



## Ambient temperature drives sex ratio and presence of pregnant females of *Anoura geoffroyi* (Phyllostomidae) bats living in temperate forests

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Phenology in animals is strongly influenced by seasonality that promotes changes in abundance of food resources and temperature. These changes may impose energetic constraints to organisms in certain seasons during the year, especially on those animals facing high energetic demands, such as nectarivorous bats. Seasonality in temperate forests could, therefore, promote migration of female nectarivorous bat to find warmer sites, thus enhancing breeding success. To test this hypothesis, we compared the proportion of females and the proportion of pregnant females of the nectarivorous bat *Anoura geoffroyi*, between months, in six different populations across temperate forests of Mexico. Bats were captured over a complete season cycle either with sweep or mist nets at the entrance or near their roosting caves, and their age, sex, and reproductive condition were recorded. We found that over 50% of bats present in the cave roosts across different populations in temperate forests of the Trans-Mexican Neovolcanic Belt of Mexico during the warmer and wetter months (April–September) were females, both pregnant and nonpregnant. In contrast, fewer than 30% of bats present in the roosting caves sampled in the colder and drier months (October–March) were females. In addition, we found that the temperature that favors the proportion of females at the studied sites was greater than 8°C. We concluded that seasonality affects sex ratio and phenology of *A. geoffroyi* in Mexican temperate forests. Our findings suggest females' migrations to lowland warmer sites to improve prenatal development.

Key words: climate change, cloud forest, nectar-feeding bats, Neotropics, pine-oak forest

La fenología en los animales está fuertemente influenciada por la estacionalidad, la cual promueve cambios en la abundancia de recursos alimenticios y la temperatura. Estos cambios pueden imponer restricciones energéticas a los organismos en ciertas estaciones a lo largo del año, especialmente en aquellos animales que se enfrentan a altas demandas energéticas, como los murciélagos nectarívoros. Por lo tanto, la estacionalidad en los bosques templados podría promover la migración de hembras de murciélagos nectarívoros para encontrar sitios más cálidos que mejoren su éxito reproductivo. Para poner a prueba esta hipótesis, comparamos la proporción de

hembras y la proporción de hembras embarazadas del murciélago nectarívoro *Anoura geoffroyi*, entre meses, en seis poblaciones diferentes en bosques templados de México. Los murciélagos fueron capturados durante un ciclo estacional completo, ya sea con redes de barrido o de niebla en la entrada o cerca de sus cuevas de percha, y se registraron su edad, sexo y condición reproductiva. Encontramos que más del 50% de los murciélagos presentes en los meses más cálidos y húmedos (abril-septiembre) eran hembras y hembras embarazadas, en diferentes poblaciones a lo largo de los bosques templados del Eje Neovolcánico Transversal Mexicano. Al contrario, en los meses más fríos y secos (octubre-marzo), menos del 30% de los murciélagos presentes en las cuevas de percha muestreadas fueron hembras. Además, encontramos que la temperatura que favorece la proporción de hembras en los sitios estudiados fue mayor a 8°C. Concluimos que la estacionalidad afecta la proporción de sexos y la fenología de *A. geoffroyi* en los bosques templados mexicanos. Nuestros hallazgos sugieren que las hembras migran a las tierras bajas (sitios más cálidos) para favorecer el desarrollo prenatal de sus crías.

Palabras clave: Bosque pino-encino, Bosque de niebla, Cambio climático, Murciélagos nectarívoros, Neotrópico

Reproductive strategies of bats vary depending on their biogeographic affinity. Temperate bats tend to have only one reproductive event per year (Barclay and Harder 2005), whereas tropical bats may have several reproductive events because they could be seasonally monoestrous, seasonally polyestrous, bimodal seasonally polyestrous, or year-round sexually active (Fleming et al. 1972; Montiel et al. 2011; Durant et al. 2013). The use of different reproductive strategies is considered an adaptation to produce offspring in energetically favorable environments during certain times of the year (Fleming et al. 1972).

The two main factors hypothesized to be determinants of reproduction in bats are food availability and ambient temperature (Kunz 1982; Arlettaz et al. 2001; Barclay et al. 2004; Mello et al. 2009; McGuire and Boyle 2013; de Carvalho et al. 2019a). In Neotropical phyllostomid bats, ambient temperature seems to be an important factor in modulating life cycles (Mello et al. 2009). Compared to their temperate bat counterparts, some Neotropical phyllostomid bats have not evolved strategies to cope with low ambient temperatures, and their elevational distributions are set by their thermal limits (Speakman and Thomas 2003; Jankowski et al. 2013; Ortega-García et al. 2017; de Carvalho et al. 2019b). Temperature also regulates plant productivity, nectar and fruit production, and the availability of prey, by limiting food resources in cold climates that prevail at high elevations (Loiselle and Blake 1991; Nicolson et al. 2007; McGuire and Boyle 2013). Metabolism of bats is also regulated by temperature, where important physiological processes like digestion, mating, and lactation require ambient temperatures around 30°C (Menaker 1962). Some phyllostomid bats can coexist and be abundant at high elevations (ca. 1,000 m.a.s.l.), but they rely on other strategies such as seasonal migrations or facultative torpor to cope with low temperatures, even if food is not a limiting factor (Dwyer 1971; Audet and Thomas 1997; Mello et al. 2008).

Although Neotropical bat species are adapted to cope with certain levels of food scarcity and have evolved to live at low temperatures, reproductive females have to face additional challenges associated with the energetic demands imposed by the sites where they live. For instance, pregnant females change their wing loading and maneuverability (Norberg and Rayner 1987; Haysen and Kunz 1996), and decrease their foraging efficiency when their energy demands increase (Barclay and

Harder 2005). Therefore, many females make seasonal movements following resource availability and the climatic conditions needed to give birth and raise their young (Angell et al. 2013). These movements, a result of their particular needs in their reproductive cycle, may determine sex proportions in the colonies they compose (Baumgarten and Vieira 1994; Senior et al. 2005; Torres-Flores et al. 2012; Ferreyra-García et al. 2018).

*Anoura geoffroyi* is an ideal biological model to evaluate the effects of temperature on sex ratio and reproductive patterns. This nectarivorous phyllostomid bat has one of the broadest temperature tolerance zones (Ortega-García et al. 2017), resulting in a large distribution together with occupancy of numerous distinct altitudinal habitats (de Carvalho et al. 2019b). Most nectarivorous bats are reproductive during the dry season of the year at different latitudes (Fleming et al. 1972; Willig 1985; Graham 1987; Stoner et al. 2003). However, recent evidence has documented for temperate forests that the presence of female bats and their reproductive events are synchronized to the warmer and wetter seasons (Ferreyra-García et al. 2018), suggesting that females make seasonal migrations following the climatic conditions needed to give birth and raise their offspring (Angell et al. 2013). In addition, there is no evidence of delayed development of embryos in *A. geoffroyi*, as has been documented for other phyllostomids (e.g., *Carollia perspicillata*, *Macrotus waterhousii*, and *Artibeus jamaicensis*—Raswailer and Badwaik 1997). We, therefore, hypothesized that a greater proportion of females would be found in the warmer and wetter seasons across different populations in temperate forests, because *A. geoffroyi* females make seasonal movements to follow the climatic conditions necessary to give birth and raise their pups. The objective of this study was to assess this prediction by evaluating the effect of temperature on the proportion of females constituting populations of *A. geoffroyi*, and the reproductive status of these females, over a complete seasonal cycle (1 year), in different temperate forests located in the Trans-Mexican Neovolcanic Belt mountain range of central Mexico.

## MATERIALS AND METHODS

*Study sites.*—We selected six populations located in temperate forests across the Trans-Mexican Volcanic Belt to

evaluate the effect of temperature variation through a complete seasonal cycle (1 year) over the reproductive activity and sex ratio of *A. geoffroyi* (Table 1). Colony 1, in Estado de Mexico, was located in a cave locally known as “La Mina,” 1.5 km N from the village San Francisco de las Tablas in the Chapa de Mota County (19°45′00.0″N, 99°30′00.0″W, 2,670 m.a.s.l.). The cave is surrounded by a well-preserved pine-oak forest dominated by *Pinus* sp. and *Quercus* sp. (Galindo-Galindo et al. 2000). Colony 2, in Jalisco, was located in a cave immersed in oak forest locally known as “La cueva del Ermitaño” near the town of Techaluta de Montenegro (20°04′51.6″N, 103°35′49.2″W, 2,422 m.a.s.l.). Colony 3, in Michoacan, was located in a volcanic cave named “La cueva de los murciélagos” (19°45′21.6″N, 101°21′28.8″W, 2,215 m.a.s.l.). The vegetation surrounding this cave was oak-pine forest dominated by *Quercus* sp. and *Ipomoea* sp. trees, and dry forest dominated by trees of the family Bombacaceae (Ferreira-García et al. 2018). Colony 4, in Morelos, was in a volcanic cave named “Chihihuiuteca,” near the city of Cuernavaca (18°58′48.0″N, 99°10′55.2″W, 1,750 m.a.s.l.). The natural vegetation surrounding this cave was dominated by tropical dry forest and deciduous oak forest. However, most of the natural vegetation has been transformed into pastures and croplands dominated by corn and tomato. Colony 5, in Tlaxcala, was in a slit in a rock outcropping located in the middle of corn croplands (19°21′00.0″N, 98°17′60.0″, 2,308 m.a.s.l.), surrounded by patches of *Pinus pseudostrobus*, *Quercus* sp., and *Cupressus benthamii* (Mendieta-Pluma 2011). Unlike all the other colonies in this study, colony 6 in Veracruz was sampled in a rural road through the gullies of the Cascadas de Texolo Ramsar Site, in the central part of the state, in the eastern foothills of El Cofre de Perote (19°24′03.6″N, 96°59′38.4″W, 1,126 m.a.s.l.). The original vegetation was montane cloud forest with temperate weather, but most of the extant remnants are in a matrix of secondary vegetation and shade-grown coffee plantations.

**Bat captures.**—We gathered data of sex and females’ reproductive condition from different bat research projects, resulting in different time spans and years. However, we obtained all data following similar procedures by capturing bats with sweep or mist nets at the entrance or near their roosting caves. We identified captured bats to species using specialized field guides (Medellín et al. 2008; Reid 2009) and standard information on each bat captured was recorded, including: weight, forearm length, sex, and age (based on the ossification of the phalanges—Brunet-Rossini and Wilkinson 2009). We assessed

reproductive condition of females as inactive (no signs of being in reproduction), pregnant, lactating, and postlactating (healed nipples). We followed guidelines approved by The American Society of Mammalogists (Sikes et al. 2016) to handle the animals; permits were issued by the Ministry of Environment and Natural Resources of Mexico (SEMARNAT) to all the authors in this study.

**Collection of temperature data.**—We obtained ambient temperature from Cuervo-Robayo et al. (2014). The climate layers are the result of the interpolation of temperature and precipitation data for the years 1910–2009, which contemplate a great number of climatic stations and had a high-resolution (30 arc sec, ~1 km). The matrix data of minimum monthly temperature was downloaded from <http://idrisi.uaemex.mx/distribucion/superficies-climaticas-para-mexico>. We used only the minimal temperature variable because these weather values are the most limiting constraints to bats for breeding. We projected the Minimum temperatures in QGIS Desktop ver. 3.0.0 and used the Point Sampling Tool plug-in to obtain the minimum temperatures. This plugin generates a new layer with the selected values of the study sites.

**Data analysis.**—To evaluate whether seasonality had an effect on the proportion of females and pregnant females constituting each locality’s population of *A. geoffroyi*, we adjusted two generalized linear models (GLMs). One for females and another for reproductive females, with a post hoc chi-square analysis for standardized coefficients and a Gaussian distribution of error. The GLM model expression was: response variable ~ month + altitude, error = Gaussian. We used the proportions of females and pregnant females as a response variable (from the total individuals captured per site), reduce the bias caused by sampling effort of each locality, whereas altitude was employed as a fixed factor because we had two localities below 2,000 m.a.s.l. (see Table 1).

We built a null model, a linear model, a logistic model, a power law model, and a piecewise model, and used a model selection approach to evaluate if there was a minimal temperature that favors the proportion of females and pregnant females at the study sites (Ficetola and Denoel 2009). We considered that piecewise models could be used to determine critical temperature values at which the proportion of females and pregnant females may drop or peak. Piecewise models investigate threshold relations and can be adjusted to data that have two distinct regions with different angular coefficients connected by a breakpoint (Lima and Mariano-Neto 2014; Rocha-Santos et al. 2016). These models

**Table 1.**—Geographic and climatic characteristics of the study sites within the Trans-Mexican Neovolcanic Belt of Mexico. Only the minimal temperature is shown as it is the most limiting variable to pup development. Abbreviations are: sampling events (Sa.E.), minimal temperature ( $T_{\min}$ ), meters above sea level (m.a.s.l.), and frequency of sampling events (F.) are present.

Site	Altitude (m.a.s.l.)	Longitude (N)	Latitude (W)	$T_{\min}$ (°C)	Sampling period	Sa.E.	F.
Edo. Mex.	2,670	19°45′00.0″	99°30′00.0″	5.90	Jan. 1990–Dec. 1991	24	Monthly
Jalisco	2,422	20°04′51.6″	103°35′49.2″	7.64	May 2016–Aug. 2017	5	Bimonthly
Michoacan	2,215	19°45′21.6″	101°21′28.8″	8.23	Sep. 2014–Sep. 2015	13	Monthly
Morelos	1,750	18°58′48.0″	99°10′55.2″	12.70	Dec. 2014–Nov. 2015	12	Monthly
Tlaxcala	2,308	19°21′00.0″	98°17′60.0″	5.31	May 2007–Apr. 2008	12	Monthly
Veracruz	1,126	19°24′03.6″	96°59′38.4″	13.69	Feb. 2015–Nov. 2016	4	Seasonally



previously were used to identify thresholds of ecological phenomena based on the relationship between deforestation percentages and plant species richness (Rocha-Santos et al. 2016).

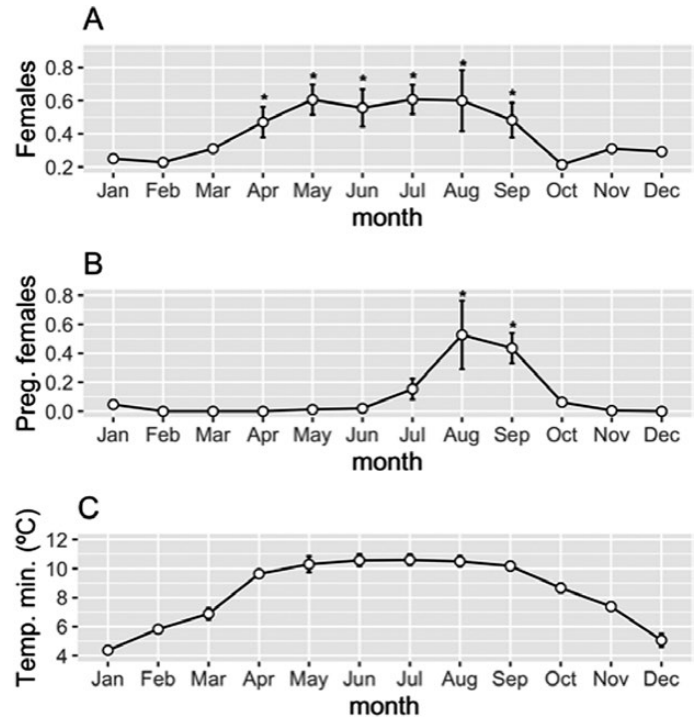
We fitted the linear model using a GLM with a Gaussian distribution and the logistic model with a binomial distribution. The power model was of the type  $y = ax^b$ , where  $a$  and  $b$  were obtained from the slope and intercept values of a linear model built with the natural logarithmic values of the response and explanatory variables. For the piecewise regression, we allowed the model to estimate the breakpoint value without any previous assumptions. We implemented all analyses in R version 3.4.2 (R Development Core Team 2013). We fitted the null, linear, and nonlinear models using the functions “glm” and “lm” of the stats package and used the “nls” function of the “powerLaw” package to fit the power law model. We used the Akaike Information Criterion adjusted to small samples (AICc) and Akaike information weights (AICc weights) to select the best model (Anderson 2008) based on its lowest AIC value, and with its weight being at least two times the weight of the second-best model. We conducted the model testing using the function “AICc” in the “AICcmodavg” package.

## RESULTS

We captured 1,110 individuals, 304 (27%) for Morelos, 275 (25%) for Estado de México, 195 (18%) for Veracruz, 130 (12%) for Michoacán, 116 (10%) for Tlaxcala, and 90 (8%) for Jalisco. Percentages of captured individuals and their sexual ratio varied across sites and seasons. We captured females all year-round only in the sites of Estado de México, Morelos, and Veracruz. Females made up 61% ( $n = 169$ ) of the total sampled individuals for Estado de México, 62% ( $n = 121$ ) for Veracruz, 51% ( $n = 157$ ) for Morelos, 42% ( $N = 55$ ) for Michoacán, 35% ( $n = 32$ ) for Jalisco, and 25% ( $n = 29$ ) for Tlaxcala. We captured males all year-round in all sites and they made up 38% ( $n = 106$ ) of the total sampled individuals for Estado de México, 38% ( $n = 74$ ) for Veracruz, 48% ( $n = 147$ ) for Morelos, 57% ( $n = 75$ ) for Michoacán, 64% ( $n = 58$ ) for Jalisco, and 75% ( $n = 87$ ) for Tlaxcala.

Overall, the populations of females displayed a monoestrous reproductive phenology across the five sampled sites (Fig. 1). The pregnancy period occurred mostly at the end of the dry season and during the rainy season—from July to September—with the peak occurring in August. Males showed a bimodal polyestrous reproductive phenology with high proportions of males with descended testes reproductively active preceding the peaks in pregnancy by a few months (April and May), with a second peak in autumn during the months of October and November.

The proportion of females and pregnant females (of the total individuals captured) increased in the months with the highest minimum temperatures (Fig. 1). The altitude of the study sites was not a significant parameter in each adjusted model (Table 2). The proportion of females present at the study sites increased as temperatures increased. The relationship could be explained either as a linear or power law response (Table 3; Fig. 2). However, minimum temperatures did not have a significant effect on the proportion of pregnant females present at the study sites.



**Fig. 1.**—Monthly mean proportion (out of the total number of individuals captured) of females (A) and pregnant females (B) of *Anoura geoffroyi* compared to monthly minimum temperature of the six study sites (C) of temperate forests within Trans-Mexican Neovolcanic Belt of Mexico. The asterisk above the mean values of each response variable indicate the months that were significantly different from the other months based on the generalized linear model (GLM) results.

## DISCUSSION

As predicted, we found a greater proportion of females and pregnant females in the warmer and wetter seasons across different populations in temperate forests of the Trans-Mexican Neovolcanic Belt of Mexico. Our results support the hypothesis that temperature is one of the main factors determining the reproductive cycle in female bats. This result suggests that females of *A. geoffroyi* make altitudinal migrations searching for ideal temperatures in which to breed and carry out subsequent embryo development. Other studies have found segregation in the roost of this species at least during a reproductive season (Willig 1985; Graham 1987; Baumgarten and Vieira 1994; Ortega and Alarcón 2008; Ferreyra-García et al. 2018).

Three hypotheses have been proposed to explain the sex bias of bats throughout the seasons. The first hypothesis suggests that females have reduced their capacity to tolerate cold ambient temperatures (McGuire and Boyle 2013). *Anoura geoffroyi* males have the capacity to use torpor when the ambient temperature drops 5°C below their body temperature, so as to maintain homeostasis (Ortega-García 2018). However, this behavior has not been reported for female phyllostomid bats. Therefore, biases in proportion of sexes in populations of *A. geoffroyi* at different times of the year could be related to the limitation of phyllostomid females to go into torpor when they are breeding in the colder months.

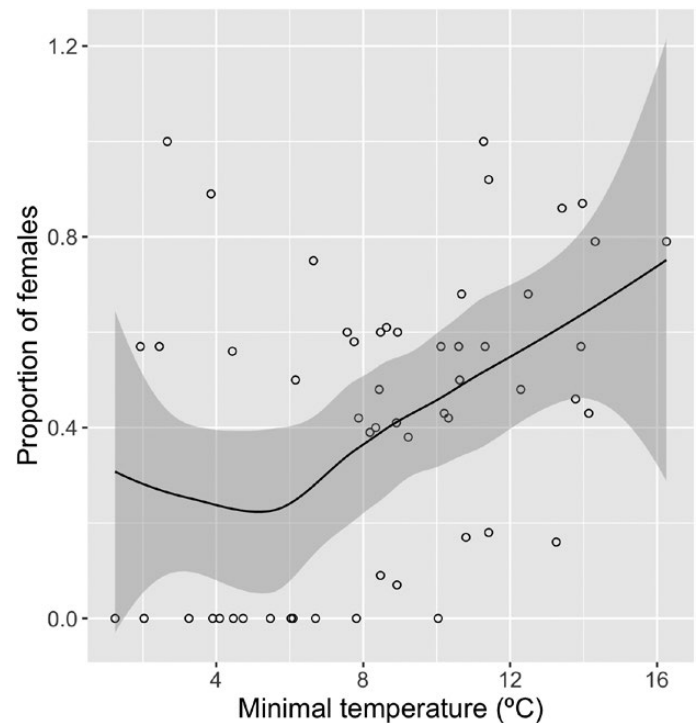
**Table 2.**—Residual deviance values of the post hoc chi-square test for the standardized coefficients of the models adjusted to explain the variation of the proportion of females and pregnant females in *Anoura geoffroyi* bats; in relation to month and altitude of temperate forests within Trans-Mexican Neovolcanic Belt of Mexico. Significant values means that the standardized coefficients of the model are different of a null model.

Response variable	Explanatory variable	Residual deviance	<i>df.</i>	<i>P</i>
Proportion of females	Month	10.51	12	> 0.001
	Altitude	0.00	1	0.84
Proportion of pregnant females	Month	1.68	12	> 0.001
	Altitude	0.00	1	0.87

**Table 3.**—Models adjusted to explain the relationship between minimal temperatures and the proportion of females in *Anoura geoffroyi* bats of temperate forests within Trans-Mexican Neovolcanic Belt of Mexico. Results on the proportion of pregnant females were not significant; therefore, are not shown here.

Variable	Model	dAICc	<i>df.</i>	Weight
Proportion of females	Linear	0.0	3	0.5219
	Power law	0.8	3	0.3578
	Null	8.0	2	0.0095
	Piecewise	3.1	5	0.1108
	Logistic	53.8	2	< 0.001

The second hypothesis proposes that bats' biased sex ratios at different times of the year could be related to intraspecific competition for food between individuals of different sexes (Ruedas et al. 1994; Cryan et al. 2000; McGuire and Boyle 2013). Food resources for *A. geoffroyi* in the temperate montane forest of the Trans-Mexican Neovolcanic Belt of Mexico generally are stable throughout the year (Ferreira-García et al. 2018). However, the biased sex ratios presented by *A. geoffroyi* at different times of the year matches those of previous studies on proportions in sex ratios of insectivorous bats in temperate forests (McGuire and Boyle 2013). Females of insectivorous temperate bats make altitudinal migrations to warmer habitats with a higher density of prey (Barclay 1991; Cryan et al. 2000; McGuire and Boyle 2013). Several studies have shown that insects are an important component in the diet of *A. geoffroyi* (Sazima 1976; Muchhala and Jarrín-V. 2002), with a slight preference in their consumption by females (Caballero-Martínez et al. 2009). We could, therefore, hypothesize that *A. geoffroyi* females make altitudinal migrations in the mountain temperate forest to find higher abundances of this resource and in search of flowering plants during the cold season. Pregnant females have been reported in August and September in a colony in Michoacán (Ferreira-García et al. 2018). At “La Mina,” (state of México) females give birth from late September to mid-November (Galindo-Galindo et al. 2000). In Trinidad, West Indies, females give birth to a single pup in late November or early December (Heideman et al. 1992). Data as to pregnancy collected in other countries suggests that seasonal breeding may vary geographically (Heideman et al. 1992). Females of *A. geoffroyi* carry their young until they reach about 50–60% of the mother's body mass (Baumgarten and Vieira 1994); lactating females are, therefore, in need of a high-nutrient diet to cope with these additional energetic demands, as proposed for tropical and temperate species (Singh and Krishna 1997; Racey and Entwistle 2000). In this sense, it is possible that pregnant females would



**Fig. 2.**—Scatterplot showing the relationship between minimal ambient temperature and proportion of females at the six studied populations of *Anoura geoffroyi* living in temperate forests across the Trans-Mexican Neovolcanic Belt. The bold line is the predicted relationship adjusted by the model power law model, the gray shadow corresponds to the standard error of the line adjusted.

move to other locations during the cold season to supplement their diet with other food items. For example, females of *Leptonycteris curasoae* consume more pollen than fruits during their reproductive season than do males (Martino et al. 2002). In *A. geoffroyi*, females also present more pollen grains in their fur than males; it has been proposed that this phenomenon might reflect a higher frequency of exploiting nectar as a food item by reproductive females (Caballero-Martínez et al. 2009). Nectarivorous bats tend to have their reproductive period during the flowering season of desirable plant species (i.e., *Ceiba*, *Ipomoea*, and *Pseudobombax*—Caballero-Martínez et al. 2009; Ferreira-García et al. 2018), especially during the dry season (Baumgarten and Vieira 1994; Stoner et al. 2003; Sperr et al. 2011, but see Zortéa 2003).

Finally, altitudinal migrations might be important for the ecology of bat species (McGuire and Boyle 2013), especially if the migration is related to find appropriate sites for breeding.

Although *A. geoffroyi* maintain resident populations in some caves in the Trans-Mexican Neovolcanic Belt of Mexico, there are fewer numbers of females present during the cold season (Ferreira-García et al. 2018), suggesting that they probably move to other roosting sites with warmer temperatures. Ecologically, this potential altitudinal movement of females during parts of the year, and the possibility that males, females, and pregnant females, might require different food items at different periods throughout the year constitute an important factor affecting their interactions with the plant species they pollinate. Differences related to sex in flower-visiting behavior and frequency, combined with differences in habitat use, dispersal, and sex-specific diet differences, could influence the number and identity of flowers encountered by males and females (Smith et al. 2019), which in turn may be a key factor in plant reproduction, especially considering the numerous plant species of which *A. geoffroyi* is a potential pollinator (Geiselman et al. 2015). Our study, therefore, highlights the importance of maintaining food and roosting resources in lowland forest of the Trans-Mexican Neovolcanic Belt of Mexico such that populations of this nectarivorous bat species may be conserved. These sex-specific altitudinal preferences may not be restricted to *A. geoffroyi*, further underscoring the importance of distinct habitats for population segments of bats that may require distinct foods at different times of the year depending on their reproductive status and condition.

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