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POSGRADO EN CIENCIAS AMBIENTALES

Effect on hemoparasite infection, song structure, and population genetics in the Black-throated sparrow (*Amphispiza bilineata*) of habitat modification at a portion of the Mexican highland plateau

Tesis que presenta

José Gerardo Ham Dueñas

Para obtener el grado de

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Director de la Tesis:

Dr. Leonardo Chapa-Vargas

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Constancia de aprobación de la tesis

La tesis ***"Effect on hemoparasite infection, song structure, and population genetics in the Black-throated sparrow (Amphispiza bilineata) of habitat modification at a portion of the Mexican highland plateau"*** presentada para obtener el Grado de Doctor en Ciencias Ambientales, fue elaborada por **José Gerardos Ham Dueñas** y aprobada el diecisiete de noviembre del dos mil diecisiete por los suscritos, designados por el Colegio de Profesores de la División de Ciencias Ambientales del Instituto Potosino de Investigación Científica y Tecnológica, A.C.

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Dedicatorias

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Resumen

Efecto en la infección de hemosporidios, estructura del canto y la genética de poblaciones en el Gorrión garganta negra (*Amphispiza bilineata*) por la modificación de hábitat en una porción del Altiplano Mexicano.

PALABRAS CLAVE. *Haemosporidios, canto, diversidad genética, aves, habitat semiárido, actividades antropogénicas.*

La comunidad científica ha buscado estrategias para evaluar los impactos de las actividades humanas en el sistema del planeta Tierra. La degradación del hábitat en las tierras secas se caracteriza por una reducción en la productividad primaria, que afecta la resiliencia de estas áreas por las presiones ambientales futuras, lo que aumenta la vulnerabilidad de la biota. El pastoreo, la agricultura y la contaminación se consideran los principales desencadenantes de la degradación del hábitat en las tierras áridas. El presente estudio tiene como objetivo evaluar los factores espaciotemporales involucrados en la infección por hemoparásitos, la estructura acústica y la diversidad genética y estructura genética poblacional del Gorrión garganta negra, en una región semiárida modificada por la extracción de especies vegetales, sobrepastoreo y contaminación por actividades mineras. Esta especie de ave tiene diferentes preferencias de hábitat en comparación con otras especies previamente estudiadas en la región. En el segundo capítulo referente a la infección hemoparasitaria, la prevalencia global de hemoparásitos fue de 22.1% y la variación presente no se asoció con el tipo de hábitat. La prevalencia y la parasitemia fueron más altas durante la época reproductiva que en la no reproductiva y, finalmente, se observó una gran diversidad de linajes de hemosporidios. En el tercer capítulo relativo a la asociación del canto y la

actividad minera, se registró una relación negativa entre las concentraciones de plomo y la densidad de arbustos con la tasa del canto y la tasa de cambio de tipos de cantos. Además, la complejidad de la sílaba en el trino y el tamaño del repertorio se asocian negativamente con las concentraciones de arsénico y plomo, respectivamente. Por lo tanto, la actividad minera tuvo una fuerte influencia en la estructura del canto en esta especie. En el último capítulo relativo a la genética de poblaciones, se encontraron altos valores de exceso de heterocigotos dentro de las poblaciones en la mayoría de los sitios, excepto en los sitios más degradados, en donde el coeficiente de endogamia fue significativamente mayor en uno de estos sitios que está expuesto a la actividad minera. No se registró ninguna estructura genética de la población para el área de estudio. Se documentó que la contaminación por metales pesados tiene el potencial de promover la pérdida de diversidad genética en una especie de ave. En general, a través del estudio actual, fue posible determinar que el gorrión garganta negra es una especie modelo ideal para investigar los cambios ambientales causados por las actividades antropogénicas. La minería fue la actividad humana más fuerte que influyó negativamente en la diversidad genética y la estructura del canto en el Gorrión garganta negra. Es esencial investigar todos los componentes biológicos en el área para conocer su valor y utilizar esta información para regular las actividades humanas que pueden afectar la biota. Además, conservar indirectamente los servicios ecosistémicos que benefician a partir de los recursos naturales de esta área.

Abstract

Effect on hemoparasite infection, song structure, and population genetics in the Black-throated sparrow (*Amphispiza bilineata*) of habitat modification at a portion of the Mexican highland plateau

KEY WORDS. *haemosporidian, song, genetic diversity, aves, human-induced changes, dryland.*

The scientific community has searched for strategies to assess the impacts of human activities on the Earth system. Habitat degradation in drylands is characterized by a reduction in primary productivity, affecting the resilience of these areas to future environmental pressures and increasing the vulnerability of the biota. Grazing, agriculture, and pollution are considered the main triggers of habitat degradation in drylands. The current study aims at evaluating spatio-temporal factors possibly involved in haemoparasite infection, song characteristics, and genetic diversity and population structure of Black-throated sparrow populations, in a semiarid region that has been modified by humans through extraction of plant species, overgrazing, and pollution from mining activities. This bird species has different habitat preferences in comparison to other, previously studied species in the region. In the 2nd chapter, overall haemosporidian prevalence was 22.1% and variation in haemoparasitism was not associated to habitat type. Prevalence and parasitaemia were higher during the breeding than the non-breeding season and finally, a high diversity of haemosporidian lineages was recorded. In the 3rd chapter, where association of song traits and mining activity was investigated, a negative relationship between lead concentrations and shrub density with song rate and song switching rate was recorded. In addition, syllable complexity in trill, and repertoire size were found to be negatively associated to arsenic and lead concentrations, respectively. Therefore, it was concluded that mining activity had the strongest influence in song structure in this bird species. In the last chapter which relates too population genetics, high values of heterozygote excess within populations were recorded in most of the sites, except in the most degraded sites, and the inbreeding coefficient was significantly higher in one of these sites, which is exposed to mining activity. No population genetic structure was recorded for the study area. It was further documented that heavy metal pollution has the potential to promote genetic diversity losses in a bird species. Overall, through the current study it was possible to determine that the Black-throated Sparrow is an ideal model species to investigate environmental changes caused by anthropogenic activities. Mining was the strongest human activity negatively influencing genetic diversity and song structure in the BTSP. It is essential to investigate all the biological components in the area to further knowledge about their value, and to use this information to regulate human activities that may affect the biota, and also, indirectly conserve ecosystem services that are benefited by the natural resources of this area.

CHAPTER 1

Introduction

Introduction

In recent decades, the scientific community has searched for strategies to assess the impacts of human activities on the Earth system. This task often involves disentangling processes occurring from local to regional scales, and relating them at the global dimension. Among the most important aspects of this process are delimiting which human-induced changes are closely linked to intense environmental alterations, and creating sustainable development strategies.

Considering the great environmental change caused during the anthropocene age, in recent years, “Planetary boundaries” were proposed by Rockström et al. (2009a, b) and Steffen et al. (2015). The purpose of these boundaries is promoting the maintenance of a safe operating Earth system. These authors defined nine planetary boundaries (Figure 1) of specific biophysical subsystems or processes with the aim of identifying their thresholds, necessary to evade severe environmental alterations within continents or at the entire planetary system scale. Some of these processes are linked to human-induced perturbation activities. Four out of nine of these processes have already exceeded their threshold. These include climate change, biosphere integrity (functional diversity and genetic diversity), biogeochemical flows, and land system change (Steffen et al. 2015; Figure 1).

In explaining some of these specific processes, land-system change considers the modification of biogeophysical processes in terrestrial biomes that have considerable feedbacks, which ultimately relate to climate. Removal and replacement of native vegetation by cropland or grazing systems are some of the main anthropogenic practices affecting this process. In addition, agriculture and grazing activities accelerate biodiversity loss (Lindenmayer and Fischer 2006).

Biosphere integrity boundary analysis also considers two components that address distinct levels of biodiversity in the Earth system. First, genetic diversity preserves unique genetic material which provides long-term potential of the biosphere to adapt to and persist during abrupt changes of abiotic factors in the Earth system. Secondly, functional diversity consists of functional traits and their value and range within given groups of organisms that influence an ecosystem (e.g. parasites, predators, etc.). Anthropogenic activities trigger biodiversity loss at both local and regional scales. Therefore, genetic and functional diversity response mechanisms appear due to environmental fluctuations, within or among species, maintaining resilience to disturbances (Rockström et al. 2009b; Steffen et al. 2015). Therefore, loss of genetic and/or functional diversity due to human activities may have serious consequences in terms of system resilience.

Moreover, there are additional planetary boundaries that have not been quantified and have the potential to modify the state of Earth system. Such is the case of novel entities (previously categorized as chemical pollution; Rockström et al. 2009), which associate to undesirable biological or geophysical effects of new or pre-existing substances (natural or modified materials) from anthropogenic sources. Persistence, mobility across scales, and repercussion on important Earth system processes of novel entities are the main characteristics that confer them their potential to affect ecological systems at the global level (Steffen et al. 2015).

The three specific planetary boundaries described above, are categorized as slow and aggregated (occurring in several locations simultaneously) processes that operate at the regional scale. Also, biosphere integrity and land-system change possess subglobal dynamics that potentially play a known significant role at the global level (Rockström et al. 2009b). In addition, interactions between boundaries are well documented (Rockström et al. 2009a; Steffen et al. 2015; Fahrig 2017). Biosphere integrity, for instance, is considered one of the two core planetary boundaries intimately connected to others (Steffen et al. 2015). Biota in ecosystems maintain an important role for the Earth system and biodiversity provides resilience, which translates into potential persistence in a specific state even when alterations in other boundaries are occurring (Rockström et al. 2009a; Freeland et al. 2011).

Research on biota processes and their responses to human activities at the local scale is fundamental because it provides data pertaining to interactions among these processes (land use change, chemical pollution, genetic and functional diversity) in specific biological models. Though interactions have been intensely studied in tropical and temperate biomes, research in dryland areas is still needed in order to understand how habitat degradation processes affect the biological components of these regions.

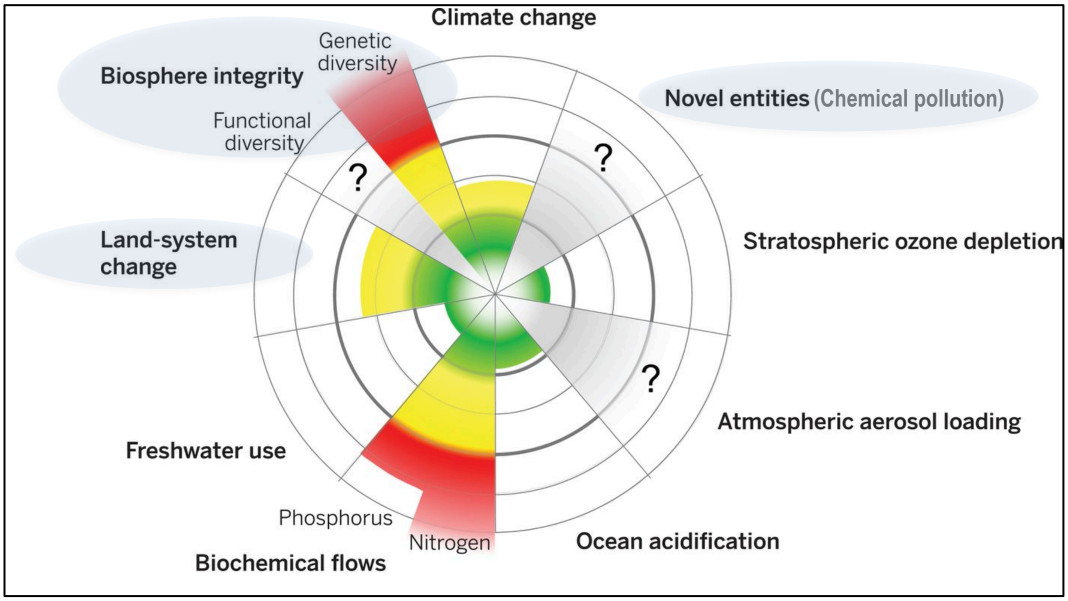

Figure 1. Status of seven from nine planetary boundaries. Green zone is the safe operating space (below the boundary), yellow represents the zone of uncertainty (increasing risk), and red is the high-risk zone. Gray represents processes for which global-level boundaries have not been quantified yet. (Taken and modified from Steffen et al. 2015).

Drylands

Drylands, which approximately cover 41% of the Earth's surface (Figure 2), are areas with limited water availability owing to a relative high evaporation rate coupled with low precipitation (Aridity Index ranges between 0.05 (hyperarid) to 0.65 (dry-subhumid). Primary productivity is positively related to water availability in these areas declining from dry sub humid, semiarid, arid, and hyper arid areas (Safriel et al. 2005). These areas are highly heterogeneous in resource distribution and other abiotic characteristics (topography, soils, solar radiation, etc.) thereby influencing the spatial patterns of vegetation distribution in the geographic area. Some species require more humid environments (e.g. woodlands), whereas others are have found their niche in open and drier areas (e.g. grasslands) (Ward 2009; Mueller et al. 2013). The interspersion of patches of variable resource availability and productivity of vegetation is a common phenomenon in drylands, which are vulnerable to degradation owing to delimited areas that concentrate soil and water resources that are also necessary for human activities (Mueller et al. 2013). Spatial structure consisting of different interspersed habitat types, influences bird species dynamics because these landscapes are typical of those in which habitat fragmentation is present. Therefore, biological dynamics may be affected (Gibbens et al. 2005; Safriel et al. 2005; Mueller et al. 2013).

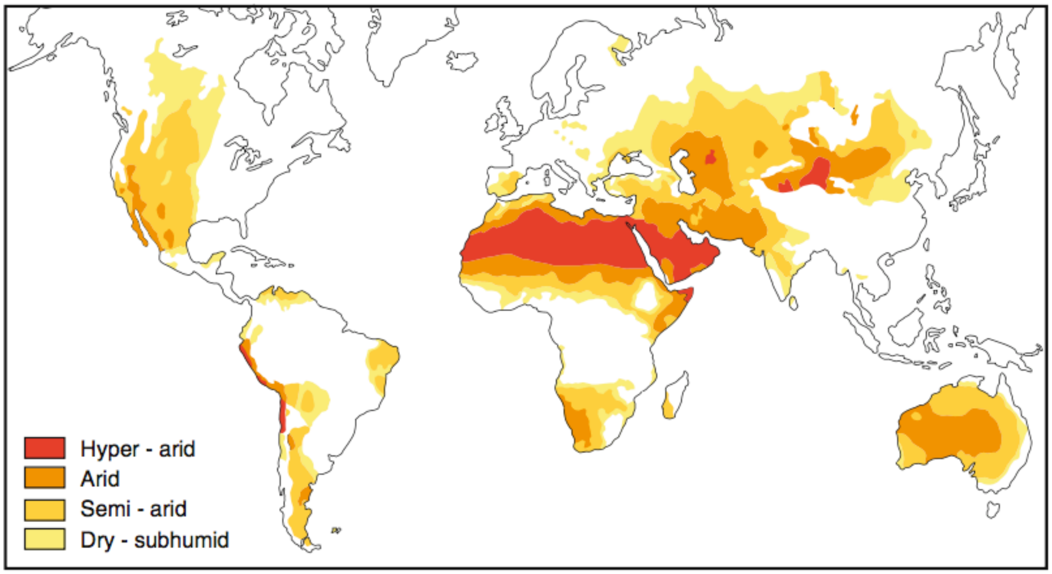

Figure 2. World distribution of drylands (taken from Mueller et al. 2013)

Land degradation in drylands

Modification of natural habitats and landscapes in drylands by anthropogenic activities may generate habitat degradation, loss, and fragmentation (Fischer and Lindenmayer 2007). While habitat loss is the nearly complete disappearance of the original ecosystem in a pristine area, degradation is a gradual deterioration in habitat quality (Lindenmayer and Fischer 2006). For the latter process, some consequences may include disruption or alteration of biological interactions, biological complexity, animal behavior, etc. Consequently, population size and reproductive rates may decrease (Fischer and Lindenmayer 2007), but in some cases an opposite response may be developed by some specific taxa (Rogers and Chown 2014). There are also deterministic processes that are closely related to habitat transformation, such as chemical pollution, hunting, and urbanization among others (Bennett & Saunders 2010). The effects of habitat degradation on organisms can be evaluated at different levels within and among populations. Changes in habitat quality may influence population health in terms of demography, genetic structure, and behavior (Lindenmayer and Fischer 2006; Amos et al. 2012).

Habitat degradation in drylands is characterized by a reduction in primary productivity, affecting the resilience of these areas to future environmental pressures and increasing the vulnerability of biota, including also people inhabiting these drylands (Safriel et al. 2005). In addition, vegetation alteration has been mostly used as an indicator of habitat degradation. This is because an important relationship between this landscape feature and biophysical processes exists (Mueller et al. 2013). Landscape heterogeneity influences the way in which wildlife exploits different types of vegetation in scattered patches. Specific landscape features such as topography, soils, elevation, etc. create these patches with specific types of vegetation (Galvin et al. 2008). Some of these patches may posses higher vegetation cover and water availability over time, thus providing an ecological safety net for wildlife (Galvin et al. 2008). However, these particular areas are also selected for human activities because these resource clusters provide suitable conditions for grazing, agriculture, and resource extraction (i.e. hunting, plant extraction, etc.) (Galvin et al. 2008; Ward 2009; Mueller et al. 2013). Therefore, grazing and agriculture are considered the main triggers of habitat degradation in drylands (Galvin et al. 2008; Mueller et al. 2013).

The highland plateau of San Luis Potosi

The highland plateau of San Luis Potosí is embedded in the south-central portion of the Chihuahua Desert, which belongs to the Mexican Xerophytic region (Rzedowski 2005) of the Chihuahua Desert. This region is dominated by semiarid drylands. The dominant vegetation communities in this area include microphyllous scrublands, semiarid grasslands, and *Yucca*-dominated scrublands (Rzedowski 1961, 2005). Natural habitats of this region have been degraded by extraction of plant species and overgrazing by cattle and goats (Garza-Hurtado 2011). These processes have created changes in the original vegetation cover, and consequently, in occupation patterns by animal species, including birds. The use of plant species such as those from the genus *Yucca sp*., and *Agave sp*., as primary products for "ixtle", a hard fiber derived from the uncultivated plants known as “lechuguilla” (*Agave lechuguilla*), and “palma samandoca” (*Yucca carnerosana*), is a common practice in this area. The region in which “ixtle” is currently exploited for commercial purposes is locally called the “Zona Ixtlera”. The fiber is converted into rope and brushes and used for a variety of purposes (Sheldon 1980). This activity has been one of the main factors promoting changes in plant cover (Rzedowski 1961, 2005; Miranda and Hernandez-X. 1963). In addition, both overgrazing and the extraction of plant species for additional commercial uses are causes of landscape modification in the highland plateau of San Luis Potosí.

Another anthropogenic factor promoting habitat transformation in the region is the mining industry, which has taken place in San Luis Potosí and in other neighboring states for at least the last 400 years (SGM 2016). Accumulation of toxic elements in soil, water, tissues of wild animals, and in humans has been recorded in this region near several mining sites (Razo et al. 2004; Chapa-Vargas et al. 2010; Martínez-Villegas et al. 2013; Espinosa-Reyes et al. 2014; Monzalvo-Santos et al. 2016). These factors are not exclusive to Mexico; habitat modification has actually occurred gradually throughout the world. Land-use changes have already had significant impacts on biodiversity and associated ecosystem services, and it has been suggested that changes in range sizes of birds in response to anthropogenic land conversions will likely be considerable (Jetz et al. 2007).

The Black Throated Sparrow (BTSP)

The Black-throated Sparrow (*Amphispiza* bilineata, BTSP) is a medium-sized bird that inhabits semiarid areas of southern United States and north-central Mexico and is a year-round resident within the Mexican territory (Figure 3). In its southernmost distribution, this species associates with different habitat types as long as creosotebush (*Larrea tridentata*) is present (Raitt and Maze 1968; Johnson et al. 2002), but it avoids urban areas (Johnson et al. 2002). This species possesses adaptations to arid regions such as its nest location, which is unrelated to surface water proximity (Coe and Rotenberry 2003), or hydration processes based on acquisition of water to maintain its balance from food items such as insects and succulent vegetation (Smyth and Bartholomew 1966).

Pidgeon et al. (2003) analyzed avian demographic patterns in a landscape context in New Mexico, using as a model the Black Throated Sparrow to document patterns of abundance and nest success among seven habitat types (mesquite, sandsage, creosotebush, whitethorn, black grama, mesa, and pinyon-juniper). They encountered higher adult abundances in mesquite habitat than in the remaining habitats. Nest success in this habitat, however, was only 10%. Creosotebush habitat also had high adult abundance, but the nest success was higher than in mesquite. Nest success in grassland habitat (mesa and black grama) was also high, but adult abundances in this habitat were lower than in the remaining habitats. They regarded mesquite habitat as a population sink, while creosotebush, black grama grassland, whitethorn, and mesa grassland were considered source habitats for the Black Throated Sparrow. In a more recent paper Pidgeon et al. (2006) found that nest success and nest density measures provide a good indicator of habitat quality for the population.

More recently, Kozma et al. (2017) reported spatio-temporal factors influencing nest survival of BTSP. According to these authors, predation pressure was the main cause of nest mortality accounting for 86% of nest failure, and daily nest survival was associated negatively with vegetation cover. Though the studies explained above can help clarify which types of vegetation can be considered high quality habitats for certain species, investment in monitoring nests could have an elevated cost in time that may approach with other tools.

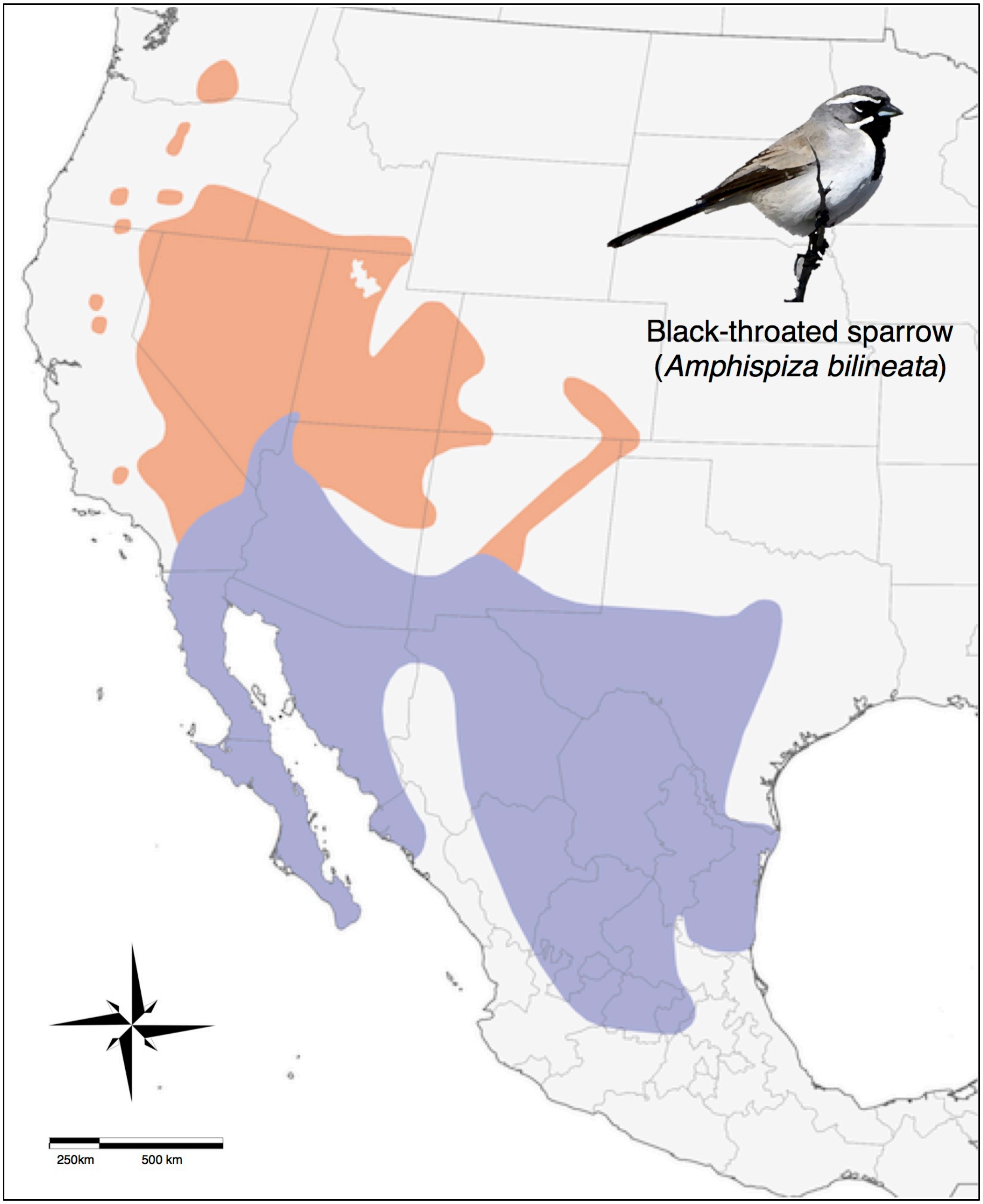

Figure 3. Map of distribution of the Black-Throated Sparrow. Orange indicates breeding range, while purple indicates year-round range. Taken from Johnson et al. (2002).

The current study aims at evaluating some factors (spatio-temporal) possibly involved in the maintenance or modification of haemoparasite infection (Chapter 2), song characteristics (Chapter 3), and genetic diversity and population structure (Chapter 4) of BTSP populations in a semiarid region that has been modified by humans through extraction of plant species, overgrazing, and pollution from mining activities. These activities could negatively influence the Black-throated Sparrow. The individual and population characteristics of BTSP examined in this PhD thesis, have rarely been studied in bird species of arid and semiarid habitats from Mexico (Garza-Hurtado 2011; Canales-Delgadillo et al. 2012; Reinoso-Pérez 2014; Monzalvo-Santos et al. 2016; Reinoso-Pérez et al. 2016).

Although habitat modification has been the subject of many conservation biology studies and is of major importance for policy and management practices, a small number of studies have focused on semiarid habitats (Fazey et al. 2005). This motivated the current research, which proposes considering these biological components (acoustic, genetics, and parasitism) simultaneously, and evaluating how ecological factors at the individual and population levels may link to the variables under study.

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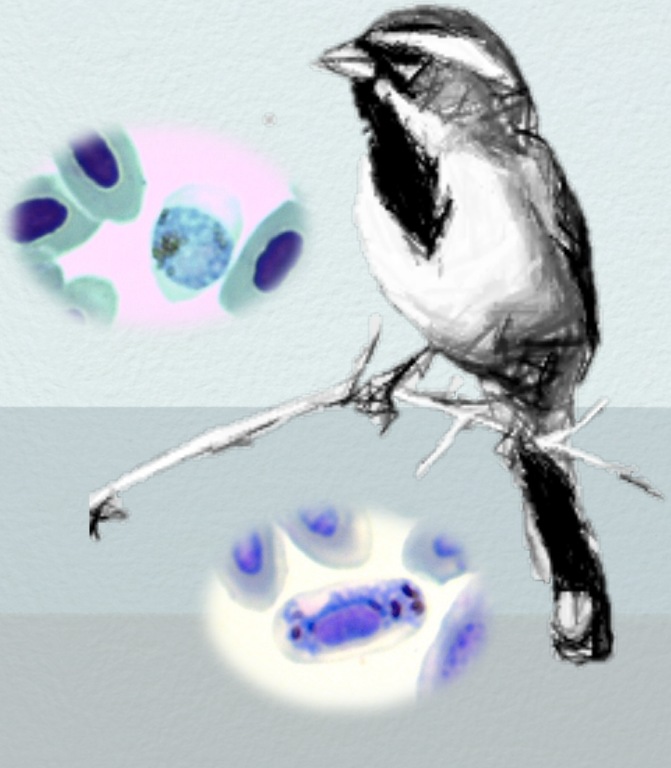
Chapter 2

Haemosporidian prevalence and parasitaemia in the Black-throated sparrow (Amphispiza bilineata) in central-Mexican dryland habitats



Haemosporidian prevalence and parasitaemia in the Black-Throated Sparrow (*Amphispiza bilineata*) in central-Mexican dryland habitats

Abstract

To date it is not well-understood how seasonality and human-induced habitat change may affect haemosporidian prevalence and parasitaemia in bird hosts in dryland habitats. We compared haemosporidian prevalence and parasitaemia between habitat types, including *Yucca*-dominated scrublands (closed habitat) and creosotebush scrublands (open habitat), and between seasons, including non-breeding (dry) and breeding (wet) in the Black-Throated Sparrow (*Amphispiza bilineata*) at semi-arid scrublands of Central Mexico. This bird species has different habitat preferences in comparison to other, previously studied species in the region; it shows higher abundances in open than in closed habitats and avoids urban areas. Overall haemosporidian prevalence was 22.1%. Prevalence and parasitaemia were higher for *Haemoproteus* sp. (*Parahaemoproteus* sp.) than *Plasmodium*. Variation in haemoparasitism was not associated to habitat type. This response differs from the previously recorded response in other bird species in the region for which haemoparasitism increases with increasing habitat degradation. Seasonality seems to be the most important driver of parasite infection for this sparrow as prevalence and parasitaemia were higher during the breeding than the non-breeding season. Two new lineages of *Haemoproteus* sp. that had not been reported before in any avian species were found through molecular diagnosis. A high diversity of haemosporidian lineages is

shared among sites. More study is needed to understand the mechanisms that associate parasitaemia, prevalence, and specific environmental factors.

Introduction

Haemosporidian parasites (Phyllum Apicomplexa, order Haemosporidia) influence the population ecology of their hosts in ways and to an extent that are not yet completely understood. Therefore, the study of factors influencing haemosporidian prevalence of infection and parasitaemia contributes to our understanding of how these associations may influence host ecology (Kiszewski et al. 2004; Valkiūnas 2004; Cox 2010). In dry land habitats, which cover a vast geographical extent, and harbor extraordinarily high diversity, Haemosporidian prevalence is highly variable (0% to 92.5%) (Blanco et al. 2001; Valera et al. 2003; Deviche et al. 2005; Fokidis et al. 2008; Barrientos et al. 2014; Reinoso-Pérez et al. 2016), and current knowledge related to the ecological factors influencing haemosporidian prevalence and parasitaemia in these habitats is still particularly limited.

Human-induced habitat change may influence parasite-host interactions in different directions (Budria and Candolin 2014). The effects of habitat modification (i.e. urbanization, deforestation, etc.) on blood parasite prevalence range from positive (Chasar et al. 2009; Delgado-V. and French 2012) to negative (Bennett et al. 1992; Tella et al. 1999; Arriero et al. 2008; Fokidis et al. 2008; Delgado-V. and French 2012; Reinoso-Pérez et al. 2016) and neutral (Fokidis et al. 2008; Belo et al. 2011). Therefore, more study covering a wide variety of environments and bird species, especially those that have been less studied, are needed.

During the last century, Mexican dryland habitats have been exposed to anthropogenic degradation (Sheldon 1980; Rzedowski 2005; Chapa-Vargas et al. 2010). In this period, yucca-dominated scrublands were modified in their plant species composition by tree extraction and overgrazing, resulting in transformation to unnatural creosotebush scrublands. The current study focused on effects of scrubland degradation in the Black-throated Sparrow (BTSP), a medium-sized bird from semiarid ecosystems of southern United States and north-central Mexico. This bird differs from other, previously-studied species from semiarid ecosystems, such as the canyon towhee (*Melozone fusca*), and the House finch (*Haemorhous mexicanus*) which had the highest haemosporidian parasitaemia in villages and in degraded scrublands (Reinoso-Pérez et al. 2016); though the BTSP associates to scrublands, it avoids urban areas and shows a preference for open habitats such as grasslands and scrublands that are dominated by creosotebush (*Larrea tridentata*). Indeed, these seem to be the highest-quality habitats for the BTSP (Pidgeon *et al.* 2003; 2006). Thus, the BTSP may benefit from some of the habitat changes that occurred in this area. Consequently, the BTSP is a good study organism for investigating how responses in haemosporidian parasitism to anthropogenic habitat modification may differ from those reported for other species with different habitat preferences. Though infection by haemosporidians has been previously reported in the BTSP (Clark and Swinehart 1969; Garza-Hurtado 2011), no effects of environmental variables were assessed.

In addition to the effects of habitat degradation, it has been reported that infection rates in birds may change through time due to climatic fluctuations. Haemosporidian transmission by vectors (Diptera: Culicidae, Hippoboscidae, and Ceratopogonidae) may be higher during the warm, rainy season (Deviche et al. 2001; Valkiūnas 2004), which lasts longer at medium latitudes, and coincides with the avian reproductive period (Beaudoin et al. 1971). Haemosporidian prevalence is reportedly higher during the breeding than in the non-reproductive season in some species (Deviche et al. 2001, 2005; Fokidis et al. 2008; Astudillo et al. 2013). For semiarid environments, it is not well known how haemosporidian parasitism changes through time.

Our objective was to evaluate the effects of habitat type on haemosporidian prevalence and parasitaemia in BTSP by comparing these variables between birds captured in moderately degraded scrublands ("*Yucca* forests") and highly degraded open scrublands (creosotebush scrubland). We also aimed at evaluating differences between seasons (breeding/wet vs. non-breeding/dry), and the potential variation in prevalence and parasitaemia among years and sites. Finally, we identified haemosporidian lineage diversity on the BTSP in our study region. We hypothesized that in moderately degraded scrublands ("*Yucca* forest"), BTSP would exhibit higher haemosporidian prevalence and parasitaemia because this has been reported as the sub-optimal habitat for this host species (Pidgeon et al. 2003, 2006). We also hypothesized that prevalence and parasitaemia would be higher during the breeding (rainy) in comparison to the post-breeding (dry) season.

Method

Study area

The highland plateau of San Luis Potosí, located in Central Mexico is dominated by semiarid climate. This area is embedded in the Mexican Xerophytic region (Rzedowski 2005) of the Chihuahua Desert. The dominant vegetation communities in this area include microphyllous scrublands, semiarid grasslands, and *Yucca* dominated scrublands (Rzedowski 2005). Microphyllous scrublands cover the largest portion of the Mexican xerophytic region and are dominated by shrubs of the genera *Larrea*, *Flourensia*, *Condalia*, and *Celtis*. Cacti species (Cactaceae) maintain high density and diversity in most of the plateau area (Rzedowski 2005).

The sites considered in the current study are located in three communal land ownership ("*ejido*") areas: La Cardoncita (LC), Presa de Santa Gertrudis (PSG), and Guadalupe Victoria (GV), and in the surroundings of Charcas (CH), a relatively large town (population size = 21,138) (INEGI, 2010) (UTM: LC= E271793.37 N2610708.64, PSG= E278059.47 N2599525.12, GV= E276057.75 N2582923.98, CH= E279261.83 N2561548.75; Zone 14N) in the municipalities of Charcas (LC, GV), and Catorce (PSG) in the state of San Luis Potosí (Figure 1). Sampling design, in terms of "stratification" was intended to represent habitat types that prevail in the region: A) highly degraded scrublands (HDS) consisting of open creosote plant communities that according to the literature (Rzedowski 2005; Garza-Hurtado 2011) and to information provided to us by local inhabitants have been modified through extraction of *Yucca* plants, and grazing by goats (herbaceous cover= 11.47%; bare ground= 79.34%; mulch= 9.19%) (Garza-Hurtado 2011). Shrubs in this habitat type are almost exclusively creosotebush (*Larrea tridentata;* density: mean= 782 shrubs/ha, SD= 315.25) and *Yucca* density is extremely low (mean= 0.75 trees/ha; SD= 4.5) (Garza-Hurtado 2011) because extraction took place during the last 50 years for fiber production locally known as “Ixtle”, which is used for the manufacture of ropes and other crafts (Miranda and Hernandez-X. 1963; Sheldon 1980; Rzedowski 2005; Garza-Hurtado 2011). For this habitat type we included three study sites located near the villages: GV, PSG, and LC (Figure 1). The distance from these study sites to its nearest village were 3.5 km, 1.8 km and 2 km, respectively. The second habitat type consisted of B) moderately degraded scrublands (MDS) that are dominated by *Yucca filifera* and *Yucca decipiens* (also known as “*Yucca* forests”; *Yucca* density: mean= 42.5 trees/ha, SD= 40; height: mean = 3.9m, SD= 1.5). As indicated by local inhabitants and corroborated through our observations in the field, this plant community only has moderate levels of grazing through the maintenance of small densities of free-living horses and cattle (herbaceous cover= 22.69%; bare ground= 39.65%; mulch= 37.66%) (Garza-Hurtado 2011) and moderate extraction of *Yucca* plants. This type of vegetation is frequent in this region, is indicative of relatively well-conserved plant communities (Rzedowski 1961), and considered as closed habitat. For this habitat type we included three study sites located near GV, PSG, and LC (Figure 1), having similar distances to the nearest village (4 km, 6.5 km, and 6.5, respectively). One additional study site is representative of moderately degraded scrublands (CH). This site is located near to a more urbanized village (3 km) and exhibits elevated soil Arsenic

(As) and Lead (Pb) concentrations (Monzalvo-Santos et al. 2016), that have originated from the intensive mining activity that has taken place for more than 400 years in the area (Figure 1).

Blood sampling

In each study site, birds were trapped using 20 ornithological mist nets (12m x 2.5m). Within each site, nets were placed at random locations and operated from dawn (6:00-7:00hrs) to just before dusk (18:00-19:00hrs) during three consecutive days. Each site was sampled both during the breeding (March-June; wet) and non-breeding (September-November; dry) seasons of 2012 to 2015. Blood samples were extracted through brachial vein puncture, and were used to obtain genetic material and to prepare blood smears. Blood samples were stored under stable conditions with Longmire buffer solution (100 mM Tris-HCl pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS) and Ethanol, and transported to the laboratory for subsequent DNA extraction. Blood smear processing was the basis for subsequent quantification of prevalence and intensity of infection (parasitaemia) in each individual.

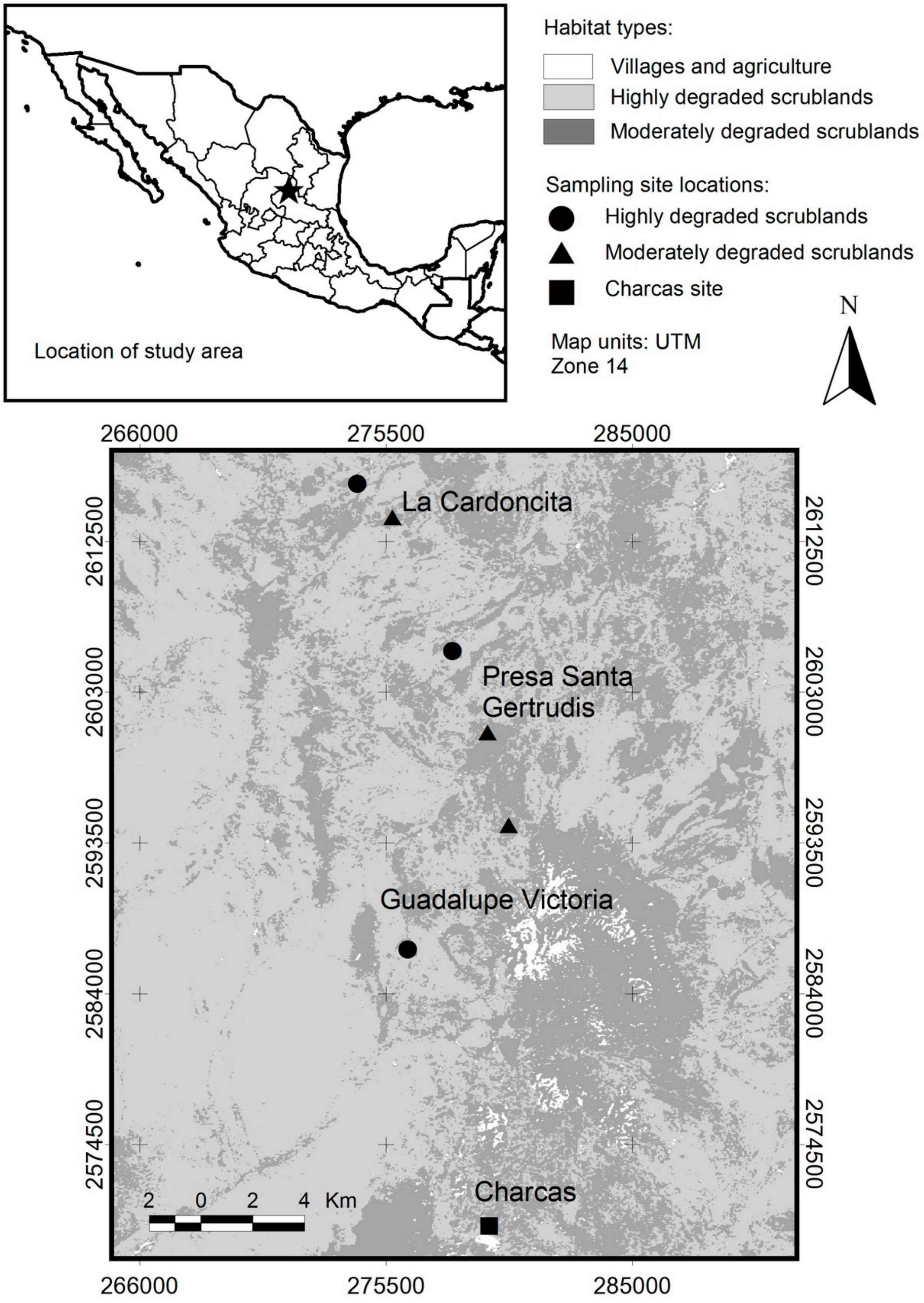


Fig. 1 Sampling sites at the highland plateau of San Luis Potosí, Mexico

Microscopy analysis

Following Valkiūnas (2004) and Santiago-Alarcon and Carbó-Ramírez (2015), blood smears were made on 25 x 75 mm slides in the field, left to dry in the shade, fixed in 100% methanol and kept in storage boxes. Subsequently, smears were stained with Giemsa stain in the laboratory in the Division of Environmental Sciences at the Institute for Scientific and Technological Research of San Luis Potosí (IPICYT). A compound microscope (Leica DM1000 LED) was used to identify and count haemosporidian parasites. Smears were initially examined with 400x magnification for general examination. Then, high magnification (1000x under the immersion objective) was used to take photos using a camera (Leica MC170 HD) mounted on the microscope. In each slide, we determined presence/absence of infection by haemosporidians, and when samples were infected, we counted the number of erythrocytes infected out of ~10,000, following a semi-automated counting protocol (Grishagin 2015); photos were processed and blood parasites and red blood cells were counted with the ImageJ v10.2 software. The number of infected erythrocytes in 10,000 was used as the measure of parasitaemia (Fokidis et al. 2008). Following the identification key (Valkiūnas 2004), parasites were identified to the genus level, and to species when possible. The following taxa were considered: *Haemoproteus* sp., *Plasmodium* sp., and *Leucocytozoon* sp., as they had been previously recorded in the region (Garza-Hurtado 2011; Reinoso-Pérez et al. 2016) and in similar habitats (Blanco et al. 2001; Deviche et al. 2005; Fokidis et al. 2008). Though extracellular haemoparasites such as *Trypanosoma*, filarial blood parasites, and others were not the focus of the current study, they were identified to the genus level and reported when found, as this information may be of interest to some readers.

DNA Extraction from blood samples

Total genomic DNA was extracted from all collected blood samples using the DNeasy blood and tissue extraction kit (Qiagen, Inc.). The quantity (µg/µl) and quality (260/280 and 260/230nm ratio) of the extractions were evaluated to verify DNA integrity with a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Inc.). All samples were used for posterior haemosporidian parasite detection, and sequencing to identify main haemosporidian lineages.

Molecular detection of haemosporidians and sequencing

A nested-PCR method proposed by Hellgren et al. (2004) was used to acquire a partial fragment of cytochrome *b* (cyt*b*). First, a 580 bp segment of the parasite mitochondrial cyt*b* gene was amplified using the initial primers HaemNFI and HaemNR3 (Hellgren et al. 2004). Then, a second PCR round was performed using primers HAEMF and HAEMR2 designed by Bensch et al. (2000), which amplify samples of *Haemoproteus* and *Plasmodium* species. The first PCR was performed on aliquots of 25 µl, which added approximately 25-50 ng of genomic DNA, 1.5 mM $MgCl_2$, 1X PCR buffer II (100 mM Tris–HCl, pH 8.3, 50 mM KCl), 0.15 mM of each deoxynucleoside triphosphates, 0.6 μM of each primer, and 0.6 U of AmpliTaq (Applied Biosystems). Thermal profiles for both PCR rounds were performed according to Hellgren et al. (2004). PCR's were carried out in C1000 and MJMini thermal cyclers (BioRad). PCR products were examined by 1% agarose gel electrophoresis and samples that amplified successfully were purified for subsequent sequencing, which was carried out in a 3130 Genetic Analyzer sequencer (Applied Biosystems).

Phylogenetic analysis

Bayesian inference was used to build a phylogenetic tree with the sequences acquired in the lab and those previously published in MalAvi (Bensch et al. 2009) and Genbank public databases, using BLAST. The nucleotide substitution model was selected with jModeltest 2.1.7 (Darriba et al. 2012) using Akaike's Information Criterion (AIC) to select the best-fit model of evolution to our DNA data. The software selected TIM2+G model considering only variation among sites on the alignment. Bayesian analysis was run in MrBayes version 3.2.2 (Ronquist et al. 2012) and two independent runs of 5×10^7 generations were conducted with four chains, sampling every 500 generations. A 50% majority-rule consensus tree was obtained, showing nodes with a posterior probability of 0.5 or more. Bayesian posterior probability values were calculated from the remaining sampled trees after 7500 (25%) burn-in samples were discarded to only include trees obtained after stationarity was reached (Ronquist and Huelsenbeck 2003). The consensus tree was displayed in FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Statistical analysis

Prevalence, mean parasitaemia, and their respective 95% confidence intervals were calculated with the Quantitative Parasitology QPweb software (Reiczige et al. 2013). For

95% confidence intervals of prevalence we used 5000 bootstrap replications through the Sterne method for confidence interval estimation (Efron 1987; Rózsa et al. 2000) and bootstrap confidence intervals were calculated according to Efron and Tibshirani (1993) and Rózsa et al. (2000). Prevalence and parasitaemia were compared between taxa (*Plasmodium* sp. vs. *Haemoproteus* sp.) using a Chi-square test, and a Generalized Linear Model (GLM) using the negative binomial distribution, respectively.

The effects of explanatory variables (habitat type, season, year, and site) on haemosporidian prevalence and parasitaemia (only parasitized samples used) were assessed through Generalized Linear Mixed Models (GLMMs, Paterson and Lello 2003) using the binomial error and the logistic link function for prevalence, and the poisson distribution for parasitaemia. Site and year were used as random factors. Data from the CH site were discarded from these analyses due to the presence of high levels of heavy metals in soil in this area. We calculated conditional R^2 values, to evaluate the fit the data (Nakagawa and Schielzeth 2013). These analyses were implemented using the "lme4" (Bates et al. 2015), MuMIn packages (Nakagawa and Schielzeth 2013; Barton 2015) in R version 3.3.1 (R Development Core Team 2011).

Results

We obtained a total of 104 BTSP blood samples. Most of the infected samples had single infections by either *Haemoproteus* sp. (*Parahaemoproteus* sp.) or *Plasmodium* sp. One individual showed a co-infection by *Haemoproteus* sp. (*Parahaemoproteus* sp.) and *Trypanosoma* sp. identified by microscopy, and it was included in all further statistical analyses as a positive sample for haemosporidians. Most infections were diagnosed by both methods (molecular diagnosis and microscopy). Blood smears were not available for eight individuals, and blood samples for molecular analyses were lacking for six individuals. For these samples, a second PCR run or examination of the entire smear was performed.

Prevalence and parasitaemia were higher in *Haemoproteus* sp. (*Parahaemoproteus* sp.) than in *Plasmodium* sp. (prevalence: $X^2 = 7.03$, df = 1, $P = 0.007$ parasitaemia: $F = 4515$, $P = 0.01$, Table 1). Four cases of infection (three by *Haemoproteus* sp., and one by *Plasmodium* sp.) were detected only through molecular diagnosis. For both taxa, *Plasmodium* sp. and *Haemoproteus* sp., gametocytes (macro and microgametocytes) were detected, and meronts were observed for *Plasmodium* sp. (Figure 2). Two juvenal BTSP individuals in their hatching year (HY) had been infected by *Haemoproteus* sp. (*Parahaemoproteus* sp.) at the time of capture, indicating that infection indeed takes place in the study area (Valkiūnas 2004). For a total of 81 samples, initial molecular screening yielded no infection and the analyses were repeated. The second PCR run confirmed that no false negatives had been obtained for these samples.

Table 1. Percentage haemosporidian prevalence and mean parasitaemia for the BTSP in scrubland sites at the highland plateau of San Luis Potosí, Mexico. n=total number of samples; SD=Standard deviation; CI= Confidence intervals. Sampling sites: CH=Charcas; GV= Guadalupe Victoria; PSG=Presa de Santa Gertrudis; LC= La Cardoncita. Habitat types: MDS=Moderately degraded scrubland; HDS=Highly degraded scrubland; rbc=Red blood cells; B=Breeding season; NB=Non-breeding season.

			Prevalence	**Parasitaemia (rbc infected / 10,000 rbc)**
Group	**Infected individuals**	**n**	**% (CI)**	**Mean (SD)**
Overall	23	104	22.1 (14.7 – 31.2)	77.8 (77.11)
Haemoproteus sp.	18	104	17.3 (10.9 – 25.9)	93.9 (197.3)
Plasmodium sp.	5	104	4.8 (1.9 – 10.9)	2.33 (0.57)
CH-MDS	2	8	25.0 (4.6 – 63.5)	-
GV-MDS	3	23	13.0 (3.6 – 32.4)	70.33 (81.9)
GV-HDS	2	11	18.2 (3.3 – 50.0)	14.5 (17.6)
PSG-MDS	9	22	40.9 (22.2 – 61.8)	38.5 (58.5)
PSG-HDS	5	22	22.7 (9.4 – 45.3)	169.8 (327.9)
LC-MDS	0	5	0	-
LC-HDS	2	13	15.4 (2.8 – 43.4)	-
MDS	12	50	24.0 (13.7 – 37.9)	49.11 (63.8)
HDS	11	54	20.4 (11.4 – 33.2)	110 (261.4)
B	17	58	29.3 (18.8 – 42.2)	95.08 (205.9)
NB	6	46	13.0 (5.8 – 25.9)	21.5 (20)

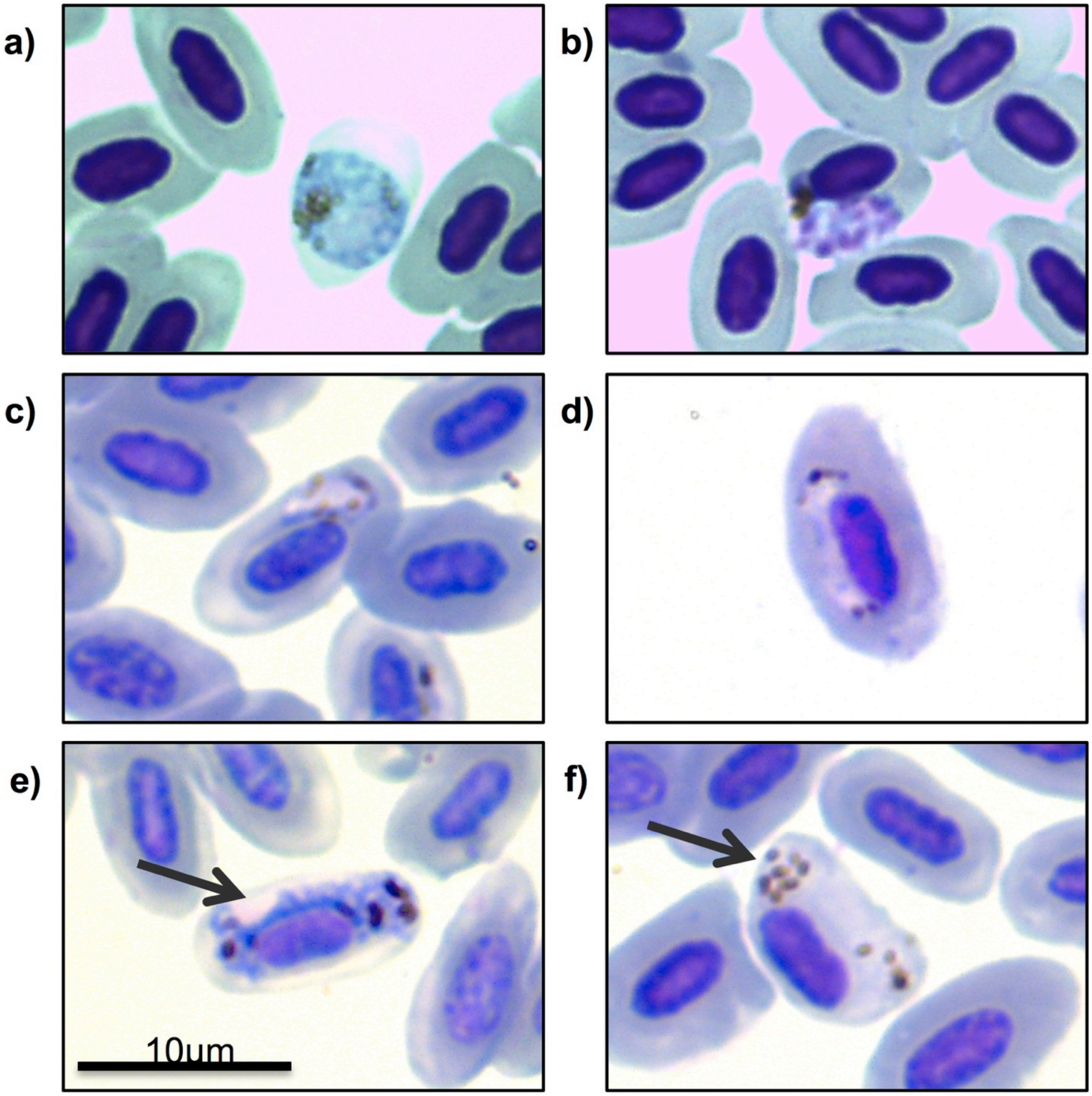


Figure 2. Blood smears with erythrocytes infected by haemosporidians: A) Microgametocyte from *Plasmodium* sp., where nucleus displacement of erythrocyte is observed; B) Meront from *Plasmodium* sp.; C) and D) immature gametocytes from *Haemoproteus* sp.; E) Macrogametocyte from *H. coatneyi*, with parasite nucleus in subterminal position (arrow); and F) Microgametocyte from *H.* coatneyi filling the erythrocyte up to it's poles (arrow) and with a dumbbell-shape

Amplicons of cytochrome *b* gene with a size of 504 bp were obtained from 20 individuals (GenBank accession numbers: KX811223 - KX811242; see Appendix 1). Two main clades were identified through the phylogenetic tree, representing *Haemoproteus* sp. (*Parahaemoproteus* sp.) and *Plasmodium* sp. (Figure 3). Two lineages whose sequences had not been previously reported corresponding to *Haemoproteus* sp. (*Parahaemoproteus* sp.) had large sequence divergence from the other known lineages (AMPBIL02: 0.82% sequence divergence, and AMPBIL01: 2.00% sequence divergence). Based on the criterion defined by Bensch et al. (2009), that a 0.2% sequence divergence (more than one nucleotide) suffices to separate lineages, our data suggest that these sequences differ from lineages previously described. The *Plasmodium* sp. clade does not define the relationship among sequences in their basal position. However, for the purpose of this study, this analysis was sufficient to distinguish three main clades within this genus (Figure 3).

We identified *H. coatneyi* through morphology analysis based on Valkiūnas (2004) (Figure 2) and on sequences acquired through laboratory analysis, which were similar to those reported in MalAvi and Genbank databases corresponding to this species. Indeed three sequences obtained from González *et al.* (2015) and one from Moens and Pérez-Tris (2016) have 99.80% and 100% matches, respectively, with respect to most sequences identified as *H. coatneyi* in our samples. *Plasmodium relictum* was detected in one individual as indicated by 100% match with sequences from web databases (GenBank and MalAvi) (Figure 3). However, identification of this species through microscopy was not completely reliable due to low parasitaemia.

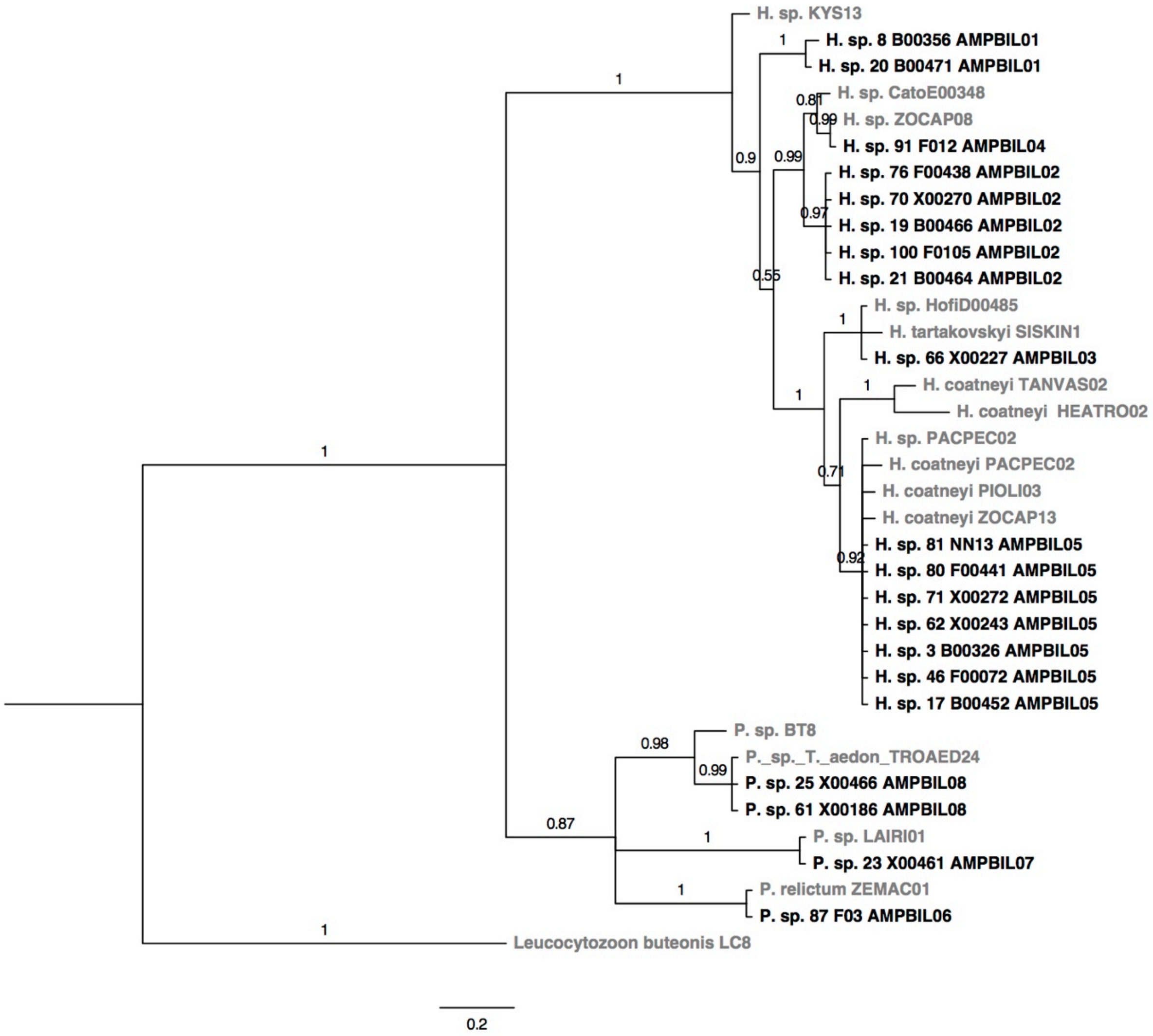


Figure 3. Phylogenetic relationships among haemosporidian lineages found in BTSP blood samples in scrubland sites at the highland plateau of San Luis Potosí, Mexico. Black fonts represent sequences acquired in this study, and grey fonts represent sequences from MalAvi and GenBank (for more details see Appendix 1). Posterior probabilities (node support) are presented in each branch. Five lineages belonging to *Haemoproteus* sp. (AMPBIL01-AMPBIL05), and three lineages belonging to *Plasmodium* sp. (AMPBIL06-AMPBIL08) were identified

Percentage and mean prevalence and parasitaemia for each level of haemosporidian taxa, site, habitat quality, and season are shown in Table 1. Generalized linear mixed models revealed that prevalence varied between seasons (z = -2.20; P = 0.02; Figure

4a; Table 2). The fit of the GLMM explaining prevalence was moderate (R^2= 0.20). Haemosporidian prevalence was higher during the breeding than the non-breeding season (Table 2, Figure 4a). The effect of habitat type on prevalence was not significant (*z* = 0.34; *P* = 0.73), but there was some variation in the data associated to site (SD = 0.42). On the other hand, the GLMM explaining parasitaemia fit reasonably well to the data (R^2= 0.83). Observed parasitaemia values for different levels of predictors (habitat and season) are shown in Table 1. Parasitaemia was higher in breeding than non-breeding season (*z* = -2.93; *P* = 0.003; Table 2, Figure 4b). Some substantial amount of variation was attributable to between-site (SD = 3.11) and between-year (SD = 1.54) differences. Finally, the effect of habitat type on parasitaemia was not significant (*z* = 0.40; *P* = 0.68).

Table 2. Parameter estimates, standard errors (SE), confidence intervals (CI; L=lower and U=Upper), and significance (*P* value) for predictor variables explaining haemosporidian prevalence and parasitaemia in BTSP in scrubland-dominated sites at the highland plateau of San Luis Potosí, Mexico. Abbreviations are as in Tables 1 and 2. Bold numbers indicate significance *P* values. * Indicate significant *P* values.

Parameter	**Level**	**Estimate**	**SE**	**CI (L)**	**CI (U)**	***P*-value**
Prevalence						
Intercept	-	-0.96	0.63	-2.21	-0.23	0.12
Habitat type	MDS	-1.54	0.70	-2.92	-2.91	0.73
Season	NB	0.24	0.72	-0.78	-1.15	0.02*
Parasitaemia						
Intercept	-	1.75	2.20	-2.55	6.07	0.42
Habitat type	MDS	1.29	3.19	-4.96	7.56	0.68
Season	NB	-0.46	0.16	-0.78	-0.15	0.003*

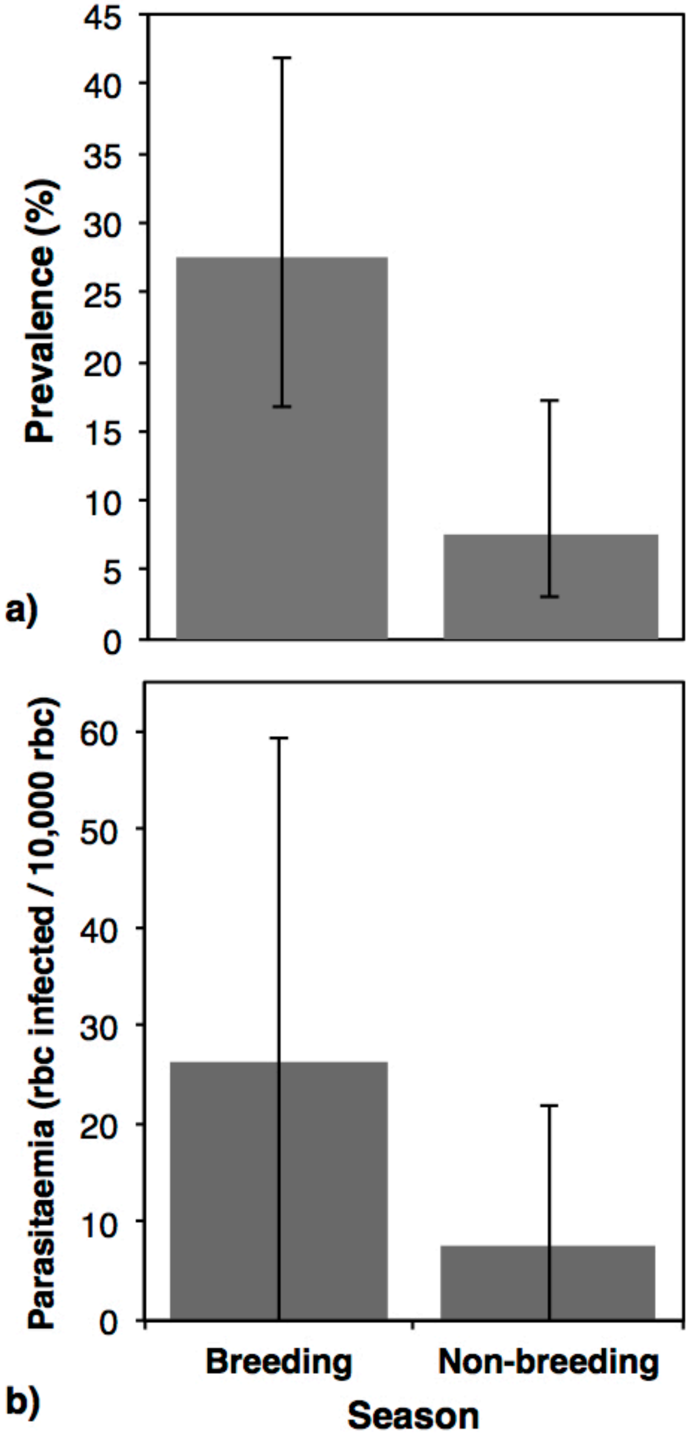


Fig. 4 Mean haemosporidian prevalence (a) and parasitaemia (b) during the breeding and non-breeding seasons in the BTSP in scrubland sites at the highland plateau of San Luis Potosí, Mexico. Error bars indicate standard errors around the mean

Discussion

Our results support the hypotheses that prevalence and parasitaemia vary between seasons. On the other hand, our hypothesis related to differences in haemosporidian prevalence and parasitaemia between “Yucca forests” (MDS) and creosotebush scrubland (HDS) was not supported by our data. The prevalence value that we recorded for BTSP is low in comparison with values previously reported for some but not all other bird species from dryland habitats (Deviche et al. 2005; Fokidis et al. 2008; Reinoso-Pérez et al. 2016). Our results are not surprising because direction and magnitude of the response may vary among bird species depending on their idiosyncrasies in terms of habitat preferences and haemoparasitism effects in different habitat types (Deviche et al. 2005; Fokidis et al. 2008; Chasar et al. 2009; Loiseau et al. 2010; Belo et al. 2012; Astudillo et al. 2013; Reinoso-Pérez et al. 2016; Hernández-Lara et al. 2017). Fokidis et al. (2008) found greater incidence of infection in rural than in urban areas in most of the species involved in a study from a semiarid area in Arizona, USA. However, the Curved-billed thrasher (*Toxostoma curvirostre*) showed no difference in haemosporidian prevalence between habitats. Patterns of intensity of haemosporidian infection in our study species also differed from those reported for other species by Reinoso-Pérez et al. (2016) in the same region; whereas we recorded no variation in parasitaemia in the BTSP in relation to habitat degradation, higher parasitaemia was recorded for the Canyon towhee and the House finch in the most degraded habitat types (village and creosotebush scrubland; Reinoso-Pérez et al. 2016). Differences in habitat preference (i.e. the BTSP avoids urban areas and is more adaptable to arid conditions than the

Canyon towhee and the House finch; Johnson et al. 2002b; Coe and Rotenberry 2003) may have something to do with these interspecific responses.

Parasitaemia may vary greatly among individuals (Valkiūnas 2004), and results inevitably associate to the stage of infection and individual health condition in which birds are captured. Therefore, some of the unexplained variation in parasitaemia that we recorded is likely related to differences among individuals in terms of the infection stage in which the capture event took place. Variation in parasitaemia may also respond to temporal and spatial variables. Dryland habitats exhibit highly significant variation in temperature and precipitation among years (Safriel et al. 2005), and these differences could account for some of this variation in parasitaemia. However, the result of the generalized mixed effects model showed that there is only a very slight variation in parasitaemia that is attributable to between-year and between-site differences. Long-term studies would help better understanding the ecological effects that influence parasitaemia. In addition, spatial variation in some environmental factors such as macro (Tella et al. 1999; Renner et al. 2016) or microhabitats (Ryckman 1960) in dryland areas could influence conditions for the proliferation of vector populations. These factors should be identified and geo-referenced, and their association to haemoparasitism should be characterized subsequently.

As described above, season was the most important factor influencing prevalence and parasitaemia in the BTSP. Extrinsic factors such as rain, which vary with season, may promote increased vector activity during the bird breeding season (Ryckman 1960; Valkiūnas 2004), and influence temporal changes in prevalence. The onset of the breeding activity in the BTSP and in most desert birds takes place in early spring or in midsummer. This period coincides with the initiation of the rainfall period (Johnson et al. 2002; Small et al. 2007). On the other hand, the higher prevalence and parasitaemia during the breeding season may also be associated to immune performance which may decrease at this time due to the increased reproductive activity (Foo et al. 2016). During reproduction, changes in reproductive hormones, specifically increased testosterone levels exert immunosuppressive effects and subsequent susceptibility to infection and proliferation. This type of response has been well documented in avian species (Deviche and Parris 2006) and other vertebrate taxa (Hillgarth and Wingfield 1997; Nordling et al. 1998; Klein 2004; Foo et al. 2016). Possible temporal changes in prevalence and parasitaemia in relation to bird immune performance should be further studied and disentangled.

We did not explore ecological and biological aspects influencing vector populations and how these may affect prevalence in BTSP. Delgado-V and French (2012) pointed out that more research on specific mechanisms influencing parasite prevalence patterns in avian populations in human-altered landscapes is needed. Understanding effects influencing dipteran abundance and diversity, as well as prevalence in vectors in relation to landscape configuration, host habitat preferences, and seasonal variation, especially in arid regions is an important topic. We must also understand the role of seasonality in a climate change context by identifying the mechanisms that influence encounter rates among vectors, parasites, and host birds (Budria and Candolin 2014). Van Riper III et al. (2014) modeled the BTSP future breeding areas and predicted gains in its distribution through southwestern United States. Considering the importance of season on risk and intensity of infection, and the potential changes on the rate of encounter owing to distributional changes of the BTSP in the future (including vectors; see Møller, 2010), we encourage investigations of possible mechanisms affecting host-vector-parasite interactions in BTSP and other bird species.

The sequences of *H. coatneyi* that we recorded match three haplotypes from samples of different bird species obtained in previous studies (González et al. 2015; Moens and Pérez-Tris 2016). Two of these reports came from neotropical migrant hosts (*Piranga rubra and P. olivaceae*: González et al. 2015) sampled in Colombia. These two intra-tropical migrant bird species have been identified functioning as hosts of *Haemoproteus* sp. in Nearctic areas (Lousiana, USA: Garvin et al. 2006). Our study area maintains an important flux of transient migrant birds (Howell and Webb 1995) that could potentially be involved in blood parasite transmission processes among resident and migrant avian populations (Garvin et al. 2006; Levin et al. 2013). Four samples from *Plasmodium* sp. also matched lineages from host species from North America (Nebraska, USA: Perkins et al. 2002; Arizona, USA: Pacheco et al. 2011; California, USA: Walther et al. 2015), one of which has also been recorded infecting resident birds and some that migrate between the Galapagos islands and North American sites (Levin et al. 2013). Finally, two *Haemoproteus* sp. samples showed similar sequences as those reported previously in the same study area (Reinoso-Pérez et al. 2016), indicating that different bird species share similar haemosporidian lineages within our study region. Consequently, future studies should investigate ecological interactions between host and parasite communities, and consider both resident and migrant bird species in the region. Such studies would help understanding potential transmission of haemoparasite lineages within and between avian populations within this important transient area.

Conclusions

A high diversity of haemosporidian lineages was present in our study area. This may indicate the presence of complex dynamics regarding transmission of haemoparasites. This diversity included two lineages not previously documented. The response by BTSP in prevalence and parasitaemia to human-altered landscapes differs from those previously recorded in other species inhabiting the region such that prevalence and parasitaemia in this species were unaffected by degree of habitat degradation. Prevalence and partasitemia, however, were higher in the breeding than the non-breeding season. Therefore, seasonality should be an important variable to consider in studies of parasitism involving this and other bird species. Year and site effects were identified as additional sources of variation in haemoparasitism. More studies would help understanding the main mechanisms that have promoted maintenance of haemosporidian community in extreme conditions such as arid environments where water sources are scarce.

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Appendix 1

Table 3. Sequences information of haemosporidian lineages obtained from this study (Sequence 1-20) and other studies (Sequence 21-35).

ID	Sample	Host	Id. Microscopy	Id. Sequencing (From Figure 3)	Country	Site	Genebank access
Seq1	B00452	*A. bilineata*	*Haemoproteus coatneyi*	B00452_AMPBIL05	Mexico	HDS_PSG	KX811223
Seq2	B00471	*A. bilineata*	*Haemoproteus sp.*	B00471_AMPBIL01	Mexico	MDS_PSG	KX811224
Seq3	B00356	*A. bilineata*	*Haemoproteus sp.*	B00356_AMPBIL01	Mexico	MDS_PSG	KX811225
Seq4	B00464	*A. bilineata*	*Haemoproteus sp.*	B00464_AMPBIL02	Mexico	MDS_PSG	KX811226
Seq5	F03	*A. bilineata*	*Plasmodium sp.*	F03_AMPBIL06	Mexico	HDS_GV	KX811227
Seq6	X00461	*A. bilineata*	*Plasmodium sp.*	X00461_AMPBIL07	Mexico	HDS_LC	KX811228
Seq7	X00186	*A. bilineata*	*Plasmodium sp.*	X00186_AMPBIL08	Mexico	MDS_CH	KX811229
Seq8	X00466	*A. bilineata*	*Haemoproteus sp.*	X00466_AMPBIL08	Mexico	HDS_LC	KX811230
Seq9	F00072	*A. bilineata*	*Haemoproteus coatneyi*	F00072_AMPBIL05	Mexico	MDS_PSG	KX811231
Seq10	F0105	*A. bilineata*	*Haemoproteus sp.*	F0105_AMPBIL02	Mexico	MDS_GV	KX811232
Seq11	F012	*A. bilineata*	*Haemoproteus sp.*	F012_AMPBIL04	Mexico	MDS_CH	KX811233
Seq12	B00466	*A. bilineata*	*Haemoproteus sp.*	B00466_AMPBIL02	Mexico	MDS_PSG	KX811234
Seq13	B00326	*A. bilineata*	*Haemoproteus coatneyi*	B00326_AMPBIL05	Mexico	HDS_PSG	KX811235
Seq14	X00243	*A. bilineata*	*Haemoproteus coatneyi*	X00243_AMPBIL05	Mexico	MDS_PSG	KX811236
Seq15	X00227	*A. bilineata*	*Haemoproteus sp.*	X00227_AMPBIL03	Mexico	MDS_PSG	KX811237
Seq16	X00270	*A. bilineata*	*Haemoproteus sp.*	X00270_AMPBIL02	Mexico	MDS_PSG	KX811238
Seq17	X00272	*A. bilineata*	*Haemoproteus coatneyi*	X00272_AMPBIL05	Mexico	MDS_PSG	KX811239
Seq18	F00438	*A. bilineata*	*Haemoproteus sp.*	F00438_AMPBIL02	Mexico	HDS_GV	KX811240
Seq19	F00441	*A. bilineata*	*Haemoproteus coatneyi*	F00441_AMPBIL05	Mexico	HDS_PSG	KX811241
Seq20	NN13	*A. bilineata*	*Haemoproteus coatneyi*	NN13_AMPBIL05	Mexico	HDS_PSG	KX811242
Seq21	Reference	*Zenaida macroura*	NA	P. relictum ZEMAC01	USA	NA	AY099032
Seq22	Reference	*Melozone fusca*	NA	H. sp. CatoE00348	Mexico	NA	KR819000
Seq23	Reference	*Zonotrichia capensis*	NA	H. sp. ZOCAP08	Colombia	NA	KC480265
Seq24	Reference	*Haemorhous mexicanus*	NA	H. sp. HofiD00485	Mexico	NA	KR818955
Seq25	Reference	*Carduelis spinus*	NA	H. tartakovskyi SISKIN1	Lithuania	NA	KM361490
Seq26	Reference	*Hemispingus atropileus*	NA	H. coatneyi HEATRO02	Colombia	NA	KF537308
Seq27	Reference	*Tangara vassorii*	NA	H. coatneyi TANVAS02	Colombia	NA	KF537285
Seq28	Reference	*Saltator maximus*	NA	H. sp. PACPEC02	Ecuador	NA	KT373859
Seq29	Reference	*Piranga rubra*	NA	H. coatneyi PACPEC02	Colombia	NA	KF537317
Seq30	Reference	*Piranga olivacea*	NA	H. coatneyi PIOLI03	Colombia	NA	KF537326
Seq31	Reference	*Zonotrichia capensis*	NA	H. coatneyi ZOCAP13	Colombia	NA	KF537327
Seq32	Reference	*Ploceus nigricollis*	NA	P. sp. BT8	USA	NA	DQ839055
Seq33	Reference	*Geospiza fuliginosa*	NA	P. sp. LAIRI01	Ecuador	NA	KC867674

Seq34	Reference	*Troglodytes aedon*	NA	P. sp. TROAED24	USA	NA	KJ620788
Seq35	Outgroup	*Buteo jamaicensis*	NA	Leucocytozoon buteonis LC8	USA	NA	HM142921

CHAPTER 3

Influence of mining activity on the Black-throated sparrow's song (Amphispiza bilineata) in Mexican drylands

Influence of mining activity on the Black-throated sparrow's song (*Amphispiza bilineata*) in Mexican drylands.

Abstract

Human disturbances may include disruption or alteration of biological interactions and animal behavior. Bioacoustics is a useful tool to assess the status of animal populations which may have been affected by the disruption of specific sound signals caused by anthropogenic disturbances, such as mining activity. Song is a potential indicator of fitness for many bird species. However, responses of acoustic traits to exposure to pollution have not been deeply studied. I evaluated to what extent the acoustic structure of the Black-throated Sparrow (BTSP) responds to exposure to mining activity in dryland habitats of the highland plateau of San Luis Potosi. A negative relationship between lead concentrations and shrub density, and two acoustic characteristics including song rate and song switching rate was recorded. In addition, two other song traits including syllable complexity in trill and number of song types (repertoire size) are negatively associated to arsenic and lead concentrations, respectively. Mining activity had a stronger influence in repertoire than spectro-temporal variables. Also habitat structure had a noteworthy effect in the BTSP's song structure, a factor that had not been considered in most previous studies. The current study provided a general perspective of how heavy metal pollution and other environmental factors associated to mining activity could influence song structure in birds.

Introduction

Effects of human-related habitat changes on organisms have been evaluated at different levels of organization, regions and environments. Some effects of these changes may include disruption or alteration of biological interactions, ecological complexity, and animal behavior (Lindenmayer and Fischer 2006; Fischer and Lindenmayer 2007). These responses are closely related to impacts such as chemical pollution, overhunting, or urbanization among others (Lindenmayer and Fischer 2006). The concept of “info-disruption” refers to alterations of chemical communication within organisms by pollution, with potentially sub-lethal or lethal effects (Lürling and Scheffer 2007; Boyd 2010). However, a different type of “info-disruption” including alterations in visual or acoustic communication between organisms should also be considered.

Bioacoustics studies provide useful alternatives for assessing the status of animal populations, which may have been affected by the disruption of specific sound signals (Gaunt and McCallum 2004; Laiolo 2010). Bird song is a well-studied sexually selected trait (Slater 2003; Marler and Slabbekoorn 2004; Catchpole and Slater 2008; Laiolo 2010). It provides functions such as territory establishment and mate attraction (Catchpole and Slater 2008). Song in birds is a function of high evolutionary value in terms of sexual selection; females can be attracted a particular type of structure in the song of a male over another. Therefore, the song is a potential indirect indicator of fitness for many bird species (Nowicki et al. 1998; Buchanan 2003).

The "nutritional-stress hypothesis" (Nowicki et al. 1998) suggests that the song learning system (neuronal and hormonal components in song memorization) and production of song are indicators of male fitness. It has been shown that certain brain structures, responsible for the vocal system, may be negatively affected when exposed to stress factors at an early stage of development, as in the case of malnutrition of fledglings. Buchanan *et al*. (2003) suggested renaming the above process the "developmental-stress hypothesis", proposing the existence of a wide spectrum of stress factors (including malnutrition), which could be related to the song learning system.

Chemical pollutants dumped in the environment by mining activity, including heavy metals and metalloids (Hg: Hallinger et al. 2010; As and Pb: Gorissen et al. 2005; Cd: Huang et al. 2015) have been identified as deterministic factors that could influence song development in vertebrate taxa, including birds. These compounds modify the reproductive system by interfering with gamete production, hormone functions, and mating system factors such as signs and behavior (Gore 2007; Shenoy and Crowley 2011). Specifically, biological and ecological effects of heavy metals and metalloids have been scarcely studied in avian species (Gorissen et al. 2005; Hallinger et al. 2010; McKay and Maher 2012). However, it is known that exposure to these pollutants may alter the acoustic structure in organisms. For instance, Hallinger et al. (2010) and McKay and Maher (2012) associated high levels of mercury (Hg) to alteration of song structure in different bird species. These studies found lower diversity of types of notes, lower tonal frequencies (Hallinger et al. 2010), higher maximum tonal frequency, and faster song rate (McKay and Maher 2012). Gorissen *et al*. (2005) reported that Great tits

(*Parus major*), inhabiting areas polluted by lead and other heavy metals, showed a limited song repertoire and less song production than those from the non-contaminated sites. Establishment of mining complexes also modifies vegetation cover in the surroundings of these human-altered areas (Almeida-Filho and Shimabukuro 2002; Moreno-de las Heras et al. 2008; Sonter et al. 2014), which in turn may also influence bird song structure (Podos et al. 2004; Boncoraglio and Saino 2007; Briefer et al. 2010).

Changes in vegetation cover across the landscape may influence the song of some bird species (Boncoraglio and Saino 2007). Attenuation and degradation of sound are factors related to some specific environmental variables such as humidity, temperature, etc. (Catchpole and Slater 2008). However, biotic factors also relate to the mode in which birds optimally transmit signals to their receivers. Sound degradation, by physical obstacles (e.g. foliage, shrubs, tree density, etc.), may shape some song features. The acoustic adaptation hypothesis (Morton 1975; Hansen 1979) suggests that songs characterized by low frequency, narrow bandwidth, long intervals among elements, and low frequency modulations would appear frequently in structurally complex habitats such as those with high plant or foliage density (Boncoraglio and Saino 2007).

Mining exploitation in the highland plateau of Mexico has taken place since the 16th century (SGM 2016). Accumulation of toxic elements in soil, water, plant, and animal tissue has been recorded in this region near several mining sites (Razo et al. 2004; Chapa-Vargas et al. 2010; Martínez-Villegas et al. 2013; Monzalvo-Santos et al. 2016). Monzalvo-Santos et al. (2016) reported high lead and arsenic concentrations in topsoil near an active mining site in the Charcas municipality in San Luis Potosí State, Mexico.

Moreover, metal contents in feather samples of three passerine bird species (Curve-billed thrasher – *Toxostoma curvirostre* -, Canyon towhee – *Melozone fusca* -, and Cactus wren – *Campylorhynchus brunneicapillus*) from this study area, correlated positively with soil metal concentrations (Monzalvo-Santos et al. 2016). Shrub densities in the surroundings of the mining complex in Charcas are lower in comparison to scrublands elsewhere in the region (unpublished data). However, no previous studies have investigated simultaneously how high metal concentrations and low plant densities in the surroundings of the mining complex in this region may affect bird song structure.

The Black-throated sparrow (*Amphispiza bilineata*) ranges from southern United States to central Mexico within the Chihuahua Desert (Howell and Webb 1995; Johnson et al. 2002). It is associated with open dryland habitats dominated by shrubs and grasses (Johnson et al. 2002; Coe and Rotenberry 2003), and shows some degree of breeding site fidelity. This species is a good study model for investigating effects of exposure to metals on song structure, because it defends relatively small home ranges, thus making it possible to associate ecological processes to very specific sites. Moreover, it has a complex song structure, and it is abundant within the immediacies of Charcas, San Luis Potosí, Mexico. Therefore, reasonably large sample sizes are relatively easy to obtain, and if changes in song structure related to exposure to metals occur, these changes could easily be detected.

Heckenlively (1967) studied the song structure and its variation in the BTSP and reported a vocal arrangement with an introductory complex consisting of an introductory note and/or complex of notes. This introductory complex is followed by a croak and/or buzz, until finally a trill is produced (Figure 1). This study also reported that the variation in song is high regarding to number of song types and syllables used, both at the individual and population level. Black-throated sparrows sing perched on the top of bushes, in intermediate shrub canopies, and at the ground (Heckenlively 1970). Additionally, it has been suggested that this species may have low or no sexual dimorphism on plumage and/or morphology (Pyle 1997). The reduction of sexually selected traits in the plumage of males could translate into a compensatory increase in the use of other male attributes such as vocalization, which could be expressed as the main male signal as a sexually selected character (Heckenlively 1967; Badyaev et al. 2002).

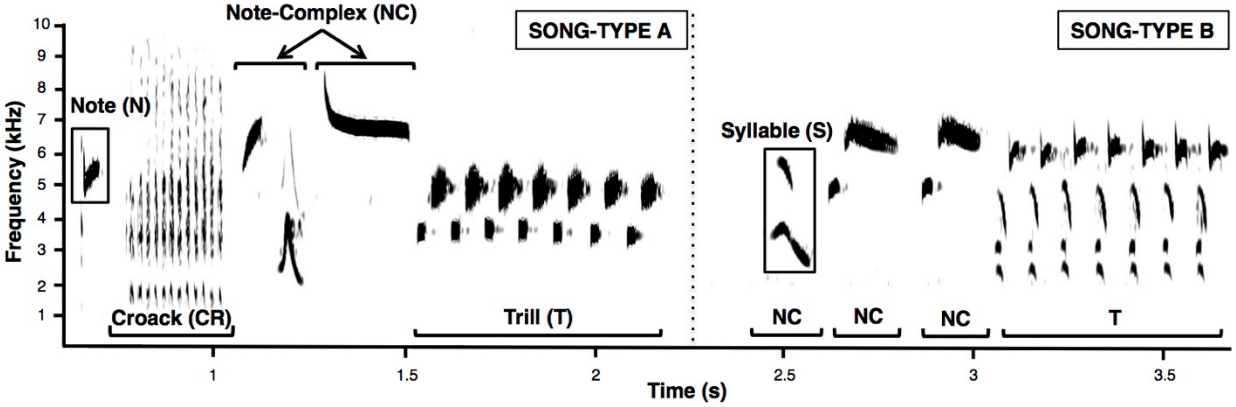

Figure 1. Spectrogram with main units and sections of BTSP’s song from one individual.

The objective of this study was to determine if the acoustic structure of the Black-throated Sparrow (BTSP) responds to exposure to mining activity in dryland habitats of the highland plateau of San Luis Potosi, Mexico. The hypothesis was that specific traits in BTSP’s song structure would differ in birds from sites with different levels of exposure to mining activity. Predictions were difficult to establish, because effects of metal exposure on song traits have not been previously investigated in this species and because the effect of exposure to metals on song structure may be confounded with that of low shrub and tree density associated with activities near to mining complex. For other bird species, shorter duration of song and reduced number of song types have been attributed to lead exposure and other heavy metals (Gorissen et al. 2005). It has also been suggested that song-trill rates (number of songs or syllables per unit of time) and song switching rate (song versatility) may be related to vocal performance and male's fitness (Podos et al. 2004). Therefore, one possibility could be that song-trill and song switching rate may change as a consequence of the combined effect of exposure to heavy metal and lowered shrub densities near the mining complex in the study area.

Method

Study area

The highland plateau of San Luis Potosí, embedded in the Mexican xerophytic region of the Chihuahua Desert (Rzedowski 2005), is located in Central Mexico. The vegetation of this region includes microphyllous scrubland, semiarid grassland, and *Yucca* dominated scrublands (*Yucca filifera* and *Yucca decipiens*; Rzedowski 2005). Three localities situated within three communal lands ("*ejido*") areas were included in this study: Presa de Santa Gertrudis (PSG), Guadalupe Victoria (GV), and Charcas (CH) (coordinates: PSG= 23.49074 N, -101.1732 W, GV= 23.34062 N, -101.19036 W, CH= 23.14810 N, -101.15593 W) (Figure 2). Different metals of commercial interest (silver, gold, lead, zinc, copper, cadmium, and iron) have been extracted for more than 400 years close to PSG and CH (Monzalvo-Santos et al. 2016; SGM 2016) but not in GV. At present, exploitation of minerals only happens near CH, where residues from mining activities are accumulated at a tailing, and where surfaces are exposed to water and wind erosion (Monzalvo-Santos et al. 2016; SGM 2016). The GV sites served as suitable reference or control sites, as mining activity has always been absent and there is no evidence of wind-borne depositions of heavy metals (Monzalvo-Santos et al. 2016).

For the purpose of this study, we chose two representative habitat types within each of the three locations; A) open habitat with an open shrub layer dominated by creosotebush (*Larrea tridentata;* density: mean= 782 shrubs/ha, SE= 99.69), and where shrubs from other genera (*Flourensia*, *Condalia*, *Celtis*, *Acacia*, *Berberis,* etc.) are present but their relative abundances is low. *Yucca* density is also low in this habitat type (mean density= 0.75 trees/ha; SD= 4.5). The second habitat type consisted of B) closed habitat dominated by *Yucca filifera* and *Yucca decipiens* (also known as "*Yucca* forest"; *Yucca* density: mean= 42.5 trees/ha, SD= 40; height: mean = 3.9m, SD= 1.5). This vegetation is indicative of a relatively well-conserved scrubland community (Rzedowski 1961). The dominant shrub genera in this habitat type are *Acacia, Flourensia*, and *Larrea*. This habitat type occurred only within GV and PSG (Figure 2).

Vegetation analysis

To evaluate the effect of habitat on bird song structure (Slabbekoorn and Smith 2002), tree and shrub density were quantified. Within each site, ten vegetation sampling points were established at random locations. In each of these points, two-11.2-m x 1.5 m transects (i.e., James and Shugart 1970) intersecting each other were established. In each transect, the number of all trees and shrubs were counted. These data were subsequently used to quantify plant density (average number of shrubs and trees per ha).

Heavy metals

Lead and arsenic concentrations in tissue of individual BTSP birds were unavailable for this study because intensive efforts to visually find and record individuals that had previously been captured to take tissue samples and to mark with color bands yielded very limited success. However, previous findings from the same study area showed that metal concentrations in soils vary significantly among study sites, and that metal concentrations in soils correlate strongly with metal concentrations in bird feathers (Monzalvo-Santos et al. 2016). Therefore, characteristic mean heavy metal (Pb and As) concentrations (mg/kg) of soil samples in each site (Monzalvo-Santos et al. 2016) were used as different levels of a factor for statistical analysis.

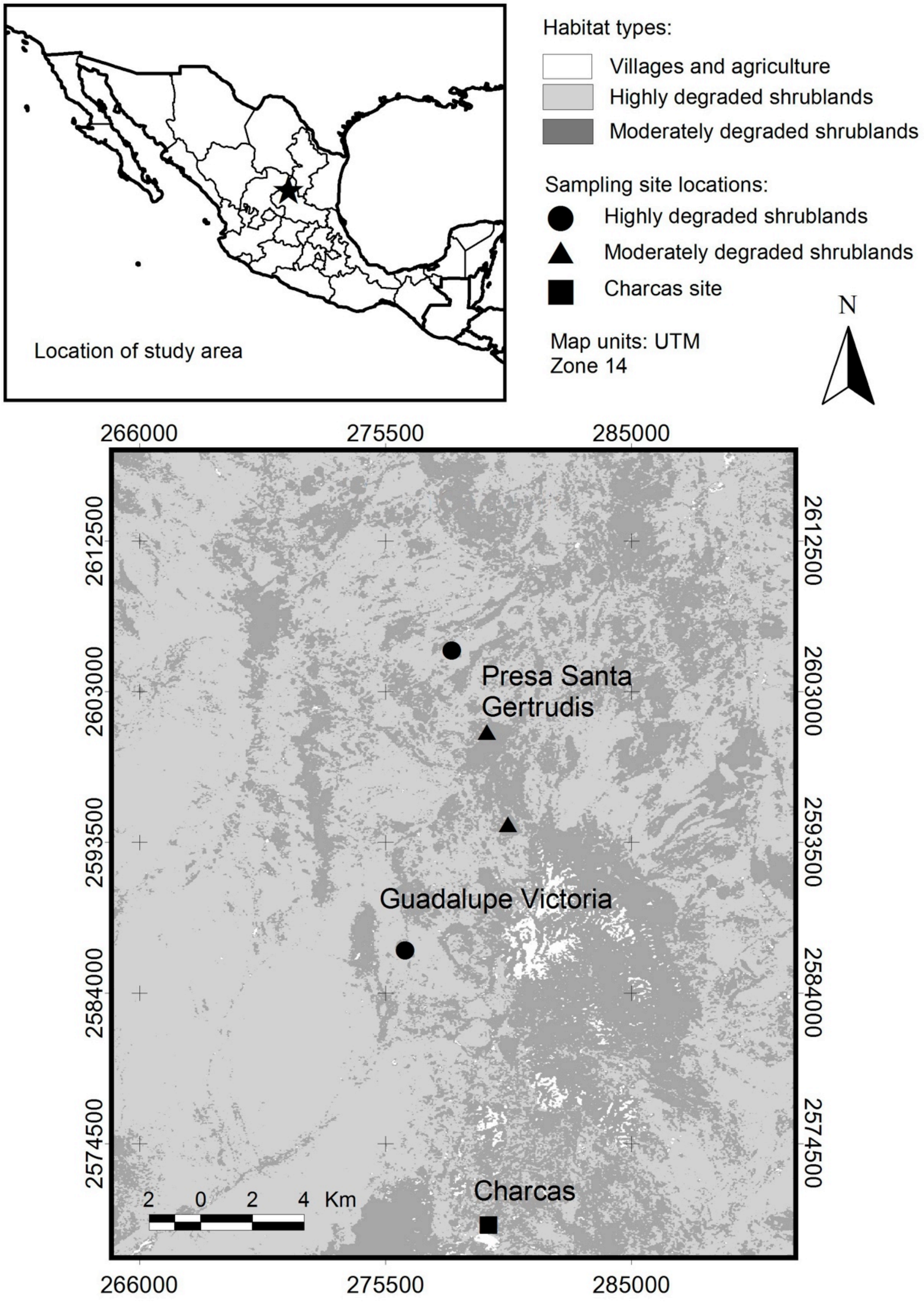

Figure 2. Map of the study area located in a portion of the Highland Plateau of San Luis Potosi. Five sites were considered: two sites categorized as moderately degraded scrubland and three as highly degraded scrubland.

Song recordings

In each study site, bird song recordings were obtained during the breeding seasons (May-July) of 2014 and 2015. For this purpose, three to four parallel 500 m long transects located at a distance of 300 to 500 m from each other were established in each site. These transects were traversed from the first hour after sunrise (6:00am – 7:00am) until noon, or until bird activity began to diminish. To prevent repetition of the recording of an individual bird previously registered, each transect was walked only once and only in one direction. Therefore, the assumption that each individual was recorded only once is reasonable. Each individual bird was recorded for at least 300 seconds or until vocalizations stopped (at least 15 songs were considered from each individual in further statistical analyses). Recordings were obtained using a Telinga twin science MK2 parabolic microphone and a Tascam DR100 MKII recorder. Signals were recorded at a sample rate of 44.1 kHz and with a sample size of 16 bits in WAV audio files.

Data analysis

Song measurements

Acoustic measurements were conducted using Raven v1.5 (Bioacoustics Research Program 2014). Spectrogram parameters were set using a 300-point (6.8 ms) Hann window (3 dB bandwidth = 211 Hz), with Discrete Fourier Transform (DFT) size of 1024 samples, and 80% overlap. Yielding time and frequency measurement precision were 1.36 ms and 43.1 Hz. Acoustic measurements were obtained for all songs recorded.

Because a large amount of variation exists in the BTSP's song (Heckenlively 1970) and because its "croack" phrase (notes in the song with the lowest frequency; Figure 2) was not easily detected on the spectrograms, the focus of the analysis concentrated on the trill, plus a few song variables (Figure 3). The following acoustic variables were quantified: a) *Spectro-temporal variables*: maximum frequency of song and trill, minimum frequency of trill, peak frequency of trill and song, bandwidth of trill, song and trill rate (number of songs and syllables per minute, respectively), and trill length (90% duration) (Figure 3). b) *Repertoire variables*: number of song types (repertoire size), number of syllables in trill, syllable complexity in trill (number of notes in each syllable or type of syllable in trill according to Heckenlively 1970) and song switching rate (song versatility). This last variable was calculated using the number of transitions to a distinct song type divided by total number of transitions (Langmore 1997; Podos 2007; Opaev 2016). The song variables described above have been frequently used in avian bioacoustic studies (e.g. Gorissen *et al*. 2005, Hallinger *et al*. 2010, McKay & Maher 2012,). Terminology and classification of each unit of the BTSP's song (song type, phrase, syllable, notes, etc.) was adopted from Heckenlively (1970). Visual identification and classification of syllables, notes, and song types were conducted for each recording through spectrograms.

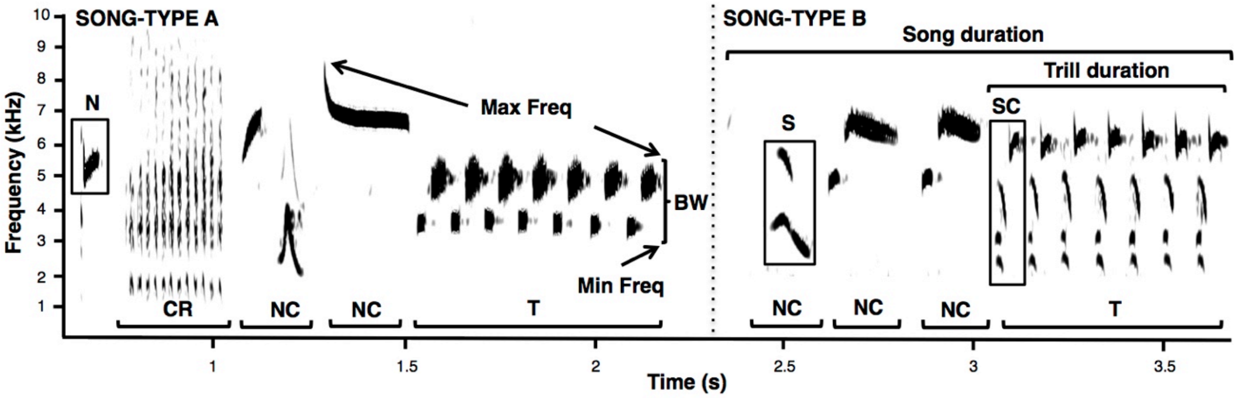

Figure 3. Spectrogram with main units, sections, and measurements of BTSP's song. A= First song, B= Second song, N= Note, S= Syllable, SC= Syllable complexity (number of notes in syllable of trill), CR= Croak, NC= Note-complex, T= Trill, BW= Bandwidth, Dashed line= transition between consecutive songs.

Statistical analysis

Generalized Linear Mixed Models (GLMMs) were used to evaluate the effects of explanatory variables in each song variable. Shrub density, tree density, and arsenic and lead concentrations from soil samples were used as independent variables. For each BTSP individual, ten songs were randomly selected. All spectro-temporal variables (max-min frequency of song and trill, peak frequency of song and trill, duration of song and trill, trill rate, and bandwidth of trill), as well as only two repertoire variables (number of syllables and syllable complexity of trill) were used in the analyses. In these analyses (Appendix, Table 1 and 2), a nested design was applied in which observations from each individual within site were used as the random factor. The remaining repertoire variables (song rate, song switching rate, and number of song types) were analyzed using only site as a random factor. Gaussian distribution was used in most of the

GLMMs, but Poisson distribution was applied to some specific response variables (number of syllables in trill, syllable complexity in trill, and number of song types).

Model selection and multi-model inference for GLMMs were performed according to Grueber et al. (2011) and Burnham and Anderson (2002). Akaike's Information Criterion corrected for small sample sizes (AIC_c) and Akaike weights (w_i) were used to evaluate the effects of predictors influencing song variables. The global models contained all the predictor variables previously described. These explanatory variables were standardized according to Gelman (2008), and then a full set of submodels was generated. Akaike differences (ΔAICc), AICc, and w_i values were used to rank models from best to least supported by the data. The top models were selected using a 95% confidence of sum of weights as a cut-off criterion for each analysis to obtain a model set to estimate model-averaged parameters (Burnham and Anderson 2002; Grueber et al. 2011). Then, model-averaged parameter estimates based on parameters from the top model sets acquired in the previous step were obtained of using the natural average as suggested by Grueber et al. (2011). For each parameter, its relative importance value (RIV) of each GLMM analysis was calculated using Akaike weights from the top model sets, where the most important value is close to 1 and the least important close to 0 (Grueber et al. 2011; Burnham 2015); their significance values (*P*-value) were also obtained. “MuMin” (Barton 2015), “lme4” (Bates et al. 2015), and “arm” libraries (Gelman and Su 2016) were used to perform GLMMs, construct model sets, perform model averaging for each set, and to generate predictions using the R software (R Development Core Team 2011).

Results

A total of 42 male individuals were recorded in all five sampling sites (GV-open= 10; GV-closed= 6; PSG-open= 8; PSG-closed= 8; CH-open= 10). Average song types for all individuals was 3.14 (SE= 0.48) and a total of 157 song types were recorded. The highest number of song types in one individual was nine and minimum one. Because a high correlation value between heavy metal levels and plant density was recorded (r > 0.6) in GLMM's analyses, a variance inflation factor was performed and confirmed multicolinearity mainly by shrub density (shrub density= 13.02; tree density= 6.76; Pb= 7.06; As= 6.04). The sets of the best-supported models whose parameters were averaged (only models within 95% confidence of sum of weights) for each GLMM analysis are presented in Table 1 (Appendix) for spectro-temporal variables, and in Table 2 (Appendix) for repertoire variables. According to model-averaged parameter estimates, the most important effects on the responses were as follows:

Spectro-temporal variables: Song rate was the only spectro-temporal variable that was strongly influenced by predictors; it decreased with increasing soil Pb concentrations (*P*= 0.013; RIV=0.93; Figure 3), and with increasing shrub density (*P*= 0.04; RIV=0.67; Figure 4; Table 3).

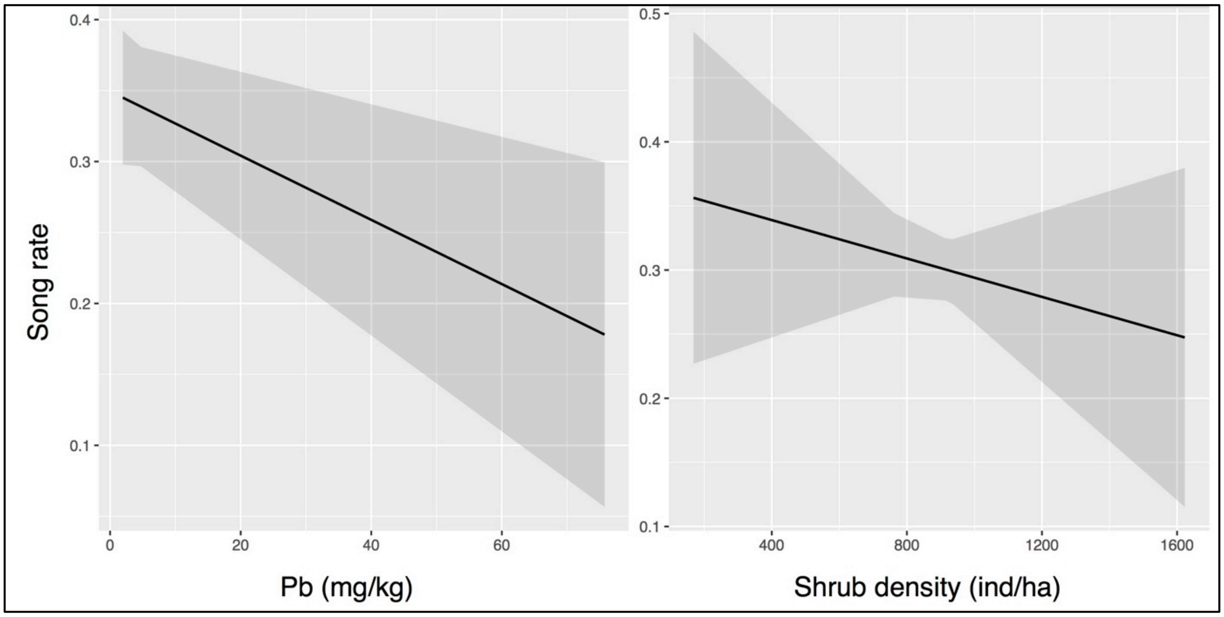

Figure 4. Effect of lead concentrations in soil and shrub density on song rate in the BTSP obtained by GLMM analysis. Gray area in plot indicates 95% confidence intervals.

Repertoire variables: syllable complexity in trill decreased with increasing concentrations of As in soil (*P*= 0.04; RIV= 0.93; Figure 5; Table 4). On the other hand, number of song types decreased with increasing Pb in soil (*P*= 0.05; RIV= 0.72; Figure 6; Table 4), and also a negative relationships between song switching rate and lead (*P*= 0.012, RIV= 0.95; Figure 7; Table 4), and shrub density (*P*= 0.06; RIV= 0.76; Figure 7; Table 4) were detected. No other repertoire variables were strongly influenced by the predictors included in the study (Table 4).

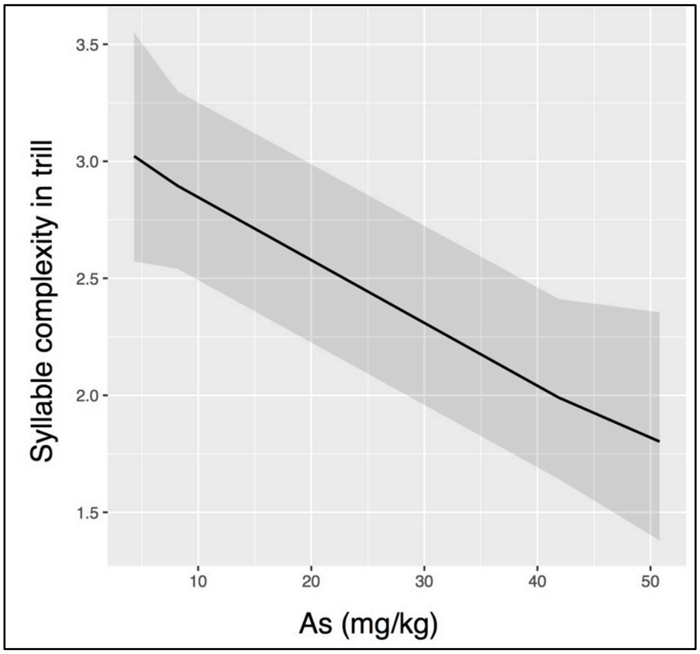

Figure 5. Effect of arsenic concentrations in soil on syllable complexity in the trill in the BTSP obtained by GLMM analysis. Gray area in plot indicates 95% confidence intervals.

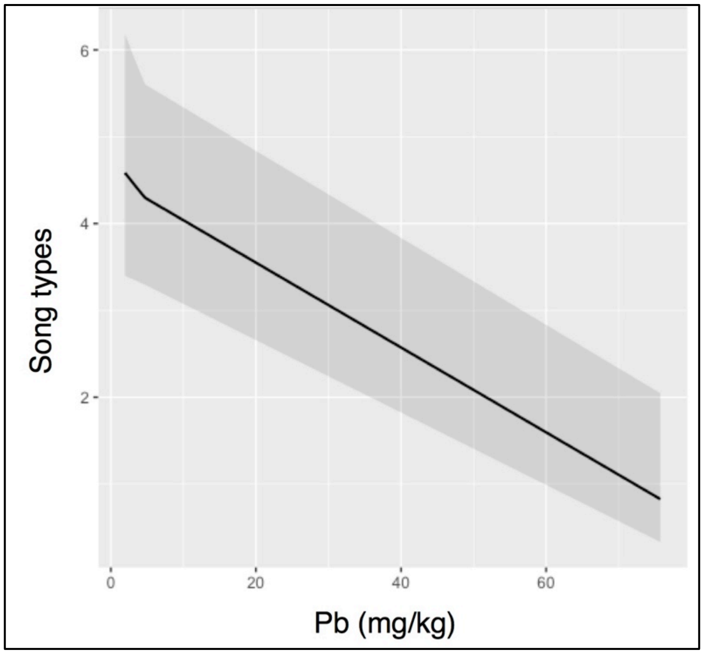

Figure 6. Effect of lead concentrations in soil on number of song types in the BTSP obtained by GLMM analysis. Gray area in plot indicates 95% confidence intervals.

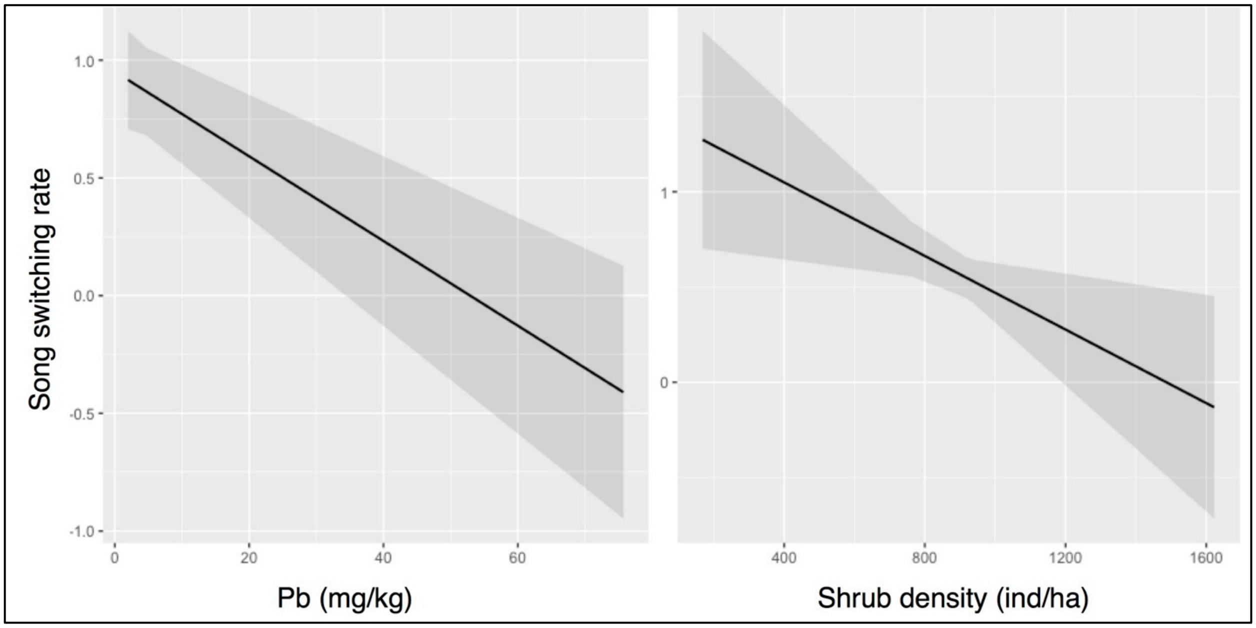

Figure 7. Effect of lead concentrations in soil and shrub density on song switching rate in the BTSP obtained by GLMM analysis. Gray area in plot indicates confidence intervals.

Discussion

Lower song rates and song switching rate were associated both with increasing soil Pb concentrations and shrub density. By contrast, lower syllable complexity in trill and number of song types were correlated only with higher As and Pb in soil.

For the current study, it was not possible to measure metal concentrations in BTSP individuals whose songs were recorded. However, it was assumed that trends in metal concentrations in the BTSP are similar to those previously reported in three passerine birds in the same study region (Monzalvo-Santos et al. 2016). This assumption is based on the similarity in the feeding behavior between BTSP and the species studied by Monzalvo-Santos and colleagues; most of the time all these four bird species obtain their food from the ground (Johnson et al. 2002) suggesting they ingest contaminated food. The lack of information of potential metal concentrations in BTSP tissue of individual birds limited the ability to disentangle potential confounding effects of vegetation density and metal contamination. In fact, it is challenging to separate the effects of exposure to human-induced pollutants from land use changes, as these changes are frequently associated (Franson and Pain 2011; Sonter et al. 2014).

A previous study by Hallinger et al. (2010) failed to register an association between song rate and exposure to Hg in four bird species. This result differs from the results of the current study consisting of higher song rates in sites having the lowest Pb concentrations. Different types of metals seem to have contrasting effects on bird song characteristics, and also the response may vary among species; however many more detailed studies are needed to be able to draw general conclusions on bird species bioacoustics behavior when exposed to heavy metals.

On the other hand, more repertoire variables than other studies (Gorissen et al. 2005; Hallinger et al. 2010; McKay and Maher 2012) were correlated to lead concentrations in soil. Syllable complexity of trill (number of notes in each syllable) on the BTSP was described by Heckenlively (1970) and may indirectly indicate how birds reduce their vocal performance by using more or less than one note in each syllable of the trill. This process might be similar to the constraint caused by using a wider bandwidth, which is also an important feature of trill performance (Podos et al. 2004). In some studies, trill performance is an important song trait to induce female attraction by display high trill rate (Vallet et al. 1998) or wide frequency bandwidth (Ballentine 2004).

The variation in number of song types (between one and nine per individual) recorded in the BTSP's population recorded on the current study is consistent with results reported by Heckenlively (1970) for a population from New Mexico. The negative relation between repertoire and Pb concentrations coincide with findings of Gorissen et al. (2005). The female preference for males with larger song repertoires has been well-studied (Nowicki et al. 2000; Nowicki et al. 2002a; Nowicki et al. 2002b; Podos et al. 2004; Catchpole and Slater 2008). The results of the current study and those of Gorissen et al. (2005) suggest that male exposure to Pb in BTSP and in other bird species could negatively affect mating success. Great number of song types (repertoire size) in males produces more female display and therefore favor sexual selection (Podos et al. 2004; Catchpole and Slater 2008).

Song switching rate (song versatility) was higher in sites with moderate or no exposure to mining. These sites also had the highest shrub density. Until now, the response of this variable to exposure to mining activity had not been assessed in other similar study. Song versatility is important for both territory defense and courtship (Langmore 1997; Podos et al. 2004; Searcy and Beecher 2009). Higher aggressive responses have been indeed reported for other species inhabiting areas polluted by metallurgic smelter (Janssens et al. 2003). The current findings, however, suggest that song switching was lower in areas exposed to mining activity, so it appears that the effect of exposure to mining activity on aggressiveness in the BTSP in scrublands of central Mexico is not similar to the response reported for other species by Janssens et al. (2003).

Habitat structure may also influence BTSP's song characteristics and the effect of habitat structure may be inevitably confounded in case of simultaneous exposure to metals. Therefore, the results of the current study relate to exposure to mining activity, which simultaneously includes both exposure to pollutants and habitat degradation. The magnitude of the effect of vegetation structure on song traits (song rate and song switching rate) is not well understood. Shrub density was autocorrelated with Pb and As concentrations in soil, and thus may constitute a confounding variable because mining is always associated with land use changes (Frouz et al. 2008; Moreno-de las Heras et al. 2008; Sonter et al. 2014). High plant extraction has indeed taken place near CH site, where the highest pollutant levels were reported (Monzalvo-Santos et al. 2016). It is indeed complicated to locate pollution sources in well-conserved plant communities in the study region. In addition land use change types that have taken place at greater distances from mining sites differ from those observed near mining complexes where the vegetation cover has been eliminated almost completely. Future studies, should aim at disentangling the potential confounding effect of habitat degradation.

Heavy metals may impair cognitive processes in several vertebrates (Burger and Gochfeld 2005; Müller et al. 2008; Khan et al. 2014; Cobbina et al. 2015) and even in invertebrates (Philips et al. 2017). However, mechanisms related to alteration of bird song by heavy metals, and by habitat alteration are not clear yet. Some possible mechanisms through which exposure to heavy metals may alter song traits in BTSP, and in other species may include: a) stress response and immunosuppression, which may be affected by chronic toxicosis by heavy metals. For instance, Pfaff et al. (2007)

reported that the heterophil:lymphocyte ratio, an indicator of physiological stress, was negatively associated with repertoire size. Additionally, Jones (2015) suggested that heterophilia responses may result from lead toxicosis in birds. However, for BTSP's no relationship between heterophil-lymphocyte ratio and lead-arsenic pollution was observed. Also considering mechanistic constraints in birds associated with developmental stress caused by anemia, also potentially caused by heavy metals is relevant (Franson and Pain 2011); b) neurological impairment may occur when arsenic and lead convert into neurotoxins (Franson and Pain 2011; Khan et al. 2014), therefore, effects in vocal performance due to learning failure may be likely (Podos et al. 2009b). High-level vocal center (HVC) is a song nuclei in the brain that is responsible for song production and for the learning pathway. Previous investigations have reported positive correlations between the size of this brain nucleus and repertoire size as an indication of male quality (Podos et al. 2004; Pfaff et al. 2007; Podos et al. 2009a). However, while no relationship between heavy metal levels and change in HVC volume have been reported, other studies have described a neurotoxic behavior in this brain region, when exposed to other pollutants (Iwaniuk et al. 2006; Markman et al. 2008). Repertoire size and song switching rate in the BTSP may be linked to song type retention through the learning process. Specifically song switching rate exhibited in the BTSP may be an example of the "anti-exhaustion hypothesis" proposed by Lambrechts and Dhondt (1988). According to this hypothesis, rapid song switch minimizes exhaustion to maintain a higher song rate. This pattern may not be present in polluted areas, and therefore, lower song type switching may occur in this zone. Data in favor of this hypothesis are scarce and experimental studies to test this hypothesis are needed. d) Cultural erosion: poor quality habitats, such as those that have been affected by pollutants, may function as sink habitats (sensu Pulliam and Danielson 1991), where cultural erosion in song may take place in comparison to source habitats. Moreover, Ortega et al. (2013) suggested a process of how habitat modification may erode bird song repertoire by the presence of invasive plants. They detected a demographic age-related bias in Chipping Sparrow populations (*Spizella passerina*) caused by degradation of habitat through the invasion of exotic plants. This alteration may change age structure (yearling-to-adult male ratio) and site fidelity. Consequently, the process of song learning may be modified so that fewer song types from neighboring males (mostly younger individuals) are learned. Environmental pollution and deforestation are also generators of poor habitat quality where younger birds may occur at higher abundances. The young-to-adult proportion in each site considered in the current study was not measured. Notwithstanding, the results of the current study demonstrate that the effects of exposure to heavy metals, and habitat degradation associated to mining activities in scrubland habitats of central Mexico affect bird song. However, these results also imply that investigation and identification of specific effects of each of these two factors, as well as specific mechanisms involved in song alteration in bird populations need to be studied at greater depth along clearly defined gradients of heavy metal pollution and vegetation composition, structure and cover. Moreover, mixed causes among probable processes described above should also be taken into account.

Identifying mechanisms linked to song structure modification was challenging owing to mixed causes (pollution and vegetation cover) that might be involved in habitat degradation. Experimental studies are needed to observe direct effects of heavy metal exposure in acoustic structure in this sparrow. Also, in further studies, sampling at individual level would reduce significantly ambiguity in how to interpret all potential environmental and anthropogenic effects involved.

Conclusions

An important effect of habitat degradation on song traits of the BTSP was detected. Specifically, changes associated to mining activity, such as heavy metal pollution and decreased shrub density, correlate with some features of BTSP song structure. Repertoire variables were the most affected in this study, where heavy metals may strongly influence syllable complexity in trill and number of song types. In addition, song-switching rate and song rate correlated with both metal pollution and lowered shrub density occurring near mining complexes. This study provided a general perspective of how heavy metal pollution and other environmental factors associated to mining activity may influence acoustic patterns in birds. Because these song traits are fundamental for the maintenance of healthy population dynamics, the contribution of the current study is significant.

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Appendix

Table 1. Model selection results for spectro-temporal variables in the BTSP. Abbreviations: k= number of parameters, AICc= Akaike information criterion adjusted for small sample sizes, ΔAICc= Akaike differences, wi Akaike weights, Null=a null model with only an intercept, but no explanatory variables, Pb= Lead concentrations in soil (mg/kg), As= Arsenic concentrations in soil (mg/kg).

Model description	k	logLik	AICc	ΔAICc	wi
Trill Peak frequency					
Pb	5	-3184.25	6378.66	0.00	0.15
Tree density	5	-3184.53	6379.21	0.56	0.11
Null	4	-3185.66	6379.43	0.77	0.10
Pb + Tree density	6	-3183.65	6379.53	0.87	0.10
Pb + Shrub density	6	-3183.82	6379.87	1.22	0.08
As	5	-3185.06	6380.29	1.63	0.07
Shrub density + Tree density	6	-3184.07	6380.37	1.71	0.06
As + Pb	6	-3184.21	6380.65	1.99	0.06
Shrub density	5	-3185.39	6380.93	2.27	0.05
As + Tree density	6	-3184.48	6381.19	2.53	0.04
Pb + Shrub density + Tree density	7	-3183.59	6381.48	2.82	0.04
As + Pb + Tree density	7	-3183.60	6381.49	2.83	0.04
As + Pb + Shrub density	7	-3183.67	6381.63	2.98	0.03
Trill min frequency					
Pb + Shrub density	6	-3048.59	6109.40	0.00	0.20
Tree density	5	-3050.05	6110.26	0.87	0.13
As + Tree density	6	-3049.03	6110.29	0.89	0.12
Pb + Shrub density + Tree density	7	-3048.24	6110.79	1.39	0.10
As + Pb + Shrub density	7	-3048.31	6110.93	1.53	0.09
Shrub density + Tree density	6	-3049.50	6111.22	1.82	0.08
As + Pb + Tree density	7	-3048.85	6112.00	2.60	0.05
Pb + Tree density	6	-3050.05	6112.31	2.92	0.05
As + Shrub density + Tree density	7	-3049.03	6112.36	2.97	0.04
Null	4	-3052.27	6112.64	3.24	0.04
As + Pb + Shrub density + Tree density	8	-3048.24	6112.87	3.47	0.03
Trill max frequency					
Null	4	-3174.55	6357.20	0.00	0.15
As + Pb	6	-3172.60	6357.42	0.22	0.14
As	5	-3173.95	6358.07	0.86	0.10
Pb	5	-3174.25	6358.66	1.45	0.07
As + Pb + Shrub density	7	-3172.29	6358.87	1.67	0.07
Pb + Shrub density + Tree density	7	-3172.37	6359.05	1.84	0.06
Pb + Shrub density	6	-3173.48	6359.18	1.98	0.06

As + Pb + Tree density	7	-3172.45	6359.19	1.99	0.06
Tree density	5	-3174.53	6359.22	2.02	0.06
Shrub density	5	-3174.54	6359.23	2.03	0.06
As + Shrub density	6	-3173.80	6359.81	2.61	0.04
As + Tree density	6	-3173.81	6359.85	2.65	0.04
As + Shrub density + Tree density	7	-3173.10	6360.51	3.30	0.03
Trill Bandwidth					
Tree density	5	-3157.97	6326.11	0.00	0.17
Null	4	-3159.49	6327.08	0.98	0.11
As + Shrub density	6	-3157.46	6327.15	1.04	0.10
Pb + Tree density	6	-3157.58	6327.38	1.28	0.09
As	5	-3158.81	6327.78	1.68	0.07
Shrub density + Tree density	6	-3157.85	6327.93	1.82	0.07
As + Tree density	6	-3157.95	6328.12	2.01	0.06
As + Pb	6	-3158.23	6328.68	2.57	0.05
Shrub density	5	-3159.27	6328.70	2.59	0.05
As + Pb + Tree density	7	-3157.33	6328.95	2.85	0.04
Pb	5	-3159.45	6329.05	2.94	0.04
As + Shrub density + Tree density	7	-3157.40	6329.09	2.98	0.04
Pb + Shrub density + Tree density	7	-3157.44	6329.18	3.08	0.04
Trill rate					
Null	4	-1244.98	2498.07	0.00	0.19
Pb	5	-1244.66	2499.48	1.42	0.09
As	5	-1244.69	2499.54	1.47	0.09
As + Pb	6	-1243.70	2499.62	1.55	0.09
As + Shrub density + Tree density	7	-1242.73	2499.75	1.69	0.08
Shrub density	5	-1244.85	2499.85	1.79	0.08
Tree density	5	-1244.98	2500.12	2.05	0.07
As + Shrub density	6	-1244.03	2500.29	2.22	0.06
As + Pb + Shrub density + Tree density	8	-1242.48	2501.35	3.28	0.04
As + Tree density	6	-1244.57	2501.37	3.30	0.04
As + Pb + Tree density	7	-1243.57	2501.44	3.37	0.04
Pb + Tree density	6	-1244.62	2501.46	3.40	0.03
Pb + Shrub density	6	-1244.65	2501.52	3.45	0.03
Duration 90% (Trill)					
Pb + Shrub density	6	398.13	-784.04	0.00	0.16
As + Pb + Tree density	7	398.99	-783.69	0.35	0.14
As + Shrub density + Tree density	7	398.78	-783.26	0.77	0.11
Tree density	5	396.48	-782.80	1.23	0.09
As + Tree density	6	397.24	-782.26	1.78	0.07
Pb + Shrub density + Tree density	7	398.23	-782.16	1.88	0.06
As + Pb + Shrub density	7	398.15	-782.01	2.03	0.06

Null	4	395.03	-781.95	2.08	0.06
Pb + Tree density	6	397.06	-781.90	2.14	0.06
Pb	5	395.91	-781.65	2.39	0.05
As + Pb + Shrub density + Tree density	8	399.01	-781.64	2.40	0.05
Shrub density + Tree density	6	396.49	-780.76	3.27	0.03
Song max frequency					
Null	4	-2942.56	5893.23	0.00	0.17
Pb + Shrub density + Tree density	7	-2939.67	5893.64	0.42	0.14
As + Pb + Shrub density + Tree density	8	-2938.94	5894.27	1.05	0.10
As	5	-2942.29	5894.74	1.51	0.08
Pb	5	-2942.33	5894.83	1.60	0.07
As + Pb	6	-2941.38	5894.99	1.76	0.07
Tree density	5	-2942.43	5895.02	1.79	0.07
Shrub density	5	-2942.54	5895.24	2.02	0.06
Pb + Shrub density	6	-2941.58	5895.38	2.16	0.06
As + Pb + Shrub density	7	-2940.97	5896.23	3.01	0.04
Pb + Tree density	6	-2942.03	5896.28	3.06	0.04
As + Shrub density	6	-2942.25	5896.73	3.50	0.03
As + Tree density	6	-2942.28	5896.79	3.56	0.03
Song peak frequency					
Null	4	-3194.83	6397.77	0.00	0.24
Shrub density	5	-3194.58	6399.32	1.55	0.11
Tree density	5	-3194.60	6399.36	1.59	0.11
As	5	-3194.75	6399.67	1.89	0.09
Pb	5	-3194.83	6399.82	2.05	0.08
As + Shrub density	6	-3194.11	6400.44	2.67	0.06
Pb + Shrub density	6	-3194.30	6400.82	3.05	0.05
Shrub density + Tree density	6	-3194.40	6401.02	3.25	0.05
Pb + Tree density	6	-3194.56	6401.33	3.56	0.04
As + Tree density	6	-3194.60	6401.43	3.65	0.04
As + Pb	6	-3194.69	6401.61	3.84	0.03
As + Pb + Shrub density	7	-3193.93	6402.15	4.38	0.03
As + Shrub density + Tree density	7	-3194.09	6402.47	4.70	0.02
Song rate					
Pb + Shrub density	5	48.42	-85.18	0.00	0.37
Pb + Shrub density + Tree density	6	48.64	-82.87	2.31	0.12
As + Pb + Shrub density	6	48.57	-82.75	2.43	0.11
Pb + Tree density	5	47.20	-82.74	2.44	0.11
As + Pb + Tree density	6	48.27	-82.14	3.04	0.08
Pb	4	45.57	-82.05	3.13	0.08
As + Pb + Shrub density + Tree density	7	48.64	-79.98	5.20	0.03
Tree density	4	44.33	-79.58	5.60	0.02

As + Pb	5	45.59	-79.51	5.68	0.02

Table 2. Model selection results for repertoire variables in the BTSP. Abbreviations: k= number of parameters, AICc= Akaike information criterion adjusted for small sample sizes, ΔAICc= Akaike differences, wi Akaike weights, Null=a null model with only an intercept, but no explanatory variables, Pb= Lead concentration in soil (mg/kg), As= Arsenic concentration in soil (mg/kg).

Model description	k	logLik	AICc	ΔAICc	wi
Number of syllables (Trill)					
Null	3	-815.65	1637.36	0.00	0.19
Tree density	4	-814.85	1637.81	0.45	0.15
As	4	-815.25	1638.61	1.24	0.10
Pb	4	-815.45	1639.01	1.65	0.08
Shrub density	4	-815.64	1639.38	2.02	0.07
Pb + Tree density	5	-814.82	1639.79	2.43	0.06
As + Tree density	5	-814.83	1639.81	2.45	0.06
Shrub density + Tree density	5	-814.85	1639.86	2.50	0.05
Pb + Shrub density	5	-814.89	1639.94	2.57	0.05
As + Shrub density	5	-814.92	1639.99	2.63	0.05
As + Pb	5	-815.23	1640.62	3.26	0.04
As + Pb + Shrub density	6	-814.35	1640.92	3.55	0.03
Pb + Shrub density + Tree density	6	-814.69	1641.60	4.23	0.02
Syllable complexity					
As	4	-580.94	1169.99	0.00	0.18
As + Shrub density	5	-580.01	1170.18	0.19	0.16
As + Pb	5	-580.03	1170.22	0.23	0.16
As + Shrub density + Tree density	6	-579.14	1170.51	0.52	0.14
As + Pb + Shrub density	6	-579.83	1171.88	1.89	0.07
As + Tree density	5	-580.94	1172.04	2.05	0.06
As + Pb + Shrub density + Tree density	7	-578.95	1172.19	2.20	0.06
As + Pb + Tree density	6	-580.03	1172.28	2.30	0.06
Null	3	-583.73	1173.53	3.54	0.03
Number of song types					
Pb + Shrub density	4	-80.74	170.55	0.00	0.25
Pb + Shrub density + Tree density	5	-80.11	171.89	1.33	0.13
Pb	3	-82.63	171.89	1.34	0.13
Null	2	-84.01	172.34	1.78	0.10
As + Pb + Shrub density	5	-80.65	172.97	2.42	0.07
As + Pb + Shrub density + Tree density	6	-79.36	173.12	2.56	0.07
As + Pb	4	-82.27	173.62	3.07	0.05
Pb + Tree density	4	-82.63	174.33	3.78	0.04

Tree density	3	-83.90	174.43	3.88	0.04
Shrub density	3	-83.98	174.59	4.04	0.03
As	3	-84.01	174.64	4.09	0.03
Song switching rate					
Pb + Shrub density	5	-15.27	42.20	0.00	0.33
Pb + Shrub density + Tree density	6	-14.44	43.27	1.07	0.19
As + Pb + Shrub density	6	-15.09	44.59	2.39	0.10
Pb	4	-17.82	44.71	2.51	0.09
As + Pb + Shrub density + Tree density	7	-13.75	44.80	2.60	0.09
As + Pb	5	-17.29	46.25	4.05	0.04
Null	3	-19.84	46.31	4.11	0.04
Pb + Tree density	5	-17.79	47.25	5.05	0.03
As + Pb + Tree density	6	-16.67	47.75	5.55	0.02

Table 3. Model-averaged parameter estimates for predictors influencing spectro-temporal variables, standard errors (SE), confidence intervals (CI; L lower and U upper), relative importance values (RIV), and significance (*P* value) for predictor variables in the BTSP. Parameter abbreviations as in Tables 1 and 2.

Parameter	**Estimate**	**SE**	**CI(L)**	**CI(U)**	**RIV**	***P*-value**
Trill peak frequency						
Intercept	4464.07	102.99	4262.21	4665.93		**<0.001**
Pb	386.69	284.37	-170.68	944.06	0.53	0.174
As	98.67	260.15	-411.22	608.56	0.25	0.704
Tree density	-279.17	229.97	-729.91	171.57	0.42	0.225
Shrub density	82.60	380.97	-664.10	829.30	0.28	0.828
Trill min frequency						
Intercept	2678.83	64.79	2551.84	2805.82		**<0.001**
Pb	407.71	283.88	-148.69	964.11	0.56	0.151
As	-118.71	244.04	-597.03	359.61	0.37	0.627
Tree density	-319.20	202.31	-715.73	77.33	0.65	0.115
Shrub density	464.10	314.87	-153.05	1081.25	0.58	0.14
Trill max frequency						
Intercept	5794.13	92.71	5612.42	5975.85		**<0.001**
Pb	411.63	314.38	-204.55	1027.81	0.49	0.19
As	-343.59	246.21	-826.15	138.98	0.51	0.16
Tree density	6.29	322.10	-625.04	637.61	0.26	0.98
Shrub density	210.08	429.09	-630.94	1051.09	0.33	0.62
Trill bandwidth						
Intercept	3114.52	98.46	2921.54	3307.50		**<0.001**
Pb	211.53	261.11	-300.25	723.31	0.28	0.418

As	-284.38	282.11	-837.32	268.56	0.4	0.313
Tree density	343.35	235.36	-117.96	804.66	0.55	0.145
Shrub density	-199.58	323.94	-834.50	435.34	0.32	0.538
Trill rate						
Intercept	17.28	1.01	15.29	19.27		**<0.001**
Pb	-1.72	3.46	-8.49	5.05	0.35	0.619
As	4.20	3.91	-3.46	11.85	0.46	0.282
Tree density	2.62	4.19	-5.60	10.84	0.32	0.532
Shrub density	3.82	4.88	-5.74	13.38	0.32	0.433
Duration 90% (Trill)						
Intercept	0.39	0.01	0.37	0.42		**<0.001**
Pb	0.07	0.05	-0.02	0.17	0.62	0.135
As	-0.06	0.05	-0.16	0.03	0.45	0.206
Tree density	-0.07	0.05	-0.16	0.02	0.65	0.135
Shrub density	0.03	0.08	-0.12	0.19	0.51	0.683
Song max frequency						
Intercept	7485.99	81.67	7325.93	7646.06		**<0.001**
Pb	517.40	422.47	-310.64	1345.44	0.54	0.221
As	-0.32	365.30	-716.31	715.67	0.36	0.999
Tree density	378.76	368.54	-343.57	1101.10	0.39	0.304
Shrub density	552.36	537.39	-500.92	1605.64	0.44	0.304
Song peak frequency						
Intercept	4588.49	102.53	4387.53	4789.45		**<0.001**
Pb	45.24	287.43	-518.12	608.60	0.25	0.875
As	141.33	261.72	-371.64	654.30	0.29	0.589
Tree density	-121.85	236.08	-584.57	340.87	0.27	0.606
Shrub density	238.86	279.18	-308.33	786.05	0.33	0.392
Song rate						
Intercept	0.30	0.01	0.28	0.33		**<0.001**
Pb	-0.13	0.05	-0.24	-0.03	**0.98**	**0.013**
As	0.01	0.05	-0.08	0.10	0.26	0.831
Tree density	0.04	0.04	-0.03	0.12	0.38	0.278
Shrub density	-0.09	0.05	-0.19	0.00	**0.67**	**0.042**

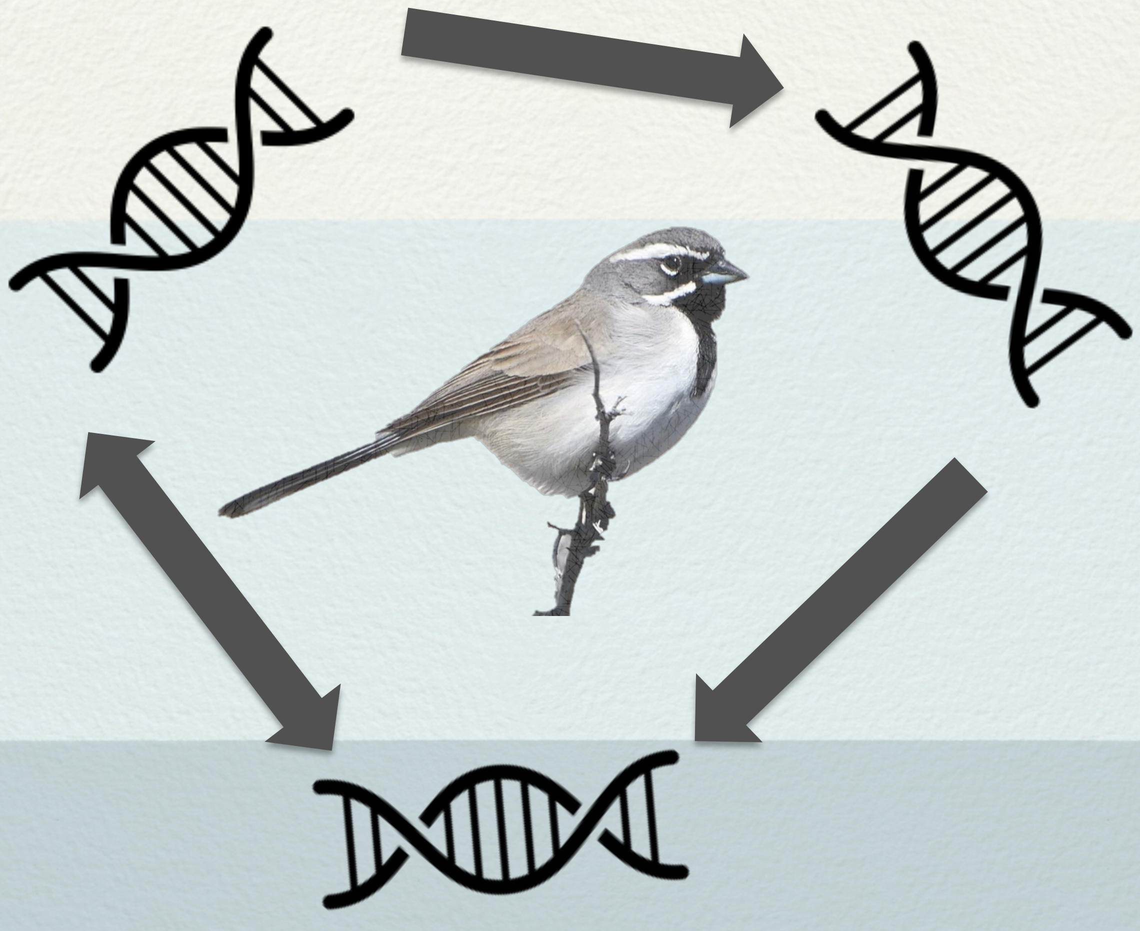
Table 4. Model-averaged parameter estimates for predictors influencing repertoire variables, standard errors (SE), confidence intervals (CI; L lower and U upper), relative importance values (RIV), and significance (*P* value) for predictor variables explaining several acoustic traits in the BTSP song. Parameter abbreviations as in Tables 1 and 2.

Parameter	Estimate	SE	CI(L)	CI(U)	**RIV**	**P-value**
Number of syllables (Trill)						

Intercept	1.82	0.05	1.72	1.92		**<0.001**
Pb	0.09	0.15	-0.20	0.37	0.3	0.539
As	0.09	0.12	-0.14	0.31	0.29	0.452
Tree density	-0.12	0.11	-0.33	0.09	0.36	0.262
Shrub density	0.09	0.15	-0.21	0.39	0.29	0.574
Syllable complexity (Trill)						
Intercept	0.91	0.03	0.85	0.98		**<0.001**
Pb	0.06	0.13	-0.20	0.32	0.38	0.628
As	-0.26	0.12	-0.50	-0.01	**0.97**	**0.040**
Tree density	-0.10	0.14	-0.37	0.17	0.35	0.460
Shrub density	-0.16	0.15	-0.45	0.13	0.47	0.268
Number of song types						
Intercept	1.10	0.11	0.89	1.32		**<0.001**
Pb	-0.84	0.44	-1.70	0.02	**0.72**	**0.055**
As	-0.08	0.40	-0.87	0.70	0.24	0.834
Tree density	-0.27	0.42	-1.09	0.55	0.27	0.519
Shrub density	-0.69	0.49	-1.65	0.27	0.52	0.160
Song switching rate						
Intercept	0.58	0.06	0.46	0.70		**<0.001**
Pb	-0.73	0.29	-1.29	-0.16	**0.95**	**0.012**
As	-0.03	0.30	-0.61	0.55	0.27	0.909
Tree density	-0.22	0.26	-0.73	0.30	0.35	0.410
Shrub density	-0.54	0.30	-1.12	0.04	**0.76**	**0.067**

CHAPTER 4

Genetic diversity and population genetic structure in the Black-throated sparrow (*Amphispiza bilineata*) at a portion of the highland plateau of San Luis Potosi, Mexico



Genetic diversity and population genetic structure in the Black-throated sparrow (*Amphispiza bilineata*) at a portion of the highland plateau of San Luis Potosi, Mexico.

Abstract

Habitat loss, fragmentation, and degradation are some of the most significant triggers of global declines of populations in several species, including birds. These processes may cause reductions in connectivity, and as a consequence, gene flow between populations may decrease through isolation, ultimately leading to loss in genetic diversity. Also, human processes such as mining activity may promote population reductions in specific taxa, and therefore, a loss of genetic diversity. Through the current study, a description of the diversity and population genetic structure of the Black-throated sparrow (BTSP) was carried out. It was also evaluated how human disturbances influence population genetics in this species. High values of heterozygote excess within populations were found in most of the sites, except in the most degraded sites. Inbreeding coefficient was significantly higher in one site, which is exposed to mining activity than in the remaining sites, suggesting a negative influence of mining activity on the local BTSP population. No population genetic structure was recorded for the study area, thus indicating that significant admixture is present among populations in the study area. This study increased the knowledge about genetic characteristics of a particular bird population

inhabiting drylands. It also documented that heavy metal pollution has the potential to promote genetic losses in a bird species.

Introduction

Understanding how human anthropogenic impacts such as land use change affect various aspects of biological diversity is still a topic that demands substantial attention from the scientific community. Habitat loss, fragmentation, and degradation are some of the most significant triggers of global declines of populations in several species (Lindenmayer and Fischer 2006; Bennett and Saunders 2010), including avian taxa (Amos et al. 2012). These processes have been particularly intense for the last two centuries, resulting in significant environmental deterioration, consequently, including also a significant defaunation process throughout the world's ecosystems (Dirzo et al. 2014).

Diverse approaches, including landscape ecology, physiology, conservation biology, behavior, bioacoustics, population genetics etc. have been developed to understand the effects of land use change and habitat degradation at different levels of organization (Turner 2001; Laiolo 2010; Haig et al. 2011; Ellis et al. 2012). Population genetics allows investigating how alteration of the landscape may alter biological processes and organism health. The number of publications on population genetics relative to landscape structure and composition has increased substantially in the last 20 years. This has allowed increasing understanding of processes involved in the retention or reduction of the population gene pool diversity (Amos et al. 2012), and therefore, identify population status in relation to land use change and habitat degradation. In addition, population genetics provide information about historical processes that could have influenced populations, likely by the reduction in population size or increase in land use change and habitat degradation (Schwartz et al. 2007; Amos et al. 2012; Nakajima et al. 2017).

Modifications that decrease the area of habitat may cause reductions in connectivity, and as a consequence, gene flow between populations may decrease through isolation, ultimately leading to loss in genetic diversity (Williams et al. 2003; Freeland et al. 2011). Dispersal abilities vary among species and a limited capacity to overcome barriers such as modified habitats may be a risk factor for population as it may lead to their decline (Collingham and Huntley 2000). Information related to habitat degradation in combination with genetic data may be informative in terms of how landscape modifications affect genetic diversity within and among populations. Biological and ecological aspects, however, must be considered because dispersal capacity is variable. Some species with high vagility (ability of organisms to changes its location or distribution over time) commonly exhibit low levels of genetic differentiation between populations, whereas in species of restricted distribution or limited home ranges the opposite may be true (Freeland et al. 2011). Demographic and physiological aspects may also be influencing factors (Hillman et al. 2014).

In the last century, Mexican dryland habitats have been severely affected through habitat modification (Rzedowski 1961; Sheldon 1980; Chapa-Vargas and Monzalvo-Santos 2012; Monzalvo-Santos et al. 2016). Expanding agriculture, plant extraction, mining, urbanization and livestock overgrazing have been the most important human activities that negatively influenced xerophytic scrublands within this region. These processes may affect population genetic structure to an unknown extent. Mining has been one of the most intensive activities in drylands (Chapa-Vargas et al. 2010; Espinosa-Reyes et al. 2014; Lester and Van Riper 2014; Monzalvo-Santos et al. 2016), directly affecting avian populations through toxicological effects (Janssens et al. 2003; Dauwe et al. 2004; Franson and Pain 2011). This human activity has a potential influence in population reduction of specific taxa and, therefore, a loss of genetic diversity (Bickham et al. 2000; DiBattista 2008).

On the other hand, spatial patterns of vegetation distribution in drylands are determined by several abiotic factors together causing a highly heterogeneous mosaic of ecosystems in the geographic space. Specifically, some species (e.g., Yucca -*Yucca sp.*-, Mesquite -*Prosopis sp.-, etc.*) require relatively higher soil moisture, while others have found a niche in more open, drier sites (i.e. grasslands, creosotebush scrubland -*Larrea tridentata*-, Mueller et al. 2013). The interspersion of patches of variable resource availability and productivity of vegetation is a common phenomenon in drylands, which are vulnerable to degradation owing to delimited areas that concentrate soil and water resources that are necessary for human activities (Mueller et al. 2013). Spatial structure consisting of different habitat types interspersed, influence species dynamics because these landscapes are typical of those in which habitat fragmentation is present. Therefore, biological dynamics may be affected (Gibbens et al. 2005; Safriel et al. 2005; Mueller et al. 2013).

Knowledge about how genetic diversity and structure of bird populations in Mexican dryland habitats respond to different environmental factors is limited. Within the region, many bird species adapt well to spatial and temporal heterogeneity of dryland conditions. By being nomadic, or having high vagility, for instance, many species such as the Worthen's Sparrow (*Spizella wortheni*) may maintain high gene flow in spite of habitat fragmentation and degradation (Canales-Delgadillo et al. 2012). However, some other species in the region tend to be more territorial (Reinoso-Pérez 2014). For these species it is still unknown what is the genetic status of their populations, and to what extent anthropogenic impacts may have affected the genetic structure of their populations.

The Black-throated Sparrow (BTSP) inhabits semiarid areas of southern United States and north-central Mexico and is a year-round resident within the Mexican territory. In its southernmost distribution, this species associates to different habitat types as long as creosotebush (*Larrea tridentata*) is present, however, urban areas are avoided by the species (Johnson et al. 2002). Pidgeon et al. (2003, 2006) suggested that areas dominated by creosotebush and grasslands are the highest quality habitats for BTSP, whereas mesquite (*Prosopis grandulosa*) habitat may constitute an ecological trap. Territories of this bird species range from 0.75 to 2.36 ha and fidelity to both breeding and winter territories is expected to be relatively high (Johnson et al. 2002).

In the field of population genetics, microsatellites are widely used nuclear markers which provide high variability within and among populations, and thus are informative in terms of genetic structure at small geographic scale (Freeland et al. 2011). The explanatory power of this variability owes to the very high mutation rate of these markers. This property confers informative capacity in terms of local genetic processes that affect populations and individuals. These markers, for instance, help detect connectivity patterns or changes that have occurred in the recent past (10-100 generations) (Selkoe and Toonen 2006). Undoubtedly, genetics may provide relevant information as to how habitat changes may affect population processes such as dispersal, gene flow, and genetic diversity (Sunnucks 2000; Dawson et al. 2010; Freeland et al. 2011).

The objective of this study was to describe the diversity and population genetic structure of the Black-throated sparrow, a resident bird species at a portion of Highland Plateau of San Luis Potosi. Because of lacking of specific environmental variables representing connectivity among sites, it was hypothesized that higher genetic diversity (higher heterozigosity and lower inbreeding values) would occur in highly degraded scrublands (open habitats) than in moderately degraded scrublands, these open habitat is the habitat in which this species occurs at higher densities and where breeding success is highest (Pidgeon et al. 2003, 2006; Kozma et al. 2017). Finally, exposure to mining activity is expected to negatively affect genetic diversity due to a higher inbreeding.

Method

Study area

The highland plateau of San Luis Potosí is embedded in the Mexican Xerophytic region (Rzedowski 2005) of the Chihuahuan Desert, characterized by semiarid climate. The dominant vegetation communities in this area include microphyllous scrublands, semiarid grasslands, and *Yucca*-dominated scrublands (Rzedowski 2005).

Four localities were considered in the current study, which are located in three communal land ("ejido") areas: La Cardoncita (LC), Presa de Santa Gertrudis (PSG), and Guadalupe Victoria (GV), and in the surroundings of Charcas (CH), a relatively large town (population size = 21,138) (INEGI, 2009) (UTM: LC = E271793.37 N2610708.64, PSG = E278059.47 N2599525.12, GV = E276057.75 N2582923.98, CH = E279261.83 N2561548.75; zone 14 N) in the municipalities of Charcas (LC, GV), and Catorce (PSG) in the state of San Luis Potosí, Mexico (Figure 1). The CH site is located near to mining complex, where mining activity has taken place for more than 400 years and soils are severely contaminated with arsenic (As) and lead (Pb) (Monzalvo-Santos et al. 2016). Sampling design in terms of "stratification" considered representative habitat types in the region: (A) highly degraded scrublands (HDS) consisting of open creosotebush plant communities that had been modified by massive extraction of *Yucca* plants in the last 50 years for construction material and fiber production (Miranda and Hernandez 1963; Sheldon 1980; Rzedowski 2005; Garza Hurtado 2011), and intensive grazing by goats (herbaceous cover = 11.47%, bare ground = 79.34%, mulch = 9.19%) (Rzedowski 1961, 2005; Garza-Hurtado 2011). Shrubs are almost exclusively creosotebush (density: mean = 782 shrubs/ha, SE = 99.69), and *Yucca* density is extremely low (mean = 0.75 trees/ha; SE = 12.64, Garza Hurtado 2011. For this habitat type, four study sites near GV, PSG, LC, and CH were included (Figure 1). The distances from these study sites to the nearest villages were 3.5, 1.8, 2, and 3 km, respectively. The second habitat type consists of (B) moderately degraded scrublands (MDS); dominated by *Yucca filifera* and *Yucca decipiens* (also known as *Yucca* forests; *Yucca* density: mean = 42.5 individuals/ha, SD = 40; height: mean = 3.9 m, SD = 1.5). This plant community has only moderate levels of grazing by horses and cattle (herbaceous cover = 22.69%; bare ground = 39.65%; mulch = 37.66%, Garza Hurtado 2011). This type of vegetation shows relatively well-conserved plant communities (Rzedowski 1961), and is considered a closed habitat. For this habitat type three study sites were included, near GV, PSG, and LC (Figure 1). These sites all have similar distances to the nearest village (4, 6.5, and 6.5 km, respectively).

Bird blood sampling for DNA extraction

BTSP individuals were captured using 20 ornithological mist nets (12 m × 2.5 m), which were placed at random locations and operated from dawn (6:00–7:00 h) to just before dusk (18:00–19:00 h) during three consecutive days. Each site was sampled both during the breeding (March–June) and non-breeding (September–November) seasons of 2012 to 2015. Blood samples were extracted through brachial vein puncture and were stored under stable conditions with Longmire buffer solution (100 mM Tris-HCl pH8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS) and Ethanol for subsequent DNA extraction.

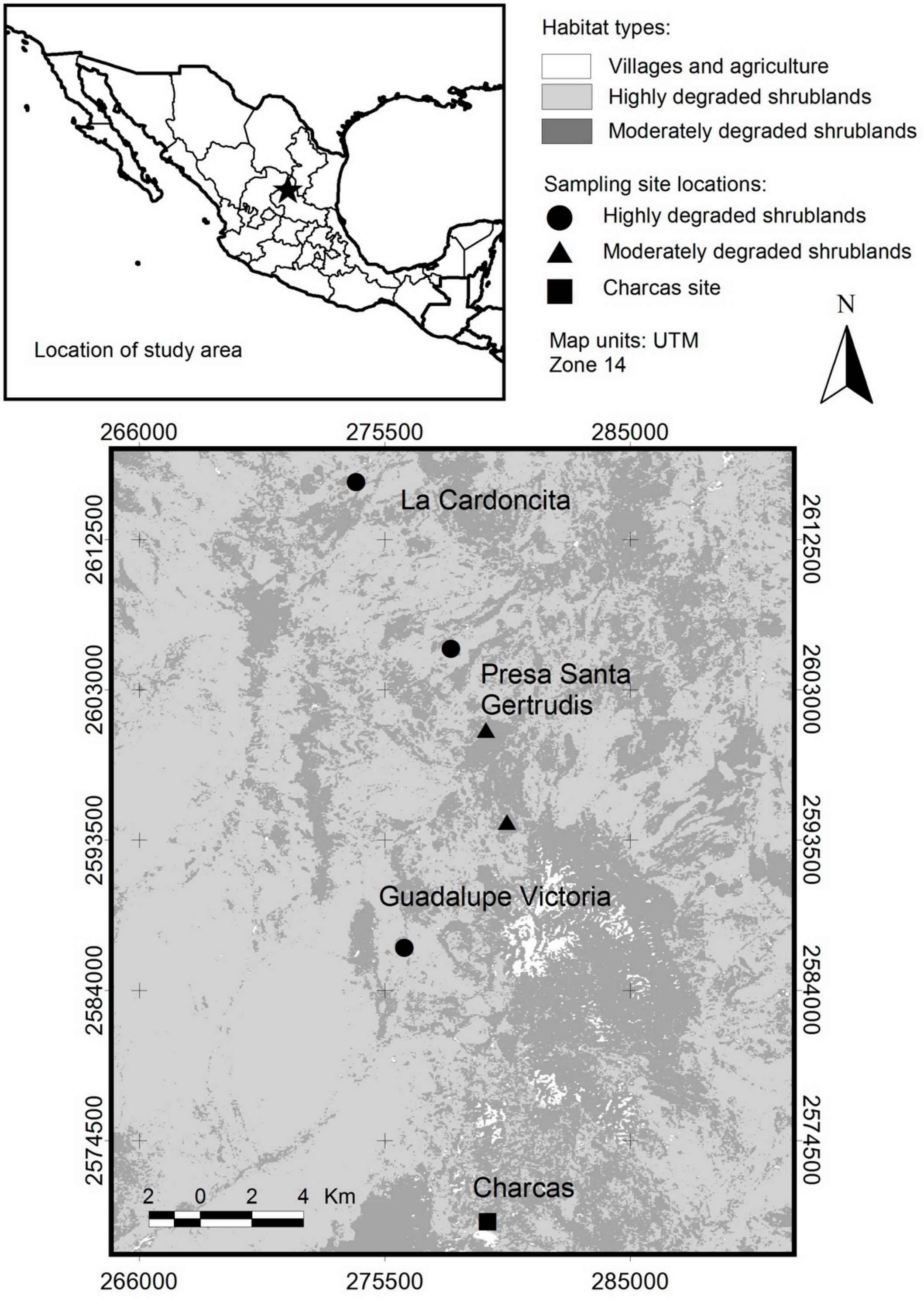


Figure 1. Map of the study area located in a portion of the Highland Plateau of San Luis Potosi. Six sites were considered: two sites categorized as moderately degraded scrubland and four as highly degraded scrubland.

DNA extraction

Genomic DNA was extracted from blood samples using DNeasy Blood and Tissue Kit (Qiagen, Inc.) according to manufacturer’s protocol. DNA quality and quantity of extractions were evaluated with a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific Inc.). Isolated DNA was used for simple sequence repeat (SSR) amplification and population genetics analyses.

SSR’s amplification and genotyping

All samples were genotyped using eight conserved avian microsatellite markers for BTSP (Piertney et al. 1998; Jeffery and Keller 2001; Hill et al. 2008; Dawson et al. 2013) (Table 1). PCR amplifications were performed in a total volume of 25 μl. Each reaction mixture contained 20 - 50ng of genomic DNA, 3 mM $MgCl_2$, 0.25 mM of each nucleotide, 0.6 μM of each primer labeled with fluorescent dyes (HEX, 6FAM or NED; Applied Biosystems), 0.6 U AmpliTaq (Applied Biosystems) and 1XPCRbuffer II (100 mM Tris–HCl, pH 8.3, 50 mM KCl). PCR profile consisted of 3 min of initial denaturation followed by 30 to 35 cycles (Table 1) of 30 s denaturation at 94 °C, 30 s annealing from 56 °C to 65 °C (Table 1), 45 s extension at 72 °C, and a final extension step of 5 min at 72 °C. PCR products were examined by 1% agarose gel electrophoresis. Fragments were diluted and separated by size on an ABI 3500 Genetic Analyser (Applied Biosystems). Allele sizes were assessed against a 500-ROX size standard (Applied Biosystems). Alleles were visualized in chromatograms and genotypes were scored using GeneMapper v5.0 (Applied Biosystems).

Table1. Cross-species microsatellite markers used in the current study. Ta= Annealing temperature, bp= base pairs, motif= repeated nucleotide pattern.

Locus	Motif	Primer sequence 5'-3'	Size range (bp)	Ta	Cycles	Reference
CAM02	(CA)16	F: GAATTAAAGAYAGCAGATGCAGG R: AGCTGATGAAATGAGAATGCAG	351-363	64	30	Dawson et al. (2013)
CAM17	(T)9 G(GT)4 CC (TG)2 (TC)3 (TG)12	F: CGGGTTGTAATCAAGAAGATGC R: CTGCGGAGCAATTAACGC	193-197	63	30	Dawson et al. (2013)
CAM23	(TG)18 (AG)5 GC (AG)3	F: CTCCACTTAGCTTGTAAATGCAC R: CCAAGRAGTGCCCTAGATGTC	129-141	65	30	Dawson et al. (2013)
Mme08	(TG) 3 TC(TG) 13	F: TCATGGAGATGGGTGAATGCC R: TGAATCAGCAGCACACACAACC	209-235	65	32	Jeffery et al. (2001)
Aca08	(AC)18	F: TAGCCACAAGCAAGACCTGA R: CTGTGACAGGAAGGGCAGTT	193-195	65	30	Hill et al. (2008)
Mme12	(CCCACA)13	F: AGGGACTGTCACTGTGGGACTGAAG R: TGGCTTTATGGAACAAGGCATC	170-184	65	30	Jeffery et al. (2001)
Aca12	(GATA)9G(GATA)5(GACA)2 (GACG)(GATA)2GATG	F: GCTTGTTTCCTGTTCCCAAA R: AATCGGATCCATAGACTTCAAA	185-275	65	33	Hill et al. (2008)
LOX1	(CTTT)30	F: ATGATGGTAAGTCTAATGAAAGC R: CCACACACATTCACTCTATTG	262-268	65	30	Piertney et al. (1998)

Genetic diversity and population structure

Allele frequencies were tested for stuttering, allele dropout, presence of null alleles, and their frequencies using MICROCHECKER v2.2.3 (Van Oosterhout et al. 2004) and FreeNA (Chapuis and Estoup 2007). Genotypic linkage disequilibrium by population and locus was tested in GENEPOP v4.2 (Raymond and Rousset 1995). Deviations from Hardy-Weinberg Equilibrium (HWE), allelic richness (Na), number of effective alleles (N_e), observed (H_o) and expected (H_e) heterozygosity were computed and used as parameters of genetic diversity within and among populations. These calculations were obtained using GenAlEx v6.5 (Peakall and Smouse 2012) and "*pegas*" package (Paradis 2010) for R v3.3.1 (R Development Core Team 2011). Heterozygote excess and its significance value was calculated for each population using GENEPOP v4.2 (Raymond and Rousset 1995).

Genetic differentiation (R_{ST}) with sum of the Squared Size Difference approach (Slatkin 1995) and gene flow, as the absolute number of migrants exchanged per generation (N_em), were calculated using Arlequin v3.5 (Excoffier and Lischer 2010). Both of these analyses were performed between six populations. Inbreeding coefficients (F_{is}) and their significance per locus and population were estimated using FSTAT v.2.9.3.2 (Goudet 1995). Genetic variance between different hierarchical levels of the population structure (within-among individuals, among populations, and among localities) by means of analysis of molecular variance (AMOVA) was determined using "*poppr*" package (Kamvar et al. 2014) in R v3.3.1 (R Development Core Team 2011).

Immigration (recent gene flow) among locations was inferred using an assignment test in GeneClass 2 (Piry et al. 2004) with the methods of Rannala and Mountain (1997) and Paetkau et al. (2006). Individuals were excluded from their location of origin when the probability of exclusion was ≥99% (α=0.01). Finally, the individual was assigned as an immigrant to the most likely population when the confidence of assignment was ≥10%.

To identify the population genetic structure of the BTSP, *a priori* and *a posteriori* group assignments were executed in distinct analyses. First, principal components analysis (PCA) was performed to summarize data on variation in allele frequencies and genetic distance (Nei's distance; Nei 1972) for each population and for individuals with their respective centroids. In this method, singular value decomposition was performed. This procedure produces eigenvectors and eigenvalues; the first eigenvector separates the individual samples explaining the largest amount of variability, while the subsequent eigenvectors explain less variability. Therefore, the first eigenvector differentiates individuals from the population that is most distinct genetically, and each subsequent eigenvector separates the next most differentiated population. This multivariate analyses was performed with "ade4" package in R v3.3.1 (R Development Core Team 2011). Secondly, a discriminant analysis of principal components (DAPC) was performed. This approach transforms the genotypes into uncorrelated components using PCA, which retains a specific number of principal components to maximize the variation among populations and to minimize the variation within population (Jombart et al. 2010). Also, a clustering algorithm with K-means and model selection by Bayesian inference criterion (BIC) was used to establish optimal number of clusters of genetically related individuals.

Results

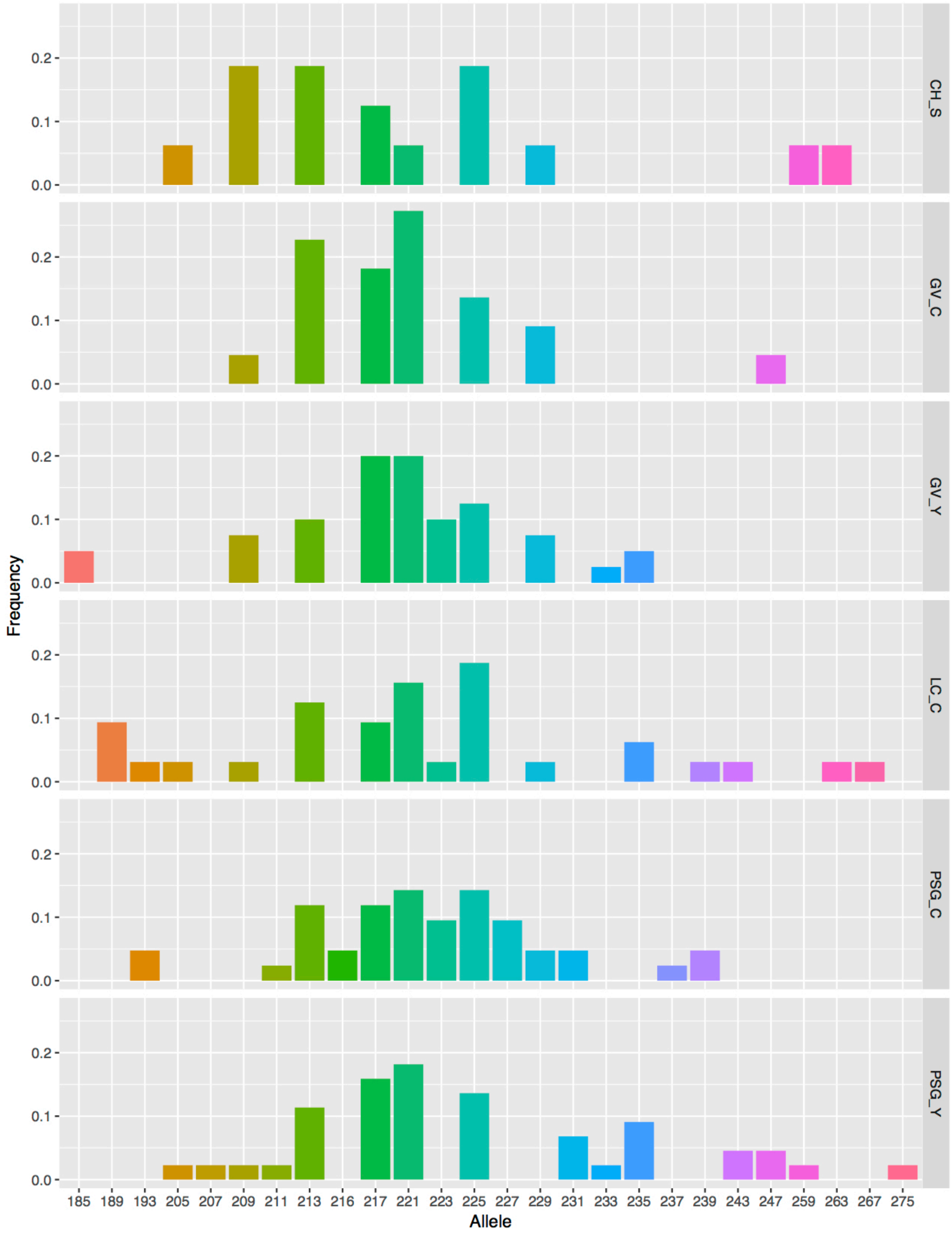
A total of 98 individual BTSP's were sampled. All samples were genotyped and characterized. One microsatellite marker (LOX1) was excluded from the analyses because it showed a high frequency of null alleles. No evidence of linkage disequilibrium was observed through the loci used in the study. The remaining seven markers were polymorphic. Their mean number of alleles was 5.45 (SE= 0.51) and their mean of number of effective alleles was 3.73 (SE= 0.36) (Table 2). Four markers (CAM17, CAM23, Mme12, and ACA12) deviated from the Hardy-Weinberg Equilibrium (Table 2). Number of alleles range from 2 (ACA08) to 16 (ACA12) and allele frequencies for each population are depicted in Figure 2.

All markers presented high heterozygosity, with the exception of CAM17, which was the marker with the lowest genetic diversity (Table 2). Mean of expected heterozygosity was 0.617 (SE= 0.034) and the highest values of heterozygote excess within populations were found in PSG-HDS (Fisher's method; *P*-value < 0.001), PSG-MDS (*P*-value < 0.001), GV-MDS (*P*-value = 0.006), and LC-HDS (*P*-value = 0.03). Non-significant values were obtained for CH (*P*-value = 0.47) and GV-HDS (*P*-value = 0.68), thus indicating that heterozygote deficiency was present in these sites. This result was associated with the estimation of the inbreeding coefficient, which was significantly higher in CH (F_{is} = 0.146; *P*-value = 0.03) than in the remaining sites.

Table 2. Genetic characterization of site and loci. Table includes sample size (*N*), observed (*Ho*) and expected (*He*) heterozygosity, number of alleles (*Na*), number effective of alleles (*Ne*), and significance level of deviations from Hardy-Weingberg Equilibrium (*P*). CH=Charcas, GV=Guadalupe Victoria, PSG=Presa de Santa Gertrudis, LC= La Cardoncita, HDS= Highly degraded scrubland, MDS= Moderately degraded scrubland, SE= Standard error.

	CH-HDS (N=8)					**GV-HDS (N=11)**					**GV-MDS (N=20)**				
Locus	***Na***	***Ne***	***Ho***	***He***	***P***	***Na***	***Ne***	***Ho***	***He***	***P***	***Na***	***Ne***	***Ho***	***He***	***P***
CAM02	5	2.67	0.63	0.63	0.08	6	5.04	0.82	0.80	0.52	6	4.04	0.75	0.75	0.72
CAM17	2	1.13	0.13	0.12	0.85	2	1.10	0.09	0.09	0.87	3	1.36	0.20	0.27	**<0.01**
CAM23	4	2.51	0.38	0.60	0.54	5	2.81	0.64	0.64	0.14	6	4.21	0.65	0.76	**<0.01**
Mme08	5	3.56	0.75	0.72	0.88	5	3.72	0.73	0.73	0.62	6	3.81	0.75	0.74	0.89
ACA08	2	1.97	0.38	0.49	0.50	2	1.98	0.55	0.50	0.74	2	1.88	0.65	0.47	0.08
Mme12	3	1.47	0.38	0.32	0.93	4	2.20	0.45	0.55	0.42	4	2.27	0.55	0.56	**<0.01**
ACA12	9	8.00	0.75	0.88	**0.02**	13	9.31	0.82	0.89	**0.01**	16	8.08	0.95	0.88	**<0.01**
Mean	4.3	3.04	0.48	0.54	-	5.3	3.74	0.58	0.60	-	6.1	3.66	0.64	0.63	-
±SE	0.92	0.88	0.09	0.10	-	1.41	1.05	0.10	0.10	-	1.75	0.85	0.09	0.08	-

	PSG-HDS (N=21)					**PSG-MDS (N=22)**					**LC-HDS (N=16)**				
Locus	***Na***	***Ne***	***Ho***	***He***	***P***	***Na***	***Ne***	***Ho***	***He***	***P***	***Na***	***Ne***	***Ho***	***He***	***P***
CAM02	7	5.19	0.80	0.81	0.13	6	4.02	0.86	0.75	**0.02**	6	5.28	0.81	0.81	0.12
CAM17	3	1.40	0.24	0.28	0.76	3	1.38	0.32	0.28	0.85	3	1.29	0.25	0.22	0.95
CAM23	6	5.31	0.62	0.81	**<0.01**	7	5.80	0.86	0.83	0.60	6	4.45	0.75	0.78	**0.02**
Mme08	7	4.01	0.86	0.75	0.82	7	4.23	0.73	0.76	0.89	5	4.06	0.75	0.75	0.49
ACA08	2	1.85	0.43	0.46	0.76	2	1.90	0.59	0.47	0.25	2	1.88	0.50	0.47	0.79
Mme12	4	2.65	0.52	0.62	0.54	5	1.90	0.50	0.47	0.74	4	2.43	0.50	0.59	0.29
ACA12	12	7.81	0.86	0.87	**<0.01**	14	10.76	0.73	0.91	**<0.01**	8	6.10	0.81	0.84	**0.01**
Mean	5.9	4.03	0.62	0.66	-	6.3	4.28	0.66	0.64	-	4.9	3.64	0.63	0.64	-
±SE	1.26	0.86	0.09	0.08	-	1.48	1.23	0.08	0.09	-	0.77	0.68	0.08	0.08	-


Frequency
0.0
0.1
0.2
CH_S
GV_C
GV_Y
LC_C
PSG_C
PSG_Y
185
189
193
205
207
209
211
213
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263
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Allele

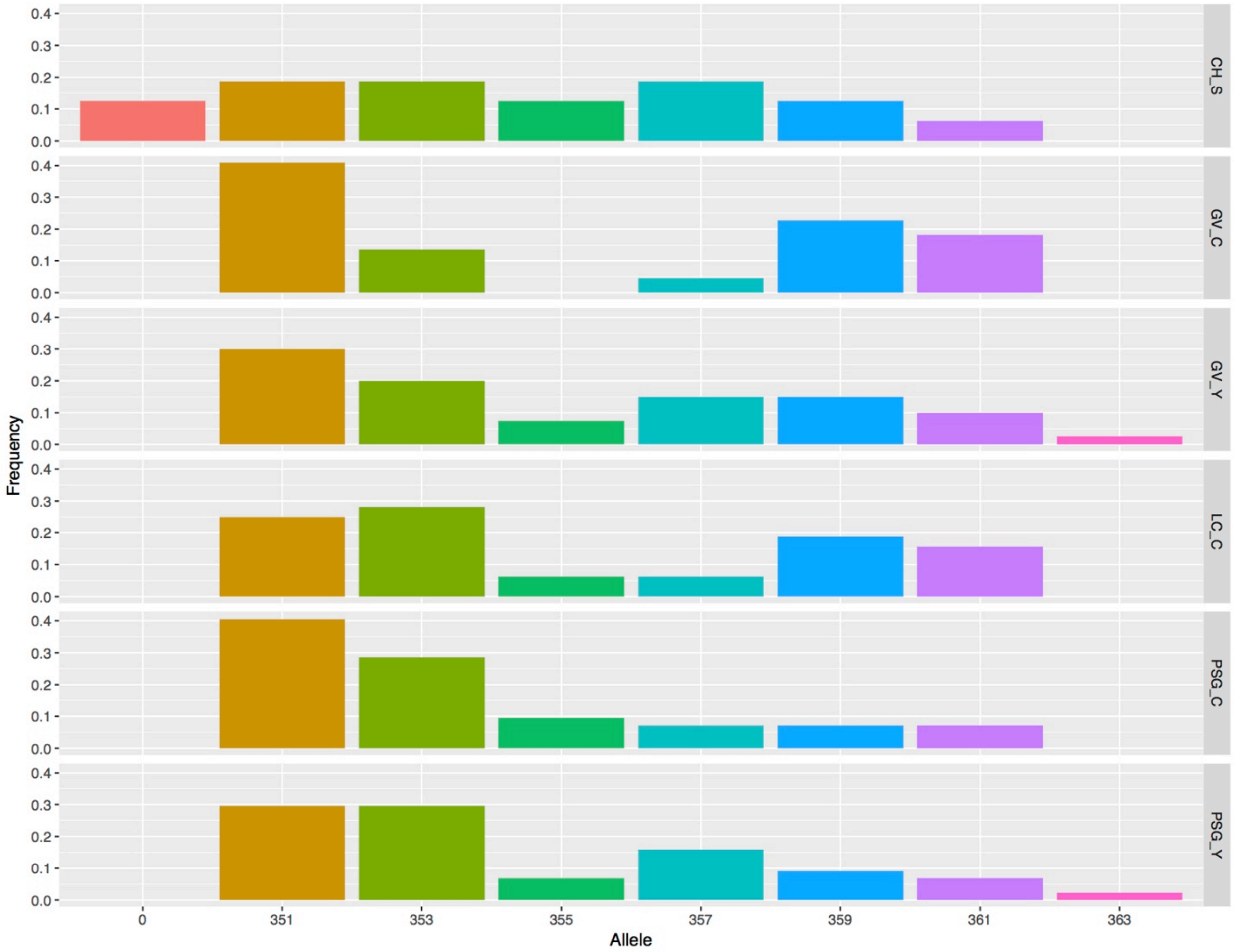

Figure 2. Histograms of allele frequencies for each site. Abbreviations: CH=Charcas, GV=Guadalupe Victoria, PSG=Presa de Santa Gertrudis, LC= La Cardoncita, HDS= Highly degraded scrubland, MDS= Moderately degraded scrubland.

AMOVA yielded lower genetic differentiation among (0%; *P*-value < 0.01) than within (~ 95.31%; *P*-value < 0.01) sites and among individuals (~ 5.2%; *P*-value < 0.01). The analysis with R_{st} values detected no genetic differentiation between BTSP populations (Table 3). PSG-MDS showed a trend of higher genetic differentiation compared to other populations, however these values were not significant (*P*-value < 0.09). Although in PSG-MDS site level of immigration appeared slightly limited, gene flow among populations was high (Table 3). The assignment test suggested that only three samples were allocated as immigrants. One bird from PSG-HDS was assigned to CH site (*Pr* = 0.003); another individual from PSG-MDS was assigned to LC-HDS (*Pr* = 0.002) and finally, one individual from LC-HDS was assigned to GV-HDS (*Pr* = 0.004).

Table 3. Pairwise genetic population differentiation (R_{ST}) between sites (below diagonal) and gene flow (N_m) exchanged between sites (above diagonal). F_{IS} (inbreeding coefficient) values and their significant values (last two columns). Numbers in bold are significant values (no significant values were recorded in R_{st}). ∞ indicate that Nm was uncertain.

	CH-HDS	**GV-HDS**	**GV-MDS**	**PSG-HDS**	**PSG-MDS**	**LC-HDS**	**F_{is}**	***P*-value**
CH-HDS	-	∞	∞	∞	∞	∞	**0.15**	**0.03**
GV-HDS	-0.02	-	∞	27.28	6.66	∞	0.06	0.23
GV-MDS	-0.01	-0.02	-	1553	6.32	∞	0.02	0.34
PSG-HDS	-0.03	0.01	0.00	-	20.43	∞	0.06	0.14
PSG-MDS	-0.02	0.07	0.07	0.02	-	106.15	0.05	0.15
LC-HDS	-0.04	-0.03	-0.01	-0.02	0.00	-	0.03	0.31

PCA of allele frequencies of individuals showed a high level of admixture between all the populations sampled (Figure 3). PCA from allelic frequencies and genetic distance (Nei's distance) were performed between populations (Figure 4). Genetic distance in the first principal component of the PCA exhibited more dissimilarity in PSG sites relative to the remaining sites (Figure 4A). However, no well-delimited clusters were identified. Similarly, the DAPC analysis failed to separate sites into genetically similar clusters (Figure 5). Optimal number of clusters estimated by Bayesian inference criterion in DAPC analysis was four, as *a posteriori* group assignment (Figure 6). However, this assignment was not related to group priors or to a specific geographic pattern.

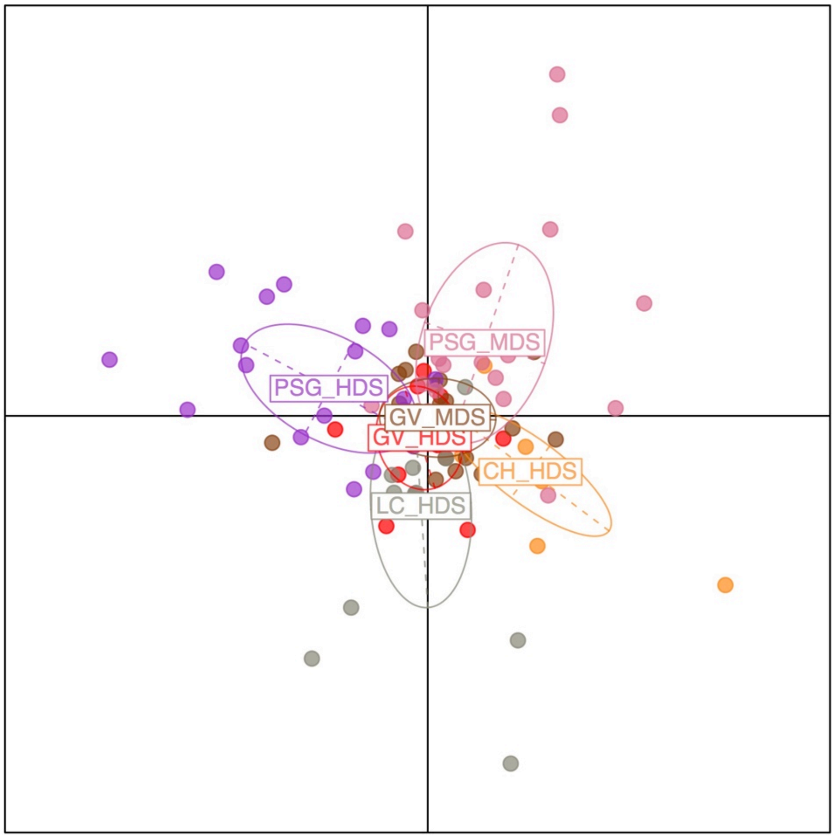

Figure 3. Results of principal components analysis (PCA) using microsatellite allele frequencies from BTSP individuals. Clusters are depicted with different colors and as ellipses (centroids). Dots represent individuals. For abbreviations see Figure 2.

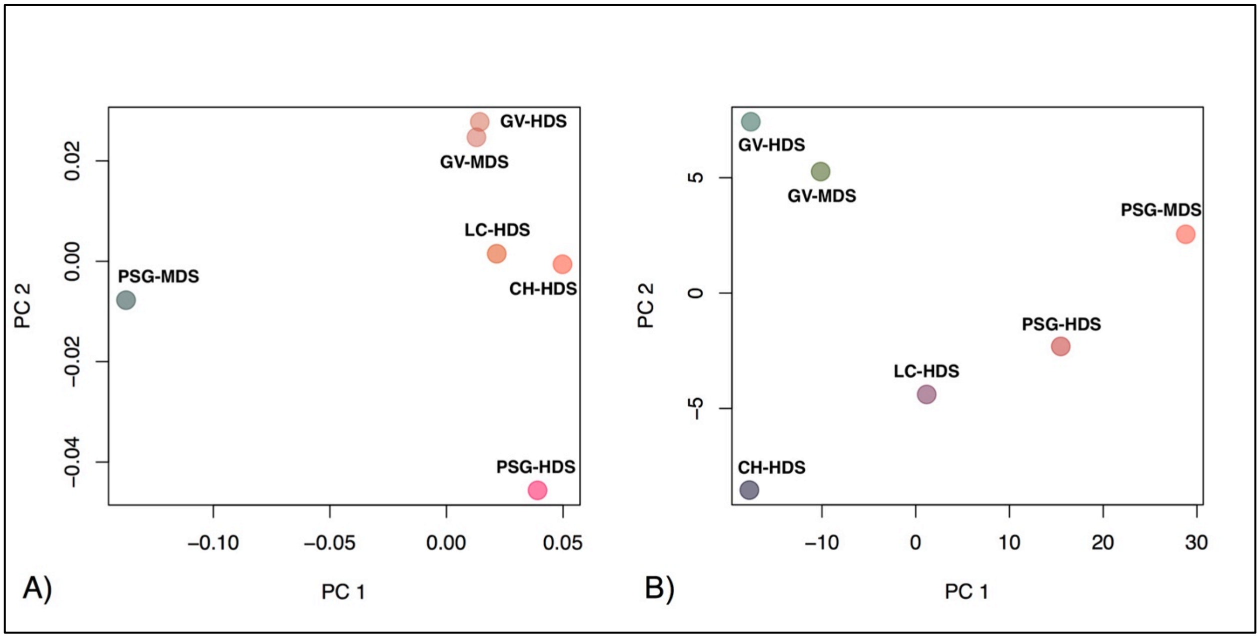

Figure 4. Principal components analysis (PCA) using A) Nei’s genetic distance and B) allele frequencies of BTSP populations. For abbreviations see Figure 2.

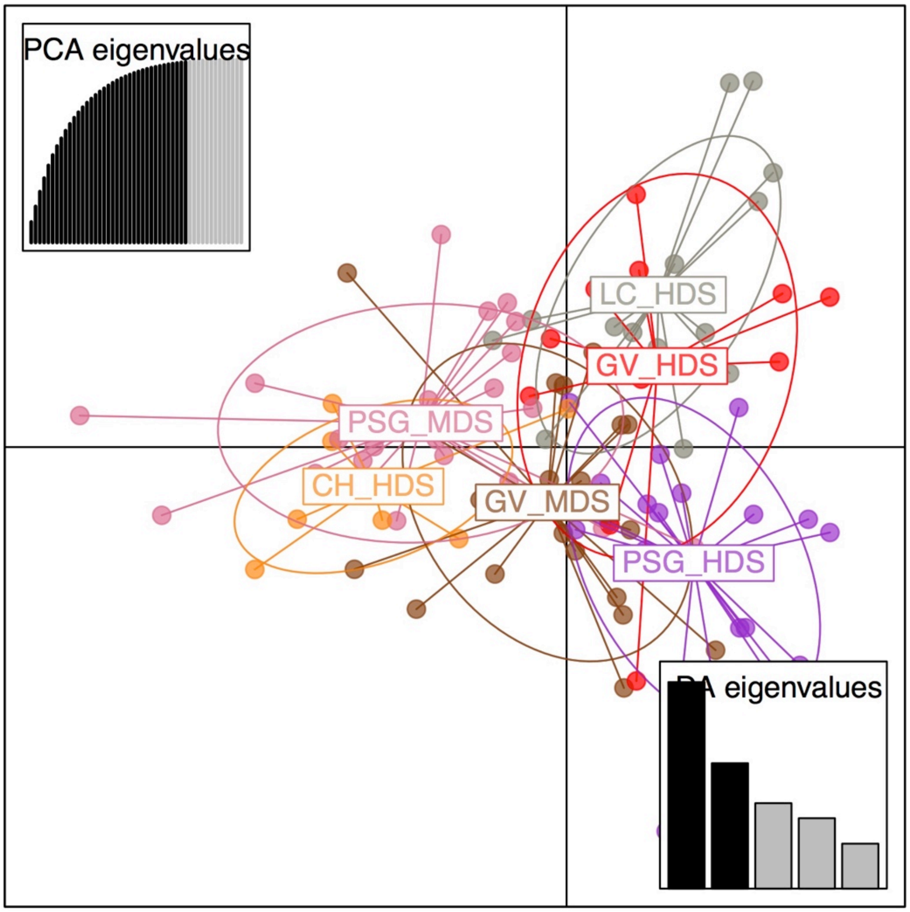

Figure 5. Scatterplot of discriminant analysis of principal components (DAPC) using microsatellite allele frequencies of BTSP individuals. The histogram in the lower part shows the first two principal components of the DAPC. The histogram in the upper part shows eigenvalues retained in the analyses. Clusters are illustrated in different colors and ellipses (centroids). Dots represent individuals. For abbreviations see Figure 2.

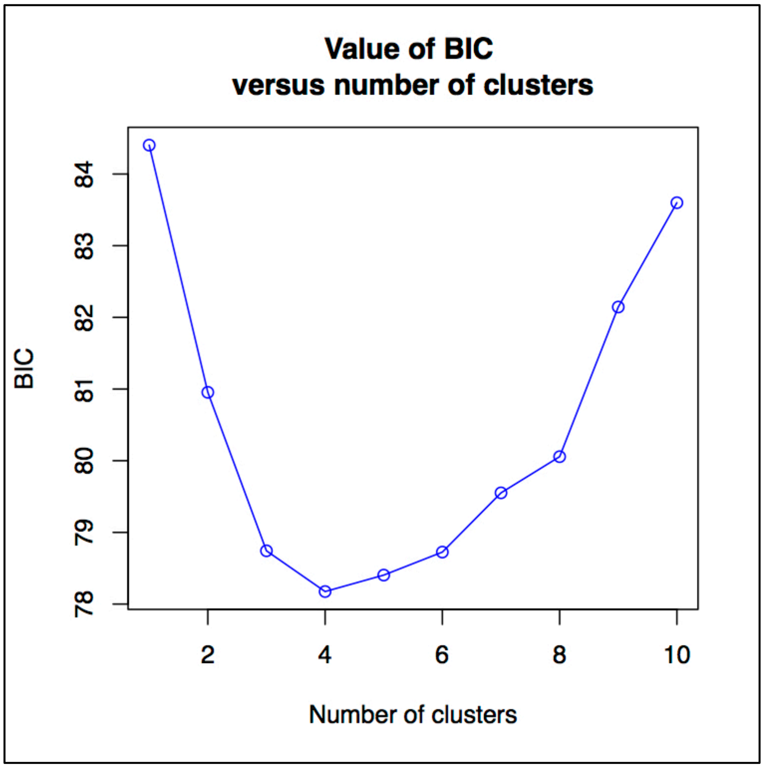

Figure 6. Inference of the number of clusters through Bayesian information criterion (BIC). The indicated number of clusters is the minimum number of clusters after which the BIC increases or decreases.

Discussion

In this study, a higher genetic diversity is reported for the BTSP compared to two passerine species (Cactus wren and Canyon towhee) (Reinoso-Pérez et al. 2014). This difference may be owed to a lower sample size and its effect on genetic parameters in Reinoso-Pérez et al. (2014) study, compared with the current study. CAM17 was the only marker with the lowest genetic diversity probably owing to strong selective pressure, also observed in Reinoso-Pérez et al. (2014). Only for two sites, CH-HDS and GV-HDS, evidence of deficiency in heterozygosity was found. Moreover, for the CH-HDS site a significantly high inbreeding coefficient (F_{is}) was also obtained. This result may be due to the strong influence of human activities on the BTSP population inhabiting this area. Intense mining activity in this site associates to unusually high heavy metal concentrations in soils and in bird feathers (Monzalvo-Santos et al. 2016). Metal exposure may negatively influence BTSP populations; a potential reduction in population size (mean in CH-HDS= 1.3 captures, LC-HDS= 5 captures, GVG-HDS= 2.75 captures, PSGG= 4.8 captures; unpublished data) through lower apparent survival of BTSP and other passerines in the region in sites affected by mining activity, which was indeed lower in comparison to unexposed sites (unpublished data), in combination with non-random mating may have contributed to the relatively high rate of inbreeding in CH site. This process reduces genetic diversity through loss of alleles, and therefore, increase homozygotes (Bickham et al. 2000; Freeland et al. 2011). Alternatively, the results of the current study might be an artifact of low sample size at the CH site. Reinoso-Pérez et al. (2014), for instance, reported high inbreeding in GV sites in *C. brunneicapillus*. However, the sample size for that study was very limited. Indeed, small sample size in genetic parameter estimations may be a source of bias (Pruett and Winker 2008; Hale et al. 2012). Notwithstanding, the two parameters that are used to calculate the inbreeding coefficient, expected and observed heterozygosity (Freeland et al. 2011), tend to have negligible bias and acceptable accuracy even if sample sizes are small (>5 individuals) when diversity is low (Pruett and Winker 2008). Therefore, the inbreeding coefficient estimated in the current study is probably unbiased.

The current study yielded non-significant genetic differentiation in terms of *Rst* values among the populations that were sampled and no spatial pattern in genetic structure related to habitat type was found. Evidence of admixture was found in all the populations with presence of potential first generation migrants (i.e., CH-HDS, LC-HDS, and GV-HDS). For the current study, the sites with low vegetation density seem to have been the origin population of dispersers. In terms of differences among habitat types, lower vegetation density was indeed the preferred habitat where nest survival of BTSP was higher (Pidgeon et al. 2003, 2006; Kozma et al. 2017). This species probably shifts habitats from low to high vegetation density, when searching for food (Shenbrot 2004; Bradbury et al. 2015). Shenbrot (2004) observed a habitat shift from densely vegetated dry river beds (wadi) in winter-spring to open flat terraces in summer-autumn in a rodent inhabiting a dryland ecosystem; the habitat types differed in their spatio-temporal availability of resources and predation pressure. Movements of BTSP flocks during winter, when birds search for food, and the distance of displacement of these birds between sites are aspects that have not been studied before. Also, BTSP's individuals out of their place of birth or of their winter home range have not been recorded yet (Johnson et al. 2002). This activity and dispersal of juveniles could promote high gene flow among populations in BTSP despite the high philopatry in this species. However, for an ongoing long-term study based on mark and recaptures in the study area, all BTSP recaptures took place at the original capture site (data unpublished), thus indicating that the species exhibits high degree of site fidelity and perhaps most dispersal corresponds to juveniles. Indeed, the genetic data provided evidence of dispersal and gene flow between sites within the study region.

Using multivariate analyses with allele frequencies and genetic distance performing DAPC, less admixture than expected to maximize variation between populations was found in PSG sites. It was difficult to explain the negligible genetic differentiation in PSG-MDS and PSG-HDS sites. Perhaps, a high concentration of resources in combination with moderate human activity in this alluvial plain are factors that may provide high plant density and food availability for BTSP (Mueller et al. 2013; Johnson et al. 2002). As a result, population dynamics may be affected such that a very high proportion of the population persists in this area (higher philopatry). Also, the probability of adaptive divergence from populations within the same area using specific phenotypic traits to exploit resources from a particular area may be possible during a long period (Dopman et al. 2002; Crispo 2008; Allen et al. 2010). However, more data related to this potential genetic differentiation are needed.

BTSP’s maintain a continuous distribution in most of the landscape, avoiding nearby urban areas (Johnson et al. 2002). Though this spatial distribution pattern may not help explain the pattern of the genetic structure of BTSP, the examination of the genetic differentiation of this bird species in a delimited geographic area is an important contribution to the knowledge on population genetics in dryland habitats. High genetic diversity is indicative of high adaptive potential. Conversely, in small populations, such as the one described for the CH site, low genetic diversity may indicate a decrease in selection response, and consequently fitness reduction due to inbreeding depression

(Gaines et al. 1997; Willi et al. 2006; Freeland et al. 2011). Consideration of landscape features in genetic analyses is required for species such as BTSP in order to identify how continuous spatial attributes may affect gene flow. Landscape genetics may be the next step to evaluate the influence of spatial features such as topography, vegetation, croplands, etc., in a local population context (Manel et al. 2003; Holderegger and Wagner 2008). Therefore, future studies should probably increase the spatial scale for sampling, and take into account specific barriers affecting this species. Finally, formation of winter flocks in BTSP should be monitored to acquire more evidence of possible dispersal of juveniles during this period and how this may affect gene flow.

Conclusions

A genetic admixture pattern was observed in BTSP, with a non-significant genetic differentiation between populations among sites, thus representing one single population rather than six populations as originally postulated. While DAPC identified four clusters, these groups were not easily recognized in both PCA and DAPC analyses. In addition, a high inbreeding value was observed at the CH site, suggesting a negative influence of mining activity on a declining BTSP population, where substantially reduced genetic diversity was detected. Other than this outcome, which was recorded only at the CH site, no negative influence of habitat degradation or fragmentation on the genetic population structure of BTSP was observed. Identification of landscape variables linked to habitat modification and natural habitat structure by using specific landscape genetic analyses are research needs for the future, in order to accurately understands their potential effect on the population genetic structure of this bird species at a much larger spatial scale. This study increased the knowledge on genetic characteristics in dryland bird populations. It also demonstrated clear detrimental effects of heavy metal pollution on the genetic loss of a bird species.

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Conclusions

General conclusions and future research directions

Through the current study it was possible to determine that the Black-throated Sparrow is an ideal model species to investigate how environmental changes caused by anthropogenic activities, such as plant replacement and extraction, overgrazing, and mining activity simultaneously affect different traits (song, genetics, and haemosparasitism) linked to biological and ecological processes pertaining to a single organism. Great haemosporidian parasite diversity was found within this single host species. Though prevalence values were moderate, a significant effect of season was found, with higher prevalence and parasitaemia during the breeding season. However, no evidence of influence of anthropogenic degradation on prevalence and parasitaemia was recorded, suggesting an opposite response in comparison with other bird species in the same study area. Contrastingly, mining activity negatively affected some acoustic features related to vocal performance and system learning in BTSP's song. This result suggests male fitness may be compromised when these song features are modified and, therefore, mating success may be diminished under exposure to intense mining activity. Finally, loss of genetic diversity was related to human activity, specifically mining activity, with high levels of inbreeding in a BTSP population inhabiting a site with a long mining history. However, no genetic population structure was detected, thus indicating poor genetic differentiation between populations. Also, some cues of bird dispersal among sites were obtained through the genetic data; a directional dispersal pattern from high-degraded areas to more conserved areas was observed. This suggests that habitat shift may occur in some individuals that search for food resources. Therefore, by simultaneously investigating different types of responses to anthropogenic degradation including infection by haemoparasites, song structure and genetic diversity, this study allowed documenting effects of different types of degradation that would otherwise not have been evident.

Overall, mining was the strongest human activity negatively influencing genetic diversity and song structure in the BTSP. Intensive land use change and heavy metal pollution have been present in this area for at least 400 years. Interestingly, fewer song types and lower genetic diversity appeared in the site most exposed to mining, suggesting that a potential reduction in population size may occur. However, the specific mechanism involved in this process is unknown as mixed processes may be occurring, such as poor recruitment of individuals and sub-lethal or lethal effects by heavy metal pollution.

More research about which landscape features influence the ecology of this bird species is needed, especially in relation to human activities. In addition, knowledge about the extension and philopatry of winter range of juveniles and adult birds is also required. This information would increase understanding about the contribution of these individuals to gene flow between sites and habitats. Also, the use of song traits in combination with genetic data as an alternative source of information may be useful to detect patterns of similarity within and among populations. One additional topic to investigate is the potential change in parasite transmission among populations in climate-change scenarios that may include shifts in species distributions.

Drylands are subject to land degradation and the great geographical extension that they encompass may significantly influence the stability of the Earth system as a whole. Information about biological and ecological processes in local scales and linkage among these processes with other biological models occurring in the same area may help better understanding the repercussion of habitat degradation at regional and global scales. Considering the local scale, for instance, it can be said that a portion of the study area included in the current study has been decreed as a “Natural Protected Area” because of the high biodiversity values that this region harbors. However, the management plan for this area does not seem to have been implemented since intense anthropogenic activities are still present. Also this region holds a high cultural importance, where the indigenous group “Wixarika” has sacred sites across this area. Ecosystem services are also important, not only for genetic-functional diversity maintenance, carbon sequestration regulation, or provisioning services to croplands and livestock, but also to preserve people’s customs and traditions. It is essential to investigate all the biological components in the area to know about their value, and to use this information to regulate human activities that may affect the biota, and also, indirectly preserve cultural expressions for specific social groups that are benefited by the natural resources of this area.