


## 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*)

A. Centeno-Cuadros<sup>1,2\*</sup>  | P. Hulva<sup>3,4\*</sup> | D. Romportl<sup>5</sup> | S. Santoro<sup>2,6</sup> | T. Stríbná<sup>3</sup> | D. Shohami<sup>1</sup> | A. Evin<sup>7</sup> | A. Tsoar<sup>1</sup> | P. Benda<sup>3,8</sup> | I. Horáček<sup>3</sup> | R. Nathan<sup>1</sup>

<sup>1</sup>Movement Ecology Laboratory, Department of Ecology, Evolution and Behavior, Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem, Israel

<sup>2</sup>Department of Molecular Biology and Biochemical Engineering, University Pablo de Olavide, Sevilla, Spain

<sup>3</sup>Department of Zoology, Charles University, Prague, Czech Republic

<sup>4</sup>Department of Biology and Ecology, University of Ostrava, Ostrava, Czech Republic

<sup>5</sup>Department of Physical Geography and Geoecology, Charles University in Prague, Prague, Czech Republic

<sup>6</sup>Department of Ethology and Biodiversity Conservation, Estación Biológica de Doñana (CSIC), Seville, Spain

<sup>7</sup>Institut des Sciences de l'Evolution, Université de Montpellier, Montpellier, France

<sup>8</sup>Department of Zoology, National Museum (Natural History), Prague, Czech Republic

## Correspondence

A. Centeno-Cuadros, Movement Ecology Laboratory, Department of Ecology, Evolution and Behavior, Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem, Israel.  
Email: acenteno@upo.es

## Present address

A. Tsoar, Nature and Parks Authority, Beer Sheva, Israel.

## 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*

\*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

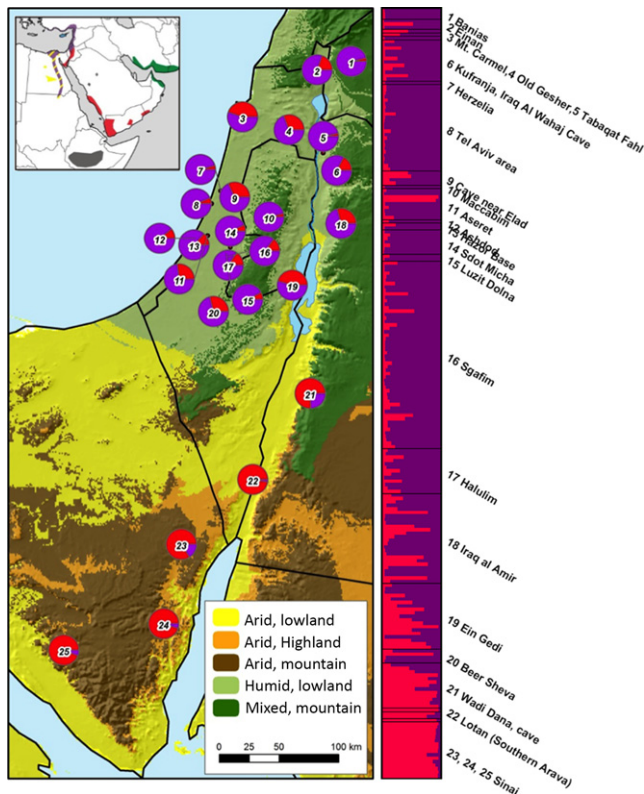
Humans are responsible for the major modification of natural ecosystems ever documented since the beginning of the Holocene (Pimm et al., 2014; Rosenzweig, 1995; Thomas et al., 2004) to such extent that the term Anthropocene had been proposed to characterize this period (Ruddiman, Ellis, Kaplan, & Fuller, 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, & Horáček, 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; Lučan 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](http://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  $-20^{\circ}\text{C}$  until processing in laboratory.

## 2.2 | DNA extraction and genotyping

Genomic DNA was isolated using the Genomic DNA Mini Kit (Genaid) 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'007 (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  $\Delta K$ , 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 DISTRICT (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 ( $1 \times 1$  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, Nogueras, & Cordeiro, 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 ( $\beta$ ) 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  $\beta$  (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 pseudo-replication 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 ( $\beta$ ) 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  $\pm$  SD:  $0.538 \pm 1.268$ ). All loci were highly variable (mean number of alleles per locus:  $10.224 \pm 2.633$ , range: 3–15; allelic richness per locus based on  $n = 6$ :  $4.238 \pm 1.299$ , 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:  $0.012 \pm 0.016$ , 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 ( $2.3 \pm 1.534$ ; range: 1–7) and populations ( $2.338 \pm 1.878$ ; range: 1–7) (Table S1). More interestingly, 11 of 18 microsatellites showed private alleles in Wadi El Feiran (Sinai) in low frequency ( $8.5 \pm 7.7\%$ ; 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  $\Delta K$  ( $\Delta K_2 = 92.0446$ ;  $\Delta K_3 = 5.0949$ ;  $\Delta K_4 = 6.7663$ ) 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.

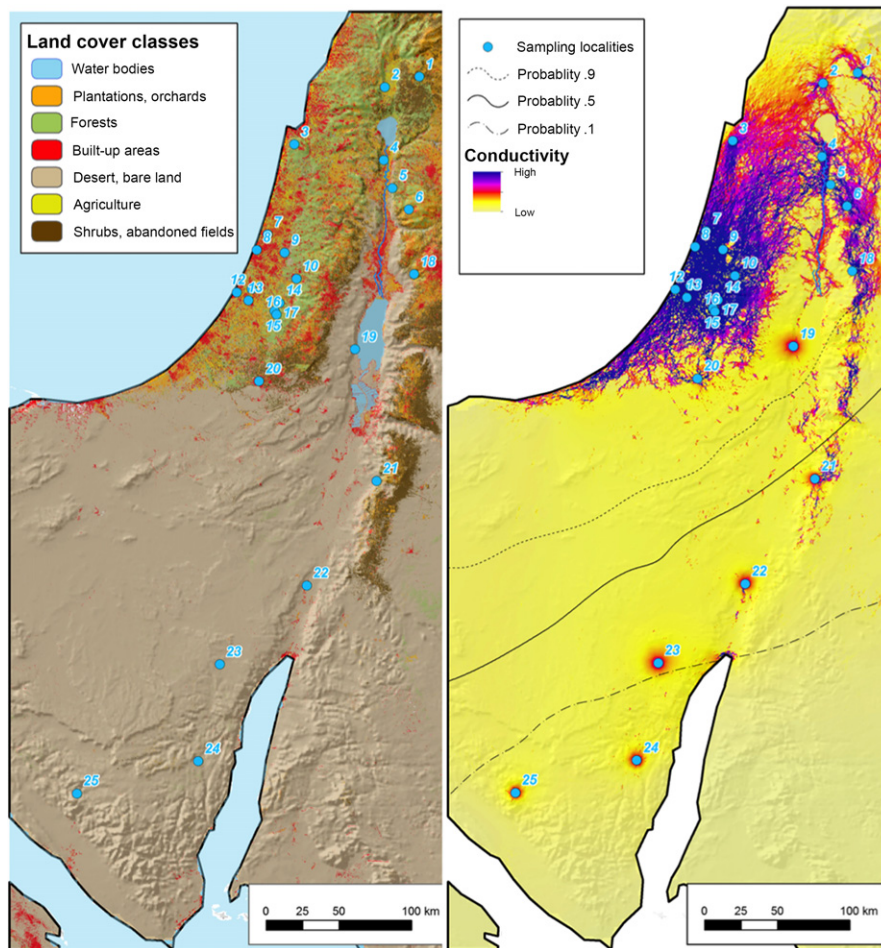
#### 3.3 | Landscape genetics

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

	<i>n</i>	<i>H<sub>E</sub></i>	<i>H<sub>O</sub></i>	HWE	<i>k</i>	AR	<i>F<sub>IS</sub></i>	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

**TABLE 1** Sample size (*n*), expected (nonbiased) (*H<sub>E</sub>*) and observed (*H<sub>O</sub>*) heterozygosities, Hardy–Weinberg equilibrium test (HWE), mean number of alleles (*k*), allelic richness (AR) based on a minimum sample size of *n* = 6, *F<sub>IS</sub>* 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

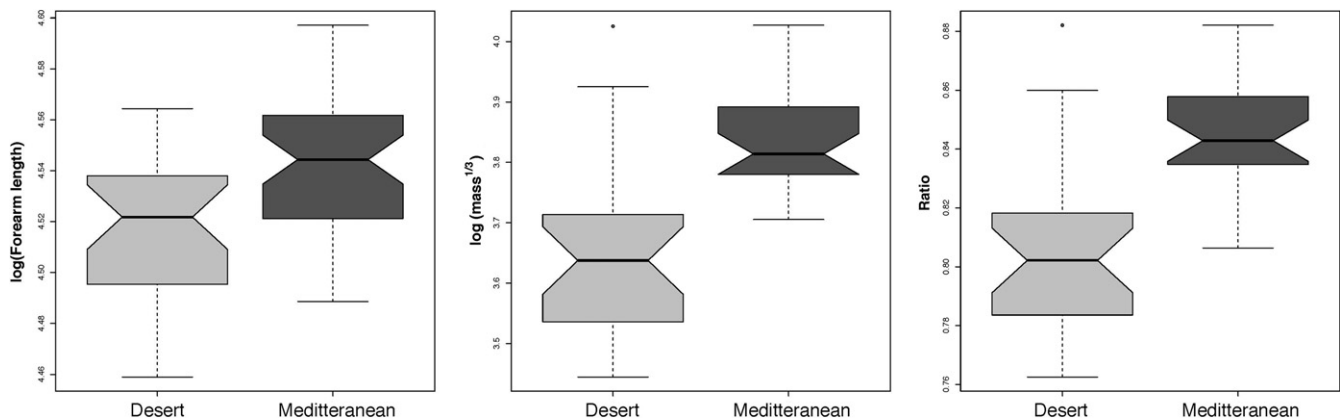
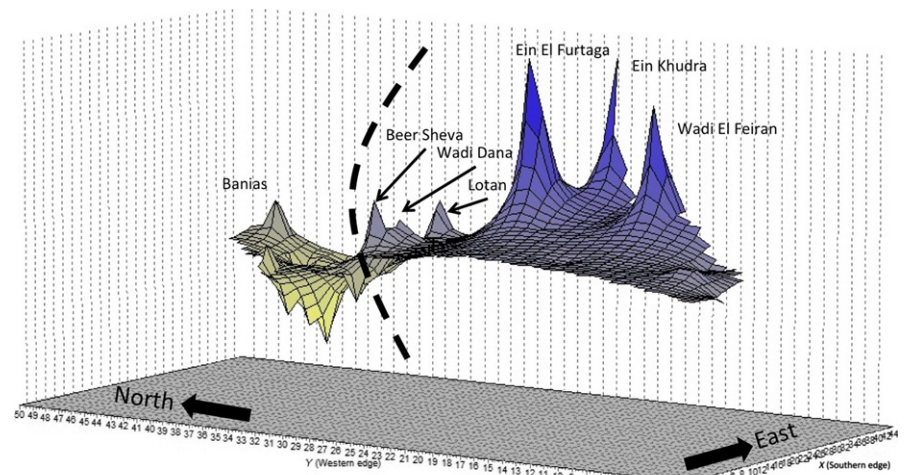


**FIGURE 2** Spatial distribution of landscape categories (left) and conductivity map (right). The conductivity map shows the output obtained in CIRCUITSCAPE using the landscape use values obtained from GPS- and radio-telemetry tracked individuals. The 0.9, 0.5 and 0.1 isolines of probability of the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

(MMRR IBD<sub>ALL</sub>:  $\beta = -0.36$ ; 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,  $\beta = -0.30$ ; 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 ( $\beta_{\text{GEO}} = -0.30$ ;  $\beta_{\text{RES}} = -0.15$ ; 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).

**FIGURE 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



**FIGURE 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.



**TABLE 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 ( $\beta$ ) and the coefficient of determination ( $R^2$ ) are given between parentheses

Model	n	Relatedness vs. Euclidean			Relatedness vs. Resistance			Relatedness vs. Euclidean vs. Resistance		
		$\beta$	$R^2$	$\beta$	$R^2$	$\beta_{\text{geo}}$	$\beta_{\text{res}}$	$\beta_{\text{geo}}$	$\beta_{\text{res}}$	$R^2$
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)	-0.30 (-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)	-0.19 (-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)	-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 (Kufanja, 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 Gesh,  $n = 1$ ; 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 (Gvoždík, Moravec, Klütsch, & Kotlík, 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 Afrotropical 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; Czaromska 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.

## ORCID

A. Centeno-Cuadros  <http://orcid.org/0000-0003-4210-1128>

## REFERENCES

- Andrianaivoarivelo, A. R., Shore, G. D., McGuire, S. M., Jenkins, R. K. B., Ramilijaona, O., Louis, E. E., & Brennenman, R. A. (2008). Characterization of 22 microsatellite marker loci in the Madagascar rousette (*Rousettus madagascariensis*). *Conservation Genetics*, 10, 1025–1028.
- Belfer-Cohen, A., & Bar-Yosef, O. (2000). Early sedentism in the Near East: A bumpy ride to village life. In I. Kuijt (Ed.), *Life in neolithic farming communities: Social organization, identity, and differentiation* (pp. 19–37). New York, NY: Plenum.
- Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., & Bonhomme, F. (2004). GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier (France).
- Benda, P., Vallo, P., Hulva, P., & Horáček, I. (2012). The Egyptian fruit bat *Rousettus aegyptiacus* (Chiroptera: Pteropodidae) in the Palearctic: Geographical variation and taxonomic status. *Biologia*, 67, 1230–1244.
- Bergmans, W. (1994). Taxonomy and biogeography of African fruit bats (Mammalia, Megachiroptera). 4. The genus *Rousettus* Gray, 1821. *Beaufortia*, 44, 79–126.
- Bernbeck, R., & Pollock, S. (2005). A cultural-historical framework. In S. Pollock & R. Bernbeck (Eds.), *Archaeologies of the middle east: Critical perspectives* (pp. 11–40). Oxford, UK: Blackwell Publishing.
- Berkes, F., Colding, J., & Folke, C. (2000). Rediscovery of traditional ecological knowledge as adaptive management. *Ecological Applications*, 10, 1251–1262.
- Carmichael, L. E., Krizan, J., Nagy, J. A., et al. (2007). Historical and ecological determinants of genetic structure in arctic canids. *Molecular Ecology*, 16, 3466–3483.

- Chapuis, M.-P., & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, 24, 621–631.
- Clarke, J. I. (1980). Contemporary urban growth. In G. H. Blake & R. I. Lawless (Eds.), *The changing Middle Eastern city* (p. 273). London: Routledge Library Editions: Society of the Middle East.
- Courtois, R., Bernatchez, L., Ouellet, J.-P., & Breton, L. (2003). Significance of caribou (*Rangifer tarandus*) ecotypes from a molecular genetics viewpoint. *Conservation Genetics*, 4, 393–404.
- Czarnomska, S. D., Jędrzejewska, B., Borowik, T., et al. (2013). Concordant mitochondrial and microsatellite DNA structuring between Polish lowland and Carpathian Mountain wolves. *Conservation Genetics*, 14, 573–588.
- Dalen, L., Fuglei, E., Hersteinsson, P., et al. (2004). Population history and genetic structure of a circumpolar species: The arctic fox. *Biological Journal of the Linnean Society*, 84, 79–89.
- Dashevsky, O., Assaf, Y., & Yovel, Y. (2017). City bats vs. country bats: Neural differences due to navigation skills in the Egyptian fruit bat *Rousettus aegyptiacus*. In *5th International Berlin Bat Meeting: Are bats special?* Berlin: Leibniz Institute for Zoo and Wildlife Research, 141 p.
- Dempster, A., Laird, N., & Rubin, D. (1977). Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society: Series B*, 39, 1–38.
- DeWoody, J., Trewin, H., & Taylor, G. (2015). Genetic and morphological differentiation in *Populus nigra* L.: Isolation by colonization or isolation by adaptation? *Molecular Ecology*, 24, 2641–2655.
- Earl, D. A., & von Holdt, B. M. (2011). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4, 359–361.
- Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, 14, 2611–2620.
- Evenari, M., Shanan, L., & Tadmor, N. (1982). Recent developments. In *The Negev: The challenge of a desert* (pp. 338–412). Cambridge: Harvard University Press.
- Evin, A., Cucchi, T., Cardini, A., et al. (2013). The long and winding road: Identifying pig domestication through molar size and shape. *Journal of Archaeological Science*, 40, 735–743.
- Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molecular Ecology Notes*, 7, 574–578.
- Fischer, J., & Lindenmayer, D. B. (2007). Landscape modification and habitat fragmentation: A synthesis. *Global Ecology and Biogeography*, 16, 265–280.
- Foote, A. D. (2012). Investigating ecological speciation in non-model organisms: A case study of killer whale ecotypes. *Evolutionary Ecology Research*, 14, 447–465.
- Fraley, C., Raftery, A. E., Murphy, T. B., & Scrucca, L. (2012). MCLUST Version 4 for R: Normal mixture modeling for model-based clustering, classification, and density estimation. Technical Report No. 597.
- Gligor, M., Ganzhorn, J. U., Rakotoniravony, D., et al. (2009). Hybridization between mouse lemurs in an ecological transition zone in southern Madagascar. *Molecular Ecology*, 18, 520–533.
- Goudet, J. (1995). FSTAT (Version 1.2): A computer program to calculate *F*-statistics. *Journal of Heredity*, 86, 485–486.
- Grimm, N. B., Faeth, S. H., Golubiewski, N. E., et al. (2008). Global change and the ecology of cities. *Science (New York, N.Y.)*, 319, 756–760.
- Guichoux, E., Lagache, L., Wagner, S., & Et, A. (2011). Current trends in microsatellite genotyping. *Molecular Ecology Resources*, 11, 591–611.
- Guillot, G., Mortier, F., & Estoup, A. (2005). GENELAND: A computer package for landscape genetics. *Molecular Ecology Notes*, 5, 712–715.
- Gvoždík, V., Moravec, J., Klütsch, C., & Kotlík, P. (2010). Phylogeography of the Middle Eastern tree frogs (*Hyla*, Hylidae, Amphibia) as inferred from nuclear and mitochondrial DNA variation, with a description of a new species. *Molecular Phylogenetics and Evolution*, 55, 1146–1166.
- Harrison, D. L., & Bates, P. J. J. (1991) *The mammals of Arabia*. Kent, UK: Harrison Zoological Museum.
- Hewitt, G. M. (1988). Hybrid zones-natural laboratories for evolutionary studies. *Trends in Ecology & Evolution*, 3, 158–167.
- Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, 405, 907–913.
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978.
- Hua, P. Y., Chen, J. P., Sun, M., et al. (2006). Characterization of microsatellite loci in fulvous fruit bat *Rousettus leschenaulti*. *Molecular Ecology Notes*, 6, 939–941.
- Hulva, P., Marešová, T., Dundarova, H., et al. (2012). Environmental margin and island evolution in Middle Eastern populations of the Egyptian fruit bat. *Molecular Ecology*, 21, 6104–6116.
- Jain, A. K. (2010). Data clustering: 50 years beyond K-means. *Pattern Recognition Letters*, 31, 651–666.
- Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics (Oxford, England)*, 23, 1801–1806.
- Jarvis, A., Reuter, H. I., Nelson, A., & Guevara, E. (2008). Hole-filled SRTM for the globe Version 4. Available from the CGIAR-CSI SRTM 90 m Database (<http://srtm.csi.cgiar.org>).
- Jha, S., & Kremen, C. (2013). Urban land use limits regional bumble bee gene flow. *Molecular Ecology*, 22, 2483–2495.
- Juste, J., Alvarez, Y., Tabarés, E., et al. (1999). Phylogeography of African fruitbats (Megachiroptera). *Molecular Phylogenetics and Evolution*, 13, 596–604.
- Juste, J., & Ibañez, C. (1993). Geographic variation and taxonomy of *Rousettus aegyptiacus* (Mammalia: Megachiroptera) in the islands of the Gulf of Guinea. *Zoological Journal of the Linnean Society*, 107, 117–129.
- Kark, S., Alkon, P. U., Safriel, U. N., & Randi, E. (1999). Conservation priorities for Chukar partridge in Israel based on genetic diversity across an ecological gradient. *Conservation Biology*, 13, 542–552.
- Kark, S., Hadany, L., Safriel, U. N., et al. (2008). How does genetic diversity change towards the range periphery? An empirical and theoretical test. *Evolutionary Ecology Research*, 10, 391–414.
- Kark, S., & van Rensburg, B. J. (2013). Ecotones: marginal or central areas of transition? *Israel Journal of Ecology & Evolution*, 52, 29–53.
- Kelt, D. A. (2011). Comparative ecology of desert small mammals: A selective review of the past 30 years. *Journal of Mammalogy*, 92, 1158–1178.
- Korine, C., Izhaki, I., & Arad, Z. (1999). Is the Egyptian fruit-bat *Rousettus aegyptiacus* a pest in Israel? An analysis of the bat's diet and implications for its conservation. *Biological Conservation*, 88, 301–306.
- Korine, C., Izhaki, I., & Makin, D. (1994). Population structure and emergence order in the fruit-bat (*Rousettus aegyptiacus*: Mammalia, Chiroptera). *Journal of Zoology*, 232, 163–174.
- Kwiecinski, G., & Griffiths, T. (1999). *Rousettus egyptiacus*. *Mammalian Species*, 611, 1–9.
- Lavee, D., & Safriel, U. N. (1989). The dilemma of cross-desert migrants – Stopover or skip a small oasis? *Journal of Arid Environments*, 17, 69–81.
- Lee-Yaw, J. A., Davidson, A., McRae, B. H., & Green, D. M. (2009). Do landscape processes predict phylogeographic patterns in the wood frog? *Molecular Ecology*, 18, 1863–1874.
- Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, 17, 183–189.
- Lučan, R. K., Bartonička, T., Jedlička, P., Řeřucha, Š., Šálek, M., Čížek, M., ... Horáček, I. (2016). Spatial activity and feeding ecology of the endangered northern population of the Egyptian fruit bat (*Rousettus aegyptiacus*). *Journal of Mammalogy*, 97, 815–822.
- Mallon, D. P. (2011). Global hotspots in the Arabian Peninsula. *Zoology in the Middle East*, 54, 13–20.



- Manel, S., Schwartz, M. K., Luikart, G., & Taberlet, P. (2003). Landscape genetics: Combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, 18, 189–197.
- Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27, 209–220.
- McRae, B. H. (2006). Isolation by resistance. *Evolution*, 60, 1551–1561.
- McRae, B. H., & Beier, P. (2007). Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 19885–19890.
- McRae, B. H., Dickson, B. G., Keitt, T. H., & Shah, V. B. (2008). Using circuit theory to model connectivity in ecology, evolution and conservation. *Ecology*, 89, 2712–2724.
- Miller, M. P. (2005). ALLELES IN SPACE (AIS): Computer software for the joint analysis of interindividual spatial and genetic information. *The Journal of heredity*, 96, 722–724.
- Miller-Butterworth, C. M., Jacobs, D. S., & Harley, E. H. (2003). Strong population substructure is correlated with morphology and ecology in a migratory bat. *Nature*, 424, 187–191.
- Morin, P. A., Parsons, K. M., Archer, F. I., et al. (2015). Geographic and temporal dynamics of a global radiation and diversification in the killer whale. *Molecular Ecology*, 24, 3964–3979.
- Moura, A. E., Kenny, J. G., Chaudhuri, R., et al. (2014). Population genomics of the killer whale indicates ecotype evolution in sympatry involving both selection and drift. *Molecular Ecology*, 23, 5179–5192.
- Munshi-South, J. (2012). Urban landscape genetics: Canopy cover predicts gene flow between white-footed mouse (*Peromyscus leucopus*) populations in New York City. *Molecular Ecology*, 21, 1360–1378.
- Musiani, M., Leonard, J. A., Cluff, H. D., et al. (2007). Differentiation of tundra/taiga and boreal coniferous forest wolves: Genetics, coat colour and association with migratory caribou. *Molecular Ecology*, 16, 4149–4170.
- Nosil, P., Vines, T. H., & Funk, D. (2005). Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, 59, 705–719.
- Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., ... Wagner, H. (2013). VEGAN: Community ecology package. R package version 2.3-0. Retrieved from <https://CRAN.R-project.org/package=vegan>
- Orsini, L., Vanoverbeke, J., Swillen, I., Mergeay, J., & De Meester, L. (2013). Drivers of population genetic differentiation in the wild: Isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Molecular Ecology*, 22, 5983–5999.
- Ortego, J., Aguirre, M. P., Nogueras, V., & Cordero, P. J. (2015). Consequences of extensive habitat fragmentation in landscape-level patterns of genetic diversity and structure in the Mediterranean esparto grasshopper. *Evolutionary Applications*, 8, 621–632.
- Peakall, R., & Smouse, P. E. (2012). GENALEX 6.5: Genetic analysis in EXCEL. Population genetic software for teaching and research – An update. *Bioinformatics (Oxford, England)*, 28, 2537–2539.
- Perevolotsky, A. (2005). Integrating landscape ecology in the conservation of Mediterranean ecosystems: The Israeli experience. *Israel Journal of Plant Sciences*, 53, 203–213.
- Pew, J., Muir, P. H., Wang, J., & Frasier, T. R. (2014). RELATED: An R package for analysing pairwise relatedness from codominant molecular markers. *Molecular Ecology Resources*, 15, 557–561.
- Pilot, M., Jedrzejewski, W., Branicki, W., et al. (2006). Ecological factors influence population genetic structure of European grey wolves. *Molecular Ecology*, 15, 4533–4553.
- Pimm, S. L., Jenkins, C. N., Abell, R., et al. (2014). The biodiversity of species and their rates of extinction, distribution, and protection. *Science (New York, N.Y.)*, 344, 1246752.
- Pond, B. A., Brown, G. S., Wilson, K. S., & Schaefer, J. A. (2016). Drawing lines: Spatial behaviours reveal two ecotypes of woodland caribou. *Biological Conservation*, 194, 139–148.
- R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Razgour, O. (2015). Beyond species distribution modeling: A landscape genetics approach to investigating range shifts under future climate change. *Ecological Informatics*, 30, 250–256.
- Razgour, O., Rebelo, H., Puechmaile, S. J., et al. (2014). Scale-dependent effects of landscape variables on gene flow and population structure in bats (C. Burridge, Ed.). *Diversity and Distributions*, 20, 1173–1185.
- Richardson, J. L., Urban, M. C., Bolnick, D. I., & Skelly, D. K. (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology & Evolution*, 29, 165–176.
- Roffler, G. H., Schwartz, M. K., Pilgrim, K. L., et al. (2016). Identification of landscape features influencing gene flow: How useful are habitat selection models? *Evolutionary Applications*, 9, 805–817.
- Rosenberg, N. A. (2003). DISTRICT: A program for the graphical display of population structure. *Molecular Ecology Notes*, 4, 137–138.
- Rosenzweig, M. (1995). *Species diversity in space and time*. Cambridge: Cambridge University Press.
- Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular ecology resources*, 8, 103–106.
- Row, J. R., Wilson, P. J., Gomez, C., et al. (2014). The subtle role of climate change on population genetic structure in Canada lynx. *Global Change Biology*, 20, 2076–2086.
- Ruddiman, W. F., Ellis, E. C., Kaplan, J. O., & Fuller, D. Q. (2015). Defining the epoch we live in. *Science*, 348, 38–39.
- Rueness, E. K., Stenseth, N. C., O'Donoghue, M., et al. (2003). Ecological and genetic spatial structuring in the Canadian lynx. *Nature*, 425, 69–72.
- Sacks, B. N., Brown, S. K., & Ernest, H. B. (2004). Population structure of California coyotes corresponds to habitat-specific breaks and illuminates species history. *Molecular Ecology*, 13, 1265–1275.
- Safi, K., Meiri, S., & Jones, K. E. (2013). Evolution of body size in bats. In F. A. Smith & S. K. Lyons (Eds.), *Animal body size: Linking pattern and process across space, time, and taxonomic group* (pp. 95–105). Chicago, IL: The University of Chicago Press.
- Seehausen, O., Takimoto, G., Roy, D., & Jokela, J. (2008). Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Molecular Ecology*, 17, 30–44.
- Shobrak, M. (2011). Bird flyways and stopover conservation sites in the Arabian Peninsula. *Zoology in the Middle East*, 54, 27–30.
- Slatkin, M. (1985). Rare alleles as indicators of gene flow. *Evolution*, 39, 53–65.
- Smith, T. B. (1997). A role for ecotones in generating rainforest biodiversity. *Science*, 276, 1855–1857.
- Stockwell, C. A., Hendry, A. P., & Kinnison, M. T. (2003). Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution*, 18, 94–101.
- Stoffberg, S., Schoeman, M. C., & Matthee, C. A. (2012). Correlated genetic and ecological diversification in a widespread southern African horseshoe bat. *PLoS ONE*, 7, e31946.
- Storfer, A., Murphy, M. A., Evans, J. S., et al. (2007). Putting the "landscape" in landscape genetics. *Heredity*, 98, 128–142.
- Thomas, C. D., Cameron, A., Green, R. E., et al. (2004). Extinction risk from climate change. *Nature*, 427, 145–148.
- Tsoar, A., Nathan, R., Bartan, Y., et al. (2011). Large-scale navigational map in a mammal. *Proceedings of the National Academy of Sciences of the United States of America*, 108, E718–E724.
- Tsoar, A., Shohami, D., & Nathan, R. (2010). A movement ecology approach to study seed dispersal and plant invasion: An overview and application of seed dispersal by fruit bats. In D. M. Richardson (Ed.), *Fifty years of invasion ecology: The legacy of Charles Elton* (pp. 101–119). London: Wiley-Blackwell.
- del Vaglio, M. A., Nicolau, H., Bosso, L., & Russo, D. (2011). A first assessment of feeding habits in the fruit bat *Rousettus aegyptiacus*

- on Cyprus island. *Hystrix: The Italian Journal of Mammalogy*, 22, 281–289.
- Varudkar, A., & Ramakrishnan, U. (2015). Commensalism facilitates gene flow in mountains: A comparison between two *Rattus* species. *Heredity*, 115, 253–261.
- Vitousek, P. M. (1997). Human domination of earth's ecosystems. *Science*, 277, 494–499.
- Waller, L. A., & Wheeler, D. C. (2012). Spatial analysis linking landscape features and genetic population structure in cougars (*Puma concolor*) in the northern Rocky Mountains. *Statistics and Its Interface*, 5, 183–193.
- Wang, J. (2002). An estimator for pairwise relatedness using molecular markers. *Genetics*, 160, 1203–1215.
- Wang, I. J. (2013). Examining the full effects of landscape heterogeneity on spatial genetic variation: A multiple matrix regression approach for quantifying geographic and ecological isolation. *Evolution: International Journal of Organic Evolution*, 67, 3403–3411.
- Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology*, 23, 5649–5662.
- Western, D. (2001). Human-modified ecosystems and future evolution. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 5458–5465.
- Wolf, J. B. W., Harrod, C., Brunner, S., et al. (2008). Tracing early stages of species differentiation: Ecological, morphological and genetic divergence of Galápagos sea lion populations. *BMC Evolutionary Biology*, 8, 150.
- Wright, S. (1943). Isolation by distance. *Genetics*, 28, 114–138.
- Wright, S. (1969). Evolution and the genetics of populations. In S. Wright (Ed.), *The theory of gene frequencies* (Vol. 2). Chicago:University of Chicago Press.
- Yom-Tov, Y., & Tchernov, E. (1988). The biogeographical history of the southern Levant. In Y. Yom-Tov & E. Tchernov (Eds.), *The zoogeography of Israel: The distribution and abundance at a zoogeographical crossroad* (pp. 159–250). Dordrecht, the Netherlands: Dr. W. Junk Publishers.

## 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;26:6224–6237. <https://doi.org/10.1111/mec.14365>