Journal of Wildlife and Biodiversity 1(2): 56-59 (2017)

by Arak University, Iran (http://jwb.araku.ac.ir/)

DOI: 10.22120/jwb.2017.28006

Short Communication

# *Eremias intermedia* or *Eremias nigrocellata*? Is morphology sufficient?

**Farahnaz Molavi[1]*, Toba M. Kalat[1], Morteza Yazdanpanah[2], Bahram H. Kiabi[2] and Haji Gh. Kami[3]**

[1]*Department of Biology, Faculty of Science, Islamic Azad University of Mashhad, Mashhad, Iran, e-mail: fm_yazdan@yahoo.com
[2]Biology Department, Faculty of Science, Shahid Beheshti University, Tehran , Iran
[3]Department of Biology, Faculty of Sciences, Golestan University, Gorgan, Iran

Received: 1 September 2017 / Revised: 7 October 2017 / Accepted: 15 October 2017 / Published online: 20 October 2017. Ministry of Sciences, Research and Technology, Arak University, Iran.

## Abstract

Markazi Province with its extended deserts and Anderson reported Eremias intermedia Strauch 1876, from Iran-Afghanistan border and Eremias nigrocellata Nikolsky 1896 from north and east of Iran. He also represented some features for the recognition of these similar species. This study that lasted about one year was aimed at identifying herpetofauna of Kharturan protected area. 15 specimens of *Eremias* have obtained their measurements in agreement with the characters of neither Eremias intermedia nor Eremias nigrocellata that was described by Anderson from Iran.

**Keywords:** Iran Lizards fauna, *Lacertidae*, Taxonomy, Morphology.

## Introduction

Widespread *Eremias* genus (Fitzinger 1834), with about 33 recognized species, includes 16 species from Iran (Rastegar-Pouyan *et al.* 2008, Rastegar-Pouyani *et al.* 2010, Mozaffari *et al.* 2011). *Eremias intermedia* Strauch, 1876 is distributed in southern central Asia and northern Afghanistan and Anderson reported it from Tajan (Tezden) river valley at the point where borders of Iran, Turkmenistan, and Afghanistan come together (Anderson 1999). Khar Turan national park is situated in Semnan province, southeast of Shahrood and parts of eastern Khorasan province. This area is comprised of three extensive plains and consists of the favorable habitat of both E. intermedia and E. noigrocellata, as previously described habitats namely sandy areas covered with semi-shrubby vegetation and loose soils respectively (Terentjev and Chernov 1949).

## Material and methods

### Study Area

The northernmost region of Khartouran protected area and 10 Km south to cross between Kalshour area of Khartouran and Shahrood-Sabzevar road. This is plain with desert shrubs such as *Ephedra* sp., *Alhagi* sp. and *Seidlitzia rosmarinus*. Six specimens were collected from this first station. Nine specimens were collected from the second station; an area situated about five Km South to Ahmad Abad, with dominant vegetation of bean-caper (*Zygophyllum eurypterum*) (Fig. 1and 2) .

Available keys (Minton 1966, Anderson 1979, Leviton 1992, Anderson 1999) were used in order to Identification based on morphologic and meristic characters, but specimens were not in agreement with any of the species and exhibit an intermediate situation between *E. intermedia* and *E. noigrocellata.*

Measurements of metric characters were made using a digital Vernier caliper (at 0.02mm accuracy) and meristic characters were explored using a dissecting microscope (7x-40x). Prevalent characters of families were studied (Table 1, Fig. 3, and Fig. 4).

**Table1.** Examined morphologic and meristic characters of *Eremias intermedia*.

| **Abbreviation** | **Character** |
|---|---|
| SVL. CL | SVL / CL |
| SVL | Length of snout to vent |
| HW | Wide of head |
| HL | Length of head |
| CL | Length of caudal |
| NS | nasal scales |
| AB | Number of scales around body |
| BE | Number of scales between eyes |
| BLEL | scales between labial and eye-right |
| BLER | scales between labial and eye-left |
| LLR | lower labial scales -right |
| LLL | supralabials scales-left |
| SLR | supralabials scales-right |
| FT4L | Fring of fourth toe-left |
| FT4R | Fring of fourth toe-right |
| T4 | Subdigital scales of the Fourth toe |
| FP | Femoral pores |
| DL | Dorsal scales Longitudinal of back |
| MD | Scales in Middorsaum |
| DT | Dorsal scales in longest transverse rows |
| VT | |
| VL | Ventral scales in longest transverse rows |

For all characters, Mean, Minimum, Maximum, and standard deviation was calculated using SPSS Ver. 16 statistical package (SPSS Inc., Chigaco, IL, USA). Kolmogorov-Smirnov test was carried out to assess the normality of character data. Homogeneity of variances was tested with the Levene test and since all characters had normal distribution and variance homogeny, sexual dimorphism was analyzed using one-way variance analysis.

**Figure 3**. *Eremias intermedia*; dorsal view of the adult specimen showing the color pattern and ventral view showing the ventral scales arrangement.

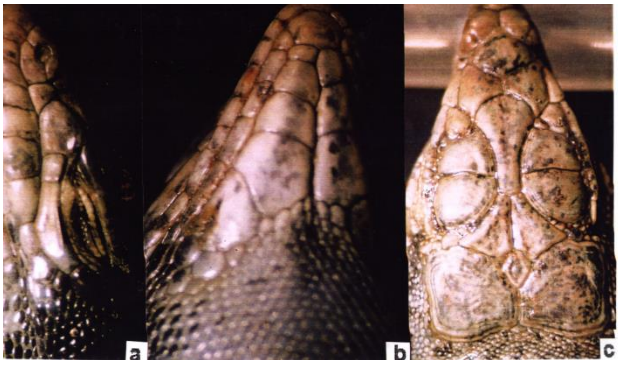

**Figure 4.** Scales of the head region of *Eremias intermedia*.

## Results and Discussion

There are only a few distinctive morphological characters that are not appropriate because they have a high CV. Results are summarized in table 2 (below). Morphological character analysis revealed the existence of sexual dimorphism in head length. The mean of this character for males is 21.79 and larger than 19.43 for females.

*E. intermedia* and *E. nigrocellata*, have an enigmatic taxonomic situation. Boulenger (1885) considered E. nigrocellata and E.

intermedia as synonymous. Nikolsky (1896), assigned *E. nigrocellata* as a subspecies of *E. intermedia* (Anderson, 1999). Anderson (1999) described them as separate valid species.

Terentjev and Chernov (1949) recorded *E. intermedia* from northeastern Iran. Anderson (1999) attributed populations of north and east of Iran to two distinct species. He regarded north and east Iran populations to *E. nigrocellata* and those that had been taken at the borders of Iran-Afghanistan- Turkmenistan to *E. intermedia*. He used these characteristics to distinguish between two species:

1-Snout-vent length/tail length (SV/T) in *E. intermedia* equals 0.53-0.60 and in *E. nigrocellata* equals 0.70-0.85.

2-Supraoculars are completely separated from frontal by a row of granules in *E. intermedia* but in *E. nigrocellata* supraoculars are in contact with frontal.

3-In *E. intermedia* there are 16-18 (mean 16.8) ventral scales in the longest transverse row compared with 17-20 (mean 19.2) in *E. nigrocellata*.

4- Subdigital scales of the Fourth toe in *E. intermedia* there is one row and in *E. nigrocellata* is two rows.

5- Tympanic shield is indistinct in *E. intermedia* but is small or distinct in *E nigrocellata*.

Anderson (1999) reexamined Guibé (1957) specimens from Mahneh (Khorasan) and Boulenger (1885) specimens from Gorgan and identified them as *E. nigrocellata*. He also examine two specimens of E. intermedia type series, that in both fourth supraoculars were completely broken up into small scales and SV/T ratio was 0.82 in adult and 0.63 in the immature specimen. In the latter specimen Supraoculars were completely separated from frontal and frontoparietal by a row of small scales, while in the adult, supraoculares are in contact with the frontal.

In this study 15specimens (9 female and 6 male) obtained from Abas Abad and Kach Chah located in Khar Turan National Park that characterize by these traits:

1- SVL/CL in females equal 0.63-0.68 and 0.62-0.67 in males.

2- The number of ventral scales in the longest transverse row in females is 16-18 (mean=17.65) and in males 18-16 (mean 17.33), (as before mentioned there is a range of character states from completely distinct to completely indistinct).

3- In none of the specimens showed no Fourth Supraocular (according to the result of Anderson,1999 for *E. intermedia*).

4- Only in two specimens do few granules separate frontal scales from frontoparietal ones and in others frontals are in contact with supraoculars.

5- Subdigital blade-like lamellae in the fourth toe are hard to examine.

So, according to this result, it seems that *E. intermedia* and *E. nigrocellata* haven’t a sharp border in their diagnostic morphologic characters and their characters exhibit a clinal pattern of variation. This fact, make their taxonomic situation obscure again. As Anderson (1999) mentioned, based on limited specimens can’t persuade that *E. intermedia* is distinct from *E. nigrocellata*. More morphological and molecular studies may offer additional insights.

## Acknowledgment

We are grateful to Mr. Mahammadian for his generous help and Mohammad Yazdanpanahi for his efforts in collecting the specimens.

## References

Anderson S.C. 1979. Preliminary key to the turtles, Lizard and Amphisbaenas of IRAN Fieldiana Zoology 65(4): 27-44.

Anderson S.C. 1999. The lizards of Iran, Vol. 15. Contributions to Herpetology. Ithaca, NY: Society for the Study of Amphibians and Reptiles.

Boulenger G.A. 1885. Catalogue of the Lizard in the British museum. Vol.1, 2.

Guibé J. 1954. Catalogue des types des lézards du Museum national d'Histoire naturelle. Bayeux: Colas.

Leviton A.E., Anderson SC, Adler K. and Minton S.A. 1992. Handbook to Middle East amphibian and reptiles. Contribution to Herpetology. Oxford, OH: Society for study of Amphibians and Reptiles.

Minton Sh.A. 1966. Acontribution to the herpetology of West Pakistan. Bulletin of the American Museum of Natural History, 134(2): 29-184.

Mozaffari O., Ahmadzadeh F. and Parham J. (2011). *Eremias papenfussi* sp. Nov., a new lacertid lizard (Sauria: Lacertidae) from Tehran, Iran. Zootaxa 31(14): 57-62.

Rastegar-Pouyani E., Rastegar-Pouyani N., Kazemi Noureini, Joger S., Wink U.M. 2010. Molecular phylogeny and evolution of the *Eremias persica* complex of the Iranian Plateau (Reptilia: Lacertidea) based on sequences of the mtDNA, Zoological Journal of the Linean Society 158: 641-660.

Rastegar-Pouyani N.R., Kami H.G., Rajabzadeh, Shafeie M., Anderson S.C. 2008. Annotated Checklist of Amphibians and Reptiles of Iran. Iranian Journal of Animal Biosystematics 4 (1): 7-30.

Terentjev P.V. and Chernov S.A. 1949. Guide to Reptile and Amphibians, SovietScience Prees, Moscow and Leningrad, 340 pp.

**Journal of Wildlife and Biodiversity 1(2):60-68 (2017)**

**by Arak University, Iran (http://jwb.araku.ac.ir/)**

**DOI: 10.22120/jwb.2017.28178**

**Research Article**

# Measuring environmental niche isolation between genetically management units of Goitered gazelle, *Gazella subgutturosa* (Guldenstadt, 1970) in Iran

**Rasoul Khosravi[1], Roya Adavoudi[2], Mansoureh Malekian[1], Mohmoud-Reza Hemami[1]***

[1]*Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran. email: mrhemami@cc.iut.ac.ir
[2]Department of Environmental Sciences, Faculty of Natural Resources, University of Tehran, Karaj, Iran.

Received: 11 September 2017 / Revised: 18 October 2017 / Accepted: 20 October 2017 / Published online: 29 October 2017. Ministry of Sciences, Research and Technology, Arak University, Iran.

## Abstract

The interactions between species and their environments can shape the distribution of spatial genetic variations. Evaluation of niche overlap and environmental dissimilarity provides valuable opportunities to investigate how niche differences contribute to genetic divergence between populations that differ in their geographical distributions and environmental conditions. Nowadays, the formerly large continuous populations of Goitered gazelle in Iran have been confined to fragmented habitats due to natural and anthropogenic factors. A statistical framework based on an ecological niche modeling at the genetical management units (MUs) level was used to compare environmental niches and evaluate the effect of niche differentiation on genetic patterns of two management units of Goitered gazelle in Central Iran. We found low values of niche overlap between the management units. The niche equivalency hypothesis revealed that the niche of MUs is more significantly distinct than expected by chance. Also, the niche similarity test for both comparisons falls within the 95% confidence limits of the null distribution. These findings demonstrate that the niche of two MUs is rarely identical, but they tend to be more similar than expected based on random predictions and environmental background, which they occur. We concluded that, besides landscape resistance and geographic distance, ecological niche isolation is another factor affecting the genetic structure of gazelle populations in Iran. Conservation planning of this vulnerable species should focus on isolated populations as separate management units and landscape linkages to maintain gene flow between the genetically similar populations.

**Keywords:** Goitered gazelle, Management unit, Niche equivalency, Niche overlap, Niche similarity.

## Introduction

There are many biological and ecological processes that can shape patterns of genetic structure in continuous or discrete populations of species including geography (Isolation by distance, IBD; Wright 1943), landscape resistance (Isolation by resistance, IBR; McRae and Beier 2007), and environment niche dissimilarity (Isolation by Environment; Wang and Summers 2010). In recent years, in the field of landscape genetics, several landscape models have been developed to examine the roles of landscape and environmental niche on gene flow (Storfer *et al.* 2010, Balkenhol *et al.* 2017).

Despite to importance of environmental niche dissimilarity as a motivator of genetic differentiations, its dynamism and strength are poorly understood (Kareiva and Marvier 2015). Populations that inhabit different niches may show the signature of genetic differentiation due to a variety of ecological processes including natural selection against immigrants, sexual selection against immigrants, reduced hybrid fitness, and biased dispersal (Wang *et al.* 2013). Hence, populations evolve traits suited to their local conditions. When populations show ecological specialization, natural selection will limit the reproductive success of individuals between different environments (Rasanen and Hendry 2008, Mosca *et al.* 2012). The pairs of populations inhabiting different ecological niches will experience reduced gene flow and greater genetic distance (Lee and Mitchell-Olds 2011, Wang *et al.* 2013).

In the absence of gene flow between demes, populations may diverge genetically while inhabiting similar environments, and hence, the specific niche is conserved (Wiens and Graham 2005). In the other hand, populations that occupy different environmental niches may show the signature of genetic differentiation. A niche includes a set of abiotic and biotic conditions that facilitate the persistence of a species (Hutchinson 1957). The two complementary concepts of the environmental niche (Grinnell 1917) and the trophic niche (Elton 1927) serve as a basis for quantifying the ecological niche (Broennimann *et al.* 2012). In recent years, researchers focused on ecological niche conservatism and niche overlap in different niche dimensions (Svendsen *et al.* 2015).

Ecological niche differences among species or populations can be analyzed to evaluate the possible ecological and evolutionary forces that shape geographical distributions, habitat preferences, and genetic structures (Raxworthy *et al.* 2007). Recently, a variety of approaches have been proposed and applied to quantify niche overlap and niche conservatism (e.g. Steiner *et al.* 2008, Medley 2010). Approaches for quantifying the niche overlap typically rely on either ordination techniques (e.g. Thuiller *et al.* 2005, Hof *et al.* 2010) or ecological niche modeling (Guisan and Thuiller 2005). Recent studies have revealed that bioclimatic variables are very useful in predicting the distribution of species and measuring environmental niches. Here, we used a statistical framework to describe and compare niches in a gridded environmental space using the ordination technique.

The Goitered gazelle (*Gazella subgutturosa*) inhabits large numbers of steppes, semi-arid and arid environments of Iran (Zachos *et al.* 2009). Gazelle populations have experienced periods of decline due to illegal hunting, drought, and habitat degradation (Zachos *et al.* 2010). Currently, almost all the existing gazelle populations are confined to protected areas surrounded by areas of human settlements and road networks. Khosravi *et al.* (2017) adopted a landscape genetics approach to investigate the spatial genetic structure and the effects of landscape features on gene flow among populations of Goitered gazelle in Iran. Their findings revealed three genetically homogeneous groups with restricted dispersal including Biduiyeh, Kolah-Qazi/Kahyaz, and Ghamishloo/Mooteh/Kalmand. They suggested that conservation measures on this vulnerable species could consider some isolated populations as separate MUs (e.g. Biduiyeh and Ghamishloo/Mooteh). The results of this study showed that a combination of IBD and IBR is affecting the genetic structure and gene flow between populations.

While they revealed new insights into the effects of landscape resistance and geographic distance on the spatial genetic structure of the populations, they did not evaluate the effects of niche isolation on genetic differentiation. We adopted a new statistical framework based on a genetically informed ecological niche approach to describe and compare niches of two gazelle's MUs including Biduiyeh and Ghamishloo/Mooteh in a gridded environmental space. Given the large distance between the MUs, we hypothesized that a difference in climate niche is one of the motivators of genetic differentiation between MUs besides IBD and IBR. Therefore, the main objective of the present work was to explore the hypothesis of the effect of climate niche differentiation on the genetic distances and the degree of niche conservatism within the Goitered gazelle's MUs in Iran.

## Material and methods

### Study area

Central Iran, with an elevation ranging from 117 to 4429 m, covers most of the distribution range of the Goitered gazelle. The mean annual temperature and precipitation are 17.6 °C and 117 mm respectively. Human activity within the landscape includes farming and livestock herding. Paved roads are one of the most important fragmenting features in this landscape (Moqanaki and Cushman 2016). Dwarf scrub vegetation is common in large areas and the dominant vegetation is composed of *Artemisia siberi*, *Astragalus gossypius*, *Zegophyllum* sp., and *Amygdalus* sp. Despite the arid environmental conditions, this part of Iran is rich in biological diversity.

There are seven Goitered gazelle populations in this area confined to the protected areas. We sampled two genetically MUs of Goitered gazelle including BID-Biduiyeh (E56$^{o}$ 20′ N29$^{o}$ 53′), and GHAM-Ghamishloo (E49$^{o}$ 95′ N36$^{o}$ 14′)/ MOT-Mooteh (E50$^{o}$ 10′ N33$^{o}$ 20′, Fig.1). Based on Moritz's (1994) definition, management units are "populations with significantly divergent alleles at either nuclear or mitochondrial loci. The previous studies (e.g. Khosravi *et al.* 2017) have confirmed that the population of Goitered gazelle in Biduiyeh, Mooteh, and Ghamishloo have significantly different alleles at either nuclear or can be considered as separate management units. The species occurrence data were obtained from a variety of sources including field surveys during genetic sampling, opportunistic direct observation, and environmental guards` direct sighting. The coordinates of all the presence points were recorded using a hand-held multichannel Global Positioning System (GPS) receiver.

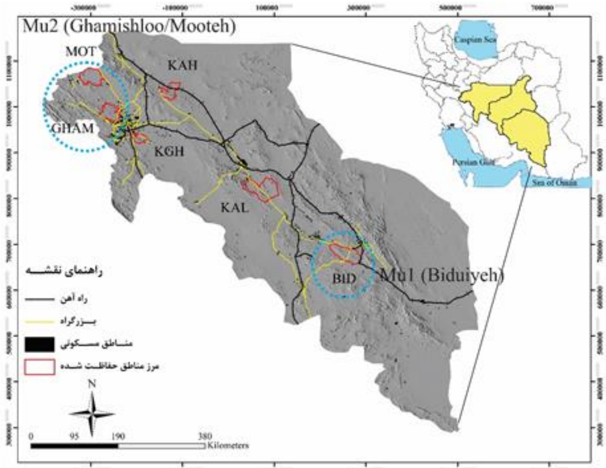

**Figure 1.** Location of the two genetically management units of *G. subgutturosa* in the study area (dash lines). MU1= Biduiyeh (BID), and MU2= Ghamishloo/Mooteh (GHAM/MOT). The red polygons show the location of protected areas and populations of Goitered gazelle. KAL=Kalmand, KGH=Kolah-Qazi, KAH=Kahyaz.

### Calculating niche overlap, equivalency, and similarity

We assessed the effects of climate niche on the genetic differentiation of the two populations (BID and GHAM/MOT) using ecological niche analysis based on the PCA-env framework proposed by Broennimann *et al.* (2012). We obtained information on climatic conditions from the WorldClim database (http://www.worldclim.org; developed by Hijmans *et al.* 2005). We used a spatial downscaling method to transfer the original 1-km resolution of WorldClim data to the target resolution of 250-m with FORTRAN using Microsoft Visual Studio (Flint and Flint 2012). This model combines a spatial gradient and inverse-distance-squared (GIDS) weighting to WorldClim data with multiple regression. The location and elevation of the new fine-resolution grid cell relative to a coarse-resolution grid cell is used to weight the parameters based on the following equation:

$$Z = \left[ \sum_{i=1}^{N} \frac{Z_i + (X - X_i)*C_x + (Y - Y_i)*C_y + (E - E_i)*C_e}{d_i^2} \right] / \left[ \sum_{i=1}^{N} \frac{1}{d_i^2} \right]$$

where Z is the estimated climatic variable at the specific location defined by easting (X) and northing (Y) coordinates and elevation (E); Zi is the climatic variable from the 1-km grid cell i; Xi, Yi, and Ei are easting and northing coordinates and elevation of the 1-km grid cell i, respectively; N is the number of 1-km grid cells in a specified search radius; Cx, Cy, and Ce are regression coefficients for easting, northing, and elevation, respectively; and di is the distance from the 250-m site to 1-km grid cell i (Flint and Flint 2012). We used a 30-km search radius to calculate bioclimatic data at the 250-m resolution (see Khosravi *et al.* 2016 for details).

As the inclusion of all 19 bioclimatic variables in the model may cause overfitting and uncertainties due to the high degree of correlation among variables, we conducted PCA to transform the climate layers into a reduced number of components (PCs). Then, the position of a kernel density of presence points in each cell in the environment space for each MUs along the principal components was compared to reflect their ecological niche differentiation and to quantify niche overlap between MUs (Broennimann *et al.* 2012). We used a smoothed density from a kernel density function to ensure that the measured overlap is independent of the resolution of the grid. Environmental space is bounded by the minimum and maximum environmental values found across the entire study region. We considered the first two axes of PCA as environmental space. In this study, the available environmental space of the MUs was defined by all pixels of the 19 climatic variables within a buffer of 20 km enclosing the species occurrence points.

Niche overlap between the MUs was computed by the method proposed by Schoener (1968). Hence, the density grids are used to compute a niche overlap between MUs as the following equation:

$$D = 1 - \frac{1}{2} \left( \sum_{ij} \left| Z_{1ij} - Z_{2ij} \right| \right)$$

Where z1ij is entity 1 occupancy and z2ij is entity 2 occupancy. This metric varies between 0 (no overlap) and 1 (complete overlap).

We computed niche equivalency and similarity using the method developed by Warren *et al.* (2008). This method uses statistical randomization (100 repetitions) to determine if the pairwise comparison of niche differentiation is statistically resulted in by chance. The niche equivalency is a one-tailed randomization test that aims to assess whether the ecological niche of two populations is significantly more equivalent than expected by chance. We also calculated niche similarity between MUs using 100 repetitions to address whether the observed niche overlap among the MUs can be attributed to their available environmental space (Broennimann *et al.* 2012). The MUs level comparisons of PCA-env were computed in R (R Development Core Team 2010) using the method developed by Broennimann *et al.* (2012).

## Results

The results of the multivariate environmental space occupied by two MUs of Goitered gazelle based on the PCA-env approach are shown in figure 2. The analysis of environmental niche properties based on PCA showed that the first axis explained 51.93% of the total variation in environmental conditions. The PC1 is mainly loaded by mean diurnal range, isothermality, temperature seasonality, precipitation seasonality, and temperature annual range. The second axis explained 34.35 % of the total variation and was loaded by the max temperature of warmest month, mean temperature of driest quarter, and precipitation of wettest quarter variables (Fig. 2 b). Niche overlap results showed great variability in the environmental space inhabited by the two populations (Fig. 3 a). In this regard, results of niche divergent analysis did not show overlap between Biduiyeh and Mooteh/Ghamishloo. (Schoener's D = 0.00; Fig. 2a).

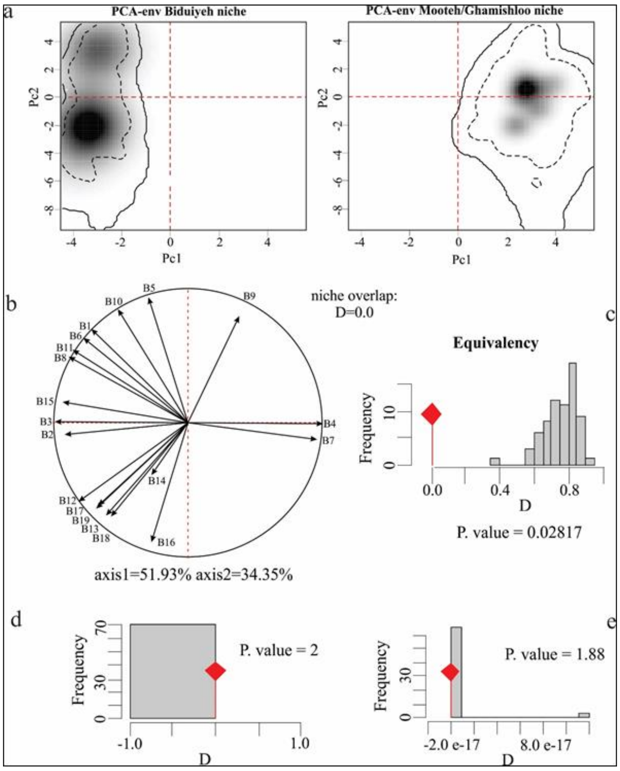

**Figure 2.** The results of niche overlap, similarity, and equivalency between two genetically populations of Goitered gazelle in climatic space using PCA-env. The PCA-env results show the niche of the MUs in two main axes with the climate conditions of the complete landscape area. (a) Niche of Biduiyeh and Mooteh/Ghamishloo along the PC1 and PC2 of the PCA analysis. Grey shading shows the kernel density of the presence points of Goitered gazelle. Also, the solid and dashed lines show 100% and 50% of the background environment space respectively. (b) The contribution of each variable on the two axes of the PCA. (c) The results of niche equivalency are based on the observed and simulated niche overlap between the populations. (d) and (e), respectively, illustrate the niche similarity of BID to MOT/GHAM and the niche similarity of MOT/GHAM to BID calculated from 100 iterations.

Using the D metric, we rejected the hypothesis of niche equivalency between the MUs ($P < 0.05$, Fig 2c) revealing that the niche of two populations is more significantly distinct than expected by chance. The results of niche similarity based on the background similarity test for both comparisons (Biduiyeh points – Mooteh/Ghamishloo background (Fig. 2d) and Mooteh/Ghamishloo point – Biduiyeh background (Fig.2 e)) falls within the 95% confidence limits of the null distributions, leading to non-rejection of the hypothesis of niche similarity ($P > 0.05$). Considering the results from niche equivalency, these findings demonstrate that the niche of two studied populations is rarely identical, but they tend to be more similar than expected based on random predictions and the broad environmental background in which they occur.

## Discussion

Here we used a robust method to quantify niche overlap between two genetically populations of Goitered gazelle in Central Iran. PCA-env is an appropriate method for evaluating the difference of niche between populations of the same species that occupy different geographic ranges and likely different climate conditions (Fitzpatrick *et al.* 2007, Steiner et al. 2008, Broennimann *et al.* 2012). Broennimann *et al.* (2012) revealed that, in comparison to other ordination techniques, PCA-env accurately quantifies the level of niche differentiation without substantial bias and is more appropriate than the species distribution model (SDMs) for investigating niche overlap. Also, Wielstra *et al.* (2012) showed that both PCA-env and ENFA are powerful methods for evaluating the niche similarity for crested newt (*Triturus cristatus*). We tested the hypothesis of environmental niche isolation as another explanation for genetic differentiation between Biduiyeh and Mooteh/Ghamishloo.

The analysis of genetic structure based on microsatellites shows that individuals of Biduiyeh and Mooteh/Ghamishloo belong to distinct genetic groups. High landscape resistance between two populations and also long geographic distance confirms the genetic differentiations between Biduiyeh and

Mooteh/Ghamishloo (Khosravi *et al.* 2017). The analysis of PCA-env highlighted that there is no niche overlap between two populations (Schoener's D = 0.00). Given the great variations in climate conditions in Iran, it is not surprising the low niche overlap between geographically distant populations of Goitered gazelle. Hence, the low niche overlap value between Biduiyeh and Mooteh/Ghamishloo reflects their different environmental constraints. Our assessment of niche equivalency based on PCA-env rejected the null hypothesis that the climate niche of populations of Goitered gazelle is equivalent. These findings show why it is not accurate to imply niche characteristics for one MU based on the niche of another MU even for populations of the same species. In addition, the results showed that the niche of Biduiyeh tends to be more similar to the niche of Mooteh/Ghamishloo than random and, thus, niche similarity cannot be rejected. We concluded that both populations do not significantly retain their environmental niche characteristics from each other. In the other words, the niche similarity results suggest that populations of Goitered gazelle in the studied landscape share more characteristics of their climate niche spaces than randomly expected. These findings suggest that while two MUs are closely related and share climate niche spaces but they are still different MUs.

The observed similarity in climate niche space between two populations suggests that they have similar environmental constraints but that a different set of variables within this environmental niche space restrict their distribution (Aguirre-Gutierrez *et al.* 2015). Niche overlap based on PCA-env analysis confirmed that there is no sign of niche conservatism or divergence between these two genetic groups. Niche conservatism is the allopatric evolution of two evolutionary-closed groups in the past (McCormack *et al.* 2010). Hence, we conclude that there is no sign of separate evolutionary events because of allopatric adaptation to isolated refuges for Biduiyeh and Moote/Ghamishloo.

A few isolated patches with suitable habitats are available between the Biduiyeh population and other populations of Goitered gazelle, which may not be enough to provide a continuous corridor for the gazelle's migration. In addition, road construction and expansion, the close proximity of this protected area to populated residential areas, and long-distance between Biduiyeh and the nearest gazelle population may have also been responsible for the isolation of this population. Based on the landscape genetics analysis, Khosravi *et al.* (2017) considered Biduiyeh and Mooteh/Ghamishloo as two management units. Based on Moritz's (1994) definition, management units are "populations with significantly divergent alleles at either nuclear or mitochondrial loci". Therefore, according to Moritz (1994), MUs address the current structure, making them more useful in addressing short-term management issues. Reduced genetic diversity in isolated populations such as Biduiyeh, will reduce the ability of the population to respond to changing environmental conditions. Hence, in future conservation plans, it should be considered the role of environmental variables in shaping the ecological niche of focus species.

In conclusion, the results of this research indicate that, besides landscape resistance and geographic distance (Khosravi *et al.* 2017), environmental niche isolation is one of the most motivators of genetic discontinuities in Goitered gazelle populations in Central Iran. In the other words, a combination of landscape resistance, geographic distance, and ecological niche divergence are affecting the gene flow of gazelle populations. Conservation planning of this vulnerable species should focus on isolated populations as separate management units and landscape linkages to maintain the gene flow between the genetically similar populations. In addition, effective conservation actions must take into account the intrinsic requirements of different management units and the main environmental drivers that shape their distributions. Therefore, different conservation interventions may be required for each management unit.

## Acknowledgment

We are grateful to Isfahan, Yazd, and Kerman provincial DOE for permission to enter to protected areas. This research was financially supported by the Iran National Science Foundation, Presidency of the Islamic Republic of Iran (Project Number 92026483).

## References

Aguirre-Gutiérrez J., Serna-Chavez H.M., Villalobos-Arambula A.R., Pérez de la Rosa J.A., Raes N. 2015. Similar but not equivalent: ecological niche comparison across closely–related Mexican white pines. Diversity and distributions 21(3): 245-257.

Balkenhol N., Cushman S., Storfer A., Waits L. 2016. Landscape genetics: concepts, methods, applications. John Wiley and Sons, pp.272.

Broennimann O., Fitzpatrick M.C., Pearman P.B., Petitpierre B., Pellissier L., Yoccoz N.G., Thuiller W., Fortin M.J., Randin C., Zimmermann, N.E. 2012. Measuring ecological niche overlap from occurrence and spatial environmental data. Global Ecology and Biogeography 21: 481–497.

Elton C.S. 1927. Animal ecology. London: Sidgwick and Jackson. pp. 130

Fitzpatrick M.C., Weltzin J.F., Sanders N.J., Dunn R.R. 2007. The biogeography of prediction error: why does the introduced range of the fire ant over-predict its native range. Global Ecology and Biogeography 16(1): 24–33.

Flint L.E., Flint A.L. 2012. Downscaling future climate scenarios to fine scales for hydrologic and ecological modeling and analysis. Ecological Process 1(1):123–140.

Graham C.H., Ron S.R., Santos J.C., Schneider C.J., Moritz C. 2004. Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. Evolution 58(8): 1781-1793.

Grinnell J. 1917. The niche-relationships of the California thrasher. The Auk 34: 427–433.

Guisan A., Thuiller W. 2005. Predicting species distribution: offering more than simple habitat models. Ecology Letters 8(9): 993–1009.

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(15): 1965–1978.

Hof C., Rahbek C., Araújo M.B. 2010. Phylogenetic signals in the climatic niches of the world’s amphibians. Ecography 33(2): 242–250.

Hutchinson G.E. 1957. Population studies – animal ecology and demography – concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology 22: 415–427.

Khosravi R., Hemami M.R., Malekian M., Flint A., Flint L. 2016. Maxent modeling for predicting potential distribution of goitered gazelle in central Iran: the effect of extent and grain size on performance of the model. Turk. Journal Zoology 40(4): 574–585.

Khosravi R., Hemami M.R., Malekian M., Silva T.L., Rezaei H.R., Brito J.C. 2016. Effect of landscape features on genetic structure of the Goitered gazelle (*Gazella subgutturosa*) in Central Iran. Conservation Genetic (https://doi.org/10.1007/s10592-017-1002-2).

Lee C.R., Mitchell-Olds T. 2011. Quantifying effects of environmental and geographical factors on patterns of genetic differentiation. Molecular Ecology 20(22): 4631–4642.

Marvier M., Kareiva P. 2015. Conservation Science: Balancing the Needs of People and Nature. Second Edition. Roberts and Company Publishers, Greenwood Village, pp. 543.

McCormack J.E., Zellmer A.J., Knowles L.L. 2010. Does niche divergence accompany allopatric divergence in Aphelocoma jays as predicted under ecological speciation? insights from tests with niche models. Evolution 64(5): 1231– 1244.

McRae B.H., Beier P. 2007. Circuit theory predicts gene flow in plant and animal populations. Proceedings of the National Academy of Sciences 104(50): 19885–19890.

Medley K.A. 2010. Niche shifts during the global invasion of the Asian tiger mosquito, Aedes albopictus Skuse (Culicidae), revealed by reciprocal distribution models. Global Ecology and Biogeography 19(1): 122–133.

Moqanaki E.M., Cushman S.A. 2016. All roads lead to Iran: Predicting landscape connectivity of the last stronghold for the critically endangered Asiatic cheetah. Animal Conservation 20(1): 29-41.

Moritz C. 1994. Defining evolutionary significant unit for conservation. Trends in Ecology and Evolution 9(10): 373-211.

Mosca E., Eckert A.J., Di Pierro E.A., Rocchini D., La Porta N., Belletti P., Neale D.B. 2012. The geographical and environmental determinants of genetic diversity for four alpine conifers of the European Alps. Molecular Ecology 21(22): 5530–5545.

Raasanen K., Hendry A.P. 2008. Disentangling interactions between adaptive divergence and gene flow when ecolo gy drives diversification. Ecology Letters 11(6): 624–636.

Raxworthy C.J., Ingram C.M., Rabibisoa N., Pearson R.G. 2007. Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day geckos (Phelsuma) from Madagascar. Systematic Biology 56(6): 907–923.

Schoener T.W. 1968. Anolis lizards of Bimini: resource partitioning in a complex fauna. Ecology 49(4): 704-726.

Steiner F.M., Schlick-Steiner B.C., VanDerWal J., Reuther K.D., Christian E., Stauffer C., Suarez A.V., Williams S.E., Crozier R.H. 2008. Combined modelling of distribution and niche in invasion biology: a case study of two invasive Tetramorium ant species. Diversity and Distributions 14(3): 538– 545.

Storfer A., Murphy M.A., Spear S. F., Holderegger R., Waits L.P. 2010. Landscape genetics: Where are we now? Molecular Ecology 19(17): 3496–514.

Svendsen G. M., Romero M. A., Williams G. N., Gagliardini D. A., Crespo E. A., Dans S. L., González R. A. 2015. Environmental Niche Overlap between Common and Dusky Dolphins in North Patagonia, Argentina. PloS one 10(6): e0126182.

Team R.C. 2016. A language and environment for statistical computing. R Foundation for statistical computing. 2015. Vienna, Austria, pp.3-36.

Thuiller W., Lavorel S., Araújo M.B. 2005. Niche properties and geographical extent as predictors of species sensitivity to climate change. Global Ecology and Biogeography 14(4): 347– 357.

Wang I.J., Summers K. 2010. Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. Molecular Ecology 19(3): 447–458.

Warren D.L., Glor R.E., Turelli M. 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution 62(11): 2868–2883.

Wielstra B., Beukema W., Arntzen J.W., Skidmore A.K., Toxopeus A.G., Raes N. 2012. Corresponding Mitochondrial DNA and Niche Divergence for Crested Newt Candidate Species. PLoS One 7(9): e46671

Wiens J.J., Graham C.H. 2005. Niche conservatism: integrating evolution, ecology and conservation biology. Annual Review of Ecology, Evolution, and Systematics 36: 519–539.

Wright S. 1943. Isolation by distance. Genetics 28(2): 114–138.

Zachos F.E., Karami M., Ibenouazi Z., Hartl G.B., Eckert I., Kirschning J. 2010. First genetic analysis of a free-living population of the threatened goitered gazelle (*Gazella subgutturosa*). Mammalin Biology 75(3): 277–282.

**Journal of Wildlife and Biodiversity 1(2): 69-78 (2017)**

**by Arak University, Iran (http://jwb.araku.ac.ir/)**

**DOI: 10.22120/jwb.2017.28260**

**Research Article**

# Human-Elephant conflict mitigation methods: A review of effectiveness and sustainability

**Ettagbor Hans Enukwa**[1,2]

[1]Department of Forest Resources, College of Agriculture and Life Science, Gyeongsang National University, Jinju 528-28, Korea.
[2]Mount Cameroon National Park, Regional Delegation of Forestry and Wildlife for the South West, P.O. Box 602, Buea, Cameroon. email: etagha@yahoo.com

Received: 08 September 2017 / Revised: 20 October 2017 / Accepted: 25 October 2017 / Published online: 3 November 2017. Ministry of Sciences, Research and Technology, Arak University, Iran.

## Abstract

Human-elephant conflicts (HEC) have resulted in a substantial loss in livelihood and human lives, consequently, the affected communities retaliate by harming or killing the elephants. Thus, measures to mitigate HEC are imperative for the successful conservation of elephants, and to ensure the coexistence of the local population with these animals. Numerous HEC mitigating methods have been implemented. I reviewed 19 different HEC mitigation methods to assess their successes and failures, as well as their sustainability. Methods that required the regular presence of humans for their implementation and functionality tended to be very tedious and unsustainable since the uptake of such methods would be very low. Also, methods that posed no threat to elephants were unsustainable as elephants become used to the methods. Approaches that are affected by weather conditions are unsustainable. Methods that were very expensive to implement are also unsustainable as the method would be abandoned when funding ends. However, electrical fencing was effective and sustainable since it doesn`t depend on the physical presence of humans for it to prevent elephant destruction, especially when a sustainable finance mechanism is assured to ensure monitoring and maintenance of the fence. Beehive fences are also very effective especially when colonization of the hives is assured, and this method is very sustainable as it is cost-effective and honey from the hives could generate income for the population. Also, methods aimed at habitat modification are sustainable since habitat improvement increases the comfort and resources elephants need to live in their habitat.

**Keywords:** Beehive fences, coexist, elephants, electrical fencing, sustainable.

## Introduction

Human-wildlife conflict (HWC) can be characterized as any interaction between humans and wildlife which negatively affects the human, the wildlife or property. Human-elephant conflict (HEC) is a major type of HWC that is considered a major challenge by conservation stakeholders. Today, HEC has become one of the biggest issues facing elephant conservationists (Stephenson 2004). Elephants cause catastrophic damage to farmers (Tchamba 1995, Ekobo 1997, Osei-Owusu and Bakker 2008), and they are considered to be more dangerous than other herbivore species, causing more deaths and injuries to humans (Sitati 2003).

HEC that causes harm to rural farmers and their property constitute a major concern to biodiversity conservation, especially across Africa and Asia (Barnes 1996, Dublin *et al.* 1997, Graham and Ochieng 2008). This conflict mostly occurs in adjacent communities that live close to the natural habitats where the elephants live.

Considering the rapid increase in the human population that has increased human needs, there has been a resultant expansion of human activities which in many cases have encroached into wildlife areas, especially by local communities living around protected areas. In situations where such areas have a significant population of elephants, HEC is bound to occur. In many cases, as a result of HEC, people lose their crops, livestock, property, and sometimes their lives. HEC has been a big problem to a huge number of people in many parts of the world.

The destruction caused by elephants irritates the affected communities, with the animals often killed, captured, or otherwise harmed in retaliation (Naughton-Treves 1998, Malima *et al.* 2004, Omondi *et al.* 2004) and in some cases, the people decide to turn a blind eye to poaching in reprisal for the damage caused by the elephants (Parker *et al.* 2007, Mwakatobe *et al.* 2014, Karidozo and Osborn 2015).

The implementation of measures to mitigate HEC is imperative to enhance the sustainability of conservation efforts and to improve the coexistence between people and wildlife. The advantages of carrying out HEC mitigating measures will evidently be both on the communities and the elephants. These advantages may include improved attitudes and tolerance of farmers towards wildlife, the decline in crop losses, human death and injury, as well a decline in the mortality of elephants during HEC (Jackson *et al.* 2008).

The aim of this review was to highlight the successes and failures of various HEC mitigating measures that have been carried out in different parts of the world. It was also aimed at assessing the strengths and weaknesses of various HEC mitigating methods.

## Material and methods

### Sources of Information

The author reviewed the results of different HEC mitigating measures that had been implemented in different parts of the world. The sources of these results were mostly articles that had been published in international peer-reviewed journals. The author focused on articles that reported findings from field research that were carried out.

These articles were gotten by searching on Google Scholar. The appropriate heading on HEC mitigating measures was typed on Google Scholar and many published articles were displayed. The author then downloaded and saved the articles that focused on HEC. Also, some articles were gotten via going through the reference list of already downloaded and saved articles. Numerous articles were gotten. The author now read through each article to select those that reported appropriately on specific HEC mitigating measures, based on practical research that was carried out.

### HEC mitigating measures

Numerous researches have been carried out on different measures to mitigate HEC. Thus, the author reviewed findings on these different measures following reported field experiments carried out by different scholars. The mitigating measures reviewed by the author include traditional, biological and physical measures. The methods include:

1) Beehive fences
2) Chilli pepper methods

- Spraying and/or shooting prepared capsicum
- Chilli fences where the chilli extract is mixed with tobacco or oil and applied on the fence.
- Chilli smoke involving the burning of chilli, tobacco and straw, dung mixture or chilli bricks creating a pungent smell.

3) Fences

  - Fence with bells or trip alarms. Polythene cord or thatching twin attached to poles used to surround farms, with cowbells or trip alarms attached to it at given distances.
  - Electrical fencing

4) Spotlight. Guards stand on watchtowers with bright torchlights and point in the elephant`s eyes.
5) Elephant drives. Trained domestic elephants (kunkies) used to drive wild elephants.
6) Fire. Bush fires are set which while burning, scare elephants away.
7) Noise or sound
- Noise from playing the drums, shouting, vehicle, tractor, loudspeakers etc
- Playing Elephant Warning Calls close to elephant herd (humankind sound)
- Playing noise of bees
- Use of firecrackers (explosives)

8) Habitat improvement
- Building manmade salt ponds
- Establish wildlife corridors between parks (increases habitat)

9) Digging trenches
10) Culling (Killing) of problem elephants
11) Contraception
12) Translocation of elephants to other areas

More experiments were reviewed on electrical fencing and chilli fences than the other HEC mitigating measures (Fig. 1), since following the extensive search that was carried out more information was available and obtained on these two methods.

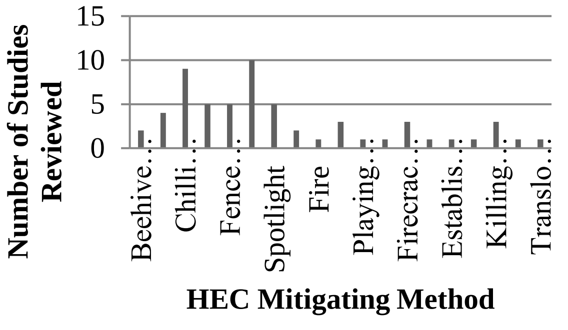

**Figure 1.** The number of studies reviewed for each Human-Elephant Conflict (HEC) mitigating method. The studies that were reviewed represent experiments that have been conducted and published in peer review journals.

## Results

### Beehive fence

Beehive fences effectively reduced HEC (Fig. 3) as crop destruction was minimized in experimental plots compared to the control plots with no fence (Lucy *et al*. 2009, Lucy *et al.* 2017). Elephants usually get scared of bees as they can easily attack them when elephants disturb or shake the fence on which beehives have been attached. This method has an advantage in that in addition to minimizing HEC, the beehives serve as income-generating activities (IGA) as honey can easily be collected and used for subsistence or sold. This method is also cheaper and easily affordable. The method was therefore highly desired by the communities.

Community members would be highly motivated to invest in this method, thus contributing to beehive fence being a sustainable approach in mitigating HEC. However, it would be very important for the users to have adequate training on the management of beehives so as to ensure adequate colonization of the hives by bees, without which, the method would be relatively ineffective. Also, the beehives should be suspended on a tightly secured fencing wire so that the beehives can swing freely (Fig. 2) especially when an elephant touches the fence, thus disturbing and releasing the bees to irritate or sting the elephant. It should as well be noted that the closer the beehives, the more effective the method would be.

### Chilli pepper methods

*Spraying and/or shooting prepared capsicum*

Capsicum prepared as a spray or as pellets when sprayed or shot at the elephants usually using dispensers, effectively deterred and scared away elephants (Fig. 3), since many elephants could easily run away when the substance hit the elephant and capsicum spread on them (Osborn and Rasmussen 1995, Ferrel 2002, Sébastien *et al*. 2010). This repellent effect of capsicum on elephants could be as a result of the substance causing effects on the animal such as burning sensation in the mucosa of the trunk, watering eyes, and trigeminal pain. However, this method could be very challenging thus limiting uptake by the community since spraying and shooting requires physical presence which is tedious and labour intensive. Also, it entails a high cost to prepare this substance, thus communities would not easily invest in it.

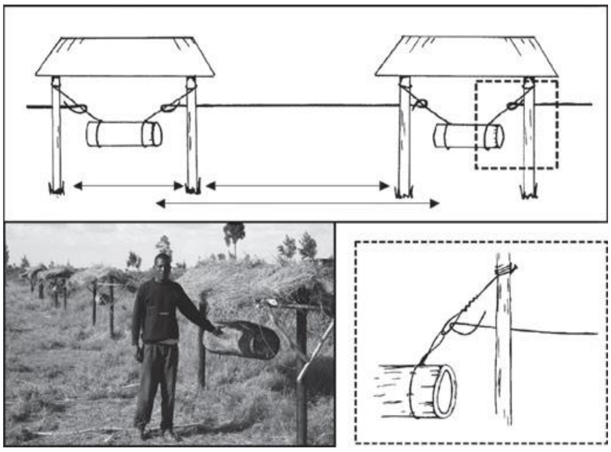

**Figure 2.** Beehive fence for the mitigation of HEC. The fence is constructed with log beehives hung under small thatched roofs (adopted from Lucy *et al.* 2009)

*Chilli fences*

89% of experiments on chilli fences effectively deterred elephants (Fig. 3), since many elephants ran away when they approached and came in contact with the fences on which chilli paste was applied (AERP HEC project 2006, John 2006, Graham and Ochieng 2008, Chelliah *et al.* 2010, Tammy *et al.* 2011, Hiten *et al.* 2012, Karidozo and Osborn 2015, Alex *et al.* 2016) compared to the control.

However, Simon and Donny (2009) reported that chilli fences had no deterrent effects on elephants, as crop-raiding by elephants was similar both in the experimental and control plots. Generally, the loss of pungency of chilli when exposed to rain or sun reduces its effectiveness and thus becomes a poor deterrent for elephants. Also, community members cannot easily adopt this method or invest in it since the cost of chilli paste is usually very expensive, and the method is labour intensive as it requires regular reapplication of chilli grease or paste on the fence. The method would only maintain its effectiveness if conservation stakeholders provide regular funding, without which it becomes unsustainable.

*Chilli smoke*

80% of experiments on chilli smoke effectively deterred elephants (Fig. 3), since the pungent smell that was created caused elephants to run away (Ferrel and Guy 2002, John 2006, Graham and Ochieng 2008; Karidozo and Osborn 2015), thus protecting farms and minimizing crop destruction compared to the control. Nevertheless, Tammy *et al.* (2011) reported that chilli smoke had no significant deterrent effects on elephants, as crop-raiding by elephants was similar both in the experimental and control plots. This method can be very successful if many of such materials are burned around the farm since the more the smoke produced the more the effectiveness of the method. This will in effect increase cost which may make the method to become very expensive for local farmers to invest in. Also, this method is highly dependent on wind, thus if the wind doesn`t blow the smoke towards the elephants, it becomes unsuccessful.

## Effects of Fences

*Electrical fencing*

The electrical fence effectively minimized HEC (Fig. 3) as the fence acts as a barrier that prevents elephants from crossing (Thouless and Sakwa 1995, Charles 1996, Caitlin *et al.* 2000, Gunaratne and Premarathne 2005, John *et al.* 2008, Colin *et al.* 2009; Graham *et al.* 2009, Tammy *et al.* 2011, Wumuyu 2012, Ahmad and Magintan 2016). Electrical fences have the capacity of not only protecting individual farms but also protecting the entire community depending on the extent of the fence. To increase its efficiency, the electrical fence needs to be well solid and maintained regularly. The construction, maintenance and enforcement cost of electrical fences is high and thus this method may only be feasible in conservation areas with adequate financial resources. Such finances would be able to create, train and ensure the functionality of a committee charged with the responsibility of regular monitoring and maintenance. A sustainable finance mechanism has, therefore, to be put in place to guarantee the sustainability of the method. For instance, Kenyan Wildlife Service (KWS) has constructed and maintained a total of 1,225kms of electrical fences nationally with 888 km within protected areas, and the cost of constructing 1km of electrical fences is about US$15,000 (Chiemelu 2004).

*Fence with bells or trip alarms*

Fence with bells and trip alarms were generally ineffective to mitigate HEC with just 40% of the experiments successfully reducing conflicts compared to the control (AERP HEC project 2006, Wahed *et al*. 2016), and 60% of the experiments unsuccessful (Caitlin *et al.* 2000 Ferrel and Guy 2002, Graham and Ochieng 2008). Generally, bells in themselves do not scare away elephants, but may only alert farmers when elephants come to their fields. Also, elephants learn and habituate to bells as they don`t face any threat or physical harm resulting from bells. However, the noise from the alarm alerts people and irritates elephants scaring them away (Wahed *et al.* 2016). This method is a cost-effective early warning approach which even though it requires regular maintenance, it does not necessarily need frequent replacements. Uptake of this method by communities would be good, especially when community members have adequate training on its maintenance.

### Spotlight

Spotlights effectively minimized HEC by preventing crop damage by elephants (Fig. 3) since the bright rays of the torchlight is directed into the eyes of the elephant, thus scaring them away (Charles 1996, AERP HEC project 2006, Graham and Ochieng 2008, Simon and Donny 2009, Tammy *et al.* 2011). The effectiveness of this method is guaranteed only when the guards chase the elephants away while directing the light into the eyes of the elephant. The torch lights must therefore be very bright and the elephants have to be close enough. This method has an advantage in that it is cost-effective, and thus the communities can easily bear the cost. However, the method is labour intensive, and thus many people would not be motivated to go through the intense stress associated with this approach. Also, the elephants may tend to come back to the farms in periods when there is no light.

### Elephant drives

50% of the experiments carried out showed that elephant drive is effective for HEC mitigation (Fig. 3), as well trained domestic elephants (kunkies) successfully drive wild elephants away (Charles 1996). However, inadequately trained domestic elephants cause wild animals to panic and run randomly, thus destroying crops the more (Tammy *et al.* 2011). Thus, for this method to be successful, the domestic animals need to be given adequate training such that they can drive wild elephants in an orderly manner, without which their actions will instead increase HEC. This method is generally not sustainable because it can only be implemented by trained wildlife experts, and it may only provide a temporal solution since the wild elephants would return to the farms when the trained domestic animals are not there.

### Fire

The only experiment conducted on fire showed that it is an effective method in mitigating HEC (Fig. 3) as elephants tend to run away from huge fires (Tammy *et al.* 2011). This method is labour intensive as it requires regular control of the fire to prevent it from extending into the nearby environment. Without control, fire may extend and destroy crops and surrounding vegetation.

## Noise or Sound

*Noise from human activities*

Only about 33% of the experiments showed that noise from human activities could mitigate HEC (Fig. 3) since elephants would be scared and thus run away from such unpleasant sounds (Charles 1996). Generally, playback of a tape containing a jumble of noises, through loudspeakers was more effective than a single noise of an animal. However, noise may cause elephants to run (escape) uncontrollably thus destroying more crops and creating more conflicts (Ferrel and Guy 2002, Tammy *et al*. 2011). Also, elephants may get used to the sound knowing it is empty threat and not escape.

*Playing Elephant Warning Calls close to elephant herd*

This method was ineffective in mitigating HEC (Fig. 3) since the elephants came back when the calls ended (Caitlin *et al.* 2000). The method requires repeated playing of the warning calls (humankind of sounds) close to the elephant herd till the elephant leaves the area.

*Playing noise of bees*

The sound of disturbed bees playing close to elephants effectively mitigated HEC (Fig. 3) as the sound scared the animals away (Lucy *et al*. 2007). However, this method can only provide a temporary solution as elephants tend to return to the area when there is no noise.

*Use of firecrackers or explosives*

This method effectively mitigated HEC (Fig. 3) as elephants become scared and run away after hearing the sound of these explosives (Charles 1996, Ferrel and Guy 2002, Graham and Ochieng 2008) especially when it occurs close to the animals. However, the method does not eradicate elephant raid as elephants may become use to the noise, or come back to the area when there is no noise.

## Habitat Improvement

*Building manmade salt ponds*

This method effectively mitigated HEC (Fig. 3), especially in areas where elephants frequently moved to communities in search of salt (Li and Ning 2003, Wahed *et al.* 2016). This indicates that habitat improvement could be a reliable way to reduce conflicts since the resources needed by the elephants would be made available to them. Conflicts mostly arise when these animals do not have the resources they need within their habitats, thus they are forced to go and search for it elsewhere. Manipulating wildlife habitat by improving or providing food, water, adequate space as well as improved habitat health could be considered by stakeholders as vital approaches to mitigate HEC.

*Establish wildlife corridors between parks (increases habitat)*

The establishment of corridors effectively mitigated HEC (Fig. 3) as elephants would easily move via corridors to other areas where they will have better habitat conditions such as food and/or water at a given period or season (Alfred *et al.* 2010). For corridors to be effective, the habitat conditions of the corridor should be similar to the preferred habitat condition in the original site where the elephants live. If the habitat conditions of corridors are not suitable for the elephants, they will always move out of the corridors to the adjacent land to obtain the resources and comfort that they need, hence creating conflicts. Also, sufficient habitat conditions should exist in the areas where the elephants are using the corridor to move to, without which the carrying capacity of the site could be exceeded (as the elephants would add to the already existing population in the new site), resulting to deterioration of the new site as well as increase conflicts.

## Digging Trenches

Dug trenches were ineffective for HEC mitigation (Fig. 3) as elephants could easily collapse the walls of trenches with their massive body weight especially in humid areas, thus crossing over them (Li and Ning 2003). Blair and Noor (1981) reported that Malaysian elephants have been known to cross over trenches of up to 2.3m wide. Trenches also have a disadvantage in that they are very expensive to construct and maintain.

### Culling (Killing) of problem elephants

Culling of selected elephants was ineffective for HEC mitigation (Fig. 3) since the remaining herd came back to the area after some time, the population increased via reproduction, and others migrate to the area, thus creating conflicts (Whyte 1993, Tchamba 1995, Hoare 2001). The killing of problem elephants by wildlife officials is a common practice in many countries, especially as the local populations affected by HEC perceive this measure as a positive step by the government to retaliate against the destruction caused by these animals. Such methods may only scare away elephants for a short time, and thus does not provide a long-term solution to HEC. Hoare (2001) reported that after killing one elephant in a herd of crop-raiding bull elephants, by the fourth night after, its radio-collared companions had returned to raid fields within one kilometre of the shooting incident. Thus culling may only improve the relation and trust between conservationists and the local communities, but it`s an ineffective approach for HEC mitigation. Instead, killing may reduce the gene pool of elephants in an area.

### Contraception

Contraception is ineffective in mitigating HEC (Fig. 3), as the approach may be damaging to the individual female and those around her, while it would require that a very huge percentage of the female elephants should be on contraceptives in order to achieve a zero population growth (Ian *et al.* 1998). This approach would therefore entail a very huge cost, thus making it not to be practically feasible and unsustainable. However, Stuart and Rudi (2001) reported that immunocontraception (inhibition of the fertilisation process) using the porcine zona pellucida (pZP) vaccine could slow down the reproductive rate of elephants. But again, for this approach to be effective, contraception should be repeated after some time when the contraceptives may have weakened and become ineffective. This is therefore a very tedious and costly process.

### Translocation of elephants to other areas

Translocation was ineffective in mitigating HEC (Fig. 3) since some of the males and females with calves left the release site and either returned home or roamed into nearby human settlements (creating conflicts) and were then poached by the local community or shot by Problem Animal Control (Noa 2009).

Noa (2009) reported higher mortality rates of translocated animals in the release site than the local population. Also, some elephants die during the translocation process, probably due to the stress they are subjected to. Thus translocation is not a sustainable approach as the elephants that return home from the release site create conflicts, whereas the translocated animals may increase HEC in the release site. Translocation also has a challenge in that it is a tedious and expensive process.

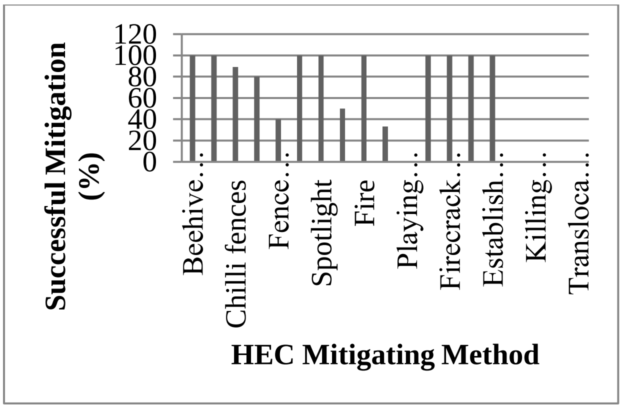

**Figure 3**. The number of studies reviewed that reported successful mitigation of Human Elephant

Conflict (HEC) for each mitigating method. An experiment is considered to have successful mitigation if it effectively scared or deterred elephants compared to the control.

The mitigation of HEC remains a major unresolved challenge for wildlife managers and stakeholders. Extensive field experiments have been carried out using different approaches to mitigate HEC. However, many of these approaches provide only temporal solutions and therefore become ineffective and unsustainable. Beehive fencing, electrical fencing, habitat manipulation and improvement could be considered the most effective and sustainable measures to mitigate HEC especially when well implemented, with adequate monitoring and maintenance carried out as the need arises.

## References

AERP HEC project 2006. Mitigating human-elephant conflict in the Amboseli ecosystem, Kenya, Summary testing of deterrents year 1.

Alex C., Nick S., John M., Julius K., Angela M., Lucas M., Humphrey P.N., Jonathan K., Raphael O., Aloyce M., Nathan H., Suzanne P., David O. 2016. Scaling-up the use of chili fences for reducing human-elephant conflict across landscapes in Tanzania. Tropical Conservation Science 9 (2): 921-930.

Alfred P.K., Curtice R.G., Lee P. 2010. Elephant use and conflict leads to Tanzania’s first wildlife conservation corridor. Pachyderm 48: 57–66.

Barnes R.F.W. 1996. The conflict between humans and elephants in the central African forests. Mammal Review 26 (2-3): 67–80.

Blair J.A.S., Noor N.M. 1981. Conflict between the Malaysian elephant and agriculture. In: Nordian, M., Latiff, A., Mahani, M.C.,Tan, S.C. (Eds.), Conservation Inputs from Life Sciences. University of Kebangsaan, Malaysia, pp. 109–133.

Caitlin E.O., Timothy R., Matthew R., Lynette A.H. 2000. Living with the modern conservation paradigm: can agricultural communities co-exist with elephants? A five-year case study in East Caprivi. Namibia. Biological Conservation 93 (3): 381-391.

Charles S. 1996. Mitigation of human-elephant conflicts in Sri Lanka. Gajah 15.

Chelliah K., Kannan G., Kundu S., Abilash N., Madhusudan A., Baskaran N., Sukumar R. 2010. Testing the efficacy of a chilli–tobacco rope fence as a deterrent against crop-raiding elephants. Current Science 99 (9): 1239-1243.

Chiemelu N. 2004. At Whose Expense? Policy Formation in Wildlife Conservation and Human Conflict in Kenya, Luce Undergraduate Environmental Fellows Program, Watson Institute for International Studies.

Colin B., Matthew G., Suzannah D., Eibleis F. 2009. The influence of electric fences on large mammal movements in the Kilombero Valley, Tanzania. African Journal of Ecology 48 (1): 280–284.

Dublin T., McShane T., Newby J. 1997. Conserving Africa`s elephants: current issues and priorities for action, WWF, Gland, Switzerland. 28 p.

Ekobo A. 1997. Elephant problem in the mungo division, littoral province (Cameroon). Pachyderm 24: 53–63.

Ferrel V.O. 2002. Capsicum oleoresin as an elephant repellent: field trials in the communal lands of Zimbabwe. Journal of Wildlife Management 66(3): 674–677.

Ferrel V.O., Guy E.P. 2002. Community-based methods to reduce crop loss to elephants: experiments in the communal lands of Zimbabwe. Pachyderm 33: 32-38.

Graham M.D., Ochieng T. 2008. Uptake and performance of farm based measures for reducing crop raiding by elephants

Loxodonta africana among small holder farms in Laikipia District, Kenya. Oryx 42 (1): 76–82.

Graham M.D., Gichohi N., Kamau F., Aike G., Craig B., Douglas-Hamilton I., Adams W. M. 2009. The Use of Electrified Fences to Reduce Human Elephant Conflict: A Case Study of the Ol Pejeta Conservancy, Laikipia District, Kenya, Working Paper 1, Laikipia Elephant Project, Nanyuki, Kenya.

Gunaratne L.H.P., Premarathne P.K. 2005. The effectiveness of electric fencing in mitigating human-elephant conflict in Sri Lanka, Research report; no. 2005-RR11, InternationalDevelopment Research Centre.

Hiten K.B., Soumen D., Anupam S., Amit S., Sanjay G., Tariq A., Dipankar G., Christy W.A. 2012. Use of Chilli Fences to Deter Asian Elephants - A Pilot Study. Gajah 36: 11-13.

Hoare R. 2001. Management implications of new research on problem elephants. Pachyderm 30: 44-48.

Ian W., Rudi A., Stuart L.P. 1998. Managing the elephants of Kruger National Park. Animal Conservation. 1 (2): 77–83.

Jackson T.P., Mosojane S., Ferreira S.M., Van Aarde R.J. 2008. Solution for elephant Loxodonta africana crop raiding in northern Botswana: moving away from symptomatic approaches. Oryx 42 (1): 83-91.

John H. 2006. Mitigation of human-elephant conflict in the Kavango-Zambezi Transfrontier Conservation Area through Community Based Problem Animal Control, with particular reference to the use of chilli peppers, Report prepared for Conservation International.

John K., Philip M., Patrick O., Patrick I.C. 2008. The performance of electric fences as elephant barriers in Amboseli, Kenya. South African Journal of Wildlife Research 38(1): 52–58.

Karidozo M., Osborn F.V. 2015. Community-based conflict mitigation trials: results of field tests of chili as elephant deterrent. Biodiversity and Endangered Species 3:144.

Li Z., Ning W. 2003. An initial study on habitat conservation of Asian elephant (*Elephas maximus*), with a focus on human elephant conflict in Simao, China. Biological Conservation 112 (3): 453–459.

Lucy E.K., Anna L., Iain D.H., Fritz V. 2009. Beehive fence deters crop-raiding elephants. African Journal of Ecology 47: 131–137.

Lucy E.K., Fredrick L., Hesron N., Emmanuel M., Iain D.H. 2017. Beehive fences as a multidimensional conflict-mitigation tool for farmers coexisting with elephants. Conservation Biology 31 (4): 743–752.

Lucy E.K., Iain D.H., Fritz V. 2007. African elephants run from the sound of disturbed bees. Current Biology 17 (19): 832-833.

Malima C., Hoare R., Blanc J. 2004. Systematic recording of human-elephant conflict: a case study in south-eastern Tanzania. Pachyderm 38: 29-38.

Mwakatobe A., Nyahongo J., Ntalwila J., Roskaft E. 2014. The impact of crop raiding by wild animalsin communities surrounding the Serengeti National Park, Tanzania. International Journal of Biodiversity and Conservation 6(9): 637-646.

Naughton-Treves L. 1998. Predicting patterns of crop damage by wildlife around Kibale National Park, Uganda. Conservation Biology 12 (1): 156-168.

Noa P.W. 2009. Spatial behaviour of translocated African elephants (Loxodonta africana) in a novel environment: using behaviour to inform conservation actions. Behaviour 146: 1171-1192.

Omondi P., Bitok E., Kagiri J. 2004. Managing human–elephant conflicts: the Kenyan experience. Pachyderm 36: 80–86.

Osborn F.V., Rasmussen L.E.L. 1995. Evidence for the effectiveness of an oleoresin

Capsicum aerosol as a repellant against wild elephants in Zimbabwe. Pachyderm 20: 55-64.

Osei-Owusu Y., Bakker L. 2008. Human-wildlife-conflict: elephant technical manual, Food and Agricultural Organization of the United Nations, Rome.

Parker G.E., Osborn F.V., Hoare R.E., Niskanen L.S. 2007. Human-elephant conflict mitigation: a training course for community-based approaches in Africa. Participant's manual, Elephant Pepper Development Trust, Livingstone, Zambia and IUCN/SSC AfESG, Nairobi, Kenya.

Sébastien L.B., Mike L.G., Nolwenn D. 2015. Repelling elephants with a chilli pepper gas dispenser: field tests and practical use in Mozambique, Zambia and Zimbabwe from 2009 to 2013. Pachyderm 56.

Sébastien L.B., Russell T., Mike L., Onias N., Marc B., Hillary M. 2010. An easy-to-use capsicum delivery system for crop-raiding elephants in Zimbabwe: preliminary results of a field test in Hwange National Park. Pachyderm 47: 80-89.

Simon H., Donny G. 2009. Reducing human–elephant conflict: do chillies help deter elephants from entering crop fields? Fauna and Flora International. Oryx 44(1): 139–146

Sitati N.W. 2003. Human-elephant conflict in the Masai Mara dispersal areas of Transmara district, PhD Thesis, University of Kent, Canterbury, UK.

Stephenson P.J. 2004. The future for elephants in Africa. Pp 133-136 in Burgess N., D'Amico Hales J., Underwood E., Dinerstein E., Olson D., Itoua I., Schipper J., Ricketts T., Newman K. (eds.), Terrestrial Ecoregions of Africa and Madagascar: A Conservation Assessment, Island Press, Washington DC, USA.

Stuart L.P., Rudi J.A. 2001. Population control: African elephants and contraception. Nature 411: 766.

Tammy E., Davies S.W., Nandita H., Joydeep C., Dhruba D., Dave J., Alexandra Z. 2011. Effectiveness of intervention methods against crop-raiding elephants. Conservation Letters 4 (5): 346–354.

Tchamb M.N. 1995. The problem elephants of Kaele: a challenge for elephant conservation in northern Cameroon. Pachyderm 19: 26–32.

Thouless, C.R. and Sakwa, J. 1995. Shocking elephants: fences and crop raiders in Laikipia District, Kenya. Biological Conservation 72 (1): 99-107.

Wahed M.A., Ullah M.R., Irfanullah H.M. 2016. Human-Elephant Conflict Mitigation Measures: Lessons from Bangladesh. IUCN, Bangladesh Country Office, Dhaka, Bangladesh. 30 pp.

Whyte I. 1993. The movement patterns of elephants in the Kruger National Park in response to culling and environmental stimuli. Pachyderm 16: 72-80. Wright S. 1943. Isolation by distance. Genetics 28(2): 114–138.

**Journal of Wildlife and Biodiversity 1(2): 79-87 (2017)**

**by Arak University, Iran (http://jwb.araku.ac.ir/)**

**DOI: 10.22120/jwb.2017.28261**

**Research Article**

# Re-evaluation of the phylogeny based on mitochondrial cytochrome *b* gene in the house shrew, *Suncus murinus-S. montanus* species complex, with special reference to Yemen and Myanmar populations

**Satoshi D. Ohdachi[1]*, Gohta Kinoshita[2], Abdul Karim Nasher[3], Takahiro Yonezawa[4], Satoru Arai[5], Fuka Kikuchi[5,6], Kyaw San Lin[7] and Saw Bawm[7]**

[1]*Institute of Low Temperature Science, Hokkaido University, Kita-ku, Sapporo 060-0819, Japan.e-mail: ohd@lowtem.hokudai.ac.jp
[2]Laboratory of Forest Biology, Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.
[3]UNEP-GEF/EPA/SGN–Socotra Program, Hadibo, Socotra Island, Rep. of Yemen.
[4]School of Life Sciences, Fudan University, SongHu Rd. 2005, Shanghai 200438, China.
[5]Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Shinjuku-ku 162-8640, Tokyo, Japan.
[6]Tokyo University of Science, Shinjuku-ku 162-8601, Tokyo, Japan.
[7]Department of Pharmacology and Parasitology, University of Veterinary Science, Yezin, Nay Pyi Taw 15013, Myanmar.

Received: 16 September 2017 / Revised: 19 October 2017 / Accepted: 20 October 2017 / Published online: 31 October 2017. Ministry of Sciences, Research and Technology, Arak University, Iran.

## Abstract

The house shrew (*Suncus murinus-S. montanus* species complex) is considered to have been unintentionally introduced by humans from their original range to other regions around the Indian Ocean and neighboring seas, but this has yet not fully been investigated. A phylogenetic tree and haplotype network were reconstructed based on the mitochondrial cytochrome *b* gene nucleotide sequences (1140 bp) of 179 individuals of house shrews from 46 localities in southern East Asia, Southeast Asia, West Asia, and islands in the western Indian Ocean. There was small genetic variation among shrews in Japan (Okinawa), southern China, Vietnam, and insular Southeast Asia. However, the shrew populations in Myanmar and Sri Lanka showed of a variety of different haplotypes. In the region of the western Indian Ocean, three interesting findings were obtained. First, the shrews on Zanzibar Island (Tanzania) shared same haplotype as those in southwestern Iran, and the haplotype was close to a group in Pakistan, despite these three regions being distantly located. Second, inferring from the haplotype network, it was suggests that the shrews in Yemen might have derived from Madagascar/Comoros populations. Third, the shrews on Réunion Island were genetically different from other populations around the western Indian Ocean but closer to Malaysia and Myanmar populations. Thus, the present study demonstrates that there have been dynamic immigration/emigration processes in the house shrews, especially for those around the western Indian Ocean. In addition, the house shrews in Myanmar may include several different species.

**Keywords:** Musk shrew, Indian Ocean, Human introduction, Immigrations, Phylogeny

## Introduction

The house shrew or musk shrew, *S. murinus* (Linnaeus, 1766) is widely distributed across southern East Asia, Southeast Asia, South Asia, West Asia, the coastal region of eastern Africa, and many islands in the Indian Ocean (Hutterer 2005, Motokawa 2015). House shrews were often unintentionally introduced by humans to non-native regions (Hutterer and Trainier 1990, Yamagata *et al.* 1995, Kurachi *et al.* 2007, Ohdachi *et al.* 2016). In addition, Ohdachi *et al.* (2016) revealed based on mitochondrial cytochrome *b* gene (cyt*b*) that it is difficult to separate *S. murinus* from *S. montanus* (Kelaart 1850), although *S. montanus* is regarded as an independent species in Sri Lanka (Phillips 1980, Meegaskumbura *et al.* 2010). Thus, "house shrew" refers to the "*Suncus murinus-S. montanus* species complex" in the present paper.

Intraspecific phylogeny of the *S. murinus-S. montanus* complex has been reported based on the mitochondrial cyt*b* sequences by Ohdachi *et al.* (2016), and unexpected distribution of haplogroups were found between distant regions. Since the previous report (Ohdachi *et al*. 2016), further samples of house shrews were obtained from Yemen and Myanmar. Hence, these individuals were added to the phylogenetic analysis, and the median-joining network was also drawn to extract more information from the sequence data. The main purpose of the present study is to reveal new insights into the phylogenetic relationships in the *S. murinus-S. montanus* complex and to infer the routes of human introduction.

## Material and methods

### Study area

Nucleotide sequences of the mitochondrial cytochrome *b* gene (1140 bp) for 10 individuals of the *S. murinus* from Yemen and Myanmar were obtained, following previous investigations (Ohdachi *et al.* 2004, Dubey *et al.* 2007, Arai *et al.* 2012). In addition to them, we cited the sequence data for 169 individuals of the *S. murinus-S. montanus* complex (including *S. murinus murinus*, *S. murinus kandianus*, *S. murinus caerulescens*, and *S. montanus*) from 44 localities (Ohdachi *et al.* 2006, Meegaskumbura et al. 2007, 2010, 2012, Lin *et al.* 2014, Ohdachi *et al.* 2016) to reconstruct a phylogenetic tree and a median-joining network. In total, 179 individuals from 46 localities were analyzed (Table S.1, Fig. 1). Two individuals of *Suncus etruscus* (Savi 1822) were used as outgroups.

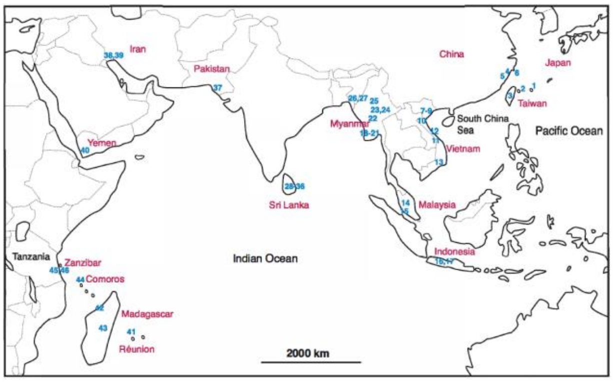

**Figure 1.** Sample localities of the house shrew (*Suncus murinus-S. montanus* species complex). The numbers correspond with those of Table S.1.

To infer the phylogenetic relationships among house shrews, the maximum likelihood (ML) tree was reconstructed using RAxMLver. 8.2.10 (Stamatakis 2014). The confidence of tree topology was evaluated by the rapid bootstrap method with 1,000 replications. A Bayesian (BI) phylogenetic tree was also reconstructed by MrBayes ver. 3.2 (Ronquist *et al.* 2012). The settings of the Metropolis Coupled Markov Chain Monte Carlo (MCMCMC) are as follows: 10,000 trees were samples for every 1,000 generations under two sets of the four simultaneous chains. The first 2,500 trees were discarded as the burn-in. Convergences of each parameter were confirmed by checking that all effective sample sizes (ESSs) were larger than 200 with Tracer 1.6 (http://tree.bio.ed.ac.uk/software/tracer/).

The dataset was analyzed for both ML and BI methods using GTR＋Γ model of nucleotide substitution, which was selected with the lowest Bayesian information criterion scores using a best fit model selection as implemented in MEGA version 5.05 (Tamura *et al.* 2011).

Taking account of the different tempos and modes of the nucleotide substitutions, the codon positions were distinguished by the partition model. The phylogenetic relationships among the three main groups in the ML tree obtained (see Fig. 2 in results) were further examined in detail by using the CODEML program implemented in the PAML ver. 4.9 (Yang 2007). For this analysis, the codon substitution (CS) + Γ model (Yang *et al.* 1998) was applied due to the better fitness of the CS + Γ model to our own sequence data than the standard nucleotide substitution model (e.g., GTR + Γ model): $AIC_{CS}+\Gamma = 8006.9$ and $AIC_{GTR}+\Gamma = 8107.0$. Because CS model is computationally too expensive, it is not practical to apply this model for a heuristic search. Therefore, the topologies within each of the three main groups were ready fixed in accordance with the result of the RAxML, and only three topologies among these three groups were examined. A median-joining network was reconstructed using the program Network ver. 5.0.0.1 (Bandelt *et al.* 1999) to infer evolutionary relationships of haplotypes and/or haplogroups.

## Results

Since the topologies of ML and BI trees were fundamentally the same, the BI tree was shown in Supplemental materials (Fig. S.2). The posterior probabilities of BI method were indicated near the corresponding nodes of the ML method (Fig. S.1). In the ML tree, three main groups (clades) were identified with high confidence values. The first was the “Suncus murinus” group, which included the majority of the house shrews. This group included two subspecies, *S. mu. murinus* and *S. mu. caerulescens* in Sri Lanka, whose subspecific ranks has been given in the previous studies (Meegaskumbura *et al.* 2007, Meegaskumbura *et al.* 2010). The second clade was the *Suncus montanus-S. murinus kandianus* species complex, which included *S. montanus, S. mu.* kandianus and the other house shrews (identified as *S. murinus*) in Kandy region. The third clade was the Nay Pyi Taw group, which consisted of two individuals from Nay Pyi Taw region, Myanmar (Loc. # 22, Fig.1). The “*Suncus murinus*” group was divided in several subgroups (Fig. S.1).

Among them, the Yemen and Madagascar and Comoros groups made a monophyletic group with high confidence (Fig. S.1). Shrews from Sri Lanka, Myanmar, and Pakistan appeared at several distant positions in the ML tree. On the contrary, shrews from Iran and Zanzibar (Tanzania) shared a single haplotype (Fig. S.1) despite distant sampling locations (Fig. 1). Shrews from Japan, China, Vietnam, and Indonesia (Java Island) formed a monophyletic subgroup. Those from central Myanmar and peninsular Malaysia formed another group, although it was statistically non-significant (Fig. S.1). Shrews from Réunion Island (an overseas department of France) in the western Indian Ocean (Loc. #41, Fig.1) were included in a monophyletic subgroup with those from East Asia, Southeast Asia, and Sri Lanka (Fig. S.1).

Among the three main groups of the ML tree (Fig. S.1), a detailed comparison was conducted between the three possible topologies of these groups (Table 1). The topology of [("*S. murinus*" group, *S. montanus-S. mu. kandianus* complex), Nay Pyi Taw group)] was selected as the most likely one, although the other two topologies could not be statistically rejected.

The branching pattern of the median-joining network (Fig. 2) was similar to the ML tree (Fig. S.1). The *Suncus montanus-S. mu. kandianus* complex and the Nay Pyi Taw group in the ML tree (Fig. S.1) were also clearly clustered in the network (Fig. 2). The network demonstrated that Yemen shrews were branched from Madagascar and Comoros shrews (Fig. 2). Shrews in Japan, China, and Indonesia were branched from the main

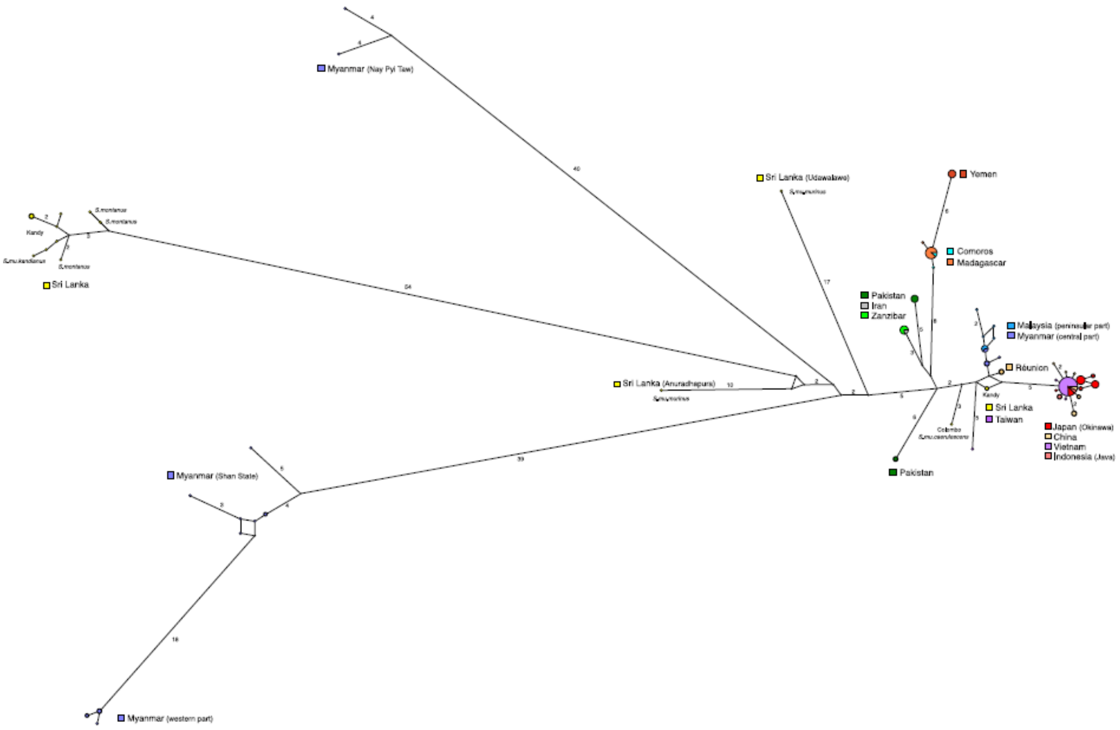

**Figure 2.** Median-joining network of the house shrews (*Suncus murinus*-*S. montanus* species complex), based on the mitochondrial cytochrome *b* gene sequences. Pie area approximately corresponds with the number of individual OTUs. Arabic numerals near branches denote the number of mutated sites. No number listed indicates a single mutation. Coloring is based on locality and is similar to those in Table S.1 and Fig S.1 A small open circle denotes a hypothetical taxonomic unit (HTU).

**Table 1.** Comparison of the tree topologies among three groups using the CS+Γ model. lnL, log-likelihood; Dli, difference of log-likelihood from the maximum likelihood tree (Tree 1), ±SE, standard deviation of the difference of log-likelihood from Tree 1; pKH, p-values of Kishino-Hasegawa test; pSH, p-values of Shimodaira-Hasegawa test; pRELL, bootstrap probabilities.

| tree | li | Dli | ±SE | pKH | pSH | pRELL |
|---|---|---|---|---|---|---|
| 1* | -3581.740 | 0.000 | 0.000 | -1.000 | -1.000 | 0.815 |
| 2 | -3584.148 | -2.403 | 2.616 | 0.179 | 0.172 | 0.110 |
| 3 | -3586.585 | -2.420 | 2.594 | 0.175 | 0.169 | 0.075 |

Tree1 = ( ("*S. murinus*" group, *S. montanus*-*S. mu. kandianus* complex), Nay Pyi Taw group)

Tree2 = ( (Nay Pyi Taw group, *S. montanus*-*S. mu. kandianus* complex), "*S. murinus*" group)

Tree3 = ( (Nay Pyi Taw group, "*S. murinus*" group), *S. montanus*-*S. mu. kandianus* complex)

See Fig. S.1 for the names of haplogroups.

haplotype that includes almost all individuals in Vietnam and some of the former regions with only a few mutations (1-3 sites) (Fig. 2). Shrews in peninsular Malaysia were branched from those in central Myanmar. In addition, the shrews of Réunion were closely located to those in central Myanmar and Malaysia (Fig. 2).

## Discussion

Ohdachi *et al.* (2016) suggested that house shrews in Japan (Okinawa), southern China and Indonesia (Java) were likely immigrated or emigrated by humans, but could not determine the direction of immigration (either from Vietnam to those regions or vice versa). In the present study, the median-joining network (Fig. 2) suggested that shrews of the former regions were probably immigrated from Vietnam since some individuals of those regions were located in peripheral positions around the main haplotype that includes most individuals from Vietnam and some other regions (Fig. 2), although we should carefully interpret the result of median-joining network for evolutionary event (Kong *et al.* 2016). From around the 10th-17th century, the sea trade between southern East Asia and Southeast Asia flourished and many Chinese and Japanese merchants formed settlements in Southeast Asia (e.g., Sakamaki 1964, Ogura 1989, Ikehata 1999, Ishii and Sakurai 1999, Schottenhammer 2007). Thus, it is thought that the house shrews moved with these trade ships.

Shrews of southwestern Iran and Zanzibar Island (Tanzania) had the same haplotype (Fig. S.1), indicating they moved between these two distant regions. Myths of Swahili people of maritime East Africa, including Zanzibar, tell that their ancestors immigrated from Persia (De Vere Allen 1993, Iejima 1993, Tominaga 2001). Therefore, house shrews may have been transported by Persians to Zanzibar. To verify this hypothesis, shrew samples from the eastern part of the Arabian Peninsula should be surveyed. The house shrews in Madagascar and Grande Comoro Island had very similar haplotype (Fig. S.1). Ancestors of the main ethnic groups in Madagascar are Austronesians originating from insular Southeast Asia that gradually mixed with Bantu peoples from East Africa (Iida *et al.* 2013, Pierron *et al.* 2017), while some Austronesians came to Madagascar via Comoros after the 6th century (Chagnoux and Haribou 1990). Therefore, it is plausible that the house shrews of Madagascar and Comoros might have immigrated with Austronesians or proto-Malagasies. However, the present study could not infer when and from where house shrews came to Madagascar and Comoros because the sampling are insufficient (Fig. S.1). In particular, samples from India were lacking. Thus, these are still open questions.

Shrews in the Yemen and Madagascar/Comoros subgroups are genetically close to each other (Fig. S.1). Furthermore, the median-joining network (Fig. 2) suggests that the house shrews in Yemen might have been derived from the population in Madagascar or Comoros, although the interpretation of median-joining network should be discreet (Kong *et al.* 2016). A similar genetic relationship between Yemen and Madagascar has been reported for the house mouse *Mus musculus* (Duplantier *et al.* 2002, Sakuma *et al.* 2016). However, in contrast to the house shrew, the direction of anthropological transportation of the house mouse is considered to have been from Yemen to Madagascar. In addition, Hadhramis, an ethnic group of Arabic people in southern Yemen, have undertaken commercial activity between Yemen and many regions around the Indian Ocean from East Africa including Madagascar to Southeast Asia (De Vere Allen 1993, Horton and Middleton 2001, Sherrif 2010, Alpers 2013). Other groups of Arab merchants have also been active in northern Madagascar (Vérin 1986). Thus, Hadhramis or other Arabs are likely to have transported the house shrew and the house mouse between Yemen and Madagascar/Comoros. Furthermore, the house shrew is called “voalavo arabo” in Malagasy language in Mahajanga, northwestern

Madagascar, which means “Arabian mouse” (Hideo Fukazawa, personal communication). This also suggests the transportation of house shrews by Hadhramis or Arab merchants between Yemen and Madagascar.

For the islands in the western Indian Ocean, the phylogenetic position of the shrews on Réunion Island (Fig. S.1) was obviously different from Madagascar, Comoros, Iran, Zanzibar subgroups, and the shrews of Réunion are phylogenetically close to those from central Myanmar and peninsular Malaysia (Fig. S.1 and 2). Many Chinese workers and merchants immigrated to Réunion from Malaya after the 19th century (Yu-Sion 2003, Alpers 2013, Kang 2015). Thus, house shrews might have immigrated to Réunion with Chinese immigrants from Malaya.

Finally, we mentioned the taxonomic status of the *S. murinus-S. montanus* complex. As clearly indicated in the phylogenetic analysis (Fig. S.1, Table 1), Nay Pyi Taw group has a unique phylogenetic position. In addition, *S. murinus kandianus* and some shrews from Kandy, Sri Lanka are included in a single clade (S. *montanus-S. mu. kandianus* species complex). If *S. montanus* is a valid species, *S. mu. kandianus* and some other shrews in Kandy should be included in it, and the Nay Pyi Taw group should be considered a new independent species. Otherwise, all of the *S. murinus-S. montanus* species complex should be referred to as a single species, *Suncus murinus*.

Thus, house shrews seemed to have been moved throughout maritime regions of the Indian Ocean and its neighboring seas by a variety of people and routes. Furthermore, an intensive re-examination of the taxonomic status of the *S. murinus-S. montanus* complex needs to be done. Additional sampling of the shrews, especially from India and eastern Arabian Peninsula, and analysis based on other sources of genetic information (especially nuclear genes) are necessary to develop the discussion of the present study.

## Acknowledgment

We would like to express our deep gratitude to the following persons for their support in discussion: Hideo Fukazawa, Keiya Hanabuchi, Keiko Takemura, and Hitoshi Suzuki. We also thank Yu Ikeyama and Keita Aoki for technical assistance. This work was partly supported by JSPS KAKENHI grants (22405037, 24405045, 25304009, and 26304035) and a grant-in-aid from the Research Program on Emerging and Re-emerging Infectious Diseases, Japan Agency for Medical Research and Development (AMED). We also appreciate Karli Lawson-Shimizu for proofing the English of an early version of the manuscript.

## References

Alpers E.A. 2013. The Indian Ocean in World History. Oxford: Oxford University Press, 192 p.

Arai S., Gu S.H., Baek L.J., Tabara K., Bennett S.N., Oh H.S., Takada N., Kang H.J., Tanaka-Taya K., Morikawa S., Okabe N., Yanagihara R., Song J.W. 2012. Divergent ancestral lineages of newfound hantaviruses harbored by phylogenetically related crocidurine shrew species in Korea. Virology 424(2): 99-105.

Bandelt H.J., Forster P., Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Phylogenetics and Evolution 16(1): 37-48.

Chagnoux H. and Haribou A. 1990. Les Comores. Paris, Presses Universitaires de France, 127 pp.

De Vere Allen J. 1993. Swahili Origins: Swahili Culture and the Shungwaya Phenomenon (Eastern African Studies). Melton, Suffolk: James Currey.

Dubey S., Salamin N., Ohdachi S.D., Barrière P., Vogel P. 2007. Molecular phylogenetics of Soricidae (Mammalia, Soricidae) reveals timing of transcontinental colonisations. Molecular Phylogenetics and Evolution 44(1): 126-137.

Duplantier J.M., Orth A., Catalan J., Bonhomme F. 2002. Evidence for a mitochondrial lineage originating from the Arabian peninsula in the Madagascar house mouse (*Mus musculus*), Heredity 89(2): 154-158.

Horton M., Middleton J. 2001. The Swahili: The Social Landscape of a Mercantile Society. Hoboken, Wiley-Blackwell, 292 p.

Hutterer R. 2005. Order Soricomorpha, In: Wilson D.E. and Reeder D.M. (Eds.) Mammal Species of theWorld.A Taxonomic and Geographic Reference. The Johns Hopkins University Press, Baltimore, 311 p.

Hutterer R., Trainier M. 1990. The immigration of the Asian house shrew (*Suncus murinus*) into Africa and Madagascar. In: Peters, G., Hutterer, R. (Eds.) Proceedings of the International Symposium on Vertebrate Biogeography and Systematics in the Tropics. Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, pp. 309-319.

Iejima H. 1993. The Civilization Created by Sea. The History of the World of the Indian Ocean. Tokyo, Asahi-Shinbun, 461 p. (in Japanese).

Iida T., Hideo F., Moriyama T. 2013. Sixty-two Chapters to Know Madagascar. Tokyo, Akashi Books, 352 p. (in Japanese).

Ikehata, S. 1999. The History of South East Asia. II (insular part). Tokyo, Yamakaka Publishing, 552 p. (in Japanese).

Ishii, Y., Sakurai, Y. 1999. The History of South East Asia. I. (continental part). Tokyo, Yamakaka Publishing. 628 p. (in Japanese).

Kang, X. 2015. Ethnic Chinese remigration from Southeast Asian countries since 1960s: a case study of Malaysian Chinese outflows. Contemporary Chinese Political Economy and Strategic Relations 1(2): 303-329.

Kong S., Sánchez-Pachecoa S.J., Murphy R.W. 2016. On the use of median-joining networks in evolutionary biology. Cladistics 32(6): 691-699.

Kurachi M., Chau B.L., Dang V.B., Dorji T., Yamamoto Y., Nyunt M.M., Maeda Y., Chhum-Phith L., Namikawa T., Yamagata T. 2007. Population structure of wild musk shrew (*Suncus murinus*) in Asia based on mitochondrial DNA variation, with research in Cambodia and Bhutan. Biochemical Genetics 45(3-4): 165-183.

Lin,X.D., Zhou R.H., Fan F.N., Ying X.H., Sun X.Y., Wang W., Holmes E.C., Zhang Y.Z. 2014. Biodiversity and evolution of Imjin virus and Thottapalayam virus in Crocidurinae shrews in Zhejiang Province, China. Virus Research 189: 114-20.

Meegaskumbura S., Meegaskumbura M., Schneider C.J. 2010. Systematic relationships and taxonomy of *S. montanu*s and *S. murinus* from Sri Lanka. Molecular Phylogenetics and Evolution 55(2): 473-87.

Meegaskumbura S., Meegaskumbura M., Schneider C.J. 2012. Re-evaluation of the taxonomy of the Sri Lankan pigmy shrew *S. fellowesgordoni* (Soricidae: Crocidurinae) and its phylogenetic relationship with *S. etruscus*. Zootaxta 3187: 57-68.

Meegaskumbura, S. H., Meegaskumbura, M. P. B., Pethiyagoda, R., Manamendra-Arachchi, K., Schneider, C. J. 2007. Crocidura hikmiya, a new shrew (Mammalia: Soricomorpha: Soricidae) from Sri Lanka. Zootaxa 1665: 19-30.

Motokawa M. 2015. *Suncus murinus* (Linnaeus, 1766). In: Ohdachi S.D., Ishibashi Y., Iwasa M.A., Fukui D., Saitoh T. (Eds.) The Wild Mammals of Japan. Second Edition. Shoukadoh, Kyoto, pp. 26-27.

Ogura S. 1989. The Japanese in the Red Seal Ships Trading Era. Tokyo, Chuko Shinsho, 219 p. (in Japanese).

Ohdachi S.D., Hasegawa M., Iwasa M.A., Abe H., Vogel P., Oshida T., Lin L.K. 2006. Molecular phylogenetics of soricid shrews (Mammalia) based on mitochondrial cytochrome *b* gene sequences: with special reference to the Soricinae. Journal of Zoology, London 270: 177-191.

Ohdachi S.D., Iwasa M.A., Nesterenko V.A., Abe H., Masuda R. and Haberl W. 2004. Molecular phylogenetics of Crocidura shrews (Insectivora) in East and Central Asia. Journal of Mammalogy 85(3):396-403.

Ohdachi S.D., Kinoshita G., Oda S., Motokawa M., Jogahara T., Arai S., Nguyen S.T., Suzuki H., Katakura K., Bawm S., Min M. Z., Thwe T.L., Gamage C.D., Hashim R., Omar H., Maryanto I., Ghadirian T., Ranorosoa M.C., Moribe J., Tsuchiya K. 2016. Intraspecific phylogeny of the house shrews, *Suncus murinus-S. montanus* species complex, based on the mitochondrial cyt *b* gene. Mammal Study 41(4): 229-238.

Phillips W.W. A. 1980. Manual of the Mammals of Sri Lanka. Part 1. 2nd edi. Unknown Binding, Wildlife and Nature Protection Society of Sri Lanka, 116 p.

Pierron D., Heiske M., Razafindrazaka H., Rakoto I., Rabetokotany N., Ravololomanga B., Rakotozafy L.M., Rakotomalala, M.M., Razafiarivony M., Rasoarifetra B., Raharijesy M.A., Razafindralambo L., Ramilisonina Fanony, F. Lejamble S., Thomas O., Mohamed A.A., Rocher C., Arachiche A., Tonaso L., Pereda-Loth V., Schiavinato S., Brucato N., Ricaut F.X., Kusuma P., Sudoyo H., Ni S., Boland A., Deleuze J.F., Beaujard P., Grange P., Adelaar S., Stoneking M., Rakotoarisoa J.A., Radimilahy C., Letellier T. 2017. Genomic landscape of human diversity across Madagascar. Proceedings of the National Academy of Sciences, USA: 114(32): 6498-6506.

Ronquist F., Teslenko M., Van Der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A., Huelsenbeck J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539-542.

Sakamaki S. 1964. Ryukyu and Southeast Asia. Journal of Asian Studies 23(3): 382-384.

Sakuma Y., Ranorosoa M.C., Kinoshita G., Shimoji H., Tsuchiya K., Ohdachi S.D., Arai S., Tanaka C., Ramino H., Suzuki H. 2016. Variation in the coat-color-controlling genes, *Mc1r* and *Asip*, in the house mouse *Mus musculus* from Madagascar. Mammal Study 41(3): 131–140.

Schottenhammer A. 2007. The East Asian maritime world 1400–1800: its fabrics of power and dynamics of exchanges. In: Schottenhammer, A. (Eds.), The East Asian Maritime World 1400–1800: its fabrics of power and dynamics of exchanges - China and Her Neibours. Harrassowitz Verlag, Wiesbaden, pp. 1-86.

Sherrif A. 2010. Dhow Cultures and the Indian Ocean: Cosmopolitanism, Commerce and Islam, Oxford University Press, 404 p.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312-1313.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance and Maximum Parsimony Methods. Molecular Biology and Evolution 28(10): 2731-2739.

Tominaga C. 2001. The Flute of Zanzibar. The History and Culture of Swahili and East African World. Tokyo, Miraisha, 238 p. (in Japanese).

Vérin P. 1986. The History of Civilisation in North Madagascar. Amsterdam, A.A. Balkema, 431 p.

Yamagata T., Ohishi K., Faruque M.O., Masangkay J.S., Ba-Loc C., Vu-Binh D., Mansjoer S.S., Ikeda H., Namikawa T. 1995. Genetic variation and geographic distribution on the mitochondrial DNA in local population of the musk shrew, *Suncus murinus*. Japanese Journal of Genetics 70(3): 321-337.

Yang Z. 2007. PAML4: a program package for phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution 24(8): 1586–1591.

Yang Z., Nielsen R., Hasegawa M. 1998. Models of amino acid substitution and applications to mitochondrial protein evolution. Molecular Biology and Evolution 15(12): 1600-1611.

Yu-Sion L. 2003. Illusion identitaire et métissageculturel chez les «Sinoi» de la Réunion. Perspectives chinoises 78: 1-13.

**Journal of Wildlife and Biodiversity 1(2): 88-93 (2017)**

**by Arak University, Iran (http://jwb.araku.ac.ir/)**

**DOI: 10.22120/jwb.2017.28295**

**Research Article**

# A survey on the current distribution and habitat suitability of the Great Bustard in West Azerbaijan, Iran

**Naser Ahmadi Sani**[1]

[1]Faculty of Agriculture and Natural Resources, Mahabad Branch, Islamic Azad University, Mahabad, Iran email: n.ahmadisani@gmail.com

Received: 20 September 2017 / Revised: 15 October 2017 / Accepted: 30 October 2017 / Published online: 06 November 2017. Ministry of Sciences, Research and Technology, Arak University, Iran.

## Abstract

Great Bustard (*Otis tarda*) is a vulnerable species is exposed to extinction risk mainly due to habitat destruction and hunting pressure. This study was conducted to assess the species' current geographical range and its habitat suitability in northwestern parts of Iran. Based on previous investigations and observations, I mapped current habitats of the species using GIS, and the important environmental variables affecting the species habitat usage were modeled by Ecological Niche Factor Analysis (ENFA). The results show that the species' geographical range is limited to the habitats of the area like Sutav, Yngijeh, Alblagh, and Qazelian Plains. ENFA analysis indicated that Great Bustard tends to live in marginal habitats and is a very sensitive species in the study area. The suitability map depicts that 6.5% of the study area is suitable for the species, however, this value is not consistent with the current habitats in terms of area and location.

**Keywords:** Habitat Assessment, GIS, *Otis tarda*, Remote Sensing.

## Introduction

Animal species are facing a serious threat due to climate change, population growth, illegal hunting, and habitat destruction (Pinto *et al.* 2005, Starkovich and Ntinou 2017).

Great Bustard (*O. tarda*) has been recently categorized as a vulnerable species on the IUCN Red List (BirdLife International 2016) while it can be regarded as a critically endangered species on a national scale (Naderi 2017). The distribution of O. tarda starts from Spain and extends to East Asia (Alonso *et al.* 2003). In the majority of Iranian reports, O. tarda has been categorized as a species posed to extinction risk. At present habitat loss due to agricultural intensification, very low abundance, leaving the nests by the females and predatory pressure is the most important cause in the declined *O. tarda* population (Pinto *et al.* 2005, Ambarli and Bilgin 2014, Naderi 2017).

Since habitat is one of the most important factors in species protection, identifying the habitat characteristics is inevitable for biodiversity conservation (Kneib *et al.* 2011, Corbane *et al.* 2015). The destruction of natural habitats due to human activities is one of the most important factors that threaten the survival of many species (Ansari 2017).

Information on the extent, distribution, and condition of natural habitats are essential for maintaining a favorable conservation status (Bell *et al.* 2015, Corbane *et al.* 2015). One of the major requirements to understand the ecology of species is the knowledge of habitat characteristics (Naderi *et al.* 2014). ENFA is one of the current methods for the assessment of habitat suitability (Engler *et al.* 2004, Rupprecht et al. 2011, Yesson *et al.* 2012). Habitat suitability modeling helps wildlife managers, with spending less time and cost, to identify threatening factors on habitat (Bahadori *et al.* 2010).

In this regard, Geographical Information System (GIS) and Remote Sensing (RS) techniques are combined with field works to map the spatial variables (Jacqain *et al.* 2005, Choi *et al.* 2011, Fattahi *et al.* 2014). RS provides opportunities for cost-effective, rapid, and repeatable habitat mapping (Nagendra et al. 2013, Bell *et al.* 2015). Therefore, it is necessary to assess the status and distribution of the species' habitats using new technologies for preserving this endangered bird.

## Material and methods

### Study Area

Bukan county, located in West Azerbaijan province in the northwest of Iran, has an area of 249,077 hectares and a population of 224,628 people (Iranian Census Report of 2011). The study area is mountainous and has a temperate climate with cold winters and hot summers. Annual rainfall in this area is about 500 mm. Figure 1 depicts different habitats of O. tarda and their position in the province and Iran.

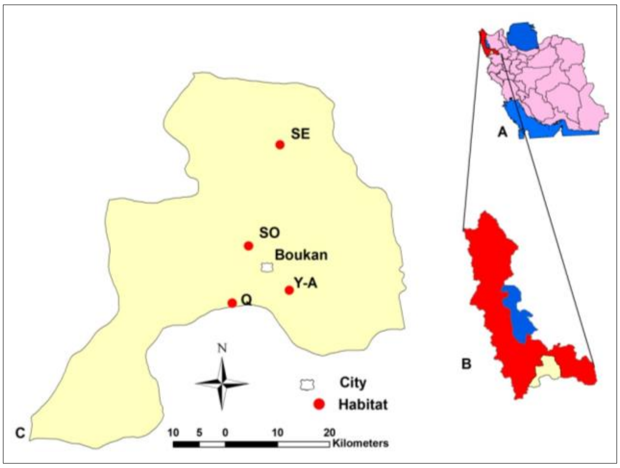

**Figure 1.** Different habitats and locations of Great Bustard in the study area (Sekanian, SO: Sutav, Y-A: Yngijeh-Alblagh, and Q: Qazlian)

As the first step, the needed data and maps were collected and the current habitats of the bird were mapped using the visual interpretation of satellite images analysis, and fieldwork by a GPS device. The area of current habitats was calculated in the GIS environment. ETM+ images of Sutav plain, as the main habitat in the study area, were analyzed for a better understanding of habitats distribution, particularly in relation to the natural, residential, and infrastructure areas such as land use/cover, roads, villages, and topography. Moreover, the images were used for mapping the sensitive areas. The biological aspects of the bird were assessed in different habitats and seasons by collecting data from previous studies, reports, and observations. In the second step, ENFA modeling approach was implemented to summarize all the environmental variables into a few uncorrelated and standardized factors. For this purpose, by studying the birds' behavior and literature review, the environmental factors effective on the distribution of the species were selected and then mapped using accessible data, GIS, and satellite data analysis. The county land-use map was produced using Landsat 8 images and the distribution map of the species was prepared using the presence point coordinates of the species presence recorded by GPS in the past years. To prepare the map of environmental variables, both types of distance and frequency calculation were used. The correlation between variables was calculated and, subsequently, the correlation matrix was obtained. The habitat suitability map was prepared and divided into desirable and undesirable classes, followed by comparing the maps of habitat suitability and habitat itself.

## Results

My data indicated that the habitat of *O. tarda* has been seriously reduced and limited to Bukan County, Iran (Fig. 2). The area of different habitats is given in Table 1. The total area of habitats is approximately 21365 ha, with Yngijeh-Alblagh as the largest habitat (Area = 9178 ha). The mainland use in this area is dry farming with wheat, peas, alfalfa, and sunflower as the main crops. This habitat has particular importance because it is a fall and winter habitat. The Sicanian as the second large habitat of the species in the study area at 7308 ha wide. The lands in this habitat are majorly cultivated with wheat, grain, pea, lentil, and alfalfa. Dry farming and traditional cultivation methods with a low human density increase the importance of this habitat. The Qazlian (with an area of about 1000 ha) and other seasonal habitats are located 4 km southwest of the border of the Simineh river.

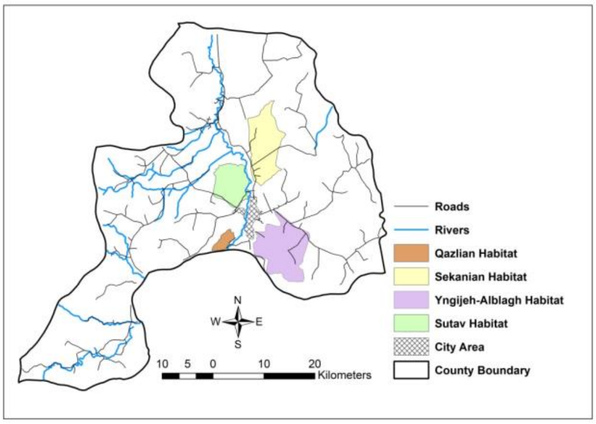

**Figure 2.** The map of roads, main rivers, current habitats, county, and city boundaries

The Qazlian is a winter habitat because of its lower elevation, being surrounded by mountains, the existence of plains and different farms, and a moderate temperature. Other low, seasonal, and immigrant lands with an area of about 4000 ha can be regarded as suitable habitats to species reproduction considering the area's topography, vegetation cover, and lands farmed with wheat and peas.

**Table 1.** Main presently occupied habitats by the Great Bustard and their area

| **Habitat** | **Area (ha)** | **Area (%)** |
|---|---:|---:|
| Sutav | 4879 | 18.5 |
| Sekanian | 7308 | 27.6 |
| Yngijeh-Alblagh | 9178 | 34.8 |
| Qazlian and others | 5000 | 19.1 |

About 65% and 32% of the area is located at elevations below 1600 and 1600-1900 m, respectively. Approximately, 62%, 27%, and 11% of the area is in the slope classes of less than 15%, 15-30%, and more than 30%, respectively. The northern and eastern slopes compared to the western and southern slopes occupy a greater area of the county. The number of villages is 173, the length of the road equals 570 km, and the city area is about 1,550 ha. According to the prepared land use map, the mainland uses of the study area are residential (4%), irrigating farming (7%), ranges (47%), and dry farming (42%).

**Habitat suitability**

The total marginality (1.28) showed that the bird tends to live in marginal habitats and choose higher environmental conditions than the average conditions in the study area. Low tolerability (0.1) and high specialization (9.2) in this study also indicate that *O. tarda* is a special species in the county (Fig. 3). In other words, this species has a low tolerance and a narrow ecological niche. In this study, according to the broken stick model, five factors explained 97% of the total species information.

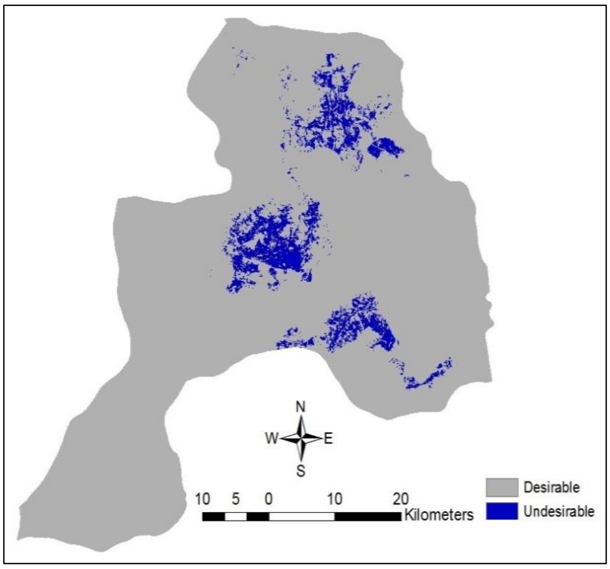

**Figure 3.** The species habitat suitability map

According to the suitability threshold of 35%, the map was divided into two desirable and undesirable classes including 93.5% and 6.5% of the county, respectively.

## Discussion

As emphasized in the Iran Great Bustard conservation action plan (Naderi 2017) the species abundance has been declined to lower than 30-40 individuals and it can be regarded as a critically endangered category on a national scale. The species is completely dependent to the farmlands with the cultivation of Poaceae and Fabaceae families. As mentioned in previous studies (Abdulkarimi and Ahmadi Sani 2012, Naderi 2017), the Sutav plain is the most important habitat of *O. tarda* in the northwest of Iran. Although Sutav Plain has the smallest area in this study, it was determined as the most important habitat because it is a productive habitat in the spring. Furthermore, despite the fact that Yngijeh-Alblagh's habitat is the greatest habitat with an area of 9178 ha, it is less important compared to other habitats.

The main cause of this phenomenon might be the destruction taken place in the past few years including the changes in land use due to its proximity to the city and a shift from traditional agriculture to mechanized one. Regarding the higher number of adult birds and reproduction, the Sutav Plain habitat is the most important habitat also in terms of birds' population (Abdulkarimi and Ahmadi 2012).

This study, in line with other works (Phua and Minowa 2005, Dong *et al.* 2013), showed the efficiency of GIS in the mapping of ecological resources and edition, management, processing, and analyzing large amounts of data in habitat assessment. As noted in some studies (Gibson 2003, Hirzel *et al.* 2006, Braunisch *et al.* 2008), ENFA analysis has the ability to compare the variables together in all presence points of species and present the best habitat for species as a map. According to previous studies (Pinto *et al.* 2005, Ambarli and Bilgin 2014), one of the major problems of *O. tarda* is a decline of its habitat area. Not paying attention to this problem in the near future would make the habitat of the species even smaller. The spatial and size incompatibility of the current habitats area with the suitability map indicates that the current habitats map was not properly surveyed. Moreover, for some reason, the birds are not safe and do not live in some areas, despite their ecological suitability. According to the results, the distribution of this species in these habitats has been wider in the past due to several reasons including hunting, climate and land-use changes, roads construction, developed residential and industrial areas, and increasing agricultural activities such as machinery traffic, plowing, irrigation, and excessive use of pesticides and herbicides.

This work shows the potential use of GIS and RS technologies to assess habitats' suitability and status in future works. Considering the increasing threats and population decline of O. tarda in Iran, it is necessary to employ new technologies in the evaluation of habitats, and also develop and protect of NGOs and local peoples to preserve the species. Moreover, it is suggested investigating habitat status and suitability in environmental studies and conservation processes for wildlife protection.

## References

Abdulkarimi R., Ahmadi Sani N. 2012. Warning to the Status of Critically Endangered Great Bustard. Journal of American Science 8 (1): 68-72.

Alonso J.C., Palacin C., Martin C.A. 2003. Status and recent trends of the Great Bustard (Otis tarda) population in the Iberian peninsula. Biological Conservation 110(2): 185-195.

Ambarli D., Bilgin C.C. 2014. Effects of landscape, land use and vegetation on bird community composition and diversity in Inner Anatolian steppes. Agriculture, Ecosystems and Environment 182: 37-46.

Ansari A. 2017. Habitat evaluation for Persian Gazelle in the Southern half of Markazi province, Iran. Journal of Wildlife and Biodiversity 1(1): 19-23.

Bahadori F., Alizadeh A., Kaboli M., Karami M., Atarod P., Mitra Sh. 2010. Habitat suitability modeling of *Sitta europaea* in northern Alborz. Journal of natural environment 63(3): 225-236.

Bell G., Neal S., Medcalf K. 2015. Use of remote sensing to produce a habitat map of Norfolk. Ecological Informatics 30: 293-299.

BirdLife International 2016. "*Otis tarda*". IUCN Red List of Threatened Species 2016.

Braunisch V., Bollmann K., Graf R.F., Hirzel A.H. 2008. Living on the edge- modelling habitat suitability for species at the edge of their fundamental niche. Ecological Modelling 214(2): 153-167.

Choi J.K., Oh H.J., Koo B.J., Ryu J.H., Lee S. 2011. Crustacean habitat potential mapping in a tidal flat using remote sensing and GIS. Ecological Modelling 222(8): 1522-1533.

Corbane C., Lang S., Pipkins K., Alleaume S., Deshayes M., Millan V.E.G., Strasser T., Borre J.V., Toon S., Michael F. 2015. Remote sensing for mapping natural habitats and their conservation status–New opportunities and challenges. International Journal of Applied Earth Observation and Geoinformation 37: 7-16.

Dong Z., Wang Z., Liu D., Li L., Ren C., Tang X., Jia M., Liu C. 2013. Assessment of habitat suitability for water birds in the West Songnen Plain, China, using remote sensing and GIS. Ecological Engineering 55:94-100.

Engler R., Guisan A., Rechsteiner L. 2004. An improved approach for predicting the distribution of rare and endangered species from occurrence and pseudo-absence data. Journal of Applied Ecology 41(2): 263-274.

Fattahi R., Ficetola G.F., Rastegar-Puyani N., Avci A., Kumlutas Y., Yousefkhani S.S. 2014. Modelling the potential distribution of the Bridled Skink, Trachylepis vittata (Olivier, 1804), in the Middle East. Zoology in the Middle East 60(3): 208-216.

Gibson L.A., Barbara A., Wilson D.M., Hill J. 2003. Modeling Habitat Suitability of the Swamp Antechinus (Antechinus minimus maritimus) in the costal heathlands of soutern Victoria. International Journal of biological Conservation 117(2): 143-150.

Hirzel A.H., Laya G.L., Helfera V., Guisana A. 2006. Evaluating habitat suitability models to predict species presences. Ecological Modeling 199(2): 142-152.

Jacqain A., Cheret V., Denux J.M., Mitcheley J., Xofis P. 2005. Habitat suitability modeling of capercaillie (*Tetrao urogallus*) using observation data. Journal of Nature Conservation 13(2): 161-169.

Kneib T., Knauer F., Kuchenhoff H. 2011. A general approach for the analysis of habitat selection. Environmental and Ecological Statistics 18(1): 1-25.

Naderi G.H., Mohammadi S., Imani A., Karami M. 2014. Habitat selection of Williams' Jerboa in Ardabil Province. Turkish Journal of Zoology 38: 432-436.

Naderi M. 2017. Great Bustard Action Plan, Iranian Department of Environment and (DOE) Arak University, 277 p.

Nagendra H., Lucas R., Honrado J.P., Jongman R.H., Tarantino C., Adamo M., Mairota, P. 2013. Remote sensing for conservation monitoring: Assessing protected areas, habitat extent, habitat condition, species diversity, and threats. Ecological Indicators 33: 45-59.

Phua M.H., Minowa M. 2005. A GIS-based multi-criteria decision making approach to forest conservation planning at a landscape scale: a case study in the Kinabalu Area, Sabah, Malaysia. Landscape and Urban Planning 71(2): 207–222.

Pinto M., Rocha P., Moreira F. 2005. Long-term trends in Great Bustard populations in Portugal suggest concentration in single high quality area. Biological Conservation 124(3): 415-423.

Rupprecht F., Oldeland J., Finckh M. 2011. Modelling potential distribution of the threatened tree species *J. oxycedrus*: how to evaluate the predictions of different modelling approaches? Journal of Vegetation Science 22(4): 647–659.

Starkovich B.M., Ntinou M. 2017. Climate change, human population growth, or both? Upper Paleolithic subsistence shifts in southern Greece. Quaternary International 428: 17-32.

Yesson C.H., Michelle L.T., Derek P.T., Andrew J.D., John G., Amy B., Julie B., Jason M.H., Alex D.R. 2012. Global habitat suitability of cold-water octocorals. Journal of Biogeography 39(7): 1278–1292.

**Journal of Wildlife and Biodiversity 1(2): 94-109 (2017)**

**by Arak University, Iran (http://jwb.araku.ac.ir/)**

**DOI: 10.22120/jwb.2017.28426**

**Research Article**

# Stress load in European ground squirrels living in habitats with high and low human impact

**Michaela Brenner, Tabea Turrini, Ilse E. Hoffmann*, Eva Millesi**

Department of Behavioural Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria. *email: ilse.hoffmann@univie.ac.at

Received: 2 September 2017 / Revised: 27 September 2017 / Accepted: 8 October 2017 / Published online: 11 November 2017. Ministry of Sciences, Research and Technology, Arak University, Iran.

## Abstract

Anthropogenic land use and its after-effects are potential sources of stress for European ground squirrel (*Spermophilus citellus*) populations, which increasingly have to cope with human impact throughout the species' range. To determine whether habitat alteration impacts the stress load of free-ranging populations in Austria, we live-trapped and faecal-sampled individuals both in a nearly unaltered steppe habitat (TD) and in a strongly altered alfalfa meadow (FB). Overall and seasonal faecal cortisol metabolite (FCM) concentrations were analysed and compared between the two study sites. FCM levels of adult males and of juveniles of both sexes were higher at FB than at TD. Adult females showed no such differences, but exhibited increased faecal progesterone metabolites (FPM) levels at both sites during June. Our results indicate that human activities affected stress load in adult males and juveniles. The altered vegetation led to highly abundant food at FB and, together with the isolation of the habitat, caused a high population density. This apparently intensified social stress in certain periods of the active season. Elevated FCM levels in both adult males and juveniles at FB coincided with the period of highest population density, when all juveniles had emerged from the natal burrows, and hibernation had not started yet. At the same time, predation pressure and human recreational activities also peaked. The highest FCM levels were found in juveniles at FB shortly after natal emergence, suggesting that this age class is most vulnerable to social stress, predation and human disturbance. The lack of a measurable stress response in adult females may be due to increased progesterone concentrations attenuating the stress-induced elevation of glucocorticoids.

**Keywords:** *Spermophilus citellus*, behavioural ecology, anthropogenic influence, faecal samples, corticosteroids, progesterone.

## Introduction

Human land use and the resulting alteration and fragmentation of natural habitat belong to the greatest threats to wildlife (e.g., Wilson 1985, Ehrlich and Wilson 1991, Soulé 1991, Dirzo *et al.* 2014). One example of a species increasingly under pressure from human impact is the European ground squirrel (*Spermophilus citellus*), a small diurnal ground-dwelling sciurid endemic to central and south-eastern Europe (Ružic 1978, Kryštufek 1999). Since the mid-20th century, the species has declined throughout its geographic range due to habitat alteration, fragmentation, and destruction caused by the spread of intensive agriculture and urbanisation (Smit and van Wijngaarden 1981). Meanwhile, *S. citellus* is listed as "Vulnerable" by the International Union for Conservation of Nsature and Natural Resources (Coroiu *et al.* 2008). In Austria, population relics occur both in the species' primary habitat (short-grass steppe and dry grassland; Kryštufek 1999, Spitzenberger and Bauer 2001) and in human-shaped landscapes (recreational sites, sports fields, runways, and other frequently mown lawns; Spitzenberger and Bauer 2001). Details on the species' life history appear elsewhere (Millesi et al. 1998, Huber *et al.* 1999, Millesi *et al.* 1999a, 1999b, Huber *et al.* 2001, Millesi *et al.* 2008a, 2008b, Strauss *et al.* 2009). Previous research has shown that European ground squirrels in human-altered habitats may achieve exceptionally high population densities, often exceeding those in unaltered habitats (Hoffmann *et al.* 2003a, 2003b). Anthropogenic land use seems to affect population dynamics, life-history traits, and movement behaviour of *S. citellus* (Hoffmann *et al.* 2003b, Hoffmann et al. 2008, Turrini *et al.* 2008).

To develop efficient conservation strategies, it is crucial to gain a better understanding of the physiological effects of human land use (Wikelski and Cooke 2006). Steroid hormones including glucocorticoids and gestagens are essential in regulating an animal's health and reproductive success, and glucocorticoid concentrations are considered to be reliable indicators of stress (Möstl and Palme 2002). High glucocorticoid levels triggered by prolonged stress affect fitness by decreasing memory and learning capacity as well as fertility and immune system (reviewed in Sapolsky *et al.* 2000). Thus, an elevated stress load of animals living in altered habitats may affect population survival. Progesterone, on the other hand, plays a key role in female reproduction by regulating ovulation, implantation, gestation, parturition, and lactation (Gellersen *et al.* 2009).

To determine possible effects on stress load, we compared glucocorticoid levels of two free-ranging *S. citellus* populations facing antipodal degrees of anthropogenic influence over the course of the active season. In addition, we analysed progesterone levels of adult and juvenile females, and discuss potential interactions with glucocorticoid levels, habitat alteration, and phases of the annual cycle.

## Material and methods

### Study area

This study was conducted during the active season from late March to mid-August 2008 on two free-living *S. citellus* populations in eastern Austria in frame of a research project. The four-year project (2006-2009) focused on five locally distinct ground squirrel habitats exposed to varying degrees of anthropogenic influence. Our criteria for a high degree of human impact included a strongly altered vegetation, substantial isolation, and frequent direct disturbance through human recreational or management activities. In contrast, low human impact was defined by a nearly natural vegetation, little isolation of the respective habitat patch, and hardly any direct human disturbance. For the purpose of this study, we selected the two habitats with the apparently highest and lowest human impact.

The strongly altered habitat patch, 'Falkenbergwiese' (hereafter FB), was an isolated 5-ha meadow in the north of Vienna (48°18'N, 16°22'E; elevation 318 m). The native dry grassland vegetation had been altered when alfalfa (*Medicago sativa*) was sowed several years before the study. The landscape adjacent to the study area consisted of a mixed oak forest (N), a huge conventionally cultivated vineyard (E), a transmitting station including buildings and transmission masts (S), and an intensively managed arable field (W). The study population was isolated from the next nearest ground-squirrel colony by the vineyard in the east. The study site belongs to an area of excursions, with daily presence of people running their dogs, hikers, bikers, and picnickers. Ground squirrels were sampled on a focal area of about 1 ha size.

The near-natural study site, 'Trausdorfer Hutweide' (hereafter TD) was a continuous steppe-like habitat measuring almost 100 ha, located west of lake Neusiedl (Burgenland; 47°48'N, 16°33'E; elevation 164m). In the decades preceding the study, the area had been used for grazing and had also served as a grassy airport. Since 1997, the site has been a protected area, with human activities limited to occasional sheep grazing and mowing it once annually. The surroundings of the study site consisted of vineyards and small fields with dust roads, constituting small-scale agricultural land use that provided connectivity to adjacent ground-squirrel populations. Except for occasional cars, equestrians and walkers, the area was undisturbed by humans. Ground squirrels were sampled on a focal area of approximately 7 ha.

## Live-trapping

Ground squirrels were captured by placing baited Tomahawk live-traps near burrows or by inserting tube traps into burrow entrances. Trapping was restricted to the animals' main activity period (10:00-15:30). Traps were observed continuously with binoculars, and a captured ground squirrel was immediately released into a funnel-shaped handling bag. Detailed descriptions of capture technique and protocol appear in Huber *et al.* (1999), Millesi *et al.* (1999b) and Hoffmann *et al.* (2008).

The following age classes were established based on weight and data from previous years: juveniles (born during the study period) and adults, comprising yearlings (born in the year before the study) and older individuals (born at least 2 years before the study). Reproductive condition of adult individuals was noted at each capture (males: testis position and scrotal pigmentation, females: condition of vulva and nipples). Body weight was measured to the nearest 1 g with a kitchen scale. Extent of moult was determined by plucking hairs. Animals were released at the locations of their capture immediately after the data recording procedure.

## Seasonal phases

Based on the seasonal activities of the different age and sex classes, we defined the following phases for each individual (means ±SE are given in parentheses).

*Adult males* (Fig. 1a)

Five seasonal phases were identified by documenting testis position, scrotal pigmentation, moult, weight and behaviour (Millesi *et al.* 1998, Strauss *et al.* 2007): (i) Mating (M; March 27 ± 0.7 d – April 17 ± 2.8 d) began with the vernal emergence of the first receptive females. Males' testes were descended and large, and the scrotum was darkly pigmented. One captured male had abdominal testes during this phase and hence was considered non-reproductive. The mating period lasted until all females were pregnant. (ii) Postmating 1 (PM1; April 18 ± 2.8 d – April 30 ± 0.7 d) was the period between the onset of testes regression until it was completed. (iii) Postmating 2 (PM2; May 01 ± 0.7 d – May 29 ± 9.9 d) included the first moult of the year and ended with the emergence of the first juveniles from the natal burrows. Although males' testes were abdominal, but they still had a pigmented scrotum. (iv) Postmating 3 (PM3; May 30 ± 9.9 d – June 25 ± 2.8 d) started when all litters had emerged aboveground and ended when the males' scrotum lost its pigmentation. (v) Prehibernation (PH; June 26 ± 2.8 d – August 13 ± 1.4 d) included the second moult and prehibernatory fattening.

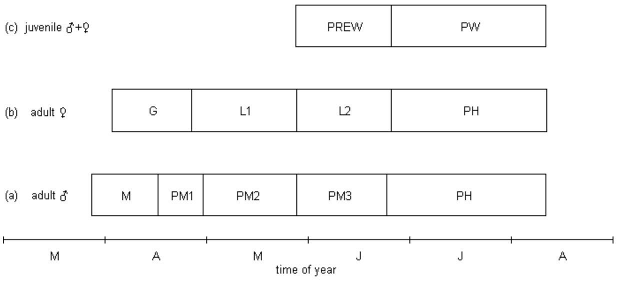

**Figure 1**. Timing of the seasonal phases in (a) adult males, (b) adult females, and (c) juveniles. Vertical lines indicate mean onset of each phase. See main text for phase definitions.

*Adult females* (Fig. 1b)

The season was divided into four phases identified by vulval and teat development as well as weight changes (Huber *et al.* 1999): (i) Gestation (G; April 2 ± 7.8 d – April 27 ± 3.5 d) was the phase in which females had a swollen and open vulva as well as darkly pigmented nipples. Females rapidly gained weight until a sudden loss of body weight, indicating parturition. (ii) Lactation 1 (L1; April 28 ± 3.5 d – May 29 ± 9.9 d) lasted from parturition until the emergence of a female's litter from the natal burrow. Lactating females showed enlarged and light-coloured teats. (iii) Lactation 2 (L2; May 30 ± 9.9 d – June 26 ± 2.8 d) started with the emergence of a female’s litter from the natal burrow and lasted until weaning. (iv) Prehibernation (PH; June 27 ± 2.8 d – August 13 ± 1.4 d) was the phase after weaning until hibernation. Teats were regressed and became dark. Prehibernation fattening occurred as reflected in a pronounced mass increase.

*Juveniles* (Fig. 1c)
Two phases (before and after weaning) were defined. As juveniles attain a threshold weight (females: 140 g, males: 146 g) before they are weaned (Hoffmann unpublished data), phases were distinguished based on body weight: (i) Preweaning (PREW; May 30 ± 1.4 d – June 26 ± 9.9 d) lasted from natal emergence when juveniles began to forage aboveground, but were still dependent on their mother. (ii) Postweaning (PW; June 27 ± 9.9 d – August 13 ± 1.4 d) was the phase after the juveniles had been weaned until the end of the study in mid-August. During this period the juveniles dispersed from their natal burrow to establish their own burrows for hibernation.

### Faecal sampling

Analyses of faecal cortisol metabolites (FCM) were used to assess glucocorticoid levels. Ground squirrels usually defecate at capture, thus, collection of faeces is a non-invasive sampling method avoiding effects of handling-related stress. Furthermore, cortisol metabolites in faeces are good indicators of circulating plasma cortisol concentrations (Mateo and Cavigelli 2005, Sheriff *et al.* 2010), and also represent pooled amounts of plasma concentrations over a certain period of time, providing an integrated measure of adrenocortical activity (Goymann *et al.* 1999). Ovarian activity in females was monitored by measuring faecal progesterone metabolites (FPM).

Fresh faeces were collected directly after excretion, immediately put in a cool box and subsequently stored at -20°C. Before analysis, samples were dried (60°C for 24 h), then pulverized. To extract metabolites from faeces, 0.1g of dry faeces was suspended in 80% methanol and then centrifuged. Hereafter, all faecal hormone metabolite concentrations are expressed in ng per g of dry weight, which has been shown to be the most robust measure with respect to dietary effects on excretion (Wasser *et al.* 1993).

### Cortisol assay

FCM levels were determined by an 11-oxoetiocholanolone-enzyme immunoassay (EIA), measuring 3alpha,11-oxo-cortisol metabolites. This EIA had been experimentally validated for *S. citellus*, revealing a delay time between hormone secretion and faecal excretion of 7.5 ± 2.5 h (mean ±SD; Strauss *et al.* 2007). FCM concentrations were assayed in duplicate. Intra- and inter-assay coefficients of variation were 14.79% and 16.73%, respectively.

### Progesterone assay

Progesterone metabolites were measured using a biotin-streptavidin EIA (Palme and Möstl 1994). The intra-assay coefficient of variation was 11.70%, the inter-assay coefficient 15.54%. For validation, we analysed progesterone concentrations in plasma and faecal samples taken from individual European ground squirrels at the same day and time in previous studies. Faecal progesterone concentrations in faeces and plasma were highly correlated ($r = 0.87$, $n = 65$, $p < 0.0001$; Strauss *et al.* unpublished data).

### Statistical analysis

Four data sets were established for statistical analyses: (1) adult males (response variable: FCM), (2) adult females (FCM, FPM), (3) juveniles (FCM), and (4) juvenile females (FPM). As Shapiro-Wilk tests revealed that data were not normally distributed, FCM and FPM values were transformed using logarithms and square roots, respectively. In each data set, variation of faecal steroid metabolites was analysed with linear mixed effects (LME) models with site, phase, and age (dataset 1 and 2), site, phase, and sex (dataset 3), and site and phase (dataset 4) as fixed main effects in the model. ID was entered as a random effect because we had repeated measures on the same individuals.

Akaike's information criterion (AIC) was used to choose the best models, starting with all main effects and all 2-way and 3-way interactions between main effects. Only the main effects and the 2-way interaction site × phase were included in the final models. Components were estimated with the restricted maximum likelihood model procedure. ANOVAs from LME models were computed using marginal (Type III) sums of squares.

In case of significant main effects with more than two levels, post-hoc pairwise comparisons were performed, using Bonferroni correction to make adjustments to the confidence interval. For paired comparisons for a significant site × phase interaction, a single factor was created from the interaction. This factor was tested by running a single factor ANOVA, using a LME model and Bonferroni post-hoc tests.

Hereafter, all data are expressed as back-transformed estimated marginal means +SE unless stated otherwise. All p-values are two tailed. The level of statistical significance was set at $\alpha = 0.05$. Data were analysed using SPSS software (SPSS for Windows, release 17.0, SPSS, Chicago, IL).

## Results

A total of 220 fecal samples from 109 individuals was collected throughout the study period (Table 1). Sex ratio was balanced among age classes at both study sites. At TD, more individuals were captured in total, but fewer were recaptured than at FB (Table 1).

### Adult males

Faecal cortisol metabolite levels were similar among yearling and older males, which hence where pooled for analyses. Adult males had significantly higher FCM levels at FB than at TD (Table 2, Fig. 2), and their FCM levels changed significantly in the course of the active season (Table 2, Fig. 3).

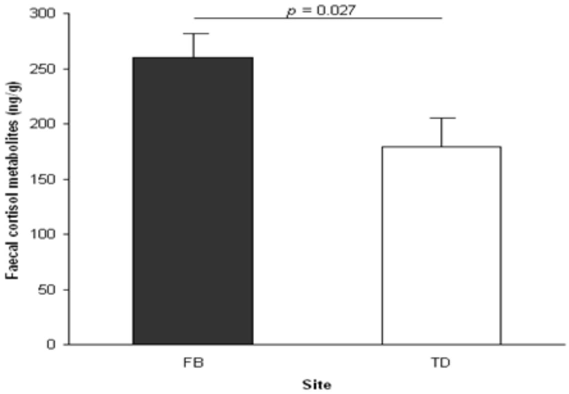

**Figure 2**. Levels of faecal cortisol metabolites in adult male *S. citellus* at the two study sites (FB: n = 15, TD: n = 18). Bars represent back-transformed estimated marginal means +SE; analysis was based on square-root transformed data.

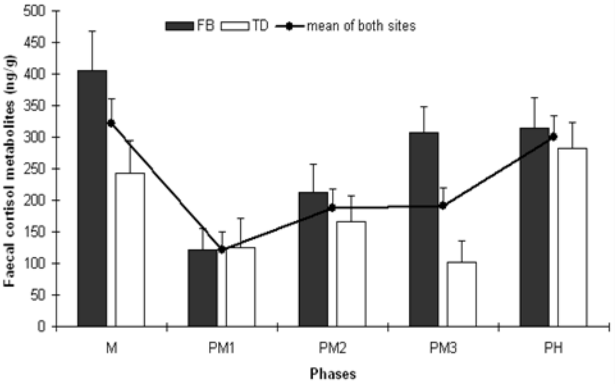

**Figure 3.** Faecal cortisol metabolite concentrations during the seasonal phases in adult male *S. citellus* at the two study sites (M: n = 4/6, PM1: n = 7/3, PM2: n = 6/6, PM3: n = 8/6, PH: n = 7/7; see main text for phase definitions). Filled circles indicate mean of both sites in the respective phase. Results are given as back-transformed estimated marginal means +SE; analysis was based on square-root transformed data. See Table 2 for ANOVA results and main text for post-hoc results.

Post-hoc tests revealed that FCM levels peaked in the mating period (320.66 + 40.87 ng/g), followed by a significant decrease in PM1 ($p<0.001$). Compared to mating, FCM levels remained low in PM2 ($p = 0.056$) and PM3 ($p = 0.042$) until PH, when they were significantly higher than in PM1 ($p < 0.001$), almost reaching values during mating.

Furthermore, there was a significant interaction between site and phase (Table 2, Fig. 3), indicating site-dependent seasonal patterns in FCM levels. Post-hoc analyses showed that within sites, compared with PM1 adult FB males exhibited elevated FCM concentrations in M ($p < 0.001$), in PM3 ($p = 0.011$), and in PH ($p = 0.024$), whereas FCM levels of adult TD males significantly increased from PM3 to PH ($p = 0.025$). Within phases, PM3 values of FB males were three times higher than those of TD males ($p = 0.012$).

## Adult females

**Table 1.** Cumulative number of *S. citellus* trapped and sampled at the two study sites (numbers of recaptured individuals in parentheses). Recapture rate refers to percentage of individuals recaptured at least once.

| | Adult | | Juvenile | | Total | Recapture rate (%) |
|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | | |
| FB (~1ha) | 15 (12) | 13 (8) | 8 (7) | 10 (8) | 46 (35) | 76 |
| TD (~7ha) | 18 (9) | 19 (7) | 14 (9) | 12 (6) | 63 (31) | 49 |

**Table 2.** Results of ANOVAs (Type III) from LME models examining effects of different predictor variables on variation in faecal cortisol metabolites in *S. citellus*, with ID as random variable for each data set.

| Data set | Fixed effects | | ANOVA |
|---|---|---|---|
| Adult males | Main effects | Site | $F_{1,29} = 5.41$, $p = 0.027$ |
| | | Phase | $F_{4,69} = 7.30$, $p < 0.001$ |
| | | Age | $F_{1,33} = 0.11$, $p = 0.743$ |
| | Interaction effect | Site × Phase | $F_{4,69} = 2.73$, $p = 0.036$ |
| Adult females | Main effects | Site | $F_{1,25} = 0.07$, $p = 0.793$ |
| | | Phase | $F_{3,48} = 0.18$, $p = 0.907$ |
| | | Age | $F_{1,22} = 0.07$, $p = 0.798$ |
| | Interaction effect | Site × Phase | $F_{3,48} = 0.32$, $p = 0.809$ |
| Juveniles | Main effects | Site | $F_{1,34} = 8.14$, $p = 0.007$ |
| | | Phase | $F_{1,74} = 4.25$, $p = 0.043$ |
| | | Sex | $F_{1,31} = 1.52$, $p = 0.227$ |
| | Interaction effect | Site × Phase | $F_{1,74} = 10.98$, $p = 0.001$ |

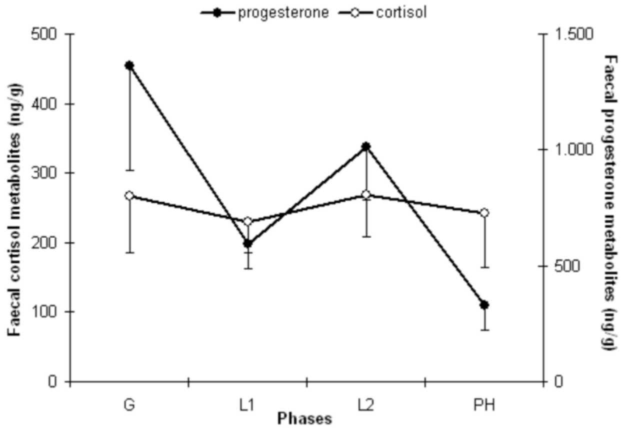

**Figure 4.** Faecal cortisol (open circles) and progesterone (closed circles) metabolite concentrations during gestation (G, n = 8), lactation 1 (L1, n = 16), lactation 2 (L2, n = 11), and prehibernation (PH, n = 7) in adult female *S. citellus*. Each data point represents the back-transformed estimated marginal mean -SE of the respective phase; analyses were based on log-transformed data. See Tables 2 and 3 for ANOVA results and main text for post-hoc results.

There was no difference in faecal cortisol metabolite levels between yearling and older females (Table 2). Faecal progesterone metabolite levels were only marginally, but not significantly higher in yearlings than in older individuals (Table 3), which hence were pooled for analyses. Neither site nor seasonal phases had significant effects on FCM levels (Table 2). While FPM levels were also similar between sites, they differed significantly among phases (Table 3, Fig. 4). Post-hoc tests showed elevated FPM concentrations both during G and L2 with significantly lower values during PH (Bonferroni pairwise comparisons: G vs. PH: p = 0.006; L2 vs. PH: p = 0.015).

## Juveniles

While there was no sex-related difference in faecal cortisol metabolite concentrations, juvenile FCM levels differed significantly between sites (Table 2) throughout the study period, with juveniles at FB having higher FCM concentrations than at TD. Furthermore, FCM values differed significantly among phases (Table 2), with elevated levels in preweaning compared to postweaning. The LME model also revealed a significant site × phase interaction (Table 2). Post-hoc tests performed on this interaction showed that FCM levels of FB juveniles in the preweaning phase were significantly higher than those of all other site × phase-categories (Bonferroni pairwise comparisons: FB PREW vs. TD PREW: p = 0.001; FB PREW vs. FB PW: p = 0.002; FB PREW vs. TD PW: p = 0.024; Fig. 5). Hence, the significant main effects of site and phase reflected elevated FCM levels of FB juveniles after natal emergence, whereas the levels after weaning were similar at both sites.

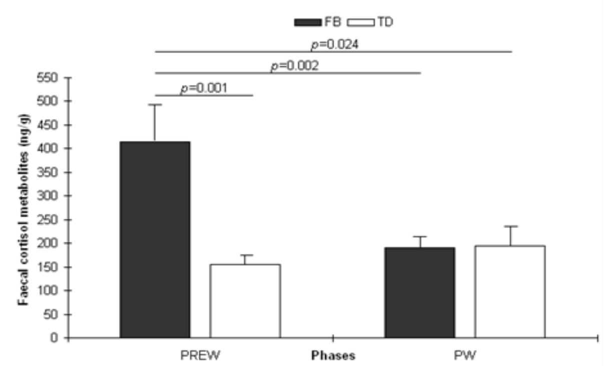

**Figure 5**. Faecal cortisol metabolites during preweaning (n = 8/15) and postweaning (n = 16/13) in juvenile *S. citellus* at the two study sites. Results are given as back-transformed estimated marginal means +SE; analysis was based on log-transformed data. P-values of post-hoc pairwise comparisons are given for each site × phase-category as compared to the FCM levels of FB juveniles during the preweaning phase.

**Table 3.** Results of ANOVAs (Type III) from LME models examining effects of different predictor variables on variation in faecal progesterone metabolites in female *S. citellus*, with ID used as random variable for each data set.

| Data set | Fixed effects | | ANOVA |
|---|---|---|---|
| Adult females | Main effects | Site | $F_{1,24}$ =1.03, $p$= 0.321 |
| | | Phase | $F_{3,48}$ = 5.49, $p$= 0.003 |
| | | Age | $F_{1,17}$ = 3.19, $p$= 0.092 |
| | Interaction effect | Site × Phase | $F_{3,48}$ = 1.00, $p$= 0.400 |
| Juvenile females | Main effects | Site | $F_{1,16}$ = 4.95, $p$= 0.041 |

| | | | |
|---|---|---|---|
| | | Phase | $F_{1,24} = 1.04$, $p=$ 0.317 |
| | Interaction effect | Site × Phase | $F_{1,24} = 0.74$, $p=$ 0.400 |

### Juvenile females

Faecal progesterone metabolites levels of juvenile females showed a site effect, with significantly higher levels at TD than at FB, but neither a phase nor an interaction effect (Table 3, Fig. 6). FPM levels at TD (144.54 + 35.11 ng/g) were almost twice as high as those at FB (75.34 + 16.71 ng/g).

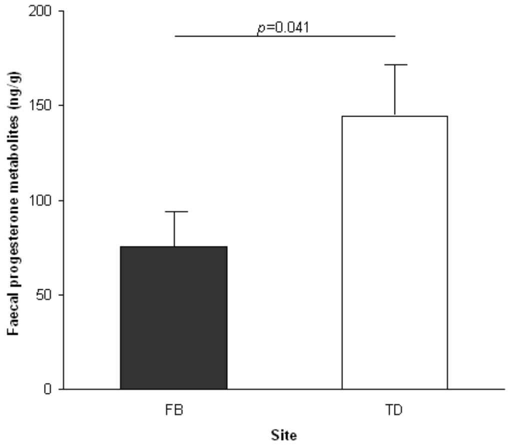

**Figure 6**. Levels of faecal progesterone metabolites in juvenile female European ground squirrels at the two study sites (FB: n = 10; TD: n = 11). Bars represent back-transformed estimated marginal means + SE; analysis was based on log-transformed data.

## Discussion

Significant site differences in faecal cortisol metabolites levels were found in adult males and in juveniles of both sexes, but not in adult females. Adult males and juveniles dwelling in the altered alfalfa meadow (FB) exhibited higher overall FCM concentrations and showed a more pronounced seasonal variation than those in the nearly natural steppe habitat (TD). In contrast, adult females had similar levels at both sites and throughout the season. Past field studies examining potential stress factors responsible for population differences in glucocorticoid levels in mammals focused on food availability (e.g., Foley *et al.* 2001, Behie *et al.* 2010), population density (e.g., Boonstra and Boag 1992, Rogovin *et al.* 2003), predation risk (e.g., Boonstra *et al.* 1998, Hik *et al.* 2001, Mateo 2007, Sheriff *et al.* 2011), and direct human disturbance (e.g., Creel *et al.* 2002, Barja *et al.* 2007). Given the high availability and abundance of alfalfa at FB throughout the active season, a lack of food (i.e., nutritional stress) was unlikely responsible for elevated FCM levels. Furthermore, another study on the same focal populations (Hoffmann *et al.* 2008) showed that both adult males and females at FB were heavier than those at TD, demonstrating that alfalfa is a nutritional food for ground squirrels (Yensen and Sherman 2003, Johnson-Nistler *et al.* 2005).

In contrast to their consexuals at TD, FB adult males showed maximum FCM concentrations during mating and a significant decrease in the subsequent PM1 phase. Mating is an energy-demanding and stressful period for reproductive males, associated with increased aggression and locomotion, resulting in a larger home range and overlap with other males, and body-mass loss (Millesi *et al.* 1998).

However, significantly elevated male glucocorticoid levels during this period have been reported only once so far, namely for *S. citellus* males at high population density (Millesi *et al.* 2004). Adult population density at FB was fivefold that of TD (43 vs. 9 individuals/ha; Hoffmann *et al.* 2008). The frequency of interactions increases with population density (Feldhamer *et al.* 1999), resulting in high glucocorticoid levels, as has been reported for several rodent species (e.g., great gerbils, Rhombomys opimus: Rogovin *et al.* 2003; meadow voles, Microtus pennsylvanicus: Boonstra and Boag 1992).

In *S. citellus*, high density during mating has been shown to increase male-male aggression and body-mass loss due to decreased foraging activity (Millesi *et al.* 2004). Thus, the higher population density at FB might have caused social stress and contributed to the increased FCM values during mating at this site. This interpretation is supported by observations of both more chases between adult males at FB than at TD and more injuries and scars in FB vs. TD males (personal observation). The high population density at FB could additionally have caused elevated FCM levels by intensifying locomotor activity in males while searching for receptive females. Indeed, despite the small habitat size of FB relative to TD, adult males in the former had home-range sizes similar to those in the latter (Turrini *et al.* 2008). As a consequence, male home ranges at FB overlapped extensively during the mating period, whereas they were more or less discrete at TD (Brenner *et al.* 2008).

At both sites, FCM levels of adult males were elevated (FB) or even peaked (TD) during prehibernation, as previously reported for *S. citellus* (Strauss *et al.* 2007). This is common in hibernating sciurids relying on fat stores (e.g., Cascade golden-mantled ground squirrel, *Callopermophilus saturatus*: Boswell et al. 1994, Golden-mantled ground squirrel, *C. lateralis*, and Belding's ground squirrel, *Urocitellus beldingi*: Nunes et al. 2006). Elevated glucocorticoid levels might reflect an endogenous seasonal change of adrenocortical activity in obligate hibernators preparing for hibernation. This elevation coincides with the onset of fattening in male *S. citellus* (Millesi et al. 1998), indicating that glucocorticoids might be crucial for accumulating lipid energy stores (King 1988). In adrenalectomised rats, fat intake and fat stores were diminished, but could be restored with corticosterone replacement (Castonguay et al. 1986); in adrenalectomised mice, corticosterone treatment even overcame inhibitory effects of leptin on food intake, body mass, and body fat (Solano and Jacobson 1999). An earlier onset of fattening of adult males at FB might have explained that their FCM values exceeded TD values threefold during PM3 in June, however, body mass data (Hoffmann unpublished) indicated no different timing. Rather, the peaking site difference during PM3 was attributable to litter emergence during this period, entailing an abrupt increase of population density.

Another cause for elevated FCM levels at FB during PM3 could have been an increased predation pressure. We noticed both a peak of attacks by common kestrels, *Falco tinnunculus* and an increasing frequency of human activities during this phase at FB, while no such changes were evident at TD (personal observations). Predation risk has frequently been linked to glucocorticoid levels of small mammals: Several studies have shown increased stress load in response to predator abundance, e.g, in snowshoe hares (*Lepus americanu*s, Boonstra *et al.* 1998, Sheriff *et al.* 2011) and Arctic ground squirrels (*Urocitellus parryii*, Hik *et al.* 2001). Nevertheless, this is not always the case (e.g., Belding's ground squirrels, *U. beldingi*: Mateo 2007). Human presence can be considered "equivalent to a form of predation risk" (Hofer and East 1998) and may therefore have a similar effect as predator abundance. During the study, FB proved to be a disturbed recreational area with frequent human presence whereas at TD, walkers passed by only occasionally (personal observations). The more the season progressed, the more human activities occurred at FB.

Adult males attack juveniles, and these interactions lead to elevated juvenile FCM levels (Strauss *et al.* unpublished). Vice versa, adult males probably also show increased adrenocortical activity in response to agonistic interactions with juveniles. Frequent juvenile-adult male encounters may have been inevitable at FB because of spatial constraints resulting from the small habitat size, whereas they were able to avoid each other at TD. In conclusion, the seasonal FCM peak in adult males at both sites seems mainly attributable to changes of adrenocortical activity related to fattening and peaking population densities. Social stress at the already densely populated FB due to juvenile emergence, together with increased predation risk and human disturbance, may have contributed to the site difference in FCM concentrations of adult males.

FCM levels were similar in male and female juveniles, indicating that sex had no influence on their glucocorticoid levels. Juveniles at FB exhibited peak FCM concentrations after natal emergence, i.e., the same timespan as adult males, exceeding the level of TD juveniles almost threefold. Besides the density-dependent social stress outlined above, the increased predation risk at FB in June most likely caused high juvenile FCM levels at this time. At FB, kestrels preyed upon small juveniles almost every day, whereas not a single kill by raptors (mainly Marsh harriers, *Circus aeruginosus*) was observed in TD (personal observations). Although the individual risk of being attacked is low in dense populations, predation attempts on nearby conspecifics are stressful events potentially increasing adrenocortical activity. Social stress, together with frequent human and dog approaches, may modify behaviour, which is in line with the fact that juvenile home ranges at FB were smaller than those at TD (Turrini *et al.* 2008). This might reflect reduced foraging distances to avoid both predator attacks and encounters with adult males and humans. The significant drop of juvenile FCM levels at FB after weaning may be explained by reduced population density (juvenile dispersal/mortality and onset of hibernation in adult females) together with diminished predation risk (sufficient juvenile size to preclude kestrel predation).

Since fattening in adult males seems to be triggered by high cortisol levels (see above), juvenile fat deposition may also depend on elevated glucocorticoids. As data collection was completed in mid-August, it is not surprising that we found no rise in FCM levels: Juveniles still invested in structural growth and not in prehibernatory fattening.

Despite apparent seasonal and site differences in population density, predator abundance and human disturbance, FCM of adult females were virtually not affected by these factors. Hence, they either did not perceive these factors as stressful or were more resistant to external stressors than adult males and juveniles. However, it cannot be excluded that large individual variation may have concealed significant differences.

As adult females are rarely involved in agonistic interactions (Strauss *et al.* unpublished), they might be less stressed by social factors. A further, not mutually exclusive, explanation would be a resilience to environmental stressors that may have evolved to ensure reproductive success (Wingfield and Sapolsky 2003). All adult females in this study were reproductively active. *S. citellus* females reproduce only once a year and bear the costs of parental effort, the latter peaking during lactation. It is therefore possible that breeding females attenuate endocrine responses that could interfere with successfully producing and rearing a litter. Consistently, captive lactating female Columbian ground squirrels (*Urocitellus columbianus*) that were exposed to a dog showed glucocorticoid concentrations lower than non-lactating females and similar to those without a stressor (Hubbs *et al.* 2000). Also, nonbreeding female Belding’s ground squirrels, *U. beldingi*, had higher glucocorticoid levels than their breeding consexuals throughout the season (Nunes *et al.* 2006). Contradictorily, elevated glucocorticoid levels have been reported for pregnant *U. beldingi* (Nunes *et al.* 2006) and lactating yellow-pined chipmunks (*Tamias amoenus*, Kenagy and Place 2000). Those studies, however, assessed total glucocorticoid concentrations in blood samples instead of FCM, and hence are of limited comparability: High total plasma concentrations may reflect high glucocorticoid-binding globulin (CBG) levels. During gestation and lactation, CBG of female rodents is usually elevated (Rosenthal *et al.* 1969a, 1969b, McDonald *et al.* 1988, Boonstra and Boag 1992, Boonstra *et al.* 2001), keeping free glucocorticoids low. As analyses from faecal samples mirror free glucocorticoid levels in plasma (Sheriff *et al.* 2010), the statistically insignificant variation of female FCM levels in our study might be due to increased CBG levels during breeding, buffering potential changes in cortisol secretion. Moreover, elevated CBG levels could be the mechanism underlying the resilience of reproductive females to external stressors. Further research is needed to clarify the role of CBG in female *S. citellus*.

Given that in both focal populations all adult females were reproductive and had similar FCM levels, it was not surprising that their faecal progesterone metabolites levels did not differ between sites either.

We did find, however, a significant variation of FPM among phases, with high levels during gestation, temporarily declining thereafter, peaking again before weaning, and finally dropping to minimum values during prehibernation. A similar pattern was found in plasma progesterone concentrations of breeding *S. citellus* females in semi-natural enclosures (Millesi *et al.* 2008a). Previous research has revealed that the second progesterone peak in *S. citellus* is caused by a non-reproductive oestrus cycle; the active luteal phase during this cycle may play a role in the fattening process of females prior to hibernation (Millesi *et al.* 2008a, 2008b). Our study supports these results. As discussed above, glucocorticoids seem to be essential for fat storage in adult males, but not in adult females. In mature female rats, progesterone treatment likely triggers altered food intake (Wade and Gray 1979), and therewith causes body weight gain due to increased fat deposition (Galletti and Klopper 1964). Accordingly, the elevation of progesterone rather than cortisol may be the initial signal for prehibernatory fattening in adult female European ground squirrels. Furthermore, the second FPM peak of adult females (between natal emergence and weaning) coincided with elevated FCM levels in adult males and juveniles in June. As experimental studies on rats showed that progesterone and its metabolite allopregnanolone attenuate the stress-induced elevation of corticosterone (Patchev *et al.* 1996), high FPM values could also explain why adult females at FB did not exhibit a likewise significant increase of FCM levels during this obviously stressful period.

Interestingly, juvenile females at TD had significantly higher FPM levels than those at FB. Combined with the lower FCM levels of TD vs. FB juveniles, this suggests that progesterone secretion in juvenile females at FB may be negatively linked to their elevated stress hormone concentrations. In non-pregnant females, progesterone is produced mainly in the ovaries by the corpus luteum and by developing follicles, and to a lesser extent in the adrenal cortex (Goodman 2009). Juvenile female *S. citellus* lack active corpora lutea prior to hibernation, but their ovaries contain secondary and tertiary follicles; the latter are fewer, but similar in size to those of adult females (Millesi *et al.* 2008b). Due to an expression of LH-receptors, granulosa cells of this follicle stage can already secrete progesterone, which has an autocrine function in further follicular development (Goodman 2009). In vitro, granulosa cells of corticosterone-treated rats showed both decreased basal progesterone secretion and reduced response to administered LH compared to those of control rats (Valli *et al.* 2000). Hence, high FCM levels at FB might have reduced progesterone release by developing follicles, potentially delaying follicle maturation. Unfortunately, no data are available on future reproductive output of juvenile females, thus evidence for this hypothesis cannot be provided.

In summary, the results of the present study suggest that anthropogenic land use raises stress load of European ground squirrels, particularly in certain periods of the annual cycle. Human interventions probably influenced stress load directly by disturbing animals and indirectly by affecting food availability and population demography. The high population density at FB may have resulted from food abundance and inhibited emigration, which in turn were consequences of human activities. Social stress elicited by the high population density in the altered habitat clearly peaked after juvenile emergence, when predation risk and direct human disturbance were also high. Further studies are needed to disentangle the different potential stressors for European ground squirrels and quantify their relative importance. Nevertheless, these stressors did not seem to affect FCM levels of adult females, either because they did not perceive them as stressors or because different physiological pathways were activated. This is supported by the fact that elevated progesterone levels in adult females coincided with elevated glucocorticoid levels in the other age and sex classes. Further investigations should assess whether the higher stress load in the human-altered habitat impacts fitness parameters such as survival by suppressing the immune system and reproductive success by interfering with reproductive function.

In conclusion, our results highlight that one way in which intensive human land use can affect small mammal populations is via mediating changes in their physiology. Therefore it is important to consider physiological mechanisms during decision-making processes in conservation.

## Acknowledgment

This study was funded by the Austrian Science Fund (FWF P18108). We thank Anna Schöbitz for her assistance in the hormone analyses.

### References

Barja I. Silván G. Rosellini S. Piñeiro A. González-Gil A. Camacho L., Illera J.C. 2007. Stress physiological responses to tourist pressure in a wild population of European pine marten. The Journal of Steroid Biochemistry and Molecular Biology 104(3-5):136–142.

Behie A.M., Pavelka M.S.M., Chapman C.A. 2010. Sources of variation in fecal cortisol levels in howler monkeys in Belize. American Journal of Primatology 72(7):600–606.

Boonstra R., Boag P.T. 1992. Spring declines in *Microtus pennsylvanicus* and the role of steroid hormones. Journal of Animal Ecology 61(2):339–352.

Boonstra R., Hik D.S., Singleton G.R., Tinnikov A. 1998. The impact of predator-induced stress on the snowshoe hare cycle. Ecological Monographs 79(5):371–394.

Boonstra R., Hubbs A.H., Lacey E.A., McColl C.J. 2001. Seasonal changes in glucocorticoid and testosterone concentrations in free-living Arctic ground squirrels from the boreal forest of the Yukon. Canadian Journal of Zoology 79(1):49–58.

Boswell T., Woods S.C., Kenagy G.J. 1994. Seasonal changes in body mass, insulin, and glucocorticoids of free-living golden-mantled ground squirrels. General and Comparative Endocrinology 96(3):339–346.

Brenner M., Turrini T., Hoffmann I.E. 2008. Does home-range overlap in the European ground squirrel increase with anthropogenic alteration? in: Book of Abstracts, II. European Ground Squirrel Meeting, Svaty Jan pod Skalou, Czech Republic, p. 15.

Castonguay T.W., Dallman M.F., Stern J.S. 1986. Some metabolic and behavioral effects of adrenalectomy on obese Zucker rats. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 251(5):923–933.

Coroiu C., Kryštufek B., Vohralík V., Zagorodnyuk I. 2008. *Spermophilus citellus*. The IUCN Red List of Threatened Species 2008: e.T20472A9204055. http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T20472A9204055.en. Downloaded on 11 September 2017.

Creel S., Fox J.E., Hardy A., Sands J., Garrott B., Peterson R.O. 2002. Snowmobile activity and glucocorticoid stress responses in wolves and elk. Conservation Biology 16(3):809–814.

Dirzo R., Young H. S., Galetti M., Ceballos G., Isaac N. J. B., Collen B. 2014. Defaunation in the Anthropocene. Science 345(6195):401–406.

Ehrlich P.R., Wilson E.O. 1991. Biodiversity studies: science and policy. Science 253(5021):758–762.

Feldhamer G.A., Drickamer L.C., Vessey S.H., Merritt J.F. 1999. Populations and life history, in: Feldhamer G.A., Drickamer L.C., Vessey S.H., Merritt J.F. (Eds.), Mammalogy: Adaptation, Diversity, and Ecology. WCB/McGraw-Hill, Boston, pp. 400–416.

Foley C.A.H., Papageorge S., Wasser S.K. 2001. Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. Conservation Biology 15(4):1134–1142.

Galletti F., Klopper A. 1964. The effect of progesterone on the quantity and distribution of body fat in the female rat. Acta Endocrinologica 46(3):379–386.

Gellersen B., Fernandes M.S., Brosens J.J. 2009. Non-genomic progesterone actions in female reproduction. Human Reproduction Update 15(1):119-138.

Goodman H.M. 2009. Basic Medical Endocrinology. London, Great Britain: Academic Press.

Goymann W., Möstl E., Van't Hof T., East M.L., Hofer H. 1999. Noninvasive fecal monitoring of glucocorticoids in spotted hyenas, *Crocuta crocuta*. General and Comparative Endocrinology 114(3):340–348.

Hik D.S., McColl C.J., Boonstra R. 2001. Why are Arctic ground squirrels more stressed in the boreal forest than in alpine meadows? ÉcoSCIENCE 8(3):275–288.

Hofer H., East M.L. 1998. Biological conservation and stress, in: Møller A.P., Milinski. M., Slater P.J.B. (Eds.), Advances in the Study of Behavior Volume 27. Academic Press, San Diego, pp. 405–525.

Hoffmann I.E., Millesi E., Huber S., Everts L.G., Dittami J.P. 2003a. Population dynamics of European ground squirrels (*Spermophilus citellus*) in a suburban area. Journal of Mammalogy 84:615–626.

Hoffmann I.E., Millesi E., Pieta K., Dittami J.P. 2003b. Anthropogenic effects on the population ecology of European ground squirrels (*Spermophilus citellus*) at the periphery of their geographic range. Mammalian Biology - Zeitschrift fur Saugetierkunde 68(2):205–213.

Hoffmann I.E., Turrini T., Brenner M. 2008. Do European ground squirrels (*Spermophilus citellus*) in Austria adjust their life history to anthropogenic influence? Lynx 39(2):241–250.

Hubbs A.H., Millar J.S., Wiebe J.P. 2000. Effect of brief exposure to a potential predator on cortisol concentrations in female Columbian ground squirrels (*Spermophilus columbianus*). Canadian Journal of Zoology 78(4):578–587.

Huber S., Millesi E., Walzl M., Dittami J., Arnold W. 1999. Reproductive effort and costs of reproduction in female European ground squirrels. Oecologia 121(1):19–24.

Huber S., Hoffmann I.E., Millesi E., Dittami J., Arnold W. 2001. Explaining the seasonal decline in litter size in European ground squirrels. Ecography 24(2):205–211.

Johnson-Nistler C.M., Knight J.E., Cash S.D. 2005. Considerations related to Richardson's ground squirrel (*Spermophilus richardsonii*) control in Montana. Agronomy Journal 97(5):1460–1464.

Kenagy G.J., Place N.J. 2000. Seasonal changes in plasma glucocorticosteroids of free-living female yellow-pine chipmunks: Effects of reproduction and capture and handling. General and Comparative Endocrinology 117(2):189–199.

King B.M. 1988. Glucocorticoids and hypothalamic obesity. Neuroscience & Biobehavioral Reviews 12(1):29–37.

Kryštufek B. 1999. *Spermophilus citellus* (Linnaeus, 1766), in: Mitchell-Jones A.J., Amori G., Bogdanowicz W., Kryštufek B., Rreijnders P.J.H., Spitzenberger F., Stubbe M., Thissen J.B.M., Vohralik V., Zima J. (Eds.), The Atlas of European Mammals. London, Academic Press

Mateo J.M., Cavigelli S.A. 2005. A validation of extraction methods for noninvasive sampling of glucocorticoids in free-living ground squirrels. Physiological and Biochemical Zoology 78(6):1069–1084.

Mateo J.M. 2007. Ecological and hormonal correlates of antipredator behavior in adult Belding’s ground squirrels (*Spermophilus beldingi*). Behavioral Ecology and Sociobiology 62(1):37–49.

McDonald I.R., Lee A.K., Than K.A., Martin R.W. 1988. Concentration of free glucocorticoids in plasma and mortality in the Australian bush rat (*Rattus fuscipes* Waterhouse). Journal of Mammalogy 69(4):740–748.

Millesi E., Huber S., Dittami J.P., Hoffmann I.E., Daan S. 1998. Parameters of mating effort and success in male European ground squirrels, *Spermophilus citellus*. Ethology 104(4):298–313.

Millesi E., Huber S., Everts L.G., Dittami J.P. 1999a. Reproductive decisions in female European ground squirrels: Factors affecting reproductive output and maternal investment. Ethology 105(2):163–175.

Millesi E., Strijkstra A.M., Hoffmann I.E., Dittami J.P., Daan S. 1999b. Sex and age differences in mass, morphology, and annual cycle in European ground squirrels, *Spermophilus citellus*. Journal of Mammalogy 80(1):218–231.

Millesi E., Hoffmann I.E., Huber S. 2004. Reproductive strategies of male European sousliks (*Spermophilus citellus*) at high and low population density. Lutra 47(2):75–84.

Millesi E., Divjak A., Strauss A. 2008a. Seasonal timing and pre-hibernation fattening in breeding and non-breeding European ground squirrels, in: Lovegrove B.G., McKechnie A.E. (Eds.), Hypometabolism in Animals: Torpor, Hibernation and Cryobiology. Interpak Books, Pietermaritzburg, pp. 291–296.

Millesi E., Strauss A., Burger T., Hoffmann I.E., Walzl, M. 2008b. Follicular development in European ground squirrels (*Spermophilus citellus*) in different phases of the annual cycle. Reproduction 136(2):205–210.

Möstl E., Palme R. 2002. Hormones as indicators of stress. Domestic animal endocrinology 23(1-2):67–74.

Nunes S., Pelz K.M., Muecke E., Holekamp K.E., Zucker I. 2006. Plasma glucocorticoid concentrations and body mass in ground squirrels: Seasonal variation and circannual organization. General and Comparative Endocrinology 146(2):136–143.

Palme R., Möstl E. 1994. Biotin-streptavidin enzyme immunoassay for the determination of oestrogens and androgens in boar feces, in: Gorog S. (Ed.), Advances in Steriod Analyses ’93: Proceedings from the 5th Symposium on the Analysis of Steroids Szombathely, Hungary, May 3-5, 1993, Szombathely, pp.111–117.

Patchev V.K., Hassan A.H.S., Holsboer F., Almeida O.F.X. 1996. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. Neuropsychopharmacology 15(6):533–540.

Rogovin Ko., Randall J.A., Kolosova I., Moshkin M. 2003. Social correlates of stress in adult males of the great gerbil, *Rhombomys opimus*, in years of high and low population densities. Hormones and Behavior 43(1):132–139.

Rosenthal H.E., Slaunwhite W.R., Sandberg A.A. 1969a. Transcortin: A corticosteroid-binding protein of plasma. X. Cortisol and progesterone interplay and unbound levels of these steroids in pregnancy. Journal of Clinical Endocrinology and Metabolism 29(3):352–367.

Rosenthal H.E., Slaunwhite W.R., Sandberg A.A. 1969b. Transcortin: A corticosteroid-binding protein of plasma. XI. Effects of estrogens or pregnancy in guinea pigs. Endocrinology 85(4):825–830.

Ružic A. 1978. Der oder das Europäische Ziesel (*Citellus citellus*) in: Niethammer J., Krapp F. (Eds.), Handbuch der Säugetiere Europas Band 1/I, Akademische Verlagsgesellschaft, Wiesbaden, pp. 123–144.

Sapolsky R.M., Romero L.M., Munck A.U. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21(1):55–89.

Sheriff M.J., Krebs C.J., Boonstra R. 2010. Assessing stress in animal populations: Do fecal and plasma glucocorticoids tell the same story? General and Comparative Endocrinology 166(3):614–619.

Sheriff M.J., Krebs C.J., Boonstra R. 2011. From process to pattern: how fluctuating predation risk impacts the stress axis of snowshoe hares during the 10-year cycle. Oecologia 166(3):1–13.

Smit C.J., van Wijngaarden A. 1981. Threatened Mammals in Europe. Wiesbaden, Germany: Akademische Verlagsgesellschaft.

Solano J.M., Jacobson L. 1999.Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA. American Journal of Physiology - Endocrinology and Metabolism 277(4): E708–E716.

Soulé M.E. 1991. Conservation: Tactics for a constant crisis. Science 253(5021):744–750.

Spitzenberger F., Bauer K. 2001. Ziesel *Spermophilus citellus* (Linnaeus, 1766), in: Spitzenberger F. (Ed.), Die Säugetierfauna Österreichs, Grüne Reihe des Bundesministeriums für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, Vienna, pp. 356–365.

Strauss A., Mascher E., Palme R., Millesi E. 2007. Sexually mature and immature yearling male European ground squirrels: A comparison of behavioral and physiological parameters. Hormones and Behavior 52(5):646–652.

Strauss A., Hoffmann I.E., Walzl M., Millesi E. 2009. Vaginal oestrus during the reproductive and non-reproductive period in European ground squirrels. Animal Reproduction Science 112(3-4):362–370.

Turrini T., Brenner M., Hoffmann I.E., Millesi E. 2008. Home ranges of European ground squirrels (*Spermophilus citellus*) in two habitats exposed to different degrees of human impact. Lynx 39(2):323–332.

Valli G., Sudha S., Ravi Sankar B., Govindarajulu P., Srinivasan N. 2000. Altered corticosterone status impairs steroidogenesis in the granulosa and thecal cells of Wistar rats. The Journal of Steroid Biochemistry and Molecular Biology 73(3-4):153–158.

Wade G.N., Gray J.M. 1979. Gonadal effects on food intake and adiposity: A metabolic hypothesis. Physiology and Behavior 22(3):583–593.

Wasser S.K., Thomas R., Nair P.P., Guidry C., Southers J., Lucas J., Wildt D.E., Monfort S.L. 1993. Effects of dietary fibre on faecal steroid measurements in baboons (*Papio cynocephalus cynocephalus*). Journal of Reproduction and Fertility 97(2):569–574.

Wikelski M., Cooke S.J. 2006. Conservation physiology. Trends in Ecology and Evolution 21:38–46.

Wilson E.O. 1985. The biological diversity crisis. BioScience 35:700–706.

Wingfield J.C., Sapolsky R.M. 2003. Reproduction and resistance to stress: When and how. Journal of Neuroendocrinology 15(8):711–724.

Yensen E., Sherman P.W. 2003. Ground squirrels, in: Feldhamer G.A., Thompson B.C., Chapman A.S. (Eds.), Wild Mammals of North America: Biology, Management, and Conservation. Johns Hopkins University Press, Baltimore, pp. 211–231.

**Journal of Wildlife and Biodiversity 1(2): 110-117 (2017)**

**by Arak University, Iran (http://jwb.araku.ac.ir/)**

**DOI: 10.22120/jwb.2017.28427**

**Research Article**

# Climate change impact on *Olneya tesota* A. Gray (Ironwood) distribution in Sonoran desert using MaxEnt Modeling approach

**Ahmad Moghaddam-Gheshlagh[1]*, Sergio Hernández-Verdugo[1], Edgar Omar Rueda-Puente[2], Jesús Soria-Ruiz[3], Saúl Parra-Terrazas[1], Antonio Pacheco-Olvera[1], Nastaran Mafakheri[4]**

[1]*Agronomy Faculty, Autonomous University of Sinaloa, Sinaloa, Mexico.
email: est.ahmad.moghaddam@uas.edu.mx
[2]Agriculture and Livestock Department (DAG), University of Sonora, Bahía Kino Highway Km 18.5. 83000 Hermosillo, Sonora, México.
[3]Geomatics Lab., National Institute of Research for Forestry Agricultural and Livestock (INIFAP). Zinacantepec 51350, Mexico.
[4]Dep. of Environmental Sciences, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran.

Received: 2 September 2017 / Revised: 27 September 2017 / Accepted: 8 October 2017 / Published online: 12 November 2017. Ministry of Sciences, Research and Technology, Arak University, Iran.

## Abstract

Based on different climatic scenarios, the distribution of the *Olneya tesota* A. Gray (Ironwood) has been modeled using the MaxEnt modeling approach in the Sonora State of Mexico. Maximum Entropy Species Distribution Modeling was used to predict distribution probability. 71,168 presence data and BIO1 to BIO19 variables of Worldclim BIOCLIM dataset for the present time, 2050 and 2070 used for modeling. The model was performed with an acceptable range of sensitivity for training data (AUC=0.927) and random prediction (AUC=0.5). The results demonstrated that the high contributed variable on the presence of the *O. tesota* A. Gray is BIO17 Precipitation of Driest Quarter (48.3%) and the low contributed variable is BIO2=Mean Diurnal Range (Mean of monthly (max temp - min temp)) (0.9%). This means that the presence of the species is highly dependent on dry months precipitation which doesn't have high fluctuations according to the used climate change scenario. Temperature fluctuations have not affected *O. tesota* A. Gray presence as it is known as a resistant species for extremely high temperatures. Therefore the probability of the presence of the species shows a significant increase on high altitudes mountains on the north-east of the Sonora state. Finally, the study concludes that climate change will affect the distribution of the *O. tesota* A. Gray as an extinction risk and the same time will help the expansion of the species presence probability on the region. And it has been encountered new regions to recommend this valuable species as a reforestation alternative for conservation and management strategy like Soyopa, Aguaprieta, and Sahuaripa municipalities among the others.

**Keywords:** Ironwood, Distribution, Climate change, Sonoran desert, Mexico.

## Introduction

Extinction and invasion risks of species is an important issue on natural resources conservation and management all over the world (Walker 2014). Climate change's impact on natural resources is an important driver of extinction and invasion risk and these kinds of studies are highly recommended to formulate more efficient bio-informatics conservation and management strategies (Peterson *et al*. 2015). Many types of research are indicating that the extinction risk is rising because of climate change impact (Thomas *et al*. 2004; Pearson *et al*. 2014) and anthropogenic activities unsustainable increasing (Leão *et al*. 2014). The global average of the extinction ratio of the species that mainly caused by climate change impact globally is around 1% of the species per year, and many important scientific journals papers like Nature Journal mention the extinction rate of the species will increase to more than 10% per year on 2050, and some of the calculating for Mexico biodiversity it will be more than the global average and even 35% of biodiversity on 2050 will extinct (Thomas *et al*. 2004; Peterson *et al*. 2015). For Mexican deserts, this problem is more visible because of agricultural expansion and other inadequate use of water resources among the others. Therefore, it is crucial to put special emphasis on desert keystone species.

For various reasons, even in developed countries, identification, sampling, and real mapping of the natural resources still remain a difficult issue for each species (Del Barrio *et al.* 2006) on actual time and their changes with the currently high climate changes fluctuations and anthropogenic activities impact on the populations and distributions of the species (Peterson *et al.* 2015). Unfortunately, Mexico as a mega-biodiverse country with more than 40% territory superficies’ as arid and semiarid zones with continuous increasing of desertification and deforestation (Vázquez-Méndez *et al.* 2008) and categorized with a high rate of desertification on international scientific communities. On the other hand, these areas’ key species’ have special importance on ecology, ecosystem, and human life (Medeiros and Drezner 2012). There are few studies about the Mexico flora species distribution with an evaluation of climate change impact on their distribution and almost there is no study about climate change impact on the distribution of desert species like O. *tesota* A. Gray (Del-Val *et al*. 2015).

Species Distribution Modeling (SDM) models are one of the useful instruments to study niche modeling, extinction and invasion risk, micro and macrohabitat suitability, and climate change impact on their distribution among the others (Araújo *et al*. 2005, Phillips *et al*. 2006). There are different models developing day by day in this area of science with using different algorithms such as BIOMAPPER (ecological niche factor analysis), GARP (genetic algorithm), GRASP (generalized linear model), SPECIES (Artificial neural network) (Austin 2007, Pearson *et al.* 2011) and many others. One of the most common presence data-only used is MaxEnt (Maximum Entropy) model (Phillip *et al.* 2004, Khanum *et al*. 2013) that has shown good results on distribution prediction and model evaluation (Matyukhina *et al*. 2015, Elia *et al.* 2015). Worldclim BioClimatic database is well-known to use as a principal database as environmental layers inputs on SDM modeling. As coming in this paper the world climate provided data from the global climate change modeling scenarios are also common for future distribution modeling of the species. And also it is proved that using the collection of different species sampling geographical coordination is also applicable for modeling for the enriching of the observational database for any SDM modeling like MaxEnt.

O. tesota A. Gray (Ironwood) is one of the high value and key species s as a principal pattern distributed on Sonoran desert ecosystem (Shupe 2005) which illegally cut by people for different uses such as handicrafts and coal production among the other purposes (Zuñiga and Suzán 2010). This species is an endemic one that is classified as being in extinction risk treat and encountered under the special protection of NOM. 056. 2010 of Mexico and it is recommended numerous times to conservation, rehabilitation, and as a reforestation alternative on the Sonoran desert (Verónica and Humberto 2014). Due to the high costs and risks of reforestation and rehabilitation on desert regions, generating useful and applicable knowledge on the local scale about the future suitable environment for its presence is necessitated as a management strategy planning (Medeiros and Drezner 2012). Unfortunately, there are only a few studies about *O. tesota* A. Gray and other desert species' actual distribution and suitable environmental conditions considering climate change impact to know future distribution (Zuñiga and Suzán 2010). Therefore, in this study, it is hypothesized that climate change or global warming is a strong threat of the extinction of *O. tesota* A. Gray on Sonoran desert. Therefore they are many questions about *O. tesota* A. Gray species that may respond by MaxEnt modeling. Our study focused on finding some confinable responses for following questions:

1-How is the actual probable presence distribution of the species on the Sonora State of Mexico according to all historical presence data of it on Sonora State? 2) How will affect global warming (climate change) in 2050 and 2070 on the distribution of the species? or whether climate change is an extinction risk treat for it? or will affect the suitability of environmental conditions for it in the future at Sonora State? 3) Which environmental factors are more important in the actual and future distribution of the species at Sonora State? And why? 4) What kinds of recommendations may give to conservation and management strategy for this species on Sonora State? Where are locally recommended reforestation or conservation areas?

Therefore, the objective of this study was to estimate actual and future probable distribution macrohabitat of *O. tesota* A. Gray (Ironwood) on the Sonora State of Mexico, using MaxEnt conventional model to study the climate change impact on its distribution to discuss efficiently conservation and management strategies of the species. The hypothesis of the study was that climate change has no significant impact on the same level of anthropogenic activities impact. Finally, it also discusses the extinction and invasion risks by regions and possible future immigration of the species by climate change and changes in its habitat suitability.

## Material and methods

### Study area

Sonora State located in the northwest of Mexico is the principal part of the global habitat of the *O. tesota* A. Gray species with 179,355 km2 area considered as an area of study. This region has an elevation from 0 m to 2,620 m of the elevation expanded from Baja California Gulf to Sierra Madre Occidental Mountains that includes 3 types of climate Variety to consider as the principal demonstrative habitat of the *O. tesota* A. Gray (Fig. 1).

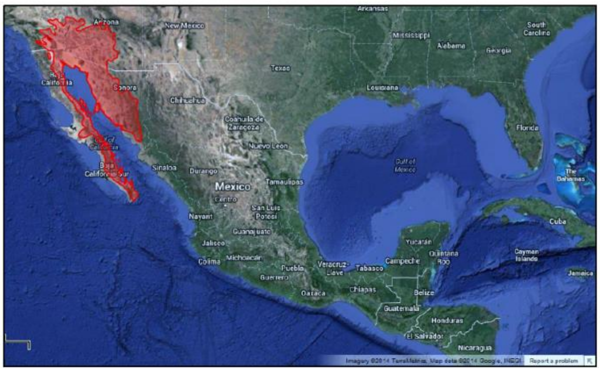

**Figure 1.** Delimitation of distribution of the species (http://globalspecies.org/kmlserver/getkml/*Olneya_tesota*/range).

The strongly altered habitat patch, 'Falkenbergwiese' (hereafter FB), was an isolated 5-ha meadow in the north of Vienna (48°18'N, 16°22'E; elevation 318 m). The native dry grassland vegetation had been altered when alfalfa (*Medicago sativa*) was sowed several years before the study. The landscape adjacent to the study area consisted of a mixed oak forest (N), a huge conventionally cultivated vineyard (E), a transmitting station including buildings and transmission masts (S), and an intensively managed arable field (W). The study population was isolated from the next nearest ground-squirrel colony by the vineyard in the east. The study site belongs to an area of excursions, with the daily presence of people running their dogs, hikers, bikers, and picnickers. Ground squirrels were sampled on a focal area of about 1 ha size.

### Presence data of species

Different sources of observation data from various projects as described in Table 1 has been used. A total of 71,161 observation data of presence only for *O. tesota* A. Gray and other species have been extracted from INIFAP

(Mexican National Institute of Research for Forestry Agricultural and Livestock), CONAFOR (Mexican National Commission Forestry), CONABIO (Mexican National Biodiversity Commission) and field sampling converted in .csv format to use in MaxEnt Modeling for the Sonora State of Mexico. The observation data have a different types of sampling methods with the different G.P.S instruments but with spatial resolution error of about 5 m, and according to environmental variables created a database 200 m×200 m resolution each pixel, the error margin was acceptable. The sampling methods for different resources were different, for example, the INIFAP database was sampled by 32 systematic stratified circle polygons per site as coming on Fig. 2.

**Table 1.** Presence-absence observation data's of *O. tesota* A. Gray on the Sonora State of Mexico.

| No. | Number and details of the sampling | Project name |
|---|---|---|
| 1 | 8,411 observation data of 18 species s on north-west of Mexico; | Sonoran Coastal areas producers association NGO, 2014. |
| 2 | 60,000 observational data of Just *O. tesota* A. Gray; | National inventory of forestry CONAFOR Project, 2004-2009 observation. |
| 3 | 6,500 observational data of 26 forestry species´ on Sonora | Martínez-Salvador M. 2013. INIFAP Project |
| 4 | 250 Personal Observation of *O. tesota* A. Gray on Hermosillo city of Sonora State | 2015. |

Present and future BioClimatic or environmental variables layers Worldclim BioClim database (consulted in 2015) from their webpage with ~ 1 km spatial resolution as commonly used in MaxEnt modeling used for environmental layers database. They have been downloaded and clipped for Sonora State with about 1km spatial resolution and then extrapolated to 200 m resolution .asci format raster layers with ArcView v.10.2 Desktop®, for all nineteen BIOCLIM (BIO1 to BIO19) as coming on Table 2 for the present time (average for 1950 to 2010), 2050 (average for 2041-2060) and (average for 2061-2080) (Khanum, *et al.* 2013). Between the existed different future climate scenarios as a base of future BioClimate data, the Greenhouse Circulation Modeling (GCM) model outputted BioClimate data are named (RCPs), Rcp85 (IPCC, 2013) as a Global extreme climate change scenario (Pearson *et al.* 2014) used for 2050 and 2070 environmental layers.

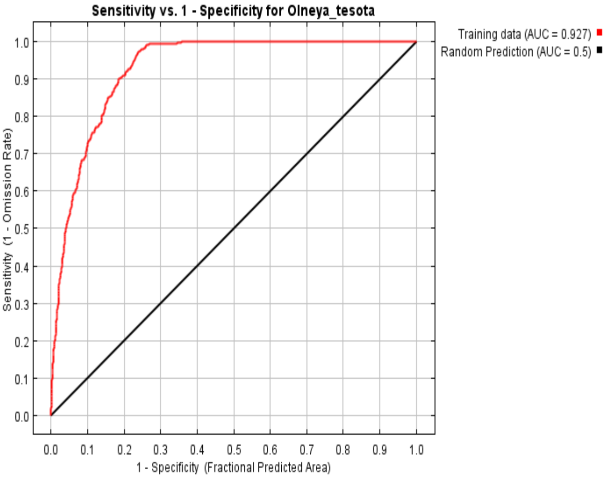

**Figure 2**. Sensitivity diagram of the MaxEnt model for *O. tesota* A. Gray.

### MaxEnt Modeling

The maximum entropy MaxEnt (version 3.3.3e) developed in 2011 (Philips *et al.* 2006, Elith 2011) used as a modeling system. MaxEnt uses presence-only data to predict the distribution probability of the species based on the theory of maximum entropy with attempting to similarity comparison of environmental conditions automatically (Matyukhina *et al.* 2015 ). The other raster calculations and mapping of the results were done with ArcView v.10.2®, to adequate observation data, probability equations among the others.

### 2.5 Model evaluation

To execute the MaxEnt model, 80% of *O. tesota* A. Gray observation data are used as a training dataset and 20% as a testing one and jackknife test used to obtain the portion of contribution and importance of the variables of each BIOCLIM variable on the *O. tesota* A. Gray distribution (Philips 2006, Khanum *et al.* 2013).

## Results

MaxEnt model performed with training data

AUC of 0.927 and random prediction AUC of 0.5 as sensitivity indices of the model demonstrate that the modeling results are acceptable (Phillips *et al.* 2006, Matyukhina *et al.* 2015) tuning of the model for the present time, 2050 and 2070 as it shown on sensitivity diagram Fig. 2 on the following as acceptable run according to various authors (Khanum *et al.* 2013). As a first result, the contribution portion of all nineteen BIOCLIM variables from Bio1 to Bio 19 has been obtained and given in Fig. 3, the calculation for 80% of the observation training data, it is observed the first high value contributed variable is BIO17 = Precipitation of Driest Quarter and lowest contributed variable is BIO2=Mean Diurnal Range (Mean of monthly (max temp - min temp)).

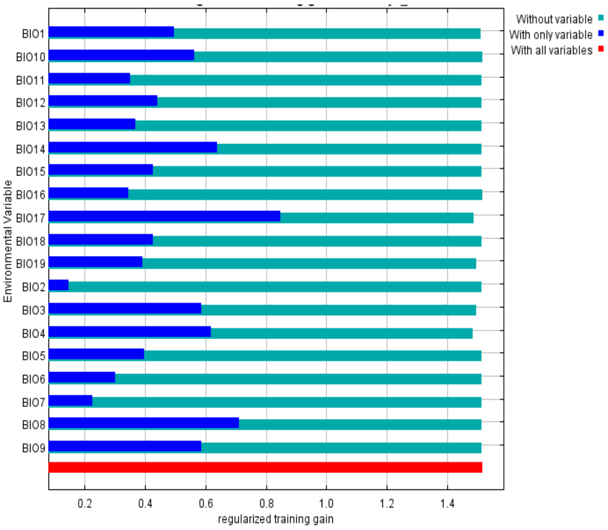

**Figure 3**. Relative predictive power of different BioClimatic variables based on Jackknife regularized training in the MaxEnt model for the species.

As it can be seen in Fig. 4 as a response to the presence of the *O. tesota* A. Gray to Bio17 as a high contributed variable, the function of the presence is close to the normal distribution curve, but for Bio2 it is not closed to it and contribution is too low. As it mentioned in the Materials and method part, BIO17 = Precipitation of Driest Quarter and it means the presence of the *O. tesota* A. Gray highly depended on dries four months of year precipitation and BIO2=Mean Diurnal Range (Mean of monthly (max temp - min temp)) as extreme rises of temperature will not affect its presence in the future that is agreed in with other studies about this species (Zuñiga and Suzán 2010, Verónica 2014).

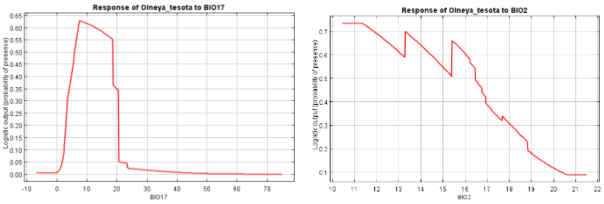

**Figure 4.** The response of the Distribution probability of *O. tesota* A. Gray.

It may discuss other BioClimatic data's contribution and response for different modeling of *O. tesota* A. Gray distribution. As a principal result, it has been obtained the predicted probability of the presence of *O. tesota* A. Gray on Sonora State for the present time which shows the present distribution is close to the coastal area where is the elevation encountered between 0m to 1000m (Zuñiga and Suzán 2010).

These results agree with different studies about *O. tesota* A. Gray population's distribution. Therefore, it is confinable modeling results for actual distribution delamination of the *O. tesota* A. Gray on the Sonora State of Mexico (Fig. 5).

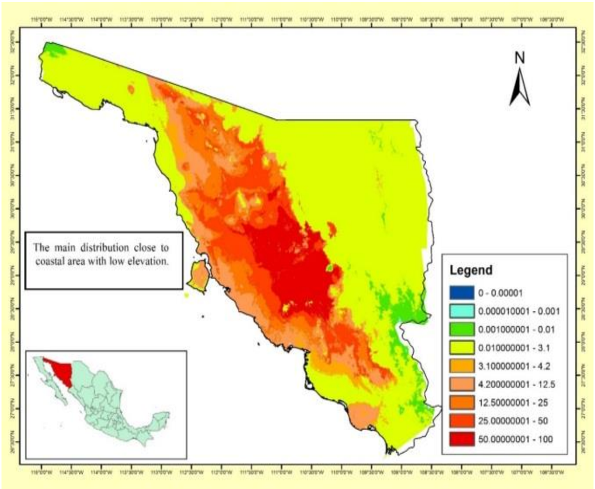

**Figure 5.** Presence probability prediction of the species, present time, Sonora State of Mexico.

The results of the simulation with the climate change scenario for 2070 is showing that the suitability of the ambient is expanding for the highly elevated areas of the Sonora State of Mexico. It means that climate change will help the species to immigrate to places with different geographical variables bypassing the time. As it showed in Fig. 6, it is observable on the map, the expansion of the distribution on the northeast part of the Sonora State mountainous areas of Sierra Occidental Madre is increased. The difference of the probability of the presence between the present time and 2070 to observe the rate of increasing the suitability of the environment for presence with from blue to red colors on Fig. 7.

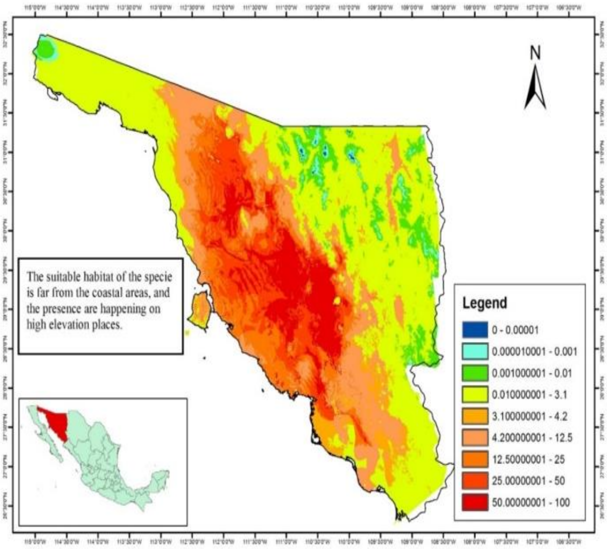

**Figure 6.** Presence probability prediction of the species on 2070, Sonora State of Mexico

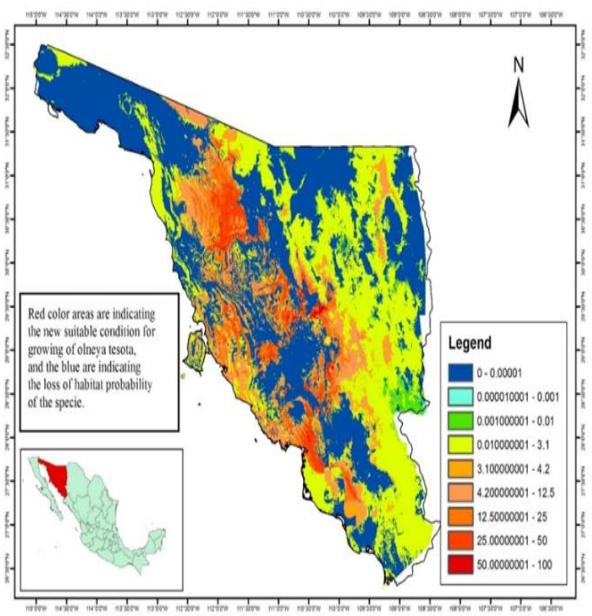

**Figure 7.** Difference of presence probability prediction of the species between 2010 and 2070, Sonora State of Mexico.

Based on Fig. 8 it can be seen that new municipalities such as Bacanora, Agua Prieta, Fronteras, Huasabas, Nacozari de Garcia, Onavas, Sahuaripa, San Pedro de la Cueva, Soyopa, Suaqui Grande and Villa Hidalgo that there is not a report about the presence of the *O. tesota* A. Gray in these regions.

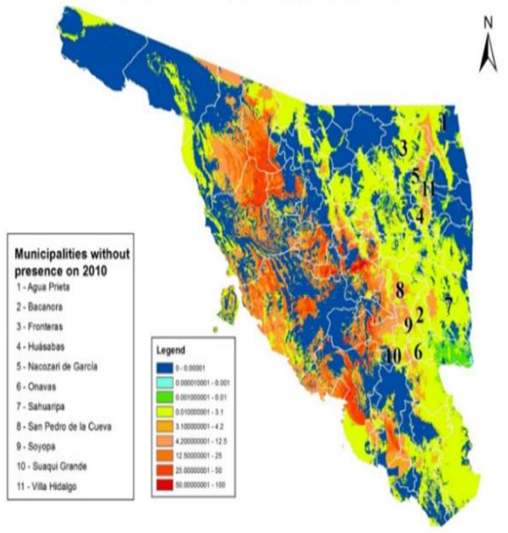

**Figure 8.** Difference of presence probability prediction of the species on 2070 in different municipalities that were not present at the present time, Sonora State of Mexico.

## Discussion

There are some studies about the evaluation of the presence adaptability of the species and generating useful information about conservation and management strategies in other regions with other species (Elia *et al.* 2015) that permit us to discuss in *O. tesota* A. Gray presenting in this region. The high priority of introducing the species is calculated by categorizing the probability of presence increases that helps to provide the rehabilitation and reforestation strategies. It may help to future studies about the species adaptation with new high altitudes among the other studies. The conclusion is according to the used climate change scenarios, climate change will affect as extinction risk threat on some part of the populations of the *O. tesota* A. Gray on the Sonora State of Mexico and it will help to the expansion of favorable environment to the growth of it in high altitudes.

We may claim that *O. tesota* A. Gray as a key species on Sonoran desert is a good alternative for ecosystem rehabilitation and reforestation from now to the future in altitudes higher (more than 1000 meters) than its recognized habitat indicator as a habitat constructor, nurse of big number of flora and fauna and protector of the ecosystem. Ultimately, *O. tesota* A. Gray will immigrate rapidly to geographically high altitudes as climate change impact its distribution, therefore it is recommended to introduce this species in other parts of Sonora as an adaptation study for conservation and management studies of the species. Moreover, we recommend the germplasm collection of the species on the negative affect estimated area as valuable genetic recourses to have the possibility of use on future rehabilitation and reforestation programs.

## Acknowledgment

We express our gratitude to the Mexican National Council for Science and Technology (CONACYT) for financial support.

## References

Barja I. Silván G. Rosellini S. Piñeiro A. Araújo M. B., Pearson R.G., Thuiller W., Erhard M. 2005. Validation of species – climate impact models under climate change. Global Change Biology 11(9):1504–1513.

Austin M. 2007. Species distribution models and ecological theory: A critical assessment and some possible new approaches. Ecological Modelling 200(1-2), pp.1–19.

Cole K.L. 1986. The lower Colorado River Valley: A Pleistocene desert. Quaternary Research 25(3): 392–400.

Elia J.D., Haig M.S., Johnson Matthew., Marcot G.B., Young R., 2015. Activity-specific ecological niche models for planning reintroductions of California condors (Gymnogyps californianus). Biological Conservation 184: 90–99.

Elith J., Phillips S.J., Hastie T., Dudík M., Chee Y.E., Yates C.J. 2011. A statistical explanation of MaxEnt for ecologists. Diversity and Distributions 17(1): 43–57.

Khanum R., Mumtaz A.S. Kumar S. 2013. Predicting impacts of climate change on medicinal Asclepiades of Pakistan using MaxEnt modeling. Acta Oecologica 49: 23–31.

Leão T.C.C., Fonesca R.C., Peres A.C., Tabarelli M. 2014. Predicting extinction risk of Brazilian Atlantic forest angiosperms. Conservation Biology 1(1): 1–11.

Martin C.A., Mcdowell L.B. 1999. Seasonal Effects on Growth of *O. tesota* following Root Pruning 337 Determining Suitable Chilling Conditions for Deciduous Fruit Trees in Iraq. Horticulture science 34(3).

Martínez-Yrízar A., Núñez S., Búrquez A. 2007. Leaf litter decomposition in a southern Sonoran Desert ecosystem, northwestern Mexico: Effects of habitat and litter quality. Acta Oecologica 32(3): 291–300

Matyukhina D.S., Miquelle G.D., Murzin AA., Pikunov G.D., Aramilev V.V., Litvinov N.M., Salkina P.G., Seryodkin V.I., Nikolaev G.I., Kostyria V.A., Gaponov V.V., Yudin G.V., Dunishenko M.Y., Smirnov N.E., Korkishko G.V., Marino J. 2015. Assessing the Influence of Environmental Parameters on Amur Tiger Distribution in the Russian Far East Using a MaxEnt Modeling Approach. Achievements in the Life Sciences, Available at: http://www.sciencedirect.com/science/article/pii/.

Medeiros A.S., Drezner T.D. 2012. Vegetation, Climate, and Soil Relationships Across the Sonoran Desert. Ecoscience 19(2), 148–160.

Meza-Rangel E., Tafoya F., Lindig-Cisneros R., Jesús Sigala-Rodríguez J., Pérez-Molphe-Balch, E. 2024. Distribución actual y potencial de las cactáceas Ferocactus histrix, Mammillaria bombycina y M. perezdelarosae en el estado de Aguascalientes, México. Acta Botanica Mexicana 108: 67–80.

Pearson R.G., Stanton J.C., Shoemaker K.T., Aiello-Lammens M.E., Ersts P.J., Horning N.F., Damien A.R., Ryu J.Ch., Yeong H., Jason M., Resit H.A. 2014. Life history and spatial traits predict extinction risk due to climate change. Nature Climate Change, 4(3): 217–221.

Peterson AT., Soberón J., Krishtalka L. 2015. A global perspective on decadal challenges and priorities in biodiversity informatics. BMC Ecology 15(1).

Peterson A.T., Ortega-Huerta M.A., Bartley J., Sanchez-CorderoV.,SoberonJ., Buddemeier R. H., Stockwell D.R.B. 2002. Future projections for Mexican faunas under global climate change scenarios. Nature 416(6881): 626–629.

Phillips S.J., Anderson R.P., Schapire R.E. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modeling 190(3–4): 231–259.

Salvador M.M., Rojas D.E. H., Saúl A., Guerrero M., Amparan J.A.P. (n.d.). Potencial productivo y zonificación para el uso y manejo de especies forestales de zonas aridas.

Shupe S.M. 2005. Multivariate characterization of Sonoran Desert vegetation in southwest Arizona using US Army field data. Plant Ecology 176(2): 215–235.

Shupe S.M., Marsh S.E. 2004. Cover and density-based vegetation classifications of the Sonoran Desert using Landsat TM and ERS-1 SAR imagery. Remote Sensing of Environment 93(1–2): 131–149.

Suzán H., Nabhan G.P., Patten, D. T. (1996). The importance of *O. tesota* as a nurse plant in the Sonoran Desert. Journal of Vegetation Science 7(5): 635–644.

Suzán H., Patten D.T., Nabhan G.P. 1997. Exploitation and conservation of Ironwood (*O. tesota*) in the Sonoran desert. Ecological Applications 7(3): 948–957.

Suzan-Azpiri H. 1994. Ecological effects of exploitation on *O. tesota* Gray and associated species in the Sonoran Desert. ProQuest Dissertations and Theses.

Suzán-Azpiri H. Sosa V.J. 2006. Comparative performance of the giant cardon cactus (Pachycereus pringlei) seedlings under two leguminous nurse plant species. Journal of Arid Environments 65(3): 351–362.

Thomas C.D., Cameron A., Green R.E., Bakkenes M., Beaumont L.J., Collingham Y.C., Erasmus B.F.N., Ferreira de Siqueira M., Grainger A., Hannah L., Hughes L., Huntley B., Van Jaarsveld A.S., Midgley G.F., Miles L., Ortega-Huerta, M.A. Townsend Peterson A., Philips O.L. and Williams S.E. 2004. Climate change and extinction risk, Nature 427(6970): 145–148.

Vásquez-méndez R., Ventura-ramos E., Domínguez-cortázar M.A. 2008. Soil Erosion Processes in Semiarid Areas Book

The Importance of Native Vegetation.

Verónica S., Humberto S. 2014. Análisis de la distribución espacial del muérdago (Phoradendron californicum) en el sur del Desierto Sonorense. Cact suc Mex 59(1):11–28.

Walker S. 2014. Applying Extinction Risk Modelling to Develop Global Conservation Priorities for Bulbous Monocots. Thesis, 65p Imperial College London University library.

Zuñiga-Tovar B., Suzán-Azpiri H. 2010. Comparative population analysis of desert ironwood (*Olneya tesota*) in the Sonoran Desert. Journal of Arid Environments 74(2): 173–178.