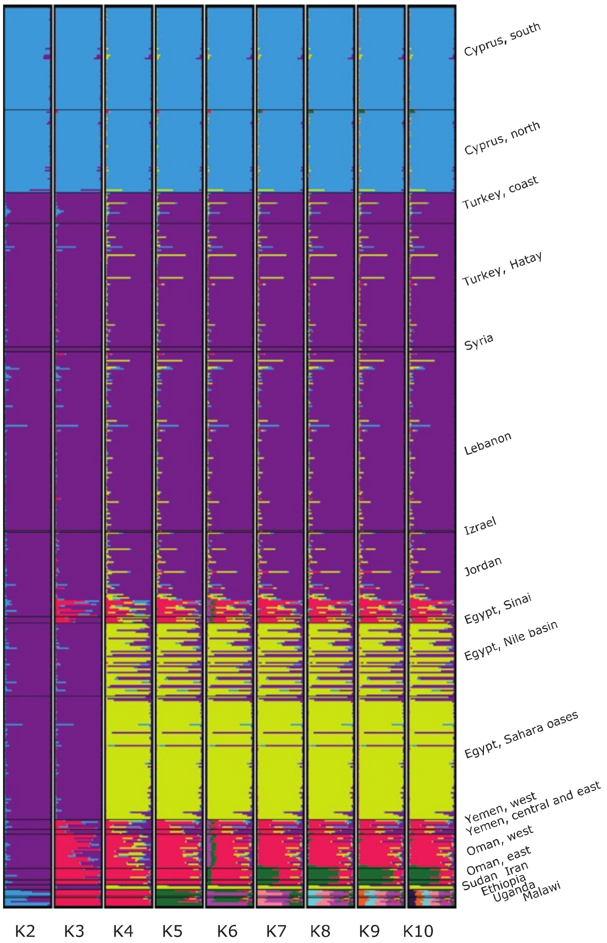

Fig. 4 The population memberships of the inspected individuals for the a priori defined number of clusters K = 2–10 inferred by the Structure software. Each genotype is represented by a horizontal line divided into K coloured segments. The length of each segment corresponds to the value of the estimated membership coefficient (Q).

domains comprising the populations from (i) the Nile basin and Saharan oases in Egypt, (ii) Sinai and southern Jordan, (iii) the Levant, including northern Jordan, Israel, Lebanon, Syria and southeast Turkey (Hatay Province) and (iv) the Mediterranean coast of Turkey and Cyprus. The analysis of the microsatellite data revealed an additional domain that included two populations from the Karpas Peninsula, which is the easternmost promontory of North Cyprus (v).

Discussion

The genetic structure at the environmental margin

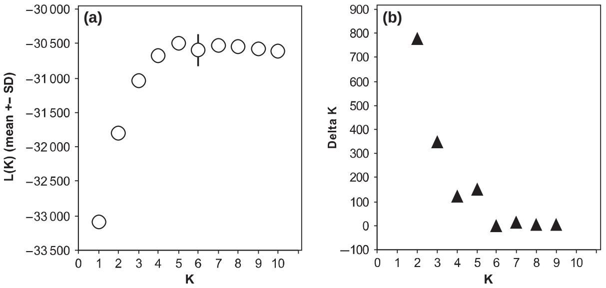

Fig. 5 The detection of the true number of clusters inferred by the Structure software using the method of Evanno et al. (2005) and displaying (a) the log probability of the data L(K) and (b) $DK = \mathrm{mean}(|L''(K)|)/SD(L(K))$ as a function of K.

Different modes of heredity in mitochondrial and nuclear markers have often resulted in different patterns obtained from both types of data in cryptic bat species complexes (Hulva et al. 2010) and even in markedly morphologically distinct lineages (Nesi et al. 2011). These conflicts can be caused by the hybridization and introgression among particular lineages or differences in the spatial behaviour between the sexes and emphasize the necessity for using a combination of both marker types. However, the results in our model taxon exhibit rough congruence between both data sets within the Middle East, although the nuclear microsatellites provided a more geographically distinct picture and different details because of the higher mutation rate. The absence of mitochondrial substructuring in comparison with the nuclear data suggests an absence of pronounced difference in the spatial behaviour of the sexes.

The results of our study indicate that the genetic variation within the study area is of relatively recent origin. Compared with the cytochrome b and NADH dehydrogenase subunit 1 sequence data available to date (Benda et al. 2012), which provide insufficient phylogeographic information, detailed sampling and using fast-evolving molecular markers including nuclear microsatellites in this study enabled us to inspect the population structure of the Egyptian fruit bat in detail. Compared with the rather homogenous population structure of cave-dwelling bat species in climax tropical ecosystems detected using comparable methodology (Chen et al. 2010; Rossiter et al. 2012), relatively pronounced isolation by distance and population diversification was observed within the studied area in the Egyptian fruit bat, which resembled the situation in tropical tree-dwelling species and indicated the occurrence of peripatric effects. This contrast may be interpreted considering that historical vicariance and gene flow, which are factors with opposing effects on population structures (Bossart & Prowell 1998), may have made different contributions in the two zones. Seasonality and Pleistocene oscillations in the climate could complicate the demographic histories of the species at higher latitudes and may favour the evolution of population substructures. Simultaneously, the cave microclimate in the Middle East was relatively stable during the Quaternary (Frumkin et al. 2000;

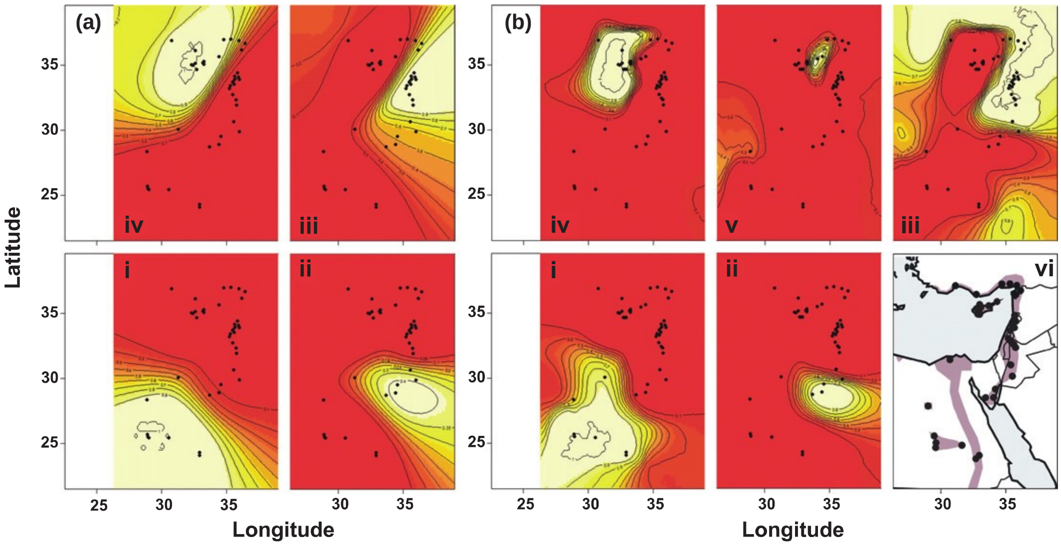

Fig. 6 The tessellation maps inferred using a landscape genetics approach with the Geneland program. The dots represent particular sampling sites, respective subpopulations are displayed by light colours and the contour lines and colour scales denote the values of the posterior probabilities of subpopulation memberships. (a) the mitochondrial sequence data and (b) the nuclear microsatellite data. Particular panels emphasize the following domains: (i) Egypt, (ii) Sinai and southern Jordan, (iii) Levant, (iv) the Mediterranean coast of Turkey and Cyprus, (v) the Karpas Peninsula in North Cyprus and (vi) a map depicting the study region and extant range of the species (violet).

Robinson et al. 2006). The varied topography of the Middle East, including uneven allocation of water sources and fluctuating hydrological regime in arid, semiarid and Mediterranean ecosystems, together with the presumably complicated and human-assisted phylogeography of fruit plants (Allen 2009), thus resulting in the mosaic distribution of vegetation associations suitable for fruit bats, could be the main factors responsible for the fragmentation and diversification at the range edge.

Phylogeography and transition zones

The results of both the mitochondrial and nuclear assays indicate a pronounced separation between East Africa and the northern range patches, coinciding with the geographic isolation of the Middle Eastern range offshoot by the Sahara desert. Moreover, the sub-Saharan samples exhibited cryptic variation within this portion of the range. Further sampling is necessary to resolve the population structure within this territory.

Individuals from Sinai and southern Jordan carry ancestral haplotypes within the northern haplogroup, and their nuclear genotypes show relationships with the African ones at low K values, which indicate the gateway role of this region in colonizing the Mediterranean basin. Both types of markers indicate the distant status of these populations within the Mediterranean region. This unit is separated from the Levantine population approximately by the territory of the Dead Sea in the north and from the Egyptian population by the Gulf of Suez and the adjacent arid habitats in the east. However, the genetic relationship to the demes in the southern Arabian Peninsula imply a recent or subrecent gene flow along the coast of the Red Sea in Saudi Arabia, although there is a gap in the species distribution in this region according to the current knowledge. This outcome stresses the role of genetic markers in tracing animal movements, because rare dispersal events may be beyond the traceability by observations, particularly in volatile and nocturnal animals. The evolution of a separate cluster comprising the eastern Arabian and Persian populations might be ascribed to the suture zone intersecting the Middle East, as demonstrated by the phylogeographic structure within the bat genus Rhinopoma (Hulva et al. 2007).

Island isolation

Within the remainder of the studied area, including the eastern Mediterranean, Nile basin and Saharan oases, a relatively homogenous population structure was detected using mitochondrial data. However, the microsatellites indicated marked differentiation of insular populations in desert oases and an oceanic island. Considering the mutation rate of both types of markers, such patterns indicate the important role of an insular environment in the nascent evolution of genetic substructures in the region.

In Egypt, the relatively basal split in the genotypes from the Saharan oases within the clustering hierarchy indicates a barrier effect of the arid territories and the isolation of particular demes in habitat islands with suitable microclimatic conditions. The population in the Nile basin appears to be composed of individuals with affiliations with both the Saharan oases and the Levant; however, marked admixture was not observed. This outcome suggests a corridor role of the Nile Valley and recent or subrecent contact with the Levantine populations, although there appears to be a recent distributional gap in the north of Sinai and the possibility of migratory behaviour in the Egyptian fruit bat. The observed gene flow between the Saharan oases and the Nile basin, as well as along the Red Sea coast, indicates at least a partial permeability of the desert barriers for the Egyptian fruit bat. One of possible explanations may involve the seasonal enhancement of passability of these region related to the phenology of the respective ecosystems. In the Levant, the species reach relatively high population densities, which are presumably connected to the high degree of gene flow and are mirrored by the homogenous population structure. The highest values of haplotype diversity (Lebanon, Hatay province in Turkey) indicate the possible microrefugial role of this region and metapopulation structure of the Egyptian fruit bat. Cyprus, the only island of oceanic origin within the studied portion of the range, bears the population with the most pronounced separate status at the level of nuclear data observable in the Middle East. The island is separated from the Turkish coast by a 75 km stretch of the Mediterranean Sea. Using the landscape genetics approach in R. aegyptiacus, the relationships between these territories indicate the colonization of Cyprus from this region of the mainland.

The native Quaternary mammalian fauna of Cyprus is characterized by a high degree of impoverishment and extreme examples of insular syndrome, including dwarf elephants and pygmy hippopotamuses (Van der Geer et al. 2010). Bats represent one of the few mammalian groups capable of naturally colonizing the region (Benda et al. 2007), and the island environment may promote peculiar site-specific evolution in some lineages, as demonstrated within the genus Pipistrellus (Hulva et al. 2010; Evin et al. 2011). The capacity of bats, the only mammals with powered flight, to cross sea barriers has been demonstrated by direct observations (Ahlén et al. 2009) and the successful colonization of remote islands by the ancestors of many insular species, which often represent the sole native mammals on islands (Fleming & Racey 2009). Genetic markers represent useful tools to measure gene flow across sea barriers. For example, marked genetic differentiation has been reported in the microchiropteran bat Myotis myotis on either side of the Strait of Gibraltar, which is a 14-km-wide sea channel between Europe and Africa (Castella et al. 2000). In a taxonomically broader study comprising of microchiropteran species, no correlation was observed between the physical ability to cross the strait (indexed by wing morphology parameters) and the level of gene flow in a particular species (García-Mudarra et al. 2009). Within the genus Rousettus, a large-scale panmixia across Madagascar, including the neighbouring islands separated by distances of up to 13 km, was demonstrated in R. madagascariensis (Goodman et al. 2010). A similar pattern involving a substantial degree of genetic mixing was also revealed in R. obliviosus in Comoros with water expanses between particular islands ranging from 40 to 80 km (Goodman et al. 2010). In our study, the genetic similarity between Rousettus populations in the eastern Arabian Peninsula and Iran indicates gene flow over the Strait of Hormuz, which is a 39-km-wide narrowing of the Persian Gulf.

However, island evolution is typical by founder demography, genetic drift and site-specific selection, which are conditioned by the isolation of the population and restriction of gene flow from the mainland (Whittaker & Fernández-Palacios 2007). These processes could lead to changes in genetic composition and decrease the genetic variation, which was the situation revealed in our study. For example, demes from Egyptian oases and Cyprus showed the lowest values of allelic richness among all the studied populations. Cypriot colonies also had the lowest values of expected heterozygosity. Two Cypriot and two Lebanese populations were characterized by inbreeding (higher F_{IS} values, observed heterozygosity lower than expected). Therefore, we concluded that the insular populations of the Egyptian fruit bat were influenced by founding events with sustained or repeated bottlenecks and genetic drift; however, assessing the contribution of a particular effect in concrete cases remains debatable (Clarke & Grant 1996). Founding events under the transilience model results in alterations in the gene frequencies (Templeton 1980), which may be the proximate mechanism behind the genetic changes demonstrated in Cyprus and Egypt by the clustering methods. Interisland differentiation and repeated bottlenecks may have occurred on Cyprus in connection with the population fragmentation caused by geomorphology and mosaic distribution of suitable habitats. This result was shown by revealing additional domain in the landscape genetics analysis on Karpas Peninsula using fast-evolving microsatellites compared with mitochondrial sequences. The abovementioned processes may theoretically gradate into founder effect speciation (Barton & Charlesworth 1984; Gavrilets & Hastings 1996). Whereas our study is an empirical illustration of this concept at the microevolutionary level, the role of the island environment in the diversification of fruit bats may also be demonstrated on larger evolutionary scales. Within the genus Rousettus, four of the ten clades with recent species statuses are island endemics, including R. bidens and R. linduensis from Sulawesi, R. obliviosus from Comoros and R. madagascariensis from Madagascar (Simmons 2005). Two lineages from islands in the Gulf of Guinea, R. a. princeps and R. a. tomensis have been granted subspecific statuses (Juste & Ibáñez 1993; Juste et al. 1996). The distributional patterns of the diversity, disparity and endemism of megabats on a worldwide scale emphasize the relationships between evolutionary hotspots and island and archipelago environments (Almeida et al. 2011). For example, 17 of the 26 total species are endemic to the Philippines (Heanney et al. 2005). It is reasonable to conclude that the isolated peripheral populations played important role in the radiation of the entire family Pteropodidae.

The complex relationships between the width and character of the geographic barriers and the degree of genetic isolation indicate that intrinsic biological factors may also play a role in the constitution of island populations and island speciation, including Cyprus, which is situated within a physically traversable distance for fruit bats from the mainland. In bats, which are gregarious and volatile mammals with long lifespans (up to 40 years), factors including the effects of spatial behaviour, navigation cues, trophic niches, population biology, social tradition and the cohesion of particular demes should be considered. Navigation cues have been studied in detail in the Egyptian fruit bat (Tsoar et al. 2011). Megabats are unique within the order Chiroptera possessing well-developed eyes with a retinal tapetum lucidum and are macrosmatic mammals with a well-developed rhinarium. The echolocation characteristic of Rousettus is useful only over a short range and is not used outside of the cave. Orientation using the Earth's magnetic field has been shown in microbats (Holland et al. 2006), but the extent to which this capacity is employed in megabats is not fully understood. Homing experiments in the Egyptian fruit bat have indicated that navigation in this context is guided primarily by distant visual landmarks together with spatial memory (Tsoar et al. 2011). Establishing a cognitive map of a visually familiar area within particular demes may promote their cohesion, especially in such markedly delimited territories as islands.

Regarding the extreme position of the eastern Mediterranean regions, including Cyprus, within the distributional pattern of the Pteropodidae family, adaptive responses to the relatively limiting abiotic conditions and island environments cannot be excluded. Adaptive evolution may also play the role in connection with founder demography (transilience model) and divergence in life-history traits boosted by founding events. In our study, the contribution of selective sweep or background selection to the loss of nuclear variation in insular demes is possible if some of the examined microsatellites were associated with fitness-related loci (Barton 2000). Further research, including genomic approaches, is necessary to test these hypotheses. In terms of the known phenotypic variations, stepped cline in body size metrics along the northwest–southeast axis, with a dominance of large forms in the northern latitudes of the Mediterranean basin, was ascertained (Benda et al. 2012). Regarding the proximate context, including the metabolic constraints of a volatile mammal with ancestry in tropical zones, it is possible to imagine the ecogeographic causality behind the evolution of this morphocline, which is interpretable within Bergman's rule.

Within the rising paradigmatic shift, the population biology level and process-based interpretations are key approaches to understanding species phenomenon, rather than static species concepts and diagnoses (Hart 2010). Our study, which contradicts the traditional subspecific alpha taxonomy of the Egyptian fruit bat, provides a useful baseline for describing biologically meaningful units within the northern range of this species. This knowledge may also be useful for frequent studies using Egyptian fruit bats as laboratory model organisms in general biology disciplines.

The role of humans

Because bats, as mammals with powered flight, are characterized by high metabolic rates, the adaptive evolution connected to feeding behaviour has played a key role in the origins of the recent diversity and distributional patterns of the order and in shaping the spatial behaviour within particular populations (Jones et al. 2005; Rojas et al. 2012). Recently, the diet of R. aegyptiacus in the Middle East has included many native or introduced agricultural plants (Korine et al. 1999; Del Vaglio et al. 2011), for example, the common fig (Ficus carica), date palm (Phoenix dactylifera), carob tree (Ceratonia siliqua) or mulberry (Morus). The history of the domestication of many of these species is connected to the Fertile Crescent and Mediterranean basin region and dates from the Neolithic revolution. The occurrence of anthropogenic roosts may also play a role in the distribution of cave-dwelling bats. The temporal coincidence between the onset of human activities in the region and the increase in the effective population size of R. aegyptiacus revealed by demographic reconstructions might support the tentative hypothesis regarding the causal relation between these phenomena. From this viewpoint, the range expansion of the species into the region and the evolution of the abovementioned patterns could have been facilitated by anthropogenic changes in the environment. This hypothesis is also corroborated by the absence of a known fossil record of R. aegyptiacus in the Mediterranean region prior to the Holocene, as reported in the Levant (Tchernov 1988). Recent demographic expansions with possibly similar roots have also been suggested by several methods in the abovementioned Comoros and Madagascar Rousettus clades (Goodman et al. 2010). However, the absence of fossils in the region prevents the calibration of the molecular clock. Further phylogeographic evidence is necessary to explicitly test the hypothesis.

However, the frugivorous bats that coexist with humans in highly populated agricultural areas often become the subject of eradication, thus raising controversial conservation issues (Korine et al. 1999). Moreover, the African populations of R. aegyptiacus have also become known as reservoirs of emerging zoonoses, that is, the Ebola and Marburg filoviruses (Pourrut et al. 2009). Regarding the high migratory capacity of these animals, genetic data shedding light to their subpopulation structure and spatial behaviour may provide valuable information regarding potential transmission routes and specify the vector role of R. aegyptiacus in the natural cycles of these viruses. Information concerning the variability within the natural range may help to assess the conservation status of particular populations within this unique faunal element in the Mediterranean basin, a region highly altered by human activities.

Acknowledgements

We thank I. Albayrak, A. Karataş, R. Lučan, H. Nicolaou, A. Reiter and M. Uhrin for assistance with the fieldwork. Karel Janko, Josef Bryja and two anonymous reviewers provided valuable comments on the previous versions of the manuscript. The project was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (IAA 601110905), the Czech Science Foundation (206/09/0888) and Institutional Research Support grant No. SVV-2012-265 206.

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P.H. designed and supervised the study and data analysis and wrote the manuscript; T.M. performed most of the microsatellite and Ch.D. of the sequencing assays; and P.H., R.B., P.B., T.B. and I.H. performed the fieldwork. This study was conducted as a component of P.H.'s postdoctoral projects at Charles University in Prague in the context of long-term multidisciplinary research on mammal model species, which focus on island biogeography, the genetics of small populations and speciation. P.H., T.M., Ch.D. and R.B. are interested mainly in molecular evolution of mammals, T.B. in ecology, and P.B. and I.H. in the biogeography and taxonomy of bats.

Data accessibility

The mitochondrial control region sequences: GenBank accession numbers JX198427–JX198505. Information regarding all analysed individuals including locality, mitochondrial sequence GenBank accession number and microsatellite genotype are available on the Dryad digital repository (doi:10.5061/dryad.k68k8).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 A list of sampling sites and their characteristics including the coordinates and number of individuals sampled, sequenced and genotyped.

Table S2 A list of the microsatellite markers and their characteristics.

Fig. S1 The relationships between the genetic and geographic distances in km for populations from the Mediterranean basin: (a) the mitochondrial data for populations with n > 1 and genetic distance measured using Φ_{ST} and (b) the nuclear microsatellite data for populations with n > 4 and genetic distances measured as F_{ST}.

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Table S1 List of sampling sites and their characteristics

country	**locality**	**N**	**E**	**N**		
Cyprus	Ahanas, Androlika	35° 00’	32° 23’	1 6	1 3	16
Cyprus	Pissouri	34° 40’	32° 42’	12	4	1 2
Cyprus	Pomos	35° 09’	32° 35’	3	1	3
Cyprus	Smigies	35° 03’	32° 20’	12	8	9
Cyprus	Episkopi, Katarina Travel garden	34° 40’	32° 54’	1	0	1
Cyprus	Episkopi, Happy Valley cave	34° 41’	32° 49’	1	0	1
Cyprus	Ergates	35° 04’	33° 14’	14	1 4	1 4
Cyprus	Gerolakkos - Alaykoy	35° 10’	33° 15’	8	0	8
Cyprus	Mammari	35° 10’	33° 12’	25	16	25
Cyprus	Afendrika	35° 39’	34° 26’	8	8	8
Cyprus	Agirdag	35° 18’	33° 15’	5	5	4
Cyprus	Yedikonuk	35° 28’	34° 02’	15	12	13
Turkey	Antalya	36° 53’	30° 45’	16	7	16
Turkey	Yakacik	36° 06’	32° 34’	3	1	3
Turkey	Adana	37° 00’	35° 18’	17	1	17
Turkey	Sayköy	36° 57’	34° 47’	19	8	19
Turkey	Cevlik	36° 07’	35° 56’	9	4	9
Turkey	Demrek, Dipsiz cave	36° 41’	36° 25’	16	5	16
Turkey	Harbiye	36° 09’	36° 08’	15	5	15
Syria	Talsh'hab	32° 42’	35° 58’	2	2	1
Syria	Ya'ar Oden	33° 12’	35° 46’	2	2	2
Lebanon	Adloun	33° 25’	35° 16’	8	4	8
Lebanon	Berquayl cave	34° 29’	36° 02’	10	2	10
Lebanon	Jeita	33° 57’	35° 39’	20	14	20
Lebanon	Jezzine, Pont Al Khalass	33° 32’	35° 35’	8	7	6
Lebanon	Mtal al Azraq	34° 25’	35° 50’	28	15	24
Lebanon	Ouataouite cave	33° 40’	35° 37’	15	3	15
Lebanon	Beirut	33° 54’	35° 29’	2	2	2
Lebanon	Nahr el Litani, Aalmane	33° 19’	35° 27’	1	1	1
Lebanon	Nahr es Safa	33° 42’	35° 28’	2	2	2
Lebanon	Aamchit, Saleh cave	34° 09’	35° 40’	12	10	11
Lebanon	Afqa cave	34° 04’	35° 54’	3	3	2
Lebanon	Antelias, Kanaan cave	33° 55’	35° 36’	9	8	9
Israel	Mt. Carmel, Ein Karmel	32° 40’	34° 58’	1	1	1
Jordan	Kufranja, Iraq Al Wahaj Cave	32° 19’	35° 43’	12	5	12
Jordan	Tabaqat Fahl	32° 27’	35° 37’	1	0	1
Jordan	Iraq al Amir, Wadi As Sir	31° 55’	35° 45’	27	14	27
Jordan	Wadi Dhana	30° 39’	35° 32’	12	9	12
Egypt	Sinai, Ain El Furtaga	29° 03’	34° 33’	1	1	1
Egypt	Sinai, Ain Hudra	28° 54’	34° 25’	1	1	1
Egypt	Sinai, Wadi El Feiran	28° 42’	33° 40’	2	2	2

Egypt	El Qahirah	30° 03’	31° 13’	6	3	6
Egypt	Aswan	24° 07’	32° 54’	22	16	22
Egypt	El A’aqab	24° 16’	32° 54’	17	2	17
Egypt	Bawiti, Bahariya Oasis	28° 21’	28° 52’	4	1	4
Egypt	Dakhla, El Qasr	25° 42’	28° 53’	45	28	45
Egypt	Dakhla, Mut	25° 29’	28° 59’	10	3	10
Egypt	El Kharga	25° 25’	30° 33’	17	13	17
Yemen	Hammam Ali	14° 41’	44° 07’	1	1	1
Yemen	Jebel Bura	14° 52’	43° 25’	1	1	1
Yemen	Ma'arib	15° 24’	45° 16’	1	1	1
Yemen	Wadi Al Lahm	15° 26’	43° 29’	1	1	1
Yemen	Wadi Dhahr	15° 27’	44° 10’	1	1	1
Yemen	Wadi Maytam	13° 52’	44° 18’	1	1	1
Yemen	Al Khuraybah, Wadi Daw'an	15° 09’	48° 26’	1	1	1
Yemen	Sah, Wadi Haramawt	15° 41’	48° 52’	1	1	1
Yemen	Hawf	16° 39’	53° 03’	1	1	1
Oman	Ain Jarziz	17° 06’	54° 05’	4	0	4
Oman	Ain Tabruq	17° 06’	54° 20’	7	0	7
Oman	Taiq cave	17° 09’	, 54° 37’	9	2	9
Oman	Al Nakhar	23° 12’	57° 13’	6	0	6
Oman	Wadi Dibab	23° 04’	58° 59’	2	0	2
Iran	Espakeh	26° 48’	60° 10’	3	3	3
Sudan	Ferka	20° 54’	30° 35’	3	0	3
Ethiopia	Tepi	7° 07’	35° 26’	2	2	2
Ethiopia	Masha	7° 52’	35° 29’	2	0	2
Uganda	unknown			1	0	1
Malawi	Mulanje Chitakali	16° 02’	35° 31’	2	2	2
Malawi	Mpalanganga estate, Zomba	15° 27’	35° 15’	1	0	1
Malawi	Zomba Plateau	15° 21’	35° 17’	2	0	2

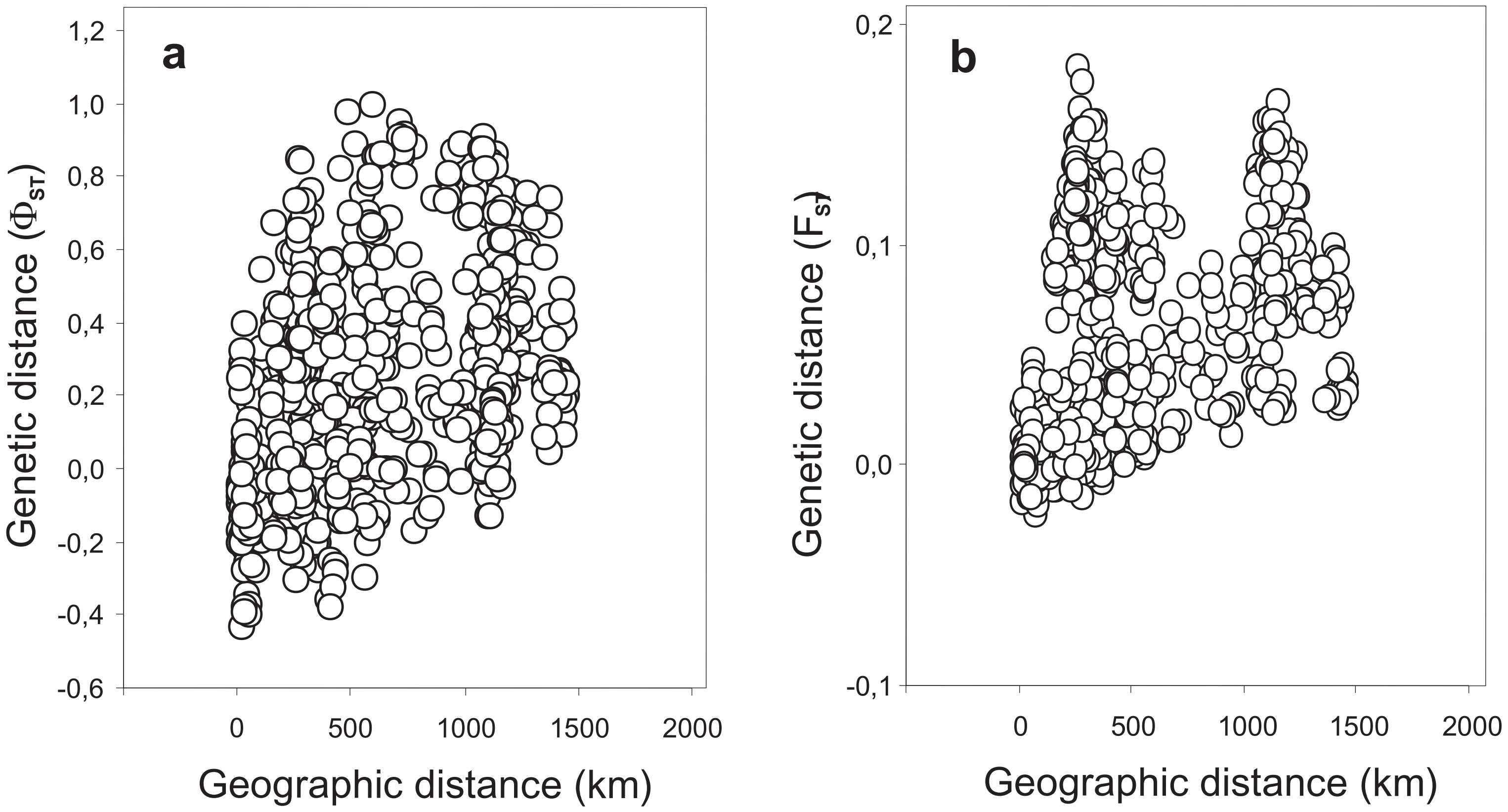
Coordinates (N, E), numbers of individuals sampled (N), number of individuals sequenced (N_s), number of individuals genotyped (N_g)

Table S2 List of microsatellite markers and their characteristics

locus name	GenBank	repeat motif	N_A	size range	source species	reference	label	T_{an}
66HDZ343	EU884014	$(CA)_{22}$	16	113 – 148	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	VIC	58
66HDZ407	EU884016	$(GT)_2$ $(GA)_3$ $(GT)_{15}$	18	129 – 173	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	NED	56
66HDZ327	EU884008	$(GT)_3$ $(GT)_{19}$	13	132 – 166	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	PET	50
66HDZ106	EU884002	$(CA)_{21}$	12	166 – 194	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	6FAM	56
66HDZ110	EU884003	$(GT)_{24}$	18	178 – 220	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	VIC	54
66HDZ413	EU884017	$(CA)_{21}$	15	205 – 300	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	NED	54
66HDZ340	EU884012	$(CA)_{14}$	18	125 – 161	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	PET	60
66HDZ117	EU884004	$(CA)_{18}$	18	154 – 194	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	NED	60
66HDZ80	EU883997	$(GT)_{14}$	17	174 – 212	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	VIC	58
66HDZ334	EU884009	$(CA)_{17}$	12	171 – 199	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	6FAM	60
66HDZ105	EU884001	$(CA)_{13}$	16	190 – 226	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	PET	58
66HDZ341	EU884013	$(CA)_9$ CG $(CA)_{13}$	14	233 – 268	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	NED	58
66HDZ82	EU883998	$(CA)_{26}$	21	226 – 280	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	VIC	60
66HDZ304	EU884006	$(GT)_{22}$	20	146 – 192	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	6FAM	62
M3-121	DQ389100	$(GT)_{15}$	21	149 – 203	*Rousettus leschenaulti*	Hua *et al*. 2006	PET	65
M3-6	DQ389097	$(GT)_{16}$	24	155 – 202	*Rousettus leschenaulti*	Hua *et al*. 2006	VIC	65
M3-8	DQ389096	$(GT)_{20}$	12	146 - 170	*Rousettus leschenaulti*	Hua *et al*. 2006	NED	65
M3-120	DQ389099	$(CA)_{13}$	15	173 – 210	*Rousettus leschenaulti*	Hua *et al*. 2006	6FAM	61
66HDZ20	EU883996	$(GT)_{20}$	17	183 – 224	*Rousettus madagascariensis*	Andrianaivoarivelo *et al*. 2008	6FAM	62
M3-1	DQ389102	$(CA)_{14}C(CA)_2$	16	191 – 220	*Rousettus leschenaulti*	Hua *et al*. 2006	6FAM	61

Number of alleles (N_A), annealing temperature (T_{an})

Fig. S1 The relationships between the genetic and geographic distances in km for populations from the Mediterranean basin. (a) Mitochondrial data, populations with n>1, genetic distance measured using Φ_{ST} b) Nuclear microsatellite data, populations with n>4, genetic distances measured as F_{ST}.



Annex III.

Received: 18 May 2016 | Accepted: 5 September 2017
DOI: 10.1111/mec.14365

ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

Habitat use, but not gene flow, is influenced by human activities in two ecotypes of Egyptian fruit bat (*Rousettus aegyptiacus*)

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Funding information
Lady Davis Fellowship Trust, Hebrew University of Jerusalem; Charles University of Prague, Grant/Award Number: SVV 260 3; Universidad Pablo de Olavide; Israel Science Foundation, Grant/Award Number:

Abstract

Understanding the ecological, behavioural and evolutionary response of organisms to changing environments is of primary importance in a human-altered world. It is crucial to elucidate how human activities alter gene flow and what are the consequences for the genetic structure of a species. We studied two lineages of the Egyptian fruit bat (Rousettus aegyptiacus) throughout the contact zone between mesic and arid Ecozones in the Middle East to evaluate the species' response to the growing proportion of human-altered habitats in the desert. We integrated population genetics, morphometrics and movement ecology to analyse population structure, morphological variation and habitat use from GPS- or radio-tagged individuals from both desert and Mediterranean areas. We classified the spatial distribution and environmental stratification by describing physical–geographical conditions and land cover. We analysed this information to estimate patch occupancy and used an isolation-by-resistance approach to model gene flow patterns. Our results suggest that lineages from desert and Mediterranean habitats, despite their admixture, are isolated by environment and by adaptation supporting their classification as ecotypes. We found a positive effect of human-altered habitats on patch occupancy and habitat use of fruit bats by increasing the availability of roosting and foraging areas. While this commensalism promotes the distribution of fruit bats throughout the Middle East, gene flow between colonies has not been altered by human activities. This discrepancy between habitat use and gene flow patterns may, therefore, be explained by the breeding system of the species and modifications of natal dispersal patterns.

KEYWORDS
circuit theory, isolation by adaptation, isolation by environment, isolation by resistance, landscape genetics, Pteropodidae

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*These authors contributed equally to this work.

ISF-FIRST 1316/05, ISF 1259/09; Grant Agency of the Academy of Sciences of the Czech Republic, Grant/Award Number: IAA 601110905; Adelina and Massimo Della Pergolla Chair of Life Sciences; Minerva Center for Movement Ecology; President of Israel Doctoral Award

1 | INTRODUCTION

Humans are responsible for the major modification of natural ecosystems ever documented since the beginning of the Holocene (Pimm et al., 2014; Rosenzweig, 1995; Thomas et al., 2004) to such extent that the term Anthropocene had been proposed to characterize this period (Ruddiman, Ellis, Kaplan, & Fuller, 2015). While many of the native species are negatively impacted because of land-use transformation or its consequences (Vitousek, 1997), others might be favoured because of their association with human-altered habitats (synanthropic or commensal species) or because they dominate the exploitation of resources over nonsynanthropic species (e.g., invasive species) (Grimm et al., 2008). Therefore, species exposed to new habitat conditions offer unique opportunities to understand the mechanisms of adaptation and gene flow of local species that generally occur within a few generations (contemporary evolution; Stockwell, Hendry, & Kinnison, 2003).

The study of adaptive evolution can be approached by combining genetic differentiation and matrix permeability (isolation by resistance, IBR) (McRae, 2006; McRae & Beier, 2007; McRae, Dickson, Keitt, & Shah, 2008), phenotypic traits (isolation by adaptation, IBA) (DeWoody, Trewin, & Taylor, 2015; Orsini, Vanoverbeke, Swillen, Mergeay, & De Meester, 2013) and/or environmental conditions (isolation by environment, IBE) (Wang & Bradburd, 2014). In highly mobile animals, behavioural factors and migration between lineages rather than selection are presumably the main factors modifying patterns at microgeographic scale (Richardson, Urban, Bolnick, & Skelly, 2014). In this sense, gene flow is a double-edged sword for local adaptations: it counteracts the effects of isolation and divergence caused by habitat fragmentation but also increases genetic variation within populations and facilitates their evolutionary potential (Lenormand, 2002; Stockwell et al., 2003). Theoretical approaches studying the role of gene flow in the evolution of population structure are based on dispersal neighbourhood, a geographic area where dispersal of most individuals in a population occurs (Wright, 1969). The analysis of landscape heterogeneity in combination with these theoretical models has greatly contributed to predict genetic differentiation and gene flow (landscape genetics) (Manel, Schwartz, Luikart, & Taberlet, 2003; Storfer et al., 2007).

Fruit bats of the genus *Rousettus* (Pteropodidae, Chiroptera) are excellent flyers with extraordinary navigational skills (Tsoar et al., 2011) and echolocation capacity (exceptional in Old World fruit bats). The echolocation enables them to inhabit caves and disperse independently of evergreen forests (Juste et al., 1999). As a consequence, *Rousettus* is the most widespread fruit bat genus and *R. aegyptiacus* is the only species of its family inhabiting both Asia and Africa including desert and Mediterranean types of climate (Benda, Vallo, Hulva, & Horacek, 2012; Harrison & Bates, 1991). The Egyptian fruit bat *Rousettus aegyptiacus* (Geoffroy, 1810) has the northernmost part of its distribution range in the Middle East and the Eastern Mediterranean. The species shows a phylogeographic structure in this area (Hulva et al., 2012) including distinct lineages associated with Mediterranean and desert Ecozones (Figure 1). The first lineage (Levantine) is distributed mainly in the coastal areas of the Eastern Mediterranean region. The second (Arabian) is distributed particularly in the coastal areas of the Arabian Peninsula with probably disjunctive range offshoot in the Sinai Peninsula and the region of the Dead Sea. Both lineages differ in body size, with the Levantine individuals being larger than the Arabians (Benda et al., 2012). These two lineages co-occur at the transition zone between the coastal Mediterranean zone and the Negev and Judean Deserts. This relatively steep transition zone, occurring due to increasing distance southwards from the Mediterranean Sea and a rain shadow effect of the Judean Hills, represents a useful model area for studying ecological differentiation. Considering the barrier effect of deserts on dispersal and the observed contribution of native agriculture and ornamental plants such as the common fig (*Ficus carica*), date palm (*Phoenix dactylifera*) or carob tree (*Ceratonia siliqua*) in the diet of northern populations of the Egyptian fruit bat (Korine, Izhaki, & Arad, 1999; Lucan et al., 2016; del Vaglio, Nicolau, Bosso, & Russo, 2011), we hypothesize that areas devoted to horticulture and associated with human settlements will play an important role in the dispersal of the species.

The Middle East is a melting pot for the admixture of the Afrotropical, Oriental and Palearctic faunas. It is characterized by the occurrence of desert areas acting as barriers for most temperate taxa that limit the colonization of Eurasia from palaeotropical species. This is especially critical for groups with relatively limited dispersal capacities such as terrestrial mammals (Yom-Tov & Tchernov, 1988). Due to the extreme abiotic conditions of deserts, transition zones between arid and humid habitats represent a suitable region to the study of ecological adaptations. Species adapted to desert areas often inhabit small and isolated patches of suitable and distant habitats surrounded by the hostile matrix frequently imposed by the desert. Theoretical models estimate the degree of genetic differentiation to be inversely correlated to species movement capacities and this has been demonstrated in sympatric species with different degree of association to human activities (e.g., Varudkar & Ramakrishnan, 2015). Therefore, vagile and human-commensal species inhabiting desert areas will vary in their genetic differentiation depending on the amount and spatial distribution of suitable (i.e., human-altered) habitats.

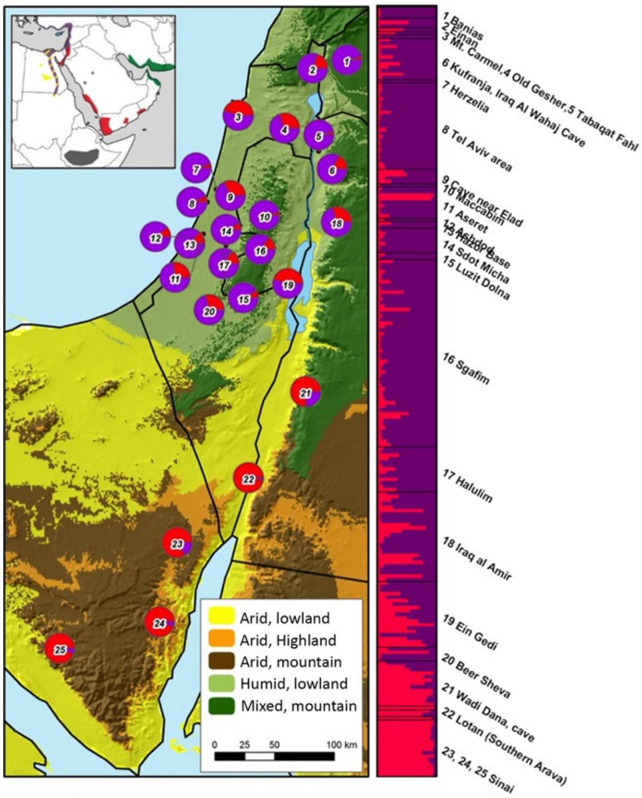


FIGURE 1 Map of study area based on physical–geographical stratification using k-means cluster analysis showing five distinct environmental zones (colour codes in lower right panel). Upper left scheme shows Middle Eastern range of Egyptian fruit bat (modified from Kwiecinski and Griffiths (1999), Benda et al. (2012) and www.iucnredlist.org). Colours denote subpopulations according to STRUCTURE analysis in Hulva et al. (2012). Pie charts mark sample locations and show the proportion of individuals assigned to Mediterranean (purple) or desert (red) clusters based on q-coefficients obtained from STRUCTURE analysis (K = 2) in this study. Bar plots on the right edge displays individual population memberships based on STRUCTURE results

Archaeological records of humans in the Middle East extend back to the Lower Palaeolithic, although the first signs of a switch from nomadism to a more sedentary, village-based life date from the Neolithic (Bernbeck & Pollock, 2005). Early signs of sedentary lifestyle in the southern Levant date back to around 13,000–10,000 years ago (Belfer-Cohen & Bar-Yosef, 2000) and ever since, human activities have intensively modified natural landscapes in both mesic and arid parts of the regions, creating a mosaic of natural and human-modified habitats with a strong influence on the colonization routes of palaeotropical fauna towards northern latitudes (Fischer & Lindenmayer, 2007; Western, 2001). Human settlements scattered throughout desert areas are artificial oases, and they may provide anthropogenic stepping stone dispersal routes. This is true ever since ancient times due to the adapted lifestyles of ancient tribes in desert areas (Berkes, Colding, & Folke, 2000). For example, the Nabateans (probably the most important Arab tribe controlling trade routes across desert areas between 4th B.P. to A.D. 1st centuries) had advanced agricultural skills based on a system of cisterns of runoff water likely used to grow date palms (among others) (Evenari, Shanan, & Tadmor, 1982). These patches of anthropogenic origin are particularly relevant for flying animals as they can effectively use these stepping stones to overcome landscape barriers and to link otherwise-isolated populations in Afrotropical and Palaearctic regions. Local habitat modifications due to human activities may, therefore, change the balance between selection and gene flow and increase the loss of adaptive genetic structure, driving taxa to the so-called speciation reversal (Seehausen, Takimoto, Roy, & Jokela, 2008).

The aim of this study was to test the association between environmental zones (IBE), land use (IBR) and phenotypic variation (IBA) with the genetic structure of the Middle Eastern subpopulations of the Egyptian fruit bat. We obtained detailed individual movements and genetic and geographic data and analysed them using models based on circuit theory representatively covering the transition area between genetically differentiated populations inhabiting Mediterranean (Levant) and desert (southern Israel, Jordan and Sinai) types of environments. The following objectives were targeted (i) to describe levels of genetic diversity and the current genetic structure of Mediterranean and desert populations using novel detailed sampling in the transition zone, microsatellite markers and circuit theory approach; (ii) to explore the link between genetic structure and habitat resistance (IBR) and environmental conditions (IBE); (iii) to explore the link between genetic and phenotypic variation (IBA); (iv) to assess the level and distribution of admixture in the contact zone between them; and (v) to evaluate the relative role of human-altered habitats on habitat use and gene flow patterns. We hypothesize that (i) the observed patterns of both lineages within the transition zone are driven by IBR and IBA and respond to environmental conditions (IBE) and that (ii) introduced plant species located in gardens and orchards included by the fruit bat in its diet are influencing gene flow patterns in desert areas. We predict that landscape resistance models wherein human-altered habitats facilitate gene flow will increase the significance and correlation between genetic and landscape distances.

2 | MATERIALS AND METHODS

2.1 | Sample collection

We used a previous set of 56 genotypes from Jordan (n = 52) and Egypt (Sinai) (n = 4) (Hulva et al., 2012) and genotyped 167 additional individuals from Egypt (Sinai) (n = 15) and Israel (n = 152) collected in 25 localities between 2005 and 2011, covering the whole transition area described between the Levantine and Arabian populations in Hulva et al. (2012) (Figure 1). These localities were scattered throughout the rainfall gradient from north to south in the studied area, ranging from 800 to 25 mm/year, respectively. All individuals were live-trapped at roosts (caves or abandoned buildings) or feeding sites using mist nets. Forearm length and body mass of the specimen were recorded. Samples for DNA extraction were obtained by wing punch or buccal swabs and stored at 20°C until processing in laboratory.

2.2 | DNA extraction and genotyping

Genomic DNA was isolated using the Genomic DNA Mini Kit (Geneaid) and DNA Blood and Tissue kit (Qiagen). Genotypes were composed by combination of 18 microsatellites (M3-1, M3-6, M3-8, M3-120 and M3-121 by Hua et al. (2006) and 66HDZ 80, 105, 106, 110, 117, 304, 327, 334, 340, 341, 343, 407 and 413 by Andrianaivoarivelo et al. (2008)) using protocols in Hulva et al. (2012). We ran negative controls in all sets of DNA extractions and PCRs to monitor contamination with foreign DNA. Allele sizes were obtained after binning microsatellite raw data by considering discrete breaks using AUTOBIN (Guichoux, Lagache, Wagner, & Et, 2011).

2.3 | Genetic diversity and structure

We first tested for the presence of null alleles in all of the 18 microsatellites using the EM algorithm (Dempster, Laird, & Rubin, 1977) implemented in the software FREENA (Chapuis & Estoup, 2007). We ran analyses of genetic diversity and population genetic structure only for those sample sites with more than five individuals ($n = 9$). Deviations from Hardy–Weinberg equilibrium (HWE) across pairs of loci and within each population were estimated using Markov chain Monte Carlo simulations and default values in GENEPOP 2007 (Rousset, 2008). Observed (H_O) and expected (H_E) heterozygosities and mean number of alleles per locus were estimated over all loci in GENETIX 4.05.2 (Belkhir, Borsa, Chikhi, Raufaste, & Bonhomme, 2004) whereas allelic richness (a measure of the number of alleles corrected by the minimum sample size [$n = 7$]) was calculated using the rarefaction procedure implemented in FSTAT v.2.9.3.2 (Goudet, 1995). We also estimated the frequency of private alleles (defined as the frequency of alleles per loci that occurred in a single population) in GENALEX v.6.501 (Peakall & Smouse, 2012). Further comparison of the frequency of private alleles with genetic structure indexes contributes to a better understanding of the level of isolation of populations. Accordingly, we first estimated differentiation indexes (F_{ST}) and their associated p-values between geographic locations using GENETIX 4.05.2 (Belkhir et al., 2004). Global F_{ST} and 95% confidence intervals were obtained using FSTAT v.2.9.3.2 (Goudet, 1995). We evaluated the effects of gene flow on genetic structure by comparing pairwise linearized genetic distances between sampling sites [F_{ST}/(1 F_{ST})] with geographic distances in logarithmic scales. To this aim, we ran a Mantel test (Mantel, 1967) and obtained p-values based on 999 permutations using the function mantel available in the VEGAN package (Oksanen et al. 2013) for R (R Core Team 2013).

We combined genetic data with geographic location in GENELAND (Guillot, Mortier, & Estoup, 2005) to consider spatial population structure. The implemented model selects the group(s) of individuals that maximize within-group Hardy–Weinberg and linkage equilibrium. First five runs with 10^6 MCMC (Markov chain Monte Carlo) iterations were used to determine the most suitable number of clusters with the following settings: thinning of 100, K values from 1 to 10, uncorrelated allele frequency model, noise blurring of coordinates of 5 km. Finally, a model with 10^7 iterations, burn-in of 10^4 and $K = 2$ (derived from initial screening) was run. We used individual-based Bayesian clustering method in STRUCTURE 2.3.2 (Falush, Stephens, & Pritchard, 2007) to estimate the number of genetically differentiated groups (K) of individuals and to identify admixed individuals and/or migrants between groups. The best K was determined in STRUCTURE HARVESTER (Earl & von Holdt, 2011) based on the highest ΔK, the second-order rate of change of log probability of the data between successive values of K (Evanno, Regnaut, & Goudet, 2005). We ran 10 replicates for each K, ranging from $K = 1$ to $K = 10$, discarding the first 100,000 steps (burn-in period) and collecting data each 100 steps (thinning) from the following 1,000,000 MCMC. Admixture model was applied. For the selected K, we averaged the estimated individual membership coefficients (q) per replicate using CLUMPP v.1.1.2 (Jakobsson & Rosenberg, 2007) and results were graphically displayed in DISTRUCT (Rosenberg, 2003). We also used coancestry indexes per individual obtained in STRUCTURE to quantify the proportion of admixed individuals in each geographic location. We categorized individuals as “pure north” or “pure south” if their q coefficient assigned to the north (Mediterranean) or south (desert) clusters was higher than 0.75 (respectively), a reasonable proportion in evolutionary/demographic history terms (one grandparent was a migrant) (Falush et al., 2007). Admixed individuals were, therefore, defined when their q value ranged between 0.25 and 0.75.

We also used the genetic landscape-shape analysis implemented in ALLELES IN SPACE (Miller, 2005) to identify landscape regions and the spatial location of their borders by considering pairwise distribution of genetic distances. A Delaunay triangulation network among sampling locations was first constructed and assigned the average residual interindividual genetic distance to each connection line. We then used the inverse distance weighted interpolation procedure to interpolate surface heights within a Cartesian grid covering the study area. These estimates were then used to build a three-dimensional surface plot where the X and Y axes draw the spatial layout of the study area and surface plot heights (Z-axis) corresponded to genetic distances.

2.4 | Morphometric variation and isolation by adaptation

We evaluated the association between genetics and phenotypic traits related with body size (IBA). Variation in phenotypic measurements ($n = 72$) (i.e., logarithm of forearm length), weight (logarithm of the cube root of the weight) and the ratio of both (body mass) of genotyped adult specimens was analysed. We tested the existence of without *a priori* groups using model-based clustering analyses (Fraley, Raftery, Murphy, & Scrucca, 2012). Differences between genetic clusters, determined by the highest membership coefficient (i.e., including the admixed individuals), were assessed using Wilcoxon rank tests and visualized using boxplots. The strength of phenotypic differences between the genetic clusters was assessed by the percentage of specimens correctly assigned to its genetic cluster by a leave-one-out cross-validated linear discriminant analysis following (Evin et al., 2013). To exclude sexual dimorphism as a bias of differences between genetic groups, significance of the interaction term of two-way ANOVAs was evaluated.

2.5 | Environmental variation and isolation by environment

The whole study area was classified based on a physical–geographical variables to explore and characterize the essential gradients of climate (annual mean temperature and precipitation) (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) and terrain (altitude and vertical heterogeneity) (Jarvis, Reuter, Nelson, & Guevara, 2008) (data sources: WorldClim v 1.4 and SRTM v4, NASA, respectively). All variables were expressed within grids of regular sizes (1 9 1 km). Then, we ran k-means cluster analysis (Jain, 2010) to classify the environmental regions (Figure 1).

To describe human modifications of the landscape in the region, we used remotely sensed data (LANDSAT ETM, USGS, 2015) and classified land cover in precise resolution (pixel size 30 m). Desert and bare areas and mosaic of plantation, orchards, shrubs and forests are dominant land cover classes according to the database followed by built-up areas, agricultural lands and water bodies. These categories were used to build landscape resistance map according to cost values based on patch occupancy of fruit bats (see below). All spatial data preparation and analysis were performed in ARCGIS 10.5 and PYTHON 2.7 software.

We tested for IBE by evaluating the correlation of Levantine (Mediterranean) and Arabian populations (see Figure 1) with the genetic relatedness of individuals between colonies while controlling for geographic Euclidean distance. To evaluate different hypotheses about the extent of genetic relatedness between the 25 study sites, we first built by random selection 100 data sets consisting of a single individual per each sampled site. Second, for each of the 100 simulated data sets, we estimated the genetic relatedness among the 25 sites using the function COANCESTRY from the RELATED package (Pew, Muir, Wang, & Frasier, 2014) in R. We selected Wang's estimator (Wang, 2002) because it showed the highest correlation coefficient ($r = .854$) between observed and expected relatedness values after simulating genotypes of known relatedness. This sequential approach generated 100 matrices of pairwise genetic relatedness values that were used to test IBE, IBD and IBR. For IBE, these 100 matrices of pairwise genetic relatedness values were used in a Partial Mantel Test using the partial.mantel function available in the VEGAN package (Oksanen et al., 2013) in R. The R-code for the whole sequential procedure is available as a supplementary material (Appendix S1). The significance of this test was estimated based on 999 permutations.

2.6 | Combining genetics, movement ecology and geography: testing isolation by resistance

The application of circuit theory to landscape genetics considers multiple potential gene flow pathways instead of a single and optimal pathway, that is, extension of isolation by distance (IBD) (Wright, 1943) to isolation by resistance (IBR, McRae, 2006; McRae & Beier, 2007; McRae et al., 2008). This approach has greatly contributed to the evaluation of the influence of human-altered habitats on population genetic structure and interpopulation dispersal of species (e.g., Ortego, Aguirre, Noguerales, & Cordero, 2015). As habitat heterogeneity was ultimately related to the spatial distribution of human settlements across the study area, we evaluated the effect of geographic distance and habitat use on genetic relatedness among individuals throughout the whole transition area and within the Mediterranean and desert clusters separately. We first built distance matrices as either geographic (Euclidean distance, null model, IBD) or as landscape-modified distance (IBR). Cost values for the IBR models were based on detailed movement data of 43 GPS- or radio-telemetry tagged Egyptian fruit bats (Tsoar, Shohami, & Nathan, 2010; Tsoar et al., 2011) captured at Sgafim cave in central Israel (31.683N, 34.910E) and tracked for 1–7 days. The 219 trees visited by these bats were mapped and identified. We used the detailed, high-resolution GIS layer of land cover created in this study to assign a specific land-use value to each visited tree and calculate the frequency of occurrence of different land uses selected by the bats. We assumed that bats from Sgafim cave, a typical Mediterranean fruit bat colony, sufficiently represent habitat preferences of foraging bats in the Mediterranean region. In desert areas, we assigned frequency values to land uses that were absent from the Mediterranean region based on our knowledge of Egyptian fruit bat foraging behaviour (e.g., palm tree plantations are the desert equivalent of fruit tree orchards in the Mediterranean region).

The landscape/resistance matrices were obtained applying circuit theory models implemented in CIRCUITSCAPE 4.0 (McRae, 2006) considering the eight-neighbour cell connection scheme (pixel size: 300 m) onto the spatially heterogeneous landscape characterized above. Patch occupancy of GPS- or radio-telemetry tagged bats was used to generate a telemetry-based landscape resistance matrix based on the high-resolution land cover GIS layer created for the whole study area (see above). We then characterized habitat conductance (i.e., patch occupancy) and obtained one matrix of pairwise landscape-modified distances between colonies. We also created a matrix of pairwise resistances within a homogenous ("flat") landscape by considering a raster layer where all cell resistance values were equal. As recommended by several authors (e.g., Jha & Kremen, 2013; Lee-Yaw, Davidson, McRae, & Green, 2009; Munshi-South, 2012), we used this matrix as controlling variable to rule out the effect of geographic distances when testing the relationship between the matrix of resistances and that of genetic relatedness (IBR). The matrix of pairwise landscape-modified distances was then used as explanatory variable of genetic relatedness using the sequential procedure explained above. The 100 matrices of pairwise genetic relatedness values were regressed one-by-one against the telemetry-based landscape distance (IBR) using the multiple matrix regression with randomization (MMRR) approach implemented in the MMRR function (Wang, 2013). The coefficient of determination (R^2) and the slope associated with the explanatory variable (b) was used to evaluate the relationship between the populations' genetic relatedness and telemetry-based distances. The same procedure was also applied when the matrix of Euclidean distances between colonies was used as explanatory variable (IBD). For each case (IBD and IBR), we obtained 100 values of R^2 and b (one for each simulated matrix of genetic relatedness) that we expressed as single 95% confidence interval (95% CI) estimates of these quantities by calculating the medians and the 2.5% and 97.5% quantiles. Our sequential procedure averts any potential issue in statistical analyses with pseudoreplication due to sampling bias of individuals from the same colony. We repeated this procedure using the whole study area and within each genetic cluster (those inferred in STRUCTURE) separately. The R-code for the whole sequential procedure and MMRR analyses is available in the Appendix S1. Resistance and Euclidean distance matrices were scaled (mean = 0, SD = 1) so their effects (b) and the relative contribution of habitat heterogeneity on gene flow patterns could be compared.

3 | RESULTS

3.1 | Genetic diversity and structure

We obtained genotypes of 223 individuals from Israel, Jordan and Egypt, 77% (n = 172) typed at 18 microsatellites and 51 samples failed in, at least, one marker (mean number of failed markers SD: 0.538 1.268). All loci were highly variable (mean number of alleles per locus: 10.224 2.633, range: 3–15; allelic richness per locus based on n = 6: 4.238 1.299, range: 2.027–6.237) and were used to describe each geographic location used for population genetic analyses (Table 1). Our analyses rejected a significant effect of null alleles at any of the 18 microsatellites used in our study (frequency of null alleles per locus: 0.012 0.016, range: 0–0.059), therefore we kept the whole set of markers for further analyses. Significant deviations of HWE across loci were only found in Tel Aviv ($p = .016$) (Table 1). On the other hand, F_{IS} values revealed a significant excess of heterozygotes in Halulim (F_{IS} = 0.037, 95% CI: 0.167, 0.021; all other 95% CI included zero values). The whole population of R. aegyptiacus in the transition area remained at HWE (all populations, $p = .939$). The number of private alleles varied between loci (2.3 1.534; range: 1–7) and populations (2.338 1.878; range: 1–7) (Table S1). More interestingly, 11 of 18 microsatellites showed private alleles in Wadi El Feiran (Sinai) in low frequency (8.5 7.7%; range: 0–22.2%), a pattern that was followed by Ein Gedi (n = 8 loci with private alleles), Iraq Al Amir and Kufranja (n = 5) (Table S1).

Despite the relative abundance of private alleles throughout different populations, there was a weak but significant genetic structure overall the transition area (overall $F_{ST} = 0.023$, 95%CI: 0.018–0.029). Using pairwise genetic distances, we found the maximum value between Tel Aviv and Wadi El Feiran ($F_{ST} = 0.078$, $p < .001$). Moreover, ca. 23% of the genetic structure between populations with more than five individuals was explained by Euclidean distance between them (Mantel test, $r = .4793$, $p = .067$).

The analysis of the output of STRUCTURE using Evanno's method highlighted $K = 2$ based on the highest DK ($DK_2 = 92.0446$; $DK_3 = 5.0949$; $DK_4 = 6.7663$) and defined two clusters in our data set (Figure 1). The spatial distribution of these two clusters was obtained in GENELAND (Figure 2) and individual coancestries (q-coefficients) are shown in Figure 1. The genetic landscape-shape analysis revealed substantial differences of population structure within Mediterranean/desert type of environment (Figure 3) and showed higher isolation among particular colonies in the desert. According to the q-coefficients obtained, we allocated most of the admixed individuals throughout the Jordan Rift Valley (Table 1, Figure 1). Thus, Ein Gedi showed the highest proportion of admixed individuals (58%, 11 of 19) followed by Kufranja (33%). Halulim, located in the southern Judean lowlands within the sharp Mediterranean-desert ecotone, came in third with 31% admixed individuals. Individual coancestries were not explained by sex (admixed males: seven of 35 [20%]; admixed females: 20 of 83 females [24%]).

3.2 | Morphometric analysis

A total of 72 specimens was analysed biometrically among which 52 specimens had both measurements (weight information was not recorded for several individuals and the weight data on the pregnant females were excluded, respectively). No groups were detected by the clustering approach for neither of the variables. However, clear phenotypic differences exist between the two genetic clusters. Specimens belonging to the Mediterranean cluster have larger forearm length ($W = 353$, $p = .002$), weigh more ($W = 69.5$, $p < .001$) and their ratio of both was also higher ($W = 74$, $p < .001$) (Figure 4). While males always show larger measurements than females (Figure S1), the sexual dimorphism appeared homogeneous between the two genetic clusters (two-way ANOVA, all $p > .5$). A total of 61.2% of the specimens can be identified to the correct genetic cluster based on their forearm length (confidence interval CI = 57–66%), 88.3% (CI = 88–90%) when the body mass is used, and 91.9% (CI = 90–92%) when the ratio of body mass and forearm length is analysed.

3.3 | Landscape genetics

The physical–geographical stratification using k-means cluster analysis led to identification of five distinct environmental zones within the focus area (Figure 1) ranging from humid Mediterranean to arid and mountainous environments. We observed a marginally significant correlation between genetic relatedness and the environmental zone while controlling for Euclidean distance (Partial Mantel test, $r = .151$; 95% CI = 0.263, 0.01). Significant correlation between genetic relatedness and Euclidean distance correcting by the environmental zone (Partial Mantel test, $r = .184$; 95% CI = 0.303, 0.093) shows the effect of dispersal distance on genetic relatedness between individuals and reinforces the IBD found at the colony level (Mantel test, $r = .4793$; $p = .067$; see above).

We found throughout the whole study area a significant decrease of related individuals as Euclidean distance increased

TABLE 1 Sample size (n), expected (nonbiased) (H_E) and observed (H_O) heterozygosities, Hardy–Weinberg equilibrium test (HWE), mean number of alleles (k), allelic richness (AR) based on a minimum sample size of n = 6, F_{IS} per geographic location and proportion (in %) of admixed individuals (based on q-coefficients estimated in STRUCTURE; see text) per location with n > 5 individuals of fruit bats

	n	H_E	H_O	HWE	k	AR	F_{IS}	Admixed (%)
Iraq al Amir	26	0.649	0.627	0.491	6.611	3.765	0.034	23.08
Wadi Dana	13	0.717	0.720	0.889	6.167	4.372	0.005	23.08
Kufranja	12	0.638	0.626	0.713	4.833	3.326	0.021	33.33
Sgafim	54	0.642	0.655	0.237	6.889	3.585	0.02	12.96
Halulim	13	0.643	0.666	0.946	5.278	3.528	0.037	30.77
Aseret	9	0.659	0.615	0.961	4.667	3.785	0.07	0
Sdot Micha	7	0.651	0.651	0.831	4.444	3.262	0.001	0
Ein Gedi	19	0.661	0.641	0.474	6.111	3.904	0.031	57.89
Tel Aviv	25	0.630	0.621	0.022	5.722	3.398	0.014	0
Wadi El Feiran	16	0.701	0.708	0.974	6.167	4.356	0.01	0

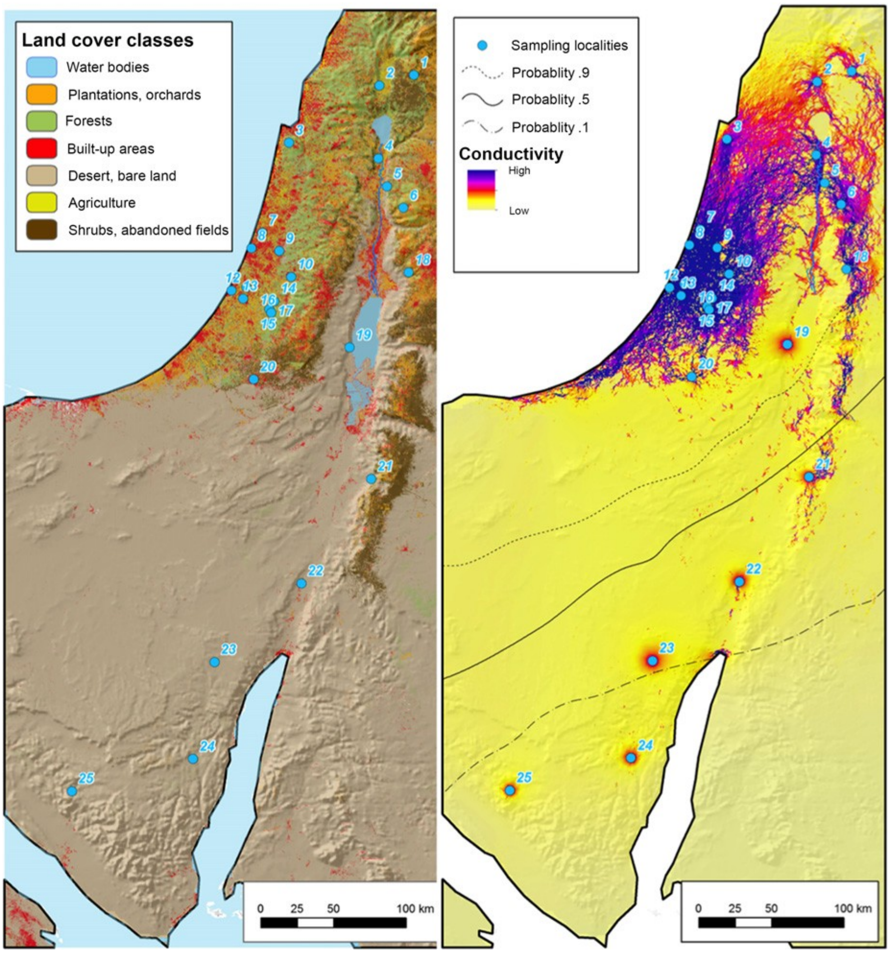
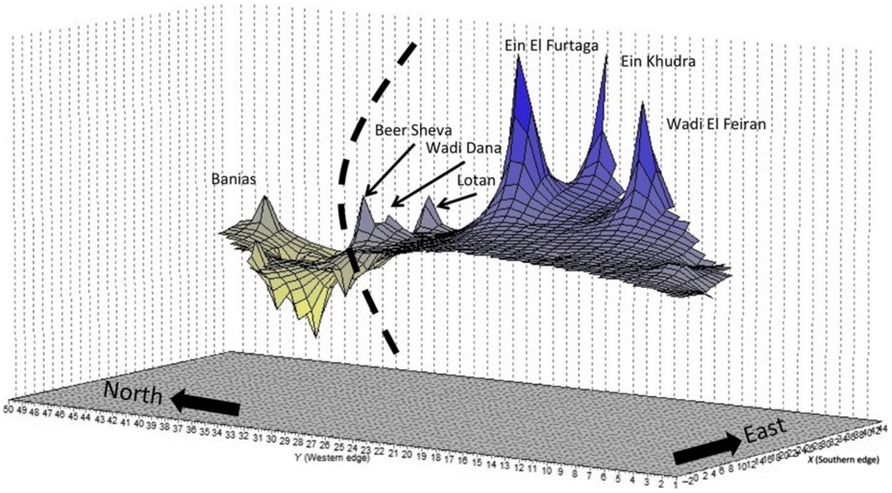
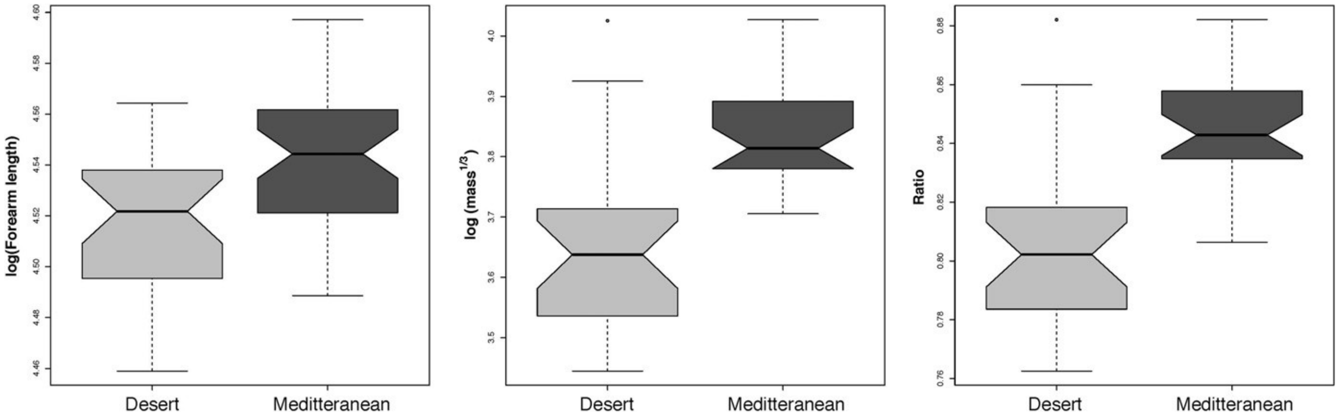


FIGURE 2 Spatial distribution of landscape categories (left) and conductivity map (right). The conductivity map shows the output obtained in CIRCUITSCAPE using the landscape use values obtained from GPS- and radio-telemetry tracked individuals. The 0.9, 0.5 and 0.1 isolines of probability of the spatial distribution of the Levantine cluster obtained in GENELAND are also depicted (right)

(MMRR IBD_{ALL}: b = 0.36; 95% CI = 0.49, 0.23; $R^2 = 0.13$; 95% CI = 0.05, 0.23; see Table 2), in close agreement with the significant IBD pattern observed at the colony level (see above). Habitat use of fruit bats, as quantified by GPS- or radio-tracked individuals, revealed a frequent use of human-modified landscapes (agriculture: 10.5%; plantation/orchards: 46.58%; built-up areas: 15.98%) compared to natural habitat patches (forest and shrubs: 26.48%; abandoned fields: 0.46%). These values were included in CIRCUITSCAPE analyses as connectivity measurements to calculate resistance matrices used in MMRR analyses. The effect of the resistance distances on genetic relatedness (IBR, b = 0.30; 95% CI = 0.41, 0.18) was similar to the isolation-by-distance pattern (IBD) although the goodness of fit of the model decreased ($R^2 = 0.09$). When both types of distances were included in the model, the effect of the geographic distance was twice that of landscape-based distance (b_{GEO} = 0.30; b_{RES} = 0.15; Table 2). The effect of the resistance distance within the Mediterranean and desert areas was negligible when compared to the Euclidean distance (see Table 2).



F I G U R E 3 Genetic landscape-shape analysis. Predicted genetic distances higher than average are displayed as peaks, lower than average are displayed as valleys. Dashed line shows the limit between northern (Levantine) and southern (Arabian) groups



F I G U R E 4 Morphometric analysis. Differences in forearm length (left), body mass (middle) and the ratio of both (right) between specimens belonging to the desert and Mediterranean genetic clusters

4 | DISCUSSION

We integrated population genetics and movement ecology to examine how genetic differentiation and habitat use of Egyptian fruit bats are associated in the Middle East. The analysis of detailed individual GPS tracks showed that fruit bats exploit human-altered habitats despite being a scarce resource in the transition area. Interestingly, we found a clear bias of habitat use of fruit bats towards human-related habitats (agriculture, orchards and built-up areas, ca. 63%), although circuit theory models in combination with kinship analysis discarded a significant effect of anthropogenic activities on gene flow patterns.

4.1 | Biomes, genetic and morphological structure

Our study provides new insights into the distribution and the genetic and morphological structure of Egyptian fruit bat within the transition zone along the Middle East. The whole population is divided into two genetic clusters along a latitudinal gradient. The northern cluster encompasses all Mediterranean areas and the northeastern side of the Jordan Rift Valley, whereas the southern cluster comprises the colonies located in the Negev and Sinai deserts and the southeastern side of the Jordan Rift Valley. These two clusters were slightly differentiated (overall $F_{ST} = 0.023$) although there were no deviations of genotype and allele frequencies at the overall study area (HWE test; not significant) suggesting that gene flow level counteracts natural selection or genetic drift. Significant deviation from HWE across loci was found in the Tel Aviv area (HWE; $p = .016$), although the only significant heterozygote excess was reported in Halulim ($F_{IS} = 0.039$; $H_E = 0.643$, $H_O = 0.666$). The Hardy–Weinberg disequilibrium reported in Tel Aviv reflects deviation from panmixia and random mating (likely due to a harem-based mating system) (see e.g., Korine, Izhaki, & Makin, 1994) although we cannot distinguish the evolutionary forces causing this pattern. The observed heterozygote excess in Halulim, however, suggests some strategy of inbreeding avoidance or differences in allelic frequencies between males and females caused by sex-biased dispersal.

T A B L E 2 Multiple matrix regression with randomization (MMRR) for genetic relatedness in relation with geographic (Euclidean) (isolation by distance) and resistance (isolation by resistance) distances. The 95% confidence intervals of the effect (b) and the coefficient of determination (R^2) are given between parentheses

		Relatedness vs. Euclidean		Relatedness vs. Resistance		Relatedness vs. Euclidean vs. Resistance		
Model	n	b	R^2	b	R^2	b_{geo}	b_{res}	R^2
Overall	25	0.36 (0.49, 0.23)	0.13 (0.05, 0.23)	0.30 (0.41, 0.18)	0.09 (0.03, 0.17)	0.30 (0.45, 0.13)	0.15 (0.30, 0.01)	0.12 (0.06, 0.21)
Mediterranean cluster	20	0.23 (0.44, 0.04)	0.05 (0.002, 0.17)	0.03 (0.40, 0.19)	0.009 (0.00002, 0.1)	0.19 (0.40, 0.10)	0.0008 (0.39, 0.23)	0.05 (0.004, 0.15)
Desert cluster	5	0.04 (0.54, 0.48)	0.03 (0.0001, 0.26)	0.07 (1.53, 1.25)	0.06 (0.0002, 0.34)	6.47 (10.3, 3.01)	6.71 (2.73, 10.46)	0.47 (0.09, 0.84)

There are also differences in population structure when both clusters are compared. For example, the southern cluster shows the highest genetic differences among colonies, revealing a higher dispersal resistance of desert environment than the northern group, as can be expected (Figure 3). This is also observed in the conductivity maps as the arid lowland habitats of Negev desert become the main geographic barrier between both clusters (Figure 2). Moreover, the distribution of admixed individuals supports this hypothesis and also reveals that the southern population may disperse to the north throughout the Jordan highlands. A closer look at the land cover reveals patches of suitable habitat in desert area, which may potentially play the role of stepping stones promoting the colonization of desert sites.

The two genetic clusters also differ morphologically, with the specimens belonging to the desert genetic cluster weighing less and having shorter forearms than the specimens belonging to the Mediterranean genetic cluster. Several explanations have been proposed to explain size and body mass geographic variation, including the Bergmann's rule suggesting that mammalian size decrease with increasing temperature, but little evidence had been found for this rule in bats at an interspecific level (Safi, Meiri, & Jones, 2013).

4.2 | The Levantine–Arabian transition

Admixed individuals are mainly gathered around the Jordan Rift, that is, in the area where both genetic clusters overlap (Kufranja, Iraq Al Amir and Ein Gedi, Table 1). Halulim, located in the sharp Mediterranean-Negev Desert ecotone of Israel that coincides with the transition area between the northern and southern clusters, also showed a high proportion of admixed individuals. In addition, Wadi El Feiran showed the highest proportion of private alleles (14 alleles in 11 of 18 loci -ca. 60%-, Table S1) which, according to Slatkin (1985), reflects the lowest gene flow rate in the study area. These results support the unique role of ecotones, or steep environmental transition zones, as either a reservoir of genetic diversity (Kark, Alkon, Safriel, & Randi, 1999; Kark et al., 2008) or a hybrid zone of divergent populations or lineages (Gligor et al., 2009; Hewitt, 1988; Kark & van Rensburg, 2013; Smith, 1997). Due to its geographic location, Ein Gedi is used by fruit bats as a foraging and potentially breeding site, which might also explain the relatively high proportion of admixed individuals (58%) in the colony. A few colonies outside of the Mediterranean-Negev desert ecotone also showed a high proportion of admixed individuals, although this likely reflects their low sample size (Mount Carmel, n = 1; Old Gesher, n = 1; Elad, n = 4).

Although the phylogeography of the Middle East is less well studied compared to Europe (Hewitt, 2000), several studies indicate a phylogeographic break between Mediterranean and Arabian lineages that follows approximately the edges of the Dead Sea Rift. For example, a contact (and possibly hybrid) zone between Hyla savignyi and H. felixarabica was reported eastward from the Dead Sea Rift (Gvozdık, Moravec, Kl€tsch, & Kotlık, 2010). The Arabian clades of R. aegyptiacus and H. felixarabica also have a similar pattern of disjunct range of two closely related lineages inhabiting Negev and western Yemen. However, this disjunction could be also caused by the lack of relevant data from the Hijaz Mountains of western Arabia. Anyway, the montane region of western Yemen is denoted as Eastern Afromontane hotspot and could play a role as a refuge and generate endemic lineages (Mallon, 2011).

The shallow genetic structure between the Mediterranean and desert lineages was largely explained by the environmental conditions and fits to an IBE pattern. IBE among highly mobile animals is reported especially in large mammals as in cosmopolitan predators such as wolves (Carmichael et al., 2007; Czarnomska et al., 2013; Musiani et al., 2007; Pilot et al., 2006) or killer whales (Foote, 2012; Morin et al., 2015; Moura et al., 2014), but also in carnivores with more restricted distribution ranges as in coyotes (Sacks, Brown, & Ernest, 2004), Arctic foxes (Carmichael et al., 2007; Dalen et al., 2004), lynx (Row et al., 2014; Rueness et al., 2003), cougars (Waller & Wheeler, 2012) or sea lions (Wolf et al., 2008) and herbivores as, for example, caribou (Courtois, Bernatchez, Ouellet, & Breton, 2003; Pond, Brown, Wilson, & Schaefer, 2016). In contrast, ecotypes in bats have been seldom studied. Evidence for this phenomenon within the order Chiroptera comes mainly from the Mediterranean region of South Africa, where differentiation of respective ecoregions promoted ecological diversification within long-fingered (Miniopterus; Miller-Butterworth, Jacobs, & Harley, 2003) and horseshoe bats (Rhinolophus; Stoffberg, Schoeman, & Matthee, 2012). Steep gradients among particular biomes and differentiation driven by Pleistocene climate oscillation is typical for regions with Mediterranean type of climate and may be a general feature of these cases and the one presented in the Egyptian fruit bat.

Our study shows the adaptive phenotypic evolution in both lineages. A smaller sized subspecies R. a. arabicus (Anderson, 1902) was ascertained in comparison to larger form in the north R. a. aegyptiacus (Geoffroy, 1810) (Benda et al., 2012; Bergmans, 1994; Juste & Ibañez, 1993). In the current study, we found an association between genetic structure and body size along the transition area, providing evidence for IBA between both lineages. The correlation between individual traits and habitat preferences is not always straightforward as adaptations are complex and they also involve behavioural and foraging ecology or physiology. Therefore, the evolution of Arabian and Mediterranean forms of fruit bats might result, therefore, from a variety of processes including natal habitat preference, philopatry, physiological adaptation driven by thermal metabolism or nutritional limitations. Although further research including landscape genomics is needed to test these hypotheses, our results provide clear evidences for IBE and IBA between both lineages and support their classification as ecotypes.

4.3 | Anthropogenic impact on gene flow and habitat use patterns

The Middle East has a rich history of human impact on the landscape and its wildlife. This region has undergone profound deep habitat transformations, especially from the second half of the 20th century, linked to the development in agriculture, urbanization and infrastructures (e.g., only during the 1960s the number of cities with 100,000 inhabitants or more in the Middle East increased from 249 to 837) (Clarke, 1980). This scenario has created a mosaic of natural and human-altered ecosystems with a clear impact on the vertebrate community (Perevolotsky, 2005). A significant IBD between colonies revealed a decrease in gene flow rates as the geographic distance increases, a pattern also observed as the average relatedness between colonies decreased with distance. This result suggests that there is limiting factor to dispersal that promotes isolation in a flying mammal that is theoretically less sensitive to habitat heterogeneity than terrestrial species as shown in the grey long-eared bat (Plecotus austriacus) (Razgour, 2015; Razgour et al., 2014). The comparison between the genetic differentiation within the distribution ranges of the Levantine and Arabian lineages (shallow in the former, strong in the latter; Figure 3) proved that colonies in the desert are more isolated and gene flow patterns vary at similar spatial scales. This pattern shows the relative importance of the landscape heterogeneity between colonies and preferences of specific habitat types by fruit bats (see Figure 2). This is in agreement with circuit theory models for the Arabian clade, showing high isolation among particular localities in arid zones of Sinai, southern Israel and southwestern Jordan. Reduction of gene flow within these areas may be ascribed to landscape and environmental properties of desert and mountain type of habitats. The Arabian ecotype inhabits rather montane and dry areas of the Arabian peninsula and Persia (Hulva et al., 2012), reaching similar type of habitats in Sinai and highlands of Jordan, which possibly mirror ancestral habitat in mountains of East Africa. Deserts are characterized by source scarcity, seasonality and patchiness when compared with more humid Ecozones (Kelt, 2011). Accordingly, desert colonies of fruit bats work as demes in a stepping stone dispersal within an insular model of population genetic structure. This is in close agreement with the role of oases as stopover sites located along bird migration flyways (Lavee & Safriel, 1989; Shobrak, 2011). The Mediterranean ecotype, on the other hand, is distributed in the Nile valley and coastal parts of the Levant and Turkey and inhabits lowlands. As the Jordan River cannot be considered a biogeographic barrier for the fruit bat, and given abrupt changes in many environmental variables in the region, we can conclude an association between genetic differentiation with environmental differences (IBE) (Wang & Bradburd, 2014). This pattern can arise independently from different processes, including natural and sexual selection and differences of fitness in hybrid offspring. Interestingly, the observed genetic differentiation could also be related to biased dispersal of individuals towards particular environments (e.g., natal habitats) or to phenotypic differences between native and immigrant individuals (Nosil, Vines, & Funk, 2005).

The exploitation of human-altered habitats by fruit bats was confirmed when detailed movement data and land-use information based on GPS- and radio-tagged individuals was used in combination with circuit theory models. Our approach based on the analysis of habitat use revealed two main conclusions. First, fruit bats are attracted to some landscape variables likely due to their foraging behaviour (e.g., plantations/orchards and forest areas). Second, habitat preferences in fruit bats are not involved in genetic differentiation between the Mediterranean and desert lineages. These seemingly mutually discriminant results are, in fact, proving that landscape genetics and resource selection function models differ in their rationale (Roffler et al., 2016). Our landscape genetics approach suggests that habitat/resource selection does not predict gene flow patterns in fruit bats. However, the movement ecology approach contributes to explain the structural connectivity of colonies throughout the transition area. Differences in the foraging behaviour (and brain activities) of fruit bats have been reported when city vs country colonies are compared (Dashevsky, Assaf, & Yovel, 2017). These authors showed that country bats fly every night to feed on a distant specific tree whereas city bats forage at many different trees. Further research should focus on foraging, social and mating behaviour in fruit bats from country and city locations and how they may influence dispersal and breeding behaviours. We hypothesize that a harem-based and promiscuous mating system (e.g., in swarming sites) might explain the observed discrepancy between habitat use and gene flow patterns.

5 | CONCLUSIONS

We found evidences for ecological differentiation (IBE) and phenotypic adaptation (IBA) of Egyptian fruit bats in relation to different habitat types, suggesting presence of two distinct ecotypes located at desert and Mediterranean areas. Proximate mechanisms behind this differentiation including, for example, microevolution of physiological and behavioural traits needs further research. We suggest that the two ecotypes probably result from historical processes (allopatric evolution and secondary contact of Mediterranean and Arabian lineages) shaped by contemporary processes such as ecological constraints and gene flow. Our results indicate that fruit bats benefit from human-mediated habitats for daily movements and foraging behaviour, while dispersal (promoter of gene flow between colonies) is probably not affected by human-altered habitats.

ACKNOWLEDGEMENTS

We thank all members of the Movement Ecology Lab as well as Nachum Ulanovsky and Yossi Yovel for their laboratory and field assistance. Logistical support for genotyping was provided by the Hebrew University Center for Genomic Technologies and the Charles University. This work was funded by the Israel Science Foundation (ISF) Grants ISF-FIRST 1316/05 and ISF 1259/09, the Grant Agency of the Academy of Sciences of the Czech Republic (IAA 601110905) and the Institutional Research Support grant No. SVV 260 313/2016, the Adelina and Massimo Della Pergolla Chair of Life Sciences and the Minerva Center for Movement Ecology (R.N.), the President of Israel Doctoral Award (D.S.), the Lady Davis Fellowship Trust and the University Pablo de Olavide (A.C-C).

DATA ACCESSIBILITY

Genotypes (n = 223) of 18 microsatellites, sampling sites and coordinates (latitude/longitude in decimal degrees) of Egyptian fruit bats analysed in this study are deposited in DRYAD (https://doi.org/10.5061/dryad.93pr2). The R-code for the whole sequential procedure written to estimate the average relatedness between colonies is available as a supplementary material (Appendix S1).

AUTHOR CONTRIBUTIONS

A.C-C and P.H. conceived and led the study and wrote the manuscript. A.C-C., T.S. and P.H. did the molecular work. A.C-C., T.S. and P.H. performed population and landscape genetics analyses. D.S. performed the analysis of telemetry data, D.R. ran GIS analyses for environmental stratification and conductivity/resistance analyses and S.S. ran the statistical models and wrote the R-code for the sequential approach available in the Appendix S1. A.T. and P.B did the fieldwork and gathered the morphometric data in Israel and in Jordan and Sinai, respectively. A.E. performed the morphometric analyses. I.H. and R.N. funded the project. All authors reviewed the final draft of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Centeno-Cuadros A, Hulva P, Romportl D, et al. Habitat use, but not gene flow, is influenced by human activities in two ecotypes of Egyptian fruit bat (Rousettus aegyptiacus). Mol Ecol. 2017;00:1–14. https://doi.org/10.1111/mec.14365

Figure S1. Forearm length (left), body mass (center) and their ratio (right) in males (M) and females (F) of *Rousettus aegyptiacus* from Desert and Mediterranean clusters.

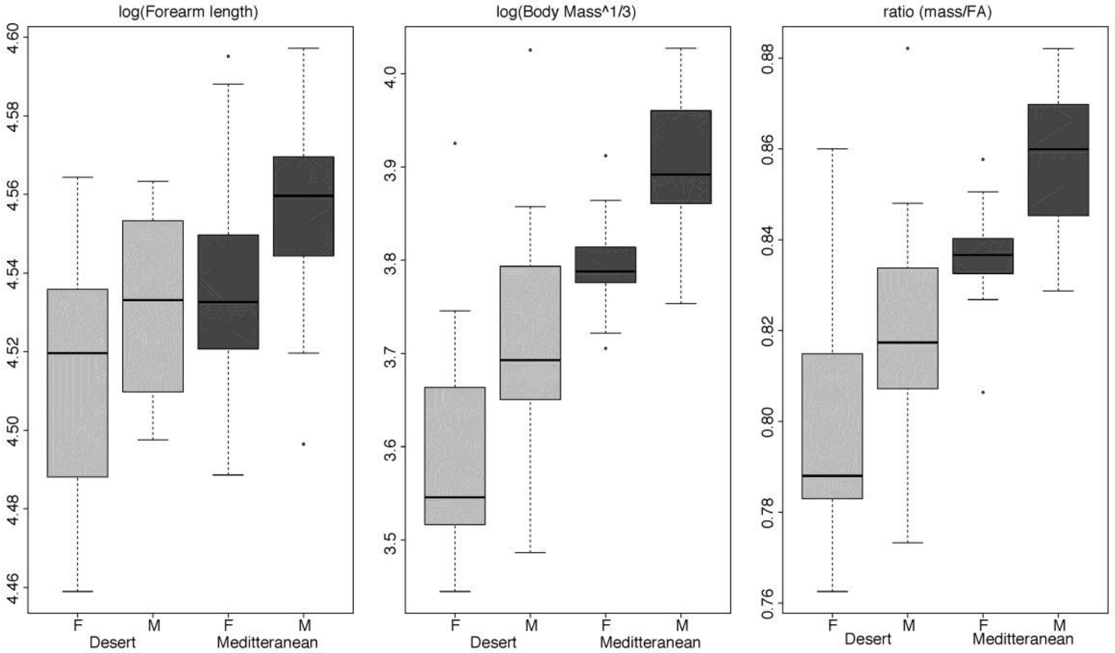

Table S1. Number of private alleles per locus and population of *Rousettus aegyptiacus* included in this study. **PAL**: number of private alleles per locus per populations. **PAP**: number of populations with private alleles per locus. **KPAP**: number of private alleles per population. **LPAP**: number of locus with private alleles per population. Aseret (**AS**), BeerSheva (**BS**), EinElFurtaga (**FUR**), EinGedi (**EG**) EinKhudra (**KHU**), Elad (**EL**) Halulim (**HA**), IraqalAmir (**IAA**), Kufranja (**KU**), Lotan (**LO**), MtCarmel (**CAR**), Sgafim (**SG**), TelAviv (**TLV**), WadiDana (**WDA**), WadiElFeiran (**WEF**).

	AS	**BS**	**FUR**	**EG**	**KHU**	**EL**	**HA**	**IAA**	**KU**	**LO**	**CAR**	**SG**	**TLV**	**WDA**	**WEF**	**PAL**	**PAP**
M3-1								1	1						1	3	3
M3-120				1				1	1						2	5	5
M3-121						1			1	1						3	3
M3-6				1					1			1		1	3	7	5
M3-8	1						1								1	3	3
Z105		1	1				1						1			4	4
Z106						1									1	2	2
Z110				1	1			1							1	4	4
Z117		1		1												2	2
Z304				1											1	2	2
Z327				1							1				1	3	3
Z334	1	1						1								3	3
Z340														1	1	2	2
Z341														2		2	1
Z343				1								1			1	3	3
Z407											1					1	1
Z413				1				1	1		1				1	5	5
Z80														1		1	1
KPAP	2	3	1	8	1	2	2	5	5	1	3	2	1	5	14		
LPAP	2	3	1	8	1	2	2	5	5	1	3	2	1	4	11		

SUPPORTING INFORMATION FILE

Habitat use, but not gene flow, is influenced by human activities in two ecotypes of Egyptian fruit bat (*Rousettus aegyptiacus*)

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1. R-code for the sequential procedure

```
# rm(list=ls())
library(sp)
library(related)
library(Matrix)
library(tseries)

set.seed(628251947)
# We use the function script detailed in Wang (2013). MMRR  performs Multiple
Matrix Regression with Randomization analysis. Y is a dependent distance matrix. X is
a list of independent distance matrices (with optional names)####

MMRR<-function(Y,X,nperm=999){
  #compute regression coefficients and test statistics
  nrowsY<-nrow(Y)
  y<-unfold(Y)
  if(is.null(names(X)))names(X)<-paste("X",1:length(X),sep="")
  Xmats<-sapply(X,unfold)
  fit<-lm(y~Xmats)
  coeffs<-fit$coefficients
  summ<-summary(fit)
  r.squared<-summ$r.squared
  tstat<-summ$coefficients[,"t value"]
  Fstat<-summ$fstatistic[1]
  tprob<-rep(1,length(tstat))
  Fprob<-1

  #perform permutations
  for(i in 1:nperm){
    rand<-sample(1:nrowsY)
    Yperm<-Y[rand,rand]
    yperm<-unfold(Yperm)
    fit<-lm(yperm~Xmats)
    summ<-summary(fit)
    Fprob<-Fprob+as.numeric(summ$fstatistic[1]>=Fstat)
    tprob<-tprob+as.numeric(abs(summ$coefficients[,"t value"])>=abs(tstat))
  }

  #return values
  tp<-tprob/(nperm+1)
  Fp<-Fprob/(nperm+1)
  names(r.squared)<-"r.squared"
  names(coeffs)<-c("Intercept",names(X))
  names(tstat)<-paste(c("Intercept",names(X)),"(t)",sep="")
  names(tp)<-paste(c("Intercept",names(X)),"(p)",sep="")
  names(Fstat)<-"F-statistic"
  names(Fp)<-"F p-value"
  return(list(r.squared=r.squared,
            coefficients=coeffs,
```

```
			tstatistic=tstat,
			tpvalue=tp,
			Fstatistic=Fstat,
			Fpvalue=Fp))
}

# unfold, called by MMRR, converts the lower diagonal elements of a matrix into a vector
unfold<-function(X){
  x<-vector()
  for(i in 2:nrow(X)) x<-c(x,X[i,1:i-1])
  return(x)
}

##################################################
#		25 sites			#
##################################################
# "eudist.txt" contains the geographic coordinates (longitude and latitude) of the 25 sampled populations
eudist<-read.table("eudist.txt",sep="\t",header=T)
# We create a 25x25 matrix where the cell i,j refers to the Euclidean distance in Km between the population i and the population j:
eudistm<-spDists(as.matrix(eudist[,2:3]),longlat=T)
# We standardize the matrix so that mean=zero and sd=1
eudistm<- (eudistm-mean(eudistm))/sd(eudistm)

# Here we call the flat matrix of resistances, i.e. calculated in CIRCUITSCAPE by assigning to all the cells in the raster layer the same value of resistance:
eudistmFlat<- read.matrix("FLAT_resistances.txt",sep="")
eudistmFlat<- (eudistmFlat-mean(eudistmFlat))/sd(eudistmFlat)#this is to standardize the matrix so that mean=zero and sd=1

# Just NORTH (Mediterranean):
eudistN<- eudist[1:20,]
eudistmN<-spDists(as.matrix(eudistN[,2:3]),longlat=T)# It calculates Euclidean distance matrix in Km
eudistmN<- (eudistmN-mean(eudistmN))/sd(eudistmN)# this is to standardize the matrix

# eudistmN with flat model:
eudistmNFlat<- read.matrix("FLAT_NORTH_CL_resistances.txt",sep="")
eudistmNFlat<- (eudistmNFlat-mean(eudistmNFlat))/sd(eudistmNFlat)

# Just SOUTH (desert):
eudistS<- eudist[21:25,]
eudistmS<-spDists(as.matrix(eudistS[,2:3]),longlat=T)# It calculates Euclidean distance matrix in Km
eudistmS<- (eudistmS-mean(eudistmS))/sd(eudistmS)# this is to standardize the matrix

# eudistmS with flat model:
```

```
eudistmSFlat<- read.matrix("FLAT_SOUTH_CL_resistances.txt",sep="")
eudistmSFlat<- (eudistmSFlat-mean(eudistmSFlat))/sd(eudistmSFlat)

################################
# Preparing the genetic data: #
################################
# rousettus.txt contains the genotype of all the 222 individuals sampled within the 25
sites
data<-read.table("rousettus.txt",stringsAsFactors=F)
data[,1]<-as.factor(data[,1])
# datal is a list with the genotypes of each of the 222 individuals sampled in each of the
25 sites
datal<-split(data,data[,1])
# cont1 will contain the genotypes of one randomy selected individual proceeding from
each site.
cont1<-matrix(0,25,37)
cont1<-as.data.frame(cont1)
# liston will be a list containing 100 cont1 (each one built by random selection with
replacement)
liston<-vector("list", 100)
for(i in 1:100){
  for(k in 1:length(datal)){
    cont1[k,]<-datal[[k]][sample(nrow(datal[[k]]),1),]
  }
  liston[[i]]<-cont1
}

# Now the matrix of genetic relatedness
#First, we transform the 100 (simulated) data sets on genotype of the 25 populations in
100 data sets of genetic relatedness between the 25 populations. We do it by using the
wang estimator within the function 'coancestry' from the 'related' package. We selected
the Wang's estimator because it showed the highest correlation coefficient (analyses not
shown here) between observed and expected relatedness values after simulating
genotypes of known relatedness.
out<-vector("list",100)
for(i in 1:100){
  out[[i]]<-
data.frame(a=as.numeric(as.integer(coancestry(liston[[i]],wang=1)$relatedness$ind1.id)
),b=as.numeric(as.integer(coancestry(liston[[i]],wang=1)$relatedness$ind2.id)),c=coanc
estry(liston[[i]],wang=1)$relatedness$wang,stringsAsFactors=FALSE)
}

# Next, we store these data sets in symmetric matrices
zmat<-vector("list",100)
for(y in 1:100){
  zmat[[y]]<-with(out[[y]],matrix(0,ncol=max(out[[y]]$b), nrow=1+max(out[[y]]$a)))
  # Then populate it with:
  zmat[[y]][with(out[[y]],cbind(a,b))]<-with(out[[y]],c)
  zmat[[y]]<-forceSymmetric(zmat[[y]])
}
```

```
# This is to standardize the genetic matrices:
for (z in 1:100){
  zmat[[z]]<- (zmat[[z]]-mean(zmat[[z]]))/sd(zmat[[z]])
}

# We repeat the entire process considering the NORTH (Mediterranean) populations:
dataN<- data[1:148,]
dataN[,1]<- as.factor(dataN[,1])
datalN<- split(dataN,dataN[,1])
datalN<- datalN[1:20]
cont1N<- matrix(0,20,37)# Note that there are 20 populations in this area
cont1N<-as.data.frame(cont1N)
listonN<-vector("list", 100)
for(i in 1:100){
  for(k in 1:length(datalN)){
    cont1N[k,]<-datalN[[k]][sample(nrow(datalN[[k]]),1),]
  }
  listonN[[i]]<-cont1N
}

outN<-vector("list",100)
for(i in 1:100){
  outN[[i]]<-
data.frame(a=as.numeric(as.integer(coancestry(listonN[[i]],wang=1)$relatedness$ind1.i
d)),b=as.numeric(as.integer(coancestry(listonN[[i]],wang=1)$relatedness$ind2.id)),c=c
oancestry(listonN[[i]],wang=1)$relatedness$wang,stringsAsFactors=FALSE)
}

zmatN<-vector("list",100)
for(y in 1:100){
  zmatN[[y]]<-with(outN[[y]],matrix(0,ncol=max(outN[[y]]$b),
nrow=1+max(outN[[y]]$a)))
  # Then populate it with:
  zmatN[[y]][with(outN[[y]],cbind(a,b))]<-with(outN[[y]],c)
  zmatN[[y]]<-forceSymmetric(zmatN[[y]])
}

# This is to standardize the genetic matrices:
for (z in 1:100){
  zmatN[[z]]<- (zmatN[[z]]-mean(zmatN[[z]]))/sd(zmatN[[z]])
}

# ...and the SOUTH (desert) populations:
dataS<- data[149:222,]
dataS[,1]<- as.factor(dataS[,1])
datalS<- split(dataS,dataS[,1])
datalS<- datalS[21:25]
cont1S<- matrix(0,5,37)# Note that there are only 5 populations in this area
cont1S<-as.data.frame(cont1S)
```

```
listonS<-vector("list", 100)
for(i in 1:100){
  for(k in 1:length(datalS)){
    cont1S[k,]<-datalS[[k]][sample(nrow(datalS[[k]]),1),]
  }
  listonS[[i]]<-cont1S
}

outS<-vector("list",100)
for(i in 1:100){
  outS[[i]]<-data.frame(a=as.numeric(as.integer(coancestry(listonS[[i]],wang=1)$relatedness$ind1.id)),b=as.numeric(as.integer(coancestry(listonS[[i]],wang=1)$relatedness$ind2.id)),c=coancestry(listonS[[i]],wang=1)$relatedness$wang,stringsAsFactors=FALSE)
}

zmatS<-vector("list",100)
for(y in 1:100){
  zmatS[[y]]<-with(outS[[y]],matrix(0,ncol=5, nrow=5))
  # Then populate it with:
  zmatS[[y]][with(outS[[y]]-20,cbind(a,b))]<-with(outS[[y]],c)
  zmatS[[y]]<-forceSymmetric(zmatS[[y]])
}

# This is to standardize the genetic matrices:
for (z in 1:100){
  zmatN[[z]]<- (zmatN[[z]]-mean(zmatN[[z]]))/sd(zmatN[[z]])
}
for (z in 1:100){
  zmatS[[z]]<- (zmatS[[z]]-mean(zmatS[[z]]))/sd(zmatS[[z]])
}

# Now we create the matrices of resistances. The file "0005_OUT_resistances.txt" contains the (symmetric) matrix with the resistance matrices calculated in CIRCUITSCAPE based on percentage time of use of different habitat types (see main text for details):
res<- read.matrix("0005_OUT_resistances.txt",sep="")
# This is to standardize the resistance matrix:
res<- (res-mean(res))/sd(res)

# Here we store the geographic, resistance, and flat matrices in a list that we will use to run the MMRR function:
Xmats<- list(geo=eudistm,res=res,res0=eudistmFlat)

# Just NORTH:
resN<- read.matrix("NORTH_CL_resistances.txt",sep="")
resN<- (resN-mean(resN))/sd(resN)

XmatsN<- list(geo=eudistmN,res=resN,res0=eudistmNFlat)
```

```
# Just SOUTH:
resS<- read.matrix("SOUTH_CL_resistances.txt",sep="")
resS<- (resS-mean(resS))/sd(resS)

XmatsS<- list(geo=eudistmS,res=resS,res0=eudistmSFlat)

################################################################
#                                               #
#        MMRR  Genetic vs. geograph. distance            #
#                                               #
################################################################
# this list will contains the outputs of the 100 MMRR analyses (one for each simulated
data set of genetic relatedness):
amr<-list()
# this vector will contain the 100 coefficients (slope) obtained by running the 100
MMRR analyses
mr.GG<- vector("numeric",100)
# this vector will contain the 100 r^2 obtained by running the 100 MMRR analyses
mrR2.GG<- vector("numeric",100)

for (i in 1:length(zmat)){
  (amr[[i]]<- MMRR(Y=zmat[[i]],X=Xmats[1],nperm=1000))
}
for (i in 1:length(amr)) mr.GG[i]<- amr[[i]]$coefficients[2]
for (i in 1:length(amr)) mrR2.GG[i]<- amr[[i]]$r.squared

# Next, we calculate the median and 2.5,97.5% quantiles:
(medianmr.GG <- median(mr.GG))
(CRI95mr.GG <-
c(as.numeric(quantile(mr.GG,0.025)),(as.numeric(quantile(mr.GG,0.975)))))
(medianmrR2.GG <- median(mrR2.GG))
(CRI95mrR2.GG <-
c(as.numeric(quantile(mrR2.GG,0.025)),(as.numeric(quantile(mrR2.GG,0.975)))))

################################################################
#                                               #
#        MMRR  Genetic vs. geograph. distance NORTH      #
#                                               #
################################################################
amrN<-list()
mr.GGN<- vector("numeric",100)
mrR2.GGN<- vector("numeric",100)

for (i in 1:length(zmatN)){
  (amrN[[i]]<- MMRR(Y=zmatN[[i]],X=XmatsN[1],nperm=1000))
}
for (i in 1:length(amrN)) mr.GGN[i]<- amrN[[i]]$coefficients[2]
for (i in 1:length(amrN)) mrR2.GGN[i]<- amrN[[i]]$r.squared

(medianmr.GGN <- median(mr.GGN))
```

```r
(CRI95mr.GGN <-
c(as.numeric(quantile(mr.GGN,0.025)),(as.numeric(quantile(mr.GGN,0.975)))))
(medianmrR2.GGN <- median(mrR2.GGN))
(CRI95mrR2.GGN <-
c(as.numeric(quantile(mrR2.GGN,0.025)),(as.numeric(quantile(mrR2.GGN,0.975)))))

#########################################################
#                                                       #
#       MMRR  Genetic vs. geograph. distance SOUTH      #
#                                                       #
#########################################################
amrS<-list()
mr.GGS<- vector("numeric",100)
mrR2.GGS<- vector("numeric",100)

for (i in 1:length(zmatS)){
  (amrS[[i]]<- MMRR(Y=zmatS[[i]],X=XmatsS[1],nperm=1000))
}
for (i in 1:length(amrS)) mr.GGS[i]<- amrS[[i]]$coefficients[2]
for (i in 1:length(amrS)) mrR2.GGS[i]<- amrS[[i]]$r.squared

(medianmr.GGS <- median(mr.GGS))
(CRI95mr.GGS <-
c(as.numeric(quantile(mr.GGS,0.025)),(as.numeric(quantile(mr.GGS,0.975)))))
(medianmrR2.GGS <- median(mrR2.GGS))
(CRI95mrR2.GGS <-
c(as.numeric(quantile(mrR2.GGS,0.025)),(as.numeric(quantile(mrR2.GGS,0.975)))))

#########################################################
#                                                       #
#           MMRR  Genetic vs. resistance          #
#                                                       #
#########################################################
amr1<-list()
mr1.GG<- vector("numeric",100)
mr1R2.GG<- vector("numeric",100)
for (i in 1:length(zmat)){
  amr1[[i]]<- MMRR(Y=zmat[[i]],X=Xmats[2],nperm=1000)
}

for (i in 1:length(amr1)) mr1.GG[i]<- amr1[[i]]$coefficients[2]
for (i in 1:length(amr1)) mr1R2.GG[i]<- amr1[[i]]$r.squared

(medianmr1.GG <- median(mr1.GG))
(CRI95mr1.GG <-
c(as.numeric(quantile(mr1.GG,0.025)),(as.numeric(quantile(mr1.GG,0.975)))))
(medianmr1R2.GG <- median(mr1R2.GG))
(CRI95mr1R2.GG <-
c(as.numeric(quantile(mr1R2.GG,0.025)),(as.numeric(quantile(mr1R2.GG,0.975)))))
```

```
################################################################
#                                          #
#              MMRR  Genetic vs. resistance NORTH       #
#                                          #
################################################################
amr1N<-list()# the list hosting the genetic vs. resistance MMRR outputs
mr1.GGN<- vector("numeric",100)
mr1R2.GGN<- vector("numeric",100)
for (i in 1:length(zmatN)){
  amr1N[[i]]<- MMRR(Y=zmatN[[i]],X=XmatsN[2],nperm=1000)
}

for (i in 1:length(amr1N)) mr1.GGN[i]<- amr1N[[i]]$coefficients[2]
for (i in 1:length(amr1N)) mr1R2.GGN[i]<- amr1N[[i]]$r.squared

(medianmr1.GGN <- median(mr1.GGN))
(CRI95mr1.GGN <-
c(as.numeric(quantile(mr1.GGN,0.025)),(as.numeric(quantile(mr1.GGN,0.975)))))
(medianmr1R2.GGN <- median(mr1R2.GGN))
(CRI95mr1R2.GGN <-
c(as.numeric(quantile(mr1R2.GGN,0.025)),(as.numeric(quantile(mr1R2.GGN,0.975))))
)

################################################################
#                                          #
#              MMRR  Genetic vs. resistance SOUTH       #
#                                          #
################################################################
amr1S<-list()
mr1.GGS<- vector("numeric",100)
mr1R2.GGS<- vector("numeric",100)
for (i in 1:length(zmatS)){
  amr1S[[i]]<- MMRR(Y=zmatS[[i]],X=XmatsS[2],nperm=1000)
}

for (i in 1:length(amr1S)) mr1.GGS[i]<- amr1S[[i]]$coefficients[2]
for (i in 1:length(amr1S)) mr1R2.GGS[i]<- amr1S[[i]]$r.squared

(medianmr1.GGS <- median(mr1.GGS))
(CRI95mr1.GGS <-
c(as.numeric(quantile(mr1.GGS,0.025)),(as.numeric(quantile(mr1.GGS,0.975)))))
(medianmr1R2.GGS <- median(mr1R2.GGS))
(CRI95mr1R2.GGS <-
c(as.numeric(quantile(mr1R2.GGS,0.025)),(as.numeric(quantile(mr1R2.GGS,0.975)))))

################################################################
#                                          #
#   MMRR  Genetic vs. Resistance vs. Geographic           #
#                                          #
################################################################
```

```
amr2<-list()
mr2.GG<- matrix(NA,100,2)
mr2R2.GG<- vector("numeric",100)
for (i in 1:length(zmat)){
  amr2[[i]]<- MMRR(Y=zmat[[i]],X=Xmats[2:3],nperm=1000)
}

for (i in 1:length(amr2)) mr2.GG[i,]<- amr2[[i]]$coefficients[c(2,3)]
for (i in 1:length(amr2)) mr2R2.GG[i]<- amr2[[i]]$r.squared

(medianmr2.GG1 <- median(mr2.GG[,1]))
(medianmr2.GG2 <- median(mr2.GG[,2]))
(CRI95mr2.GG1 <-
c(as.numeric(quantile(mr2.GG[,1],0.025)),(as.numeric(quantile(mr2.GG[,1],0.975)))))
(CRI95mr2.GG2 <-
c(as.numeric(quantile(mr2.GG[,2],0.025)),(as.numeric(quantile(mr2.GG[,2],0.975)))))
(medianmr2R2.GG <- median(mr2R2.GG))
(CRI95mr2R2.GG <-
c(as.numeric(quantile(mr2R2.GG,0.025)),(as.numeric(quantile(mr2R2.GG,0.975)))))

##################################################################
#                                               #
#   MMRR  Genetic vs. resistance vs. geograph. NORTH       #
#                                               #
##################################################################
amr2N<-list()
mr2.GGN<- matrix(NA,100,2)
mr2R2.GGN<- vector("numeric",100)
for (i in 1:length(zmatN)){
  amr2N[[i]]<- MMRR(Y=zmatN[[i]],X=XmatsN[2:3],nperm=1000)
}

for (i in 1:length(amr2N)) mr2.GGN[i,]<- amr2N[[i]]$coefficients[c(2,3)]
for (i in 1:length(amr2N)) mr2R2.GGN[i]<- amr2N[[i]]$r.squared

(medianmr2.GG1N <- median(mr2.GGN[,1]))
(medianmr2.GG2N <- median(mr2.GGN[,2]))
(CRI95mr2.GG1N <-
c(as.numeric(quantile(mr2.GGN[,1],0.025)),(as.numeric(quantile(mr2.GGN[,1],0.975)))
))
(CRI95mr2.GG2N <-
c(as.numeric(quantile(mr2.GGN[,2],0.025)),(as.numeric(quantile(mr2.GGN[,2],0.975)))
))
(medianmr2R2.GGN <- median(mr2R2.GGN))
(CRI95mr2R2.GGN <-
c(as.numeric(quantile(mr2R2.GGN,0.025)),(as.numeric(quantile(mr2R2.GGN,0.975))))
)

##################################################################
#                                               #
```

```
#   MMRR  Genetic vs. resistance vs. geograph. SOUTH       #
#                                                    #
############################################################
amr2S<-list()
mr2.GGS<- matrix(NA,100,2)
mr2R2.GGS<- vector("numeric",100)
for (i in 1:length(zmatS)){
  amr2S[[i]]<- MMRR(Y=zmatS[[i]],X=XmatsS[2:3],nperm=1000)
}

for (i in 1:length(amr2S)) mr2.GGS[i,]<- amr2S[[i]]$coefficients[c(2,3)]
for (i in 1:length(amr2S)) mr2R2.GGS[i]<- amr2S[[i]]$r.squared

(medianmr2.GG1S <- median(mr2.GGS[,1]))
(medianmr2.GG2S <- median(mr2.GGS[,2]))
(CRI95mr2.GG1S <-
c(as.numeric(quantile(mr2.GGS[,1],0.025)),(as.numeric(quantile(mr2.GGS[,1],0.975))))
)
(CRI95mr2.GG2S <-
c(as.numeric(quantile(mr2.GGS[,2],0.025)),(as.numeric(quantile(mr2.GGS[,2],0.975))))
)
(medianmr2R2.GGS <- median(mr2R2.GGS))
(CRI95mr2R2.GGS <-
c(as.numeric(quantile(mr2R2.GGS,0.025)),(as.numeric(quantile(mr2R2.GGS,0.975)))))
```

2. **Resistance distances between 25 colonies of fruit bats estimated using CIRCUITSCAPE (filename: “FLAT_resistances”).**

```
0       0.0692294680.0881439040.1716723040.2519573780.165081924
        0.1058090660.1195212690.1076584440.1090289130.116043818
        0.1307011430.1194867910.1127168130.1151952870.116335002
        0.1168796220.2243399780.3732091310.19210632  0.593878369
        0.7197824180.8564837181.0527950381.128623801

0.0692294680        0.0555216240.1423654220.22337236  0.137524717
        0.0743627620.0881686880.0763114220.0777926520.084808967
        0.0994810320.0882727850.0815127720.0839998610.085144169
        0.0857001770.1959874720.3427813590.1610113880.563439181
        0.6891087220.8257060081.0219915961.097807447

0.0881439040.0555216240       0.13865782  0.2179678350.13307881
        0.0565624510.0708833650.05912207  0.0611410520.06808147
        0.0828117010.0716308850.06493283  0.0674475730.068610457
        0.0692068770.1889521130.3288720150.1447982460.549451578
        0.6743239970.8105671031.0067650541.082536907

0.1716723040.1423654220.13865782  0       0.2885818020.205960215
        0.1516830680.1651898040.1532893130.1544382120.161489016
        0.1761237130.1648983420.1581017820.1605700630.16170244
        0.1622317490.2662981080.4175943050.2373562890.638311826
        0.7645084640.90133992  1.0976834561.173528391

0.2519573780.22337236  0.2179678350.2885818020       0.270255838
        0.2282780280.2415078190.2295930230.2304086640.237456632
        0.2520476570.2408040380.2339767460.2364196820.237538355
        0.2380340520.3339459440.4911828670.31291222  0.711956251
        0.8388365050.9759713081.1723898821.248272488

0.1650819240.1375247170.13307881  0.2059602150.2702558380
        0.1418303830.1547109520.1428189370.1431877410.150161786
        0.1646811260.1534107280.1465548960.1489505850.150047437
        0.1504839210.2309518250.3996459160.2249023820.620421814
        0.7485614770.8862542371.0828107781.158762647

0.1058090660.0743627620.0565624510.1516830680.2282780280.141830383
        0       0.0438198010.0380644140.0440609130.04831997  0.063407029
        0.0524869480.0467336130.0492488140.0505300130.051256998
        0.1932341020.3178507360.12736374  0.5376565690.660475679
        0.7957925830.9917604711.06741682

0.1195212690.0881686880.0708833650.1651898040.2415078190.154710952
        0.0438198010       0.0467183430.0509447570.05148043  0.066881757
        0.0563035930.0517895310.0541755260.0555507160.056314299
        0.2052428160.3255331910.1323873570.5447031440.666753142
        0.8017113730.9975894271.073200672

0.1076584440.0763114220.05912207  0.1532893130.2295930230.142818937
        0.0380644140.0467183430       0.03920338  0.0444499810.059523011
        0.0485203470.0424815330.0450875110.0463771260.047152432
```

0.1934646810.3150608390.1234635170.5348001930.657253126
0.7924091790.98833739 1.063973823

0.1090289130.0777926520.0611410520.1544382120.2304086640.143187741
0.0440609130.0509447570.03920338 0   0.0402932890.054812641
0.0433101760.0348783930.0378950790.0390397620.039876916
0.1927435710.3092264430.1169460770.5294764630.651587447
0.7866205850.9825202781.058142406

0.1160438180.0848089670.06808147 0.1614890160.2374566320.150161786
0.04831997 0.05148043 0.0444499810.0402932890   0.039360588
0.0295473770.03380833 0.0347821110.0364203380.036870493
0.1993618910.3119488920.1116360930.52872925 0.648817243
0.78281819 0.9784538011.05394329

0.1307011430.0994810320.0828117010.1761237130.2520476570.164681126
0.0634070290.0668817570.0595230110.0548126410.0393605880
0.0388933330.0474195270.0475813260.0490918730.048881897
0.2136662330.3248788050.1215770830.54027311 0.659504038
0.7930470790.98856411 1.063994043

0.1194867910.0882727850.0716308850.1648983420.2408040380.153410728
0.0524869480.0563035930.0485203470.0433101760.029547377
0.0388933330   0.0351009240.0349221390.0364972190.036319429
0.2023240350.3130559850.1096767560.5285570880.647825113
0.7814046990.9769322831.052367519

0.1127168130.0815127720.06493283 0.1581017820.2339767460.146554896
0.0467336130.0517895310.0424815330.0348783930.03380833
0.0474195270.0351009240   0.0249608690.0268567680.029603287
0.19544282 0.3061468210.1084989650.5247094760.645537918
0.7799866260.9757410631.051290276

0.1151952870.0839998610.0674475730.1605700630.2364196820.148950585
0.0492488140.0541755260.0450875110.0378950790.034782111
0.0475813260.0349221390.0249608690   0.0195930930.026857173
0.1976843360.3070965240.1073529330.5248823560.645279023
0.7795287760.97523376 1.050758154

0.1163350020.0851441690.0686104570.16170244 0.2375383550.150047437
0.0505300130.0555507160.0463771260.0390397620.036420338
0.0490918730.0364972190.0268567680.0195930930   0.026071125
0.1987189660.3076339920.1079047250.5255346090.645967236
0.7802503730.9759648261.051493985

0.1168796220.0857001770.0692068770.1622317490.2380340520.150483921
0.0512569980.0563142990.0471524320.0398769160.036870493
0.0488818970.0363194290.0296032870.0268571730.0260711250
0.19896895 0.3063118090.10452958 0.5236169330.643765869
0.7779547680.9736485091.049167308

0.2243399780.1959874720.1889521130.2662981080.3339459440.230951825
0.1932341020.2052428160.1934646810.1927435710.199361891
0.2136662330.2023240350.19544282 0.1976843360.198718966

```
        0.19896895  0        0.4353467860.2718347590.6554935830.787869389
        0.9274349231.1244543381.200638578

0.3732091310.3427813590.3288720150.4175943050.4911828670.399645916
        0.3178507360.3255331910.3150608390.3092264430.311948892
        0.3248788050.3130559850.3061468210.3070965240.307633992
        0.3063118090.4353467860        0.3703382140.7179884250.862371706
        1.0080210341.2065801781.283536934

0.19210632  0.1610113880.1447982460.2373562890.31291222  0.224902382
        0.12736374  0.1323873570.1234635170.1169460770.111636093
        0.1215770830.1096767560.1084989650.1073529330.107904725
        0.10452958  0.2718347590.3703382140        0.5657434580.679689226
        0.8130232421.0086969761.084209452

0.5938783690.5634391810.5494515780.6383118260.7119562510.620421814
        0.5376565690.5447031440.5348001930.5294764630.52872925
        0.54027311  0.5285570880.5247094760.5248823560.525534609
        0.5236169330.6554935830.7179884250.5657434580        0.852442276
        1.0493365761.2600189691.343058262

0.7197824180.6891087220.6743239970.7645084640.8388365050.748561477
        0.6604756790.6667531420.6572531260.6515874470.648817243
        0.6595040380.6478251130.6455379180.6452790230.645967236
        0.6437658690.7878693890.8623717060.6796892260.8524422760
        0.9586473211.1895101211.282318884

0.8564837180.8257060080.8105671030.90133992  0.9759713080.886254237
        0.7957925830.8017113730.7924091790.7866205850.78281819
        0.7930470790.7814046990.7799866260.7795287760.780250373
        0.7779547680.9274349231.0080210340.8130232421.049336576
        0.9586473210        0.98099242  1.11172218

1.0527950381.0219915961.0067650541.0976834561.1723898821.082810778
        0.9917604710.9975894270.98833739  0.9825202780.978453801
        0.98856411  0.9769322830.9757410630.97523376  0.975964826
        0.9736485091.1244543381.2065801781.0086969761.260018969
        1.1895101210.98099242  0        0.987315204

1.1286238011.0978074471.0825369071.1735283911.2482724881.158762647
        1.06741682  1.0732006721.0639738231.0581424061.05394329
        1.0639940431.0523675191.0512902761.0507581541.051493985
        1.0491673081.2006385781.2835369341.0842094521.343058262
        1.2823188841.11172218  0.9873152040
```

3. Longitude and latitude coordinates of the 25 colonies used in this study (filename: “eudist”).

pop	lon	lat
1	35.782088	33.13935
2	35.570183	33.075225
3	34.901664	31.601153
4	35.01	32.72
5	35.562464	32.623206
6	35.616553	32.450018
7	35.716547	32.316719
8	34.844675	32.162414
9	34.777819	32.066158
10	34.95075	32.049531
11	35.023022	31.888236
12	35.749849	31.916719
13	34.747458	31.824914
14	34.655314	31.804381
15	34.726944	31.754406
16	34.918589	31.739481
17	34.888222	31.688208
18	35.383572	31.451192
19	34.791467	31.252981
20	35.518178	30.635015
21	35.086775	29.988264
22	34.9	31.666667
23	34.549931	29.500008
24	34.416639	28.900007
25	33.666659	28.700005

4. “Flat” resistance distances between 25 colonies.

```
0       0.7875668041.4337504760.9535147390.9808541031.055564413
        1.1186034841.3706386931.4820863571.3084749211.328852672
        1.2846191171.4578344931.5915798831.4743573631.396639726
        1.4173748911.4171496361.5382782481.6459062991.771748891
        1.4193140021.9085099532.1208451342.196288912
0.7875668040        1.3631825620.8642708950.91284104  0.993114811
        1.0584501651.29621711  1.4089143291.2353585391.257247989
        1.2188806671.3863871811.5203647651.4032288741.325609497
        1.3465101831.34799318  1.4682813391.5765798081.70221446
        1.34853196  1.8388841622.05119846  2.126631973
1.4337504761.3631825620        1.2127073671.1790103571.127662298
        1.1101275251.0359329961.0500703240.9155387140.83459226
        1.0285574440.8272334980.9319302310.7959207040.71068818
        0.64841229  0.8938477430.85690565  1.0627671741.160859957
        0.6107642721.2855501971.4951397951.569234428
0.9535147390.8642708951.2127073670        0.7402498210.848384315
        0.9236948691.1420889051.2557721581.08199451  1.105348054
        1.0753197241.2349373091.3691878321.2521396711.174551717
        1.1956722541.1993140631.3185663421.4277659311.553121989
        1.1977904381.6896702691.9019568011.977376697
0.9808541030.91284104  1.1790103570.7402498210        0.753029582
        0.8525212791.1232221721.23193307  1.0577259831.075208879
        1.0256992161.2045028081.3379102861.2205320761.142491305
        1.1630386071.1606471021.28290625  1.3898212821.515887087
        1.1648552621.6527484581.8651067211.940561864
1.0555644130.9931148111.1276622980.8483843150.7530295820
        0.7372941021.0915876741.1938105641.0192171391.028783314
        0.9527717621.1575729051.2898371061.1720149141.093377546
        1.11313928  1.1025166531.2288914011.3327203821.459744727
        1.11454548  1.5970292991.8094844971.884987176
1.1186034841.0584501651.1101275250.9236948690.8525212790.737294102
        0       1.0992462381.1940246131.0200550811.0197523580.893665567
        1.1463162461.2768609671.1584531621.0794249591.097858939
        1.07395035  1.2067731831.3051980091.4338947741.098655514
        1.5719107231.7845327621.860117551
1.3706386931.29621711  1.0359329961.1420889051.1232221721.091587674
        1.0992462380        0.8053493380.7652863410.8973679261.09826057
        0.9890962421.1392675661.0306968310.9731078521.001204813
        1.09923883  1.1694109341.3116363081.4266136711.00884369
        1.5585078281.7697242991.844618108
1.4820863571.4089143291.0500703241.2557721581.23193307  1.193810564
        1.1940246130.8053493380        0.82731926  0.9287045391.171309831
        0.9572036531.1158318671.0152038580.9805036891.008328432
        1.14267471  1.1919051211.3463926031.4573998511.018568114
        1.5874634681.7982555851.872940748
1.3084749211.2353585390.9155387141.08199451  1.0577259831.019217139
        1.0200550810.7652863410.82731926  0       0.7489167721.001773373
        0.8760239191.0259777910.9147885810.8477056270.878950763
```

```
     0.9864541911.0531675731.1983699241.3124905320.886883396
     1.4440275631.6551637821.730018175
1.3288526721.2572479890.83459226  1.1053480541.0752088791.028783314
     1.0197523580.8973679260.9287045390.7489167720        0.972192288
     0.83899791  0.9820389230.8623296760.7539566630.795984213
     0.9207570790.9832860911.1335539491.2464120310.803014004
     1.3774945161.5885338891.663340744
1.2846191171.2188806671.0285574441.0753197241.0256992160.952771762
     0.8936655671.09826057  1.1713098311.0017733730.9721922880
     1.0864801371.2107280031.0904331991.0116331841.024756626
     0.9488024451.1063746651.1822662971.3179151841.02336332
     1.4589494011.6722587981.748181457
1.4578344931.3863871810.8272334981.2349373091.2045028081.157572905
     1.1463162460.9890962420.9572036530.8760239190.83899791
     1.0864801370        0.7266935220.6331983880.7698986580.774166603
     0.9959946060.9797855531.1698956051.2688546010.790463903
     1.3932149431.6026750621.676705055
1.5915798831.5203647650.9319302311.3691878321.3379102861.289837106
     1.2768609671.1392675661.1158318671.0259777910.982038923
     1.2107280030.7266935220        0.7039556190.9037915260.894041071
     1.1075967041.0653784921.2680318161.3617999060.906230575
     1.4835414521.6923782051.766101585
1.4743573631.4032288740.7959207041.2521396711.2205320761.172014914
     1.1584531621.0306968311.0152038580.9147885810.862329676
     1.0904331990.6331983880.7039556190        0.7679375210.752067711
     0.9839781540.94160113  1.1451385461.2392735010.766298724
     1.3612831161.5701896661.643947382
1.3966397261.3256094970.71068818  1.1745517171.1424913051.093377546
     1.0794249590.9731078520.9805036890.8477056270.753956663
     1.0116331840.7698986580.9037915260.7679375210        0.596009238
     0.9100750170.9172957021.0981386091.2019181630.62575297
     1.3289116541.5390110311.613355875
1.4173748911.3465101830.64841229  1.1956722541.1630386071.11313928
     1.0978589391.0012048131.0083284320.8789507630.795984213
     1.0247566260.7741666030.8940410710.7520677110.5960092380
     0.9100773180.8939422961.0872901431.1874992450.468788994
     1.3128683981.5225933541.596754085
1.4171496361.34799318  0.8938477431.1993140631.1606471021.102516653
     1.07395035  1.09923883  1.14267471  0.9864541910.920757079
     0.9488024450.9959946061.1075967040.9839781540.910075017
     0.9100773180        0.9331722181.0017500261.1386748590.903854999
     1.2810033221.4946572441.570749117
1.5382782481.4682813390.85690565  1.3185663421.28290625  1.228891401
     1.2067731831.1694109341.1919051211.0531675730.983286091
     1.1063746650.9797855531.0653784920.94160113  0.917295702
     0.8939422960.9331722180        1.0028974821.0734118060.885635568
     1.18775366  1.3951875981.46822901
1.6459062991.5765798081.0627671741.4277659311.3898212821.332720382
     1.3051980091.3116363081.3463926031.1983699241.133553949
     1.1822662971.1698956051.2680318161.1451385461.098138609
```

```
        1.0872901431.0017500261.0028974820       0.9680238341.080534689
        1.1574369361.3810629551.462046906
1.7717488911.70221446  1.1608599571.5531219891.5158870871.459744727
        1.4338947741.4266136711.4573998511.3124905321.246412031
        1.3179151841.2688546011.3617999061.2392735011.201918163
        1.1874992451.1386748591.0734118060.9680238340       1.18064588
        1.0048711731.2462962151.335715268
1.4193140021.34853196  0.6107642721.1977904381.1648552621.11454548
        1.0986555141.00884369  1.0185681140.8868833960.803014004
        1.02336332  0.7904639030.9062305750.7662987240.62575297
        0.4687889940.9038549990.8856355681.0805346891.18064588  0
        1.3060455721.51578307  1.589950139
1.9085099531.8388841621.2855501971.6896702691.6527484581.597029299
        1.5719107231.5585078281.5874634681.4440275631.377494516
        1.4589494011.3932149431.4835414521.3612831161.328911654
        1.3128683981.2810033221.18775366  1.1574369361.004871173
        1.3060455720       0.9922244161.119858336
2.1208451342.05119846  1.4951397951.9019568011.8651067211.809484497
        1.7845327621.7697242991.7982555851.6551637821.588533889
        1.6722587981.6026750621.6923782051.5701896661.539011031
        1.5225933541.4946572441.3951875981.3810629551.246296215
        1.51578307  0.9922244160       0.998140857
2.1962889122.1266319731.5692344281.9773766971.9405618641.884987176
        1.8601175511.8446181081.8729407481.7300181751.663340744
        1.7481814571.6767050551.7661015851.6439473821.613355875
        1.5967540851.5707491171.46822901  1.4620469061.335715268
        1.5899501391.1198583360.9981408570
```

5. “Flat” resistance distances between colonies from the Northern cluster (filename: “FLAT_NORTH_CL_resistances”).

```
0       0.7875668041.1878900850.9808541031.0555644131.118603484
        1.3706386991.4820863571.3084749181.3288526711.457834491
        1.5915798841.4743573621.3966397271.4173748911.419314002
        1.4337504561.2846191171.4171496351.538278194

0.7875668040        1.0980031260.91284104  0.9931148111.058450165
        1.29621711  1.4089143251.2353585381.2572479891.386387115
        1.5203647481.4032288691.3256094971.3465101831.34853196
        1.3631825621.2188806671.34799318  1.468281372

1.1878900851.0980031260        1.0026527851.0370643921.089491013
        1.1750158161.3046562761.1333637821.1741168671.303635446
        1.4402659491.3241942351.2481399451.2707963311.273765026
        1.2907839981.1952987321.29099267  1.401941714

0.9808541030.91284104  1.0026527850        0.7530295820.85252128
        1.1232221651.2319330711.0577259871.0752088761.204502805
        1.3379102921.2205320811.1424913041.1630386111.164855254
        1.1790103541.0256992171.1606471031.282906249

1.0555644130.9931148111.0370643920.7530295820        0.7372941
        1.0915876731.1938105631.0192171391.0287833151.157572904
        1.2898371051.1720149141.0933775461.11313928  1.114545482
        1.1276622970.9527717621.1025166521.228891401

1.1186034841.0584501651.0894910130.85252128  0.7372941    0
        1.0992461781.1940246131.0200551281.01975233  1.14631609
        1.27686097  1.1584531621.0794249491.09785894  1.098655514
        1.1101275250.8936655671.0739503461.206773183

1.3706386991.29621711  1.1750158161.1232221651.0915876731.099246178
        0       0.8053493390.7652863410.8973679260.9890962421.139267566
        1.0306968310.9731078521.0012048121.00884369  1.035932995
        1.09826057  1.0992388011.169410933

1.4820863571.4089143251.3046562761.2319330711.1938105631.194024613
        0.8053493390        0.82731926  0.9287045140.9572036341.115832061
        1.0152038560.9805036791.0083284321.0185681141.050070204
        1.1713098311.14267471  1.191905125

1.3084749181.2353585381.1333637821.0577259871.0192171391.020055128
        0.7652863410.82731926  0        0.7489167720.8760239191.025977791
        0.91478858  0.8477056150.87895071  0.8868833960.915538709
        1.0017735260.9864541911.053167573

1.3288526711.2572479891.1741168671.0752088761.0287833151.01975233
        0.8973679260.9287045140.7489167720        0.83899791  0.982038923
        0.8623296760.7539566630.7959842130.8030140040.83459226
        0.9721922880.9207570790.983286091
```

```
1.4578344911.3863871151.3036354461.2045028051.1575729041.14631609
	0.9890962420.9572036340.8760239190.83899791	0	0.726693522
	0.6331983620.7698985670.7741665990.7904639020.827233609
	1.0864801370.9959946060.979785673

1.5915798841.5203647481.4402659491.3379102921.2898371051.27686097
	1.1392675661.1158320611.0259777910.9820389230.7266935220
	0.7039558440.9037915260.8940410710.9062305760.931930231
	1.2107279961.1075967041.065378492

1.4743573621.4032288691.3241942351.2205320811.1720149141.158453162
	1.0306968311.0152038560.91478858	0.8623296760.633198362
	0.7039558440	0.7679375210.7520677110.7662987240.795920704
	1.0904331990.9839781540.94160113

1.3966397271.3256094971.2481399451.1424913041.0933775461.079424949
	0.9731078520.9805036790.8477056150.7539566630.769898567
	0.9037915260.7679375210	0.5960092380.62575297	0.71068818
	1.0116335850.9100750170.917295702

1.4173748911.3465101831.2707963311.1630386111.11313928	1.09785894
	1.0012048121.0083284320.87895071	0.7959842130.774166599
	0.8940410710.7520677110.5960092380	0.4687889940.64841229
	1.0247566260.9100773180.893942296

1.4193140021.34853196	1.2737650261.1648552541.1145454821.098655514
	1.00884369	1.0185681140.8868833960.8030140040.790463902
	0.9062305760.7662987240.62575297	0.4687889940	0.610764272
	1.0233633540.9038549390.885635568

1.4337504561.3631825621.2907839981.1790103541.1276622971.110127525
	1.0359329951.0500702040.9155387090.83459226	0.827233609
	0.9319302310.7959207040.71068818	0.64841229	0.6107642720
	1.0285574450.8938477430.85690565

1.2846191171.2188806671.1952987321.0256992170.9527717620.893665567
	1.09826057	1.1713098311.0017735260.9721922881.086480137
	1.2107279961.0904331991.0116335851.0247566261.023363354
	1.0285574450	0.9488024451.106374665

1.4171496351.34799318	1.29099267	1.1606471031.1025166521.073950346
	1.0992388011.14267471	0.9864541910.9207570790.995994606
	1.1075967040.9839781540.9100750170.9100773180.903854939
	0.8938477430.9488024450	0.933172168

1.5382781941.4682813721.4019417141.2829062491.2288914011.206773183
	1.1694109331.1919051251.0531675730.9832860910.979785673
	1.0653784920.94160113	0.9172957020.8939422960.885635568
	0.85690565	1.1063746650.9331721680
```

6. “Flat” resistance distances between colonies from the Southern cluster (filename: “FLAT_SOUTH_CL_resistances”).

```
0 0.9680238359 1.157436936 1.381062955 1.462046906
0.9680238359 0 1.004871071 1.246296215 1.335715268
1.157436936 1.004871071 0 0.9922242391 1.119858271
1.381062955 1.246296215 0.9922242391 0 0.9981408565
1.462046906 1.335715268 1.119858271 0.9981408565 0
```

7. Genotypes of fruit bats analyzed in this study (filename: “rousettus”).

```
1       190     190     184     184     191     195     163     198     155     160     217
        217     181     181     194     194     179     179     162     164     150     150
        191     191     152     156     250     250     125     125     151     154     229
        229     184     198

1       206     210     182     184     173     195     163     163     155     160     209
        213     178     179     196     198     179     185     164     170     150     150
        186     191     152     154     255     255     125     125     154     173     225
        229     184     184

1       208     208     0       0       173     193     163     182     158     160     203
        213     166     178     194     198     179     179     162     166     147     147
        186     191     152     157     250     259     127     127     154     160     229
        229     184     198

2       190     208     0       0       191     193     163     163     160     162     209
        222     181     181     196     198     176     179     158     166     150     150
        186     191     142     156     250     250     125     127     160     166     229
        229     184     198

2       190     206     182     184     173     189     163     182     153     155     209
        211     181     184     196     196     179     179     162     164     147     147
        191     191     144     160     250     253     127     136     149     166     225
        229     184     184

2       210     210     184     184     173     189     163     182     155     155     211
        211     176     178     196     196     179     185     162     164     150     150
        191     191     142     152     250     250     125     143     158     162     223
        227     184     184

3       208     210     184     184     187     187     163     198     155     167     209
        216     174     179     196     196     176     179     164     166     147     150
        191     191     151     156     253     259     125     127     154     154     227
        229     184     198

3       208     210     182     184     173     191     163     198     153     153     209
        216     178     178     194     194     176     179     162     164     147     150
        191     191     140     142     253     253     125     125     160     164     225
        229     184     184

3       198     206     182     182     173     193     163     163     155     158     0
        0       178     181     192     200     179     179     164     164     150     150
        186     191     152     156     250     253     141     143     151     160     225
        229     184     194

3       208     210     182     182     187     191     163     163     155     164     209
        216     176     178     192     196     179     179     164     176     147     150
        191     191     142     152     250     253     125     138     158     162     0
        0       184     184
```

```
3       190   208   184   184   191   193   163   163   155   155   217
        222   176   178   196   196   179   179   166   166   150   150
        191   191   142   156   253   255   125   138   160   166   225
        229   184   198

3       190   210   182   184   187   193   163   163   155   160   203
        213   174   178   196   200   179   179   166   174   147   150
        191   191   156   157   253   255   125   141   158   164   221
        223   184   184

3       190   212   0     0     187   193   163   198   158   162   222
        224   178   179   196   205   0     0     0     0     150   150
        186   191   142   156   255   259   125   127   158   169   229
        229   184   198

3       190   190   182   184   173   189   163   185   155   155   203
        216   178   179   196   196   179   179   162   162   147   150
        191   191   142   144   255   259   127   129   154   160   225
        227   184   184

3       190   208   184   184   187   191   163   163   160   160   209
        211   178   181   196   196   176   179   162   178   150   150
        191   191   142   142   253   253   125   127   154   154   227
        229   184   198

3       206   210   182   186   187   193   163   182   155   155   211
        213   178   178   196   198   179   179   158   162   147   147
        191   191   142   142   253   255   141   143   154   169   221
        229   184   198

3       190   212   182   184   191   191   163   163   153   158   217
        222   176   181   198   198   179   179   162   162   150   150
        191   191   142   152   253   255   125   127   154   154   225
        227   184   184

3       190   208   0     0     191   191   163   163   153   160   211
        216   178   179   196   200   0     0     0     0     150   150
        191   191   144   152   250   253   125   138   158   164   229
        231   184   198

3       190   210   182   184   187   195   163   163   155   155   203
        224   178   184   194   196   176   179   164   164   147   150
        191   191   137   142   253   255   125   143   154   160   221
        227   184   198

4       194   206   182   184   187   195   163   163   155   158   211
        213   181   184   194   196   179   179   158   162   147   150
        191   191   146   156   250   253   127   127   145   154   212
        223   184   198

5       205   206   0     0     189   193   163   163   153   162   211
        217   176   179   194   196   179   185   162   176   147   150
```

```
        191   191   142   142   251   253   127   143   154   166   223
        229   184   198

6       0     0     182   184   173   187   163   163   158   158   209
        211   176   178   194   198   179   179   158   164   147   150
        191   191   142   151   248   250   125   127   154   164   223
        227   184   198

7       0     0     182   182   191   193   163   194   155   158   213
        222   176   178   194   196   179   179   162   166   147   150
        191   191   142   152   0     0     125   127   151   162   217
        229   198   198

7       190   208   182   184   173   193   163   182   158   160   209
        220   178   178   196   196   179   179   162   164   147   150
        191   191   152   152   253   253   125   125   154   164   225
        229   184   184

7       190   215   182   184   173   193   163   163   158   162   201
        216   176   179   196   196   179   185   162   162   147   150
        191   191   156   156   250   253   127   127   151   160   223
        229   184   194

7       206   208   182   184   173   189   163   163   158   162   216
        222   178   179   194   198   179   185   162   162   147   150
        191   191   142   142   0     0     127   144   154   154   227
        229   184   198

7       190   206   184   184   187   193   163   182   155   155   216
        217   174   174   194   196   179   179   166   166   147   150
        191   191   142   142   253   253   125   127   154   154   225
        227   184   194

7       198   210   182   184   191   191   180   198   155   155   209
        211   0     0     194   198   179   179   162   164   147   147
        191   191   140   142   0     0     125   127   154   154   223
        229   0     0

7       190   190   182   184   173   191   163   198   155   160   211
        217   0     0     194   194   179   185   162   164   147   150
        191   191   142   142   0     0     125   127   154   154   227
        229   184   198

7       208   208   182   184   173   191   182   190   153   155   201
        209   181   181   194   196   179   179   162   162   147   147
        191   191   146   152   0     0     125   138   154   164   223
        227   184   184

7       210   210   182   182   191   191   163   182   158   158   211
        217   0     0     194   196   179   179   162   164   150   150
        191   191   137   142   0     0     125   127   154   156   223
        229   184   198
```

```
7     194   210   182   184   173   187   182   182   153   160   211
      216   176   178   194   196   179   179   155   158   150   150
      191   191   142   144   253   253   125   125   154   154   227
      229   184   184

7     190   190   184   191   180   191   182   196   155   162   213
      217   178   179   194   196   179   179   162   178   150   150
      191   191   142   154   255   255   125   125   158   164   227
      234   184   184

7     190   208   182   184   173   187   182   194   155   158   216
      217   174   178   198   198   179   185   166   166   147   147
      186   191   142   154   250   253   127   138   151   154   223
      229   184   198

8     190   210   182   184   187   195   163   163   158   160   211
      216   176   181   194   198   179   179   164   176   0     0
      186   191   140   142   253   253   127   127   154   154   223
      227   184   198

9     190   190   184   184   173   191   163   198   155   160   209
      211   178   178   196   200   179   179   162   170   147   150
      191   191   142   146   250   255   129   141   151   166   229
      229   184   184

9     190   206   182   182   173   187   182   198   155   155   209
      213   166   184   196   196   179   185   166   174   150   150
      191   191   142   156   253   255   125   125   166   171   223
      229   184   184

9     206   210   182   182   189   191   200   200   160   160   211
      213   178   178   196   200   179   179   162   162   150   150
      191   191   156   156   250   251   125   127   154   160   227
      229   184   184

9     0     0     182   184   173   191   163   163   160   160   213
      216   178   179   194   196   179   179   162   164   147   150
      191   191   142   142   253   255   127   127   158   160   227
      229   184   198

9     205   210   182   184   187   191   196   198   158   158   211
      216   181   181   194   196   179   179   162   162   147   150
      191   191   156   156   250   253   125   125   154   154   225
      229   184   184

9     206   206   184   184   173   187   163   182   155   162   216
      220   176   178   196   200   179   185   162   164   147   150
      191   191   144   146   250   253   125   127   164   164   223
      229   184   198

9     190   210   182   184   173   173   163   163   158   160   211
      217   178   178   194   196   179   179   162   162   147   150
```

```
        191   191   142   156   248   255   125   141   154   158   223
        231   184   184

9       194   212   182   182   173   187   163   163   155   158   211
        213   176   178   198   200   179   179   162   162   147   150
        191   191   144   144   253   253   125   125   164   164   223
        227   184   198

9       190   206   182   184   193   193   163   163   158   160   211
        211   176   178   194   196   179   191   162   162   147   150
        191   191   146   160   250   255   125   125   151   160   227
        227   184   198

9       205   210   182   182   173   187   163   163   155   158   216
        222   176   178   196   196   176   179   162   164   147   150
        191   191   152   156   250   253   125   141   166   171   229
        229   184   198

9       210   210   182   184   191   191   163   198   155   160   209
        211   176   179   196   200   179   185   164   174   150   150
        191   191   142   152   250   255   125   144   151   164   225
        227   184   184

9       190   190   182   182   173   173   163   163   155   162   209
        211   176   178   194   196   179   185   162   170   147   150
        186   191   142   152   255   259   125   144   154   164   223
        225   184   184

9       190   190   182   184   191   191   163   163   155   160   213
        213   174   178   196   196   179   179   162   164   147   150
        191   191   152   156   253   253   125   127   164   164   223
        227   184   198

9       206   210   182   182   173   191   163   182   155   160   197
        216   176   178   194   196   179   179   158   162   150   150
        186   196   152   157   250   255   125   127   151   166   225
        227   184   184

9       194   208   184   184   173   173   163   163   158   160   203
        213   178   178   196   196   179   185   158   162   147   150
        191   191   144   152   253   259   125   127   164   173   227
        231   184   184

9       206   208   184   184   173   189   163   163   153   155   211
        216   176   178   194   200   179   179   162   170   147   147
        191   191   144   152   250   255   127   141   158   158   223
        229   198   198

9       190   210   182   184   173   187   163   163   155   162   216
        216   178   178   196   196   179   179   162   162   147   150
        191   191   142   146   250   255   125   125   154   164   229
        229   184   198
```

```
9       190    190    182    184    187    193    182    196    158    160    216
        217    176    178    198    200    179    179    162    164    150    150
        191    191    142    152    250    250    125    125    151    164    227
        229    184    184

9       206    212    182    184    187    195    163    198    153    155    211
        213    176    179    196    198    179    179    162    176    147    150
        186    191    152    157    250    255    125    125    154    164    223
        229    184    184

9       210    210    184    184    187    191    163    182    158    164    211
        217    176    178    194    196    179    185    162    174    150    150
        191    191    140    142    253    255    125    127    160    160    225
        227    184    184

9       206    206    182    184    191    191    163    163    153    160    209
        216    174    178    196    198    179    179    164    174    150    150
        191    191    142    142    250    259    125    144    162    166    229
        229    184    198

9       210    210    182    182    187    191    163    182    158    160    211
        216    176    178    198    198    176    179    164    174    150    150
        186    191    154    157    253    255    125    143    151    164    229
        229    184    184

9       190    206    184    184    187    191    163    182    155    160    213
        222    178    178    196    196    179    179    164    170    150    150
        191    191    142    142    250    250    125    143    154    158    227
        229    184    184

9       206    206    182    182    191    193    163    182    158    160    211
        216    176    178    194    196    179    179    162    174    150    150
        186    191    142    142    255    255    125    127    149    160    223
        225    184    184

9       190    206    184    184    173    193    163    182    158    158    211
        213    178    178    194    200    179    191    164    166    147    150
        191    191    144    148    250    250    141    141    160    169    229
        229    184    184

10      198    210    182    184    172    173    163    200    151    155    216
        217    178    181    196    196    179    185    162    164    147    150
        191    191    144    148    250    255    125    127    154    162    225
        229    198    198

10      190    198    182    184    187    195    163    182    158    158    213
        222    178    192    196    196    179    179    162    162    147    150
        191    191    146    148    255    255    125    127    154    154    221
        223    184    184

10      210    210    182    184    187    193    163    198    158    158    209
        222    176    178    194    194    179    179    162    164    147    150
```

```
        191   191   137   144   251   251   125   125   154   166   227
        229   184   198

10      206   210   184   184   173   173   163   163   153   158   211
        216   179   179   196   198   179   179   162   166   147   150
        191   191   140   156   250   255   125   138   154   154   219
        229   184   184

11      205   210   182   182   173   191   182   200   158   158   211
        216   176   178   194   196   179   179   162   162   147   150
        191   191   142   142   250   255   127   144   154   164   225
        225   184   184

12      206   210   182   182   187   191   163   182   158   160   201
        213   176   178   196   200   179   179   164   178   150   150
        191   191   142   157   250   253   125   127   151   160   223
        225   174   184

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     229  184  184

22   190  194  182  184  173  191  163  200  155  162  217
     220  178  178  196  196  176  185  162  162  147  150
     186  191  142  142  250  253  127  127  158  164  227
     229  184  198

22   190  206  184  184  173  189  186  194  158  160  203
     216  178  178  196  196  179  179  162  164  150  150
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        186  191  152  154  253  255  125  125  154  160  221
        225  184  194

22      210  210  182  184  187  191  163  163  153  155  209
        222  178  178  196  198  179  179  162  178  150  150
        191  191  146  157  255  255  127  129  154  166  223
        225  184  184

22      210  212  182  182  173  193  163  200  153  160  203
        211  176  178  196  196  179  179  158  164  150  150
        186  191  142  156  250  259  125  127  160  160  223
        229  184  184

22      198  210  0    0    173  173  163  163  158  162  203
        217  178  178  192  196  0    0    0    0    147  150
        191  191  142  142  250  255  125  144  151  154  229
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22      206  212  182  182  173  189  163  163  158  160  216
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22      205  210  184  184  173  191  163  163  153  158  213
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        229  184  184

22      206  210  182  184  191  191  163  182  155  155  213
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        186  191  156  156  253  253  125  127  154  166  225
        229  184  184

22      198  210  182  184  173  195  163  182  155  162  209
        211  178  178  194  194  179  179  158  162  147  147
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        229  184  198
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	229	184	198								
22	208	208	184	184	173	193	163	163	158	158	203
	213	178	179	196	196	179	179	162	164	147	147
	191	191	142	152	253	253	127	138	151	154	223
	225	184	184								
22	212	212	182	184	173	195	163	182	155	158	211
	217	174	178	196	198	176	179	162	178	147	150
	0	0	152	152	0	0	125	127	154	160	0
	0	184	198								
22	210	212	182	184	173	197	182	198	155	155	216
	217	179	181	196	196	179	179	162	162	147	150
	186	191	148	156	253	255	125	125	154	160	223
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	191	191	142	157	253	255	125	125	154	158	225
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	217	181	181	194	198	179	179	162	162	147	150
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22	190	198	182	184	189	191	163	182	155	158	209
	213	179	181	196	196	176	179	162	174	150	150

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        191   191   142   154   250   255   125   129   164   169   227
        229   184   198

22      208   210   184   184   191   191   163   182   155   162   203
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        191   193   142   144   253   255   125   125   151   164   225
        231   184   184

22      210   210   182   182   173   187   163   182   158   158   211
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22      205   210   184   184   191   193   163   163   155   160   216
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        229   184   194

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22      208   208   182   184   187   191   163   163   158   160   213
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        229   184   184

22      208   208   182   182   173   195   163   163   158   164   213
        222   179   181   198   200   176   179   162   178   150   150
        191   196   142   142   250   253   125   127   164   164   223
        227   184   184

22      190   206   182   184   173   193   163   198   160   164   213
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        225   184   194

22      205   206   184   184   189   195   163   163   155   155   209
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22      206   206   182   184   187   195   163   182   155   158   213
        213   178   179   194   196   179   179   162   162   150   150
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        231   184   198
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22	206	210	182	182	173	187	163	194	158	158	211
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	191	191	152	154	250	250	125	127	154	158	225
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	229	184	198								
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	222	166	178	194	196	172	179	168	180	147	150
	191	191	148	154	253	255	127	134	158	162	225
	227	184	184								
25	194	210	173	182	195	195	163	163	155	162	201
	216	178	178	194	196	179	179	164	164	147	147

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        193   199   148   156   255   259   125   127   151   158   221
        229   184   184

25      198   210   173   186   173   193   163   163   153   155   222
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        191   193   144   156   253   255   125   133   151   160   221
        229   184   194

25      190   202   182   184   189   189   163   196   146   158   216
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        191   199   144   157   253   255   125   125   151   154   225
        229   184   198

25      206   208   182   182   189   193   163   186   158   160   217
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        191   191   144   148   253   255   123   134   149   151   225
        231   184   194

25      205   210   182   186   189   195   163   163   146   155   213
        217   178   184   194   200   178   185   162   162   147   147
        191   191   140   154   255   255   129   136   151   158   219
        225   184   194

25      194   197   173   182   191   191   163   163   153   153   217
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        191   191   146   152   253   253   138   138   151   158   217
        225   184   196

25      194   206   182   186   173   193   163   182   158   160   217
        226   166   179   194   196   178   185   162   164   147   147
        191   191   144   152   251   255   127   141   160   164   227
        229   184   198

25      202   206   182   182   193   195   163   163   146   158   216
        222   172   178   196   200   179   185   162   164   147   147
        191   191   140   144   253   255   134   143   151   162   221
        225   184   184

25      206   208   182   184   189   191   163   190   146   153   216
        217   178   178   203   203   179   179   162   164   147   150
        191   191   140   156   255   255   125   133   154   158   227
        227   184   198

25      206   206   181   186   187   195   163   186   158   158   216
        220   166   178   196   200   179   185   162   164   147   147
        191   191   140   156   251   253   125   125   154   160   223
        231   184   184

25      194   194   173   181   189   197   163   163   158   164   211
        216   166   166   196   203   179   179   162   162   147   150
        191   193   140   146   253   255   125   138   154   154   223
        231   184   196
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25	202	210	173	182	189	189	163	178	155	162	217
	217	178	179	203	203	178	179	162	162	147	147
	191	191	146	154	255	255	125	136	154	154	231
	231	184	196								

25	194	194	182	182	191	193	163	178	162	164	201
	222	178	181	194	198	178	179	164	168	150	150
	191	191	146	146	253	253	129	136	151	151	219
	223	184	184								

8. **Table S1.** Number of private alleles per locus and population of *Rousettus aegyptiacus* included in this study. PAL: number of private alleles per locus per populations. PAP: number of populations with private alleles per locus. KPAP: number of private alleles per population. LPAP: number of locus with private alleles per population. Aseret (AS), BeerSheva (BS), EinElFurtaga(FUR), EinGedi (EG) EinKhudra (KHU), Elad (EL) Halulim (HA), IraqalAmir (IAA), Kufranja (KU), Lotan (LO), MtCarmel (CAR), Sgafim (SG), TelAviv (TLV), WadiDana (WDA), WadiElFeiran (WEF).

	AS	**BS**	**FUR**	**EG**	**KHU**	**EL**	**HA**	**IAA**	**KU**	**LO**	**CAR**	**SG**	**TLV**	**WDA**	**WEF**	**PAL**	**PAP**
M3-1								1	1						1	3	3
M3-120				1				1	1						2	5	5
M3-121						1			1	1						3	3
M3-6				1					1			1		1	3	7	5
M3-8	1						1								1	3	3
Z105		1	1				1						1			4	4
Z106						1									1	2	2
Z110				1	1			1							1	4	4
Z117		1		1												2	2
Z304				1											1	2	2
Z327				1							1				1	3	3
Z334	1	1						1								3	3
Z340														1	1	2	2
Z341														2		2	1
Z343				1								1			1	3	3
Z407											1					1	1
Z413				1				1	1		1				1	5	5
Z80														1		1	1
KPAP	2	3	1	8	1	2	2	5	5	1	3	2	1	5	14		
LPAP	2	3	1	8	1	2	2	5	5	1	3	2	1	4	11		

9. **Figure S1.** Forearm length (left), body mass (center) and their ratio (right) in males (M) and females (F) of *Rousettus aegyptiacus* from Desert and Mediterranean clusters.