Journal of Mammalogy, 84(1):106–116, 2003

DEPENDENCE ON CACTI AND AGAVES IN NECTAR-FEEDING BATS FROM VENEZUELAN ARID ZONES

JAFET M. NASSAR,* HARALD BECK, LEONEL DA S. L. STERNBERG, AND THEODORE H. FLEMING

Instituto Venezolano de Investigaciones Científicas, Centro de Ecología, Apartado Postal 21827, Caracas 1020-A, Venezuela (JMN)
Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124, USA (JMN, HB, LSLS, THF)

We used stable carbon and nitrogen isotope analyses to test the hypothesis that nectar-feeding bats *Leptonycteris curasoae* and *Glossophaga longirostris* depend on cacti and agaves as food sources in Venezuelan arid zones and to compare their trophic positions. We measured the isotopic compositions of muscle tissue in the 2 species during 1 year at 3 arid locations. Overall carbon isotopic composition ($\partial^{13}C$) of *L. curasoae* (−11.76‰) and *G. longirostris* (−13.28‰) resembled values characteristic of columnar cacti and agaves (−12.47‰), which have in common the crassulacean acid metabolism (CAM) photosynthetic pathway. *L. curasoae* appears to be more dependent on cacti and agaves (98% CAM in the diet) than *G. longirostris* (85% CAM in the diet). CAM dependence, as we designate dependence on cacti and agaves, was evidenced across sites. Level of CAM dependence slightly varied over the year only in *G. longirostris*. We concluded that the 2 species of bats mainly rely on CAM plants in Venezuelan arid zones. Overall nitrogen isotopic composition ($\partial^{15}N$) did not differ between *L. curasoae* (15.87‰) and *G. longirostris* (15.37‰). Although our results suggest that the 2 bats occupy the same trophic position, no conclusive evidence supported this observation. The strong interdependence between these bats and their host CAM plants in northern South America suggests that a disturbance affecting 1 component of the interaction would have a strong effect on the other.

Key words: arid zones, CAM plants, carbon, *Glossophaga longirostris*, *Leptonycteris curasoae*, nectar-feeding bats, nitrogen, stable isotopes, Venezuela

---

Many studies addressing the reproductive biology of chiropterophilous (bat pollinated) cacti and century plants (agaves) have placed special emphasis on determining the role of bats in their pollination. In agaves, bats can be responsible for a large proportion of the fruit set (Arizaga et al. 2000; Howell 1979; Howell and Roth 1981), but diurnal pollen vectors (e.g., bees, wasps, butterflies, hawkmoths, and birds) can be equally or more important in some circumstances (Slauson 2000). In cacti, dependence on bat pollination increases latitudinally from the subtropics (Alcorn et al. 1959, 1962; Fleming et al. 1996, 2001; Sahley 1996) to the tropics (Casas et al. 1999; Nassar 1991; Nassar et al. 1997; Petit 1995; Tschapka et al. 1999; Valiente-Banuet et al. 1996, 1997a, 1997b; B. Rivera-Marchand, pers. comm.), where pollination of several species is almost exclusively attributed to flower-visiting bats. Thus, many tropical chiropterophilous cacti can be considered obligate mutualists with nectar-feeding bats. However, the extent to which nectar-feeding bats can be considered obligate mutualists with cacti and agaves remains an open question. Specialization on these plants as food sources by bats in tropical arid zones might be expected due to their year-round

* *Correspondent: jnassar@oikos.ivic.ve*

production of nectar, pollen, and fruits (Petit 1997; Sosa and Soriano 1996; J. M. Nassar, in litt.).

The southern long-nosed bat, *Leptonycteris curasoae* (Phyllostomidae, Glossophaginae), and Miller's long-tongued bat, *Glossophaga longirostris* (Phyllostomidae, Glossophaginae), are 2 nectar-feeding bats closely associated with arid environments in the New World. *L. curasoae* is a large bat (21.6–29.1 g—Swanepoel and Genoways 1979). It has a disjunct distribution, from southwestern United States to northern Guatemala, northern South America, and the Dutch Antilles (Arita and Humphrey 1988). *G. longirostris* is considerably smaller (11.5–14.6 g—Swanepoel and Genoways 1979). This species is distributed in northern South America, including the Dutch Antilles, Trinidad, and nearby Caribbean islands (Webster and Handley 1986). The 2 species are the primary pollinators and seed dispersers of several columnar cacti in northern South America and the Lesser Antilles (Fleming and Nassar 2002; Nassar et al. 1997; Petit 1995; Sosa and Soriano 1993, 1996). *G. longirostris* also disperses the seeds of plants in Moraceae and Elaeocarpaceae (Ruiz et al. 1997; Sosa and Soriano 1993). Relatively few insect parts have been found in fecal samples of *L. curasoae* and *G. longirostris* (Petit 1997; Ruiz et al. 1997; Sosa and Soriano 1993, 1996). There is thus no conclusive evidence indicating whether these bats actively feed on insects or whether insects are occasionally consumed during feeding on plant parts.

Under certain circumstances, stable-isotope analysis can be a practical technique for determining the food materials assimilated and incorporated into tissues of an animal, from which inferences about food choices at different times can be made. This is possible because several investigators have observed that the isotopic composition of tissues from several organisms closely follows the isotopic composition of their diet (see Peterson and Fry 1987 for a review).

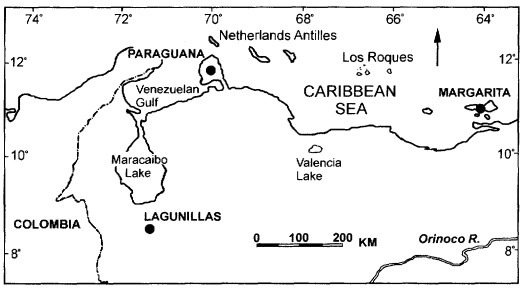

FIG. 1.—Sites in Venezuela where muscle tissue samples of *Leptonycteris curasoae* and *Glossophaga longirostris* were collected to conduct carbon and nitrogen isotope analysis.

In this study we examined the diets of *L. curasoae* and *G. longirostris* in arid zones of Venezuela by measuring both carbon ($\partial^{13}C$) and nitrogen ($\partial^{15}N$) isotopic composition in their muscle tissues. Estimates of $\partial^{13}C$ were used to infer relative importance of cacti and agaves in the diet of bats, whereas $\partial^{15}N$ estimates were used to compare trophic level between the 2 species. Isotope analyses of bat tissues were conducted for 1 year in 3 widely separated localities. Our goals were to estimate levels of dependence on columnar cacti and agaves by *L. curasoae* and *G. longirostris* and to compare the relative trophic positions of these species. Our working hypotheses were that the 2 species of bats depend on columnar cacti and agaves as food sources in Venezuelan arid regions and that, of the 2 species, a higher average trophic level would be expected in *G. longirostris* if it exhibits the same insect-feeding habits as its congener *G. soricina*.

MATERIALS AND METHODS

Study sites.—Field studies were conducted every 3 months from October 1996 to July 1997. Bat muscle tissue was collected from 3 localities where the ranges of *L. curasoae* and *G. longirostris* overlap (Fig. 1): Piedra de Rivero Cave, Comején (11°13′N, 64°14′W; 0–20 m above sea level), on the Macanao Peninsula of Margarita Island in Nueva Esparta State; Guano Cave (11°54′N, 69°57′W; 0–20 m above sea level), 2 km N of Buenavista and Piedra Honda Cave

(11°05′N, 69°60′W), 3.9 km NW of San José de Cocodite, on the Paraguaná Peninsula, Falcón State; and Laguna de Caparú (08°30′N, 71°20′W; 800–850 m above sea level), 3 km SE of San Juan de Lagunillas, Mérida State. We will refer to sites as Margarita, Paraguana, and Lagunillas. The 3 localities are in arid and semiarid regions of Venezuela, which have annual mean temperatures >24°C, mean annual precipitation <800 mm, and irregular rains, occurring in 1 or 2 peaks of rainfall per year (Sarmiento 1976). Vegetation at these localities consists mainly of spiny shrubs and bushes and an abundance of cacti and leguminous trees (Huber and Alarcón 1988).

Sampling procedures.—During each sampling period, 4–7 bats of each species were captured with mist nets at each site, except during the July sampling period at Lagunillas, when only 1 specimen of *L. curasoae* was captured. Altogether, 64 *L. curasoae* and 71 *G. longirostris* were collected. Sex and reproductive condition were recorded for each specimen. Unless pregnant, individuals were euthanized by cervical dislocation, in accordance with the American Society of Mammalogists guidelines for the capture, handling, and care of mammals (www.mammalsociety.org/committees/commanimalcare-use/98acucguidelines.pdf). Pectoral muscle samples (about 3 g) were obtained from each bat by dissecting muscle tissue using a scalpel and scissors. Muscle slices were wrapped in aluminum foil, appropriately labeled, and temporarily stored in a cooler with ice until we deposited them in a freezer. Finally, all tissues were dried at 60°C in a laboratory oven (Thelco, Jouan Inc., Winchester, Virginia) and stored in dry conditions until isotope analysis. All specimens were preserved and stored in the reference collection of the Biology Museum of Instituto de Zoología Tropical, Universidad Central de Venezuela, Caracas, Venezuela.

In addition, we collected and dried nocturnal insects (Lepidoptera) and flower tissue (pollen, anthers, and petals) from several plant species in the study sites that we presumed or knew were used by bats. These species included 3 columnar cacti, *Stenocereus griseus*, *Cereus repandus*, and *Pilosocereus lanuginosus* (Cactaceae), 1 century plant, *Agave cocui* (Agavaceae), 1 kapok tree, *Ceiba* (Bombacaceae), and 1 bignonia, *Crescentia cujete* (Bignoniaceae). Insects were trapped using light traps in the field, and floral tissue was collected fresh from plants. Both insects and flowers were dried in a laboratory oven and stored under dry conditions until isotope analysis.

Isotope analysis.—Carbon isotopic composition in muscle tissue of bats can be used to determine the relative importance of columnar cacti and agaves in the diet of nectar-feeding bats, thanks to the particular photosynthetic mechanism these plants use. Most plant species, including kapok trees and bignonias, use the fundamental process of CO_2 assimilation named the Calvin photosynthetic pathway or C_3 photosynthesis. Other species, including columnar cacti and agaves, use a variant of the C_3 mechanism called crassulacean acid metabolism (CAM) photosynthetic pathway. This mode of CO_2 assimilation is characterized by the synthesis of organic acids in darkness, a process related to water conservation in dry environments (Lawlor 2001). It has been demonstrated that CAM plants differ from C_3 plants in the ratio of ^{13}C to ^{12}C of their tissues (Bender 1971; Osmond et al. 1973; Smith and Epstein 1971). This difference in isotopic ratios is brought about by the difference in the primary carboxylation reaction between C_3 and CAM plants. C_3 plants use ribulose bisphosphate carboxylase as the primary carboxylation enzyme, which discriminates against ^{13}C to a greater degree than phosphoenolpyruvate carboxylase, the primary carboxylase enzyme used by CAM plants (Lambers et al. 2000).

The obligate CAM condition characteristic of columnar cacti and agaves present in the study sites sampled (Díaz and Medina 1984; Olivares 1984) allowed us to assume that differences in carbon isotopic composition among digestible and nondigestible plant tissues within species should be relatively small compared with the large differences reported between C_3 and CAM plants. Caution must be taken, however, when inferring the relative importance of CAM plants in bats' diet. Many tropical grasses and some monocot crops (e.g., corn and sugarcane) use another variant of the C_3 photosynthesis, the C_4 photosynthetic pathway, which generates ^{13}C:^{12}C ratios similar to those found in CAM species (Lawlor 2001). This was not a problem in our study because we did not find C_4 species offering food resources attractive to bats in any of the locations and they are not reported in the floristic lists that include the study sites (Rico et al. 1996; Smith and Salazar 1991; R. Windfield, in litt.).

Nitrogen isotope analysis can be used to determine the relative trophic position of bats because animals occupying higher trophic levels should have a higher enrichment of ^{15}N in their tissues (DeNiro and Epstein 1978). It is not yet well understood why there is a trophic level effect, but it is suspected that nitrogen excretion involving glutamine and serine reactions may discriminate against ^{15}N more so than ^{14}N (Minagawa and Wada 1984).

The rate of carbon turnover in the muscle tissue of small mammals (half life = 27.6 days) allows an estimation of their diet composition only during the last 30–45 days of activity before capture (Tieszen et al. 1983). We assumed that a similar rate of carbon turnover occurs in muscles of bats.

Carbon and nitrogen isotopic composition of muscle tissue were determined using standard methods described by DeNiro and Epstein (1978) and Sealy et al. (1987), respectively. Samples of about 15 mg of ground muscle were combusted at 800°C for 3 h in Vycor ampoules (Corning Glass, Pittsburgh, Pennsylvania) with 1 g of cupric oxide (Aldrich Chemicals, Milwaukee, Wisconsin), 1 g of copper, and about 50 mg of silver foil. The same procedure was used with insect and plant tissues, using 20 mg of tissue. Carbon dioxide and nitrogen were cryogenically purified from the combustion products in vacuum. The $\partial^{13}C$ and $\partial^{15}N$ values were estimated with a micromass spectrometer (PRISM, Manchester, United Kingdom). Stable-isotope compositions for carbon and nitrogen are expressed using delta (∂) notation in parts per thousand (‰):

$$\partial X\ (‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 10^3$$

where X is ^{13}C or ^{15}N and R is the relevant ratio ^{13}C:^{12}C or ^{15}N:^{14}N. Standards used for $\partial^{13}C$ and $\partial^{15}N$ values were Peedee belemnite marine limestone (United States Department of Commerce, Gaithersburg, Maryland) and atmospheric nitrogen (collected from air), respectively. Analytical precision of these measurements was ±0.1‰ (*SD*) for both elements.

Data analysis.—For each dependent variable ($\partial^{13}C$ and $\partial^{15}N$), we fit a general linear model with 4 fixed effects and 1 interaction: species, sex, site, and time and species × time (SAS Institute Inc. 2001). We were unable to find a single transformation to stabilize within-class variance completely. This is expected, however, because the concentrations measured in the tissue samples represent a composite of the isotopic composition characteristic and specific to different food sources used by these bats (Bender 1971; Osmond et al. 1973; Smith and Epstein 1971). Because the conventional *F*-test for assessing significance of effects is known to be robust to some departure from this assumption (Manly 1997), we proceeded with the analysis on untransformed values.

We used a mass-balance equation to estimate the proportional contribution of CAM and C_3 carbon to the diet of each species of bat (Fleming 1995):

$$\partial_S - 1 = p_I\partial_I + (1 - p_I)\partial_{II},$$

where ∂_S is the ∂ value in the tissue sample being analyzed, p_I is the fraction of the diet composed of food type I (CAM plants) with a value of ∂_I, and the rest of the diet is assumed to come from food type II (C_3 plants) with a value of ∂_{II}. We used a 1‰ correction factor (1 subtracted from ∂_S) because, in general and particularly in the case of mammals, it has been determined that there is a general trophic enrichment of up to 1‰ in ^{13}C between the tissues of the animal and its food (DeNiro and Epstein 1981; Ehleringer et al. 1986; Peterson and Fry 1987; M. J. DeNiro and S. Epstein, in litt.). This difference is probably brought about by fractionations occurring during respiration (Peterson and Fry 1987). Isotopic data are presented as mean ± 1 *SE*.

Results

The overall carbon isotopic composition ($\partial^{13}C$) of muscle tissue in *L. curasoae* (−11.76 ± 0.18‰) and *G. longirostris* (−13.28 ± 0.29‰) closely matched the average $\partial^{13}C$ measured for CAM plants (−12.47 ± 0.20‰; Table 1). The average $\partial^{13}C$ obtained for C_3 plants was much lower (−24.86 ± 1.7‰) than the value obtained for CAM species and resembled that found for insects (−24.82 ± 2.62‰). Based on these estimates and assuming that the primary source of carbon in the diet of these bats comes from plant material, the relative contribution of CAM plants to the diet of *L. curasoae* and *G. longirostris* was 98%

TABLE 1.—Stable carbon ($\partial^{13}C$) and nitrogen ($\partial^{15}N$) isotope composition (mean ± 1 *SE*) in bat muscle tissue for 2 bat species from arid Venezuelan locations and for their potential food sources.

Category	*n*	Isotopes	
		Carbon $\partial^{13}C$ (‰)	Nitrogen $\partial^{15}N$ (‰)
Leptonycteris curasoae	64	−11.76 ± 0.18	15.87 ± 0.39
Sex			
Male	47	−11.53 ± 0.18	15.93 ± 0.45
Female	17	−12.38 ± 0.47	15.70 ± 0.77
Locality			
Margarita	24	−11.43 ± 0.23	18.98 ± 0.52
Paraguana	25	−12.39 ± 0.37	14.62 ± 0.22
Lagunillas	15	−11.23 ± 0.19	12.97 ± 0.32
Glossophaga longirostris	71	−13.28 ± 0.29	15.37 ± 0.45
Sex			
Male	34	−13.13 ± 0.47	15.57 ± 0.75
Female	37	−13.41 ± 0.35	15.18 ± 0.53
Locality			
Margarita	23	−13.54 ± 0.66	19.35 ± 0.48
Paraguana	24	−13.20 ± 0.37	15.64 ± 0.39
Lagunillas	24	−13.11 ± 0.45	11.28 ± 0.24
Food sources			
CAM plants	4	−12.47 ± 0.20	10.41 ± 2.62
C_3 plants	2	−24.86 ± 1.7	11.69 ± 3.87
Insects	2	−24.82 ± 2.62	11.53 ± 0.88

and 85%, respectively. Despite strong contributions of CAM food products to the diets of bats, our analysis revealed a significant species effect ($F = 18.75$, *d.f.* = 1, $P < 0.0001$), *L. curasoae* having a higher $\partial^{13}C$ than *G. longirostris*. On the other hand, there was no significant species effect on $\partial^{15}N$ levels in muscle ($F = 0.69$, *d.f.* = 1, $P = 0.41$; Table 1). Nitrogen isotopic composition of the plants examined was highly variable (10.83 ± 4.8‰), with values as low as 5.18‰ in *A. cocui* and as high as 17.13‰ in *P. lanuginosus*. Average $\partial^{15}N$ obtained for insects was lower (11.5 ± 0.88‰) than levels found in bats (Table 1).

No sex effect ($F = 3.32$, *d.f.* = 1, $P = 0.07$) was detected for $\partial^{13}C$, but it was detected for $\partial^{15}N$ ($F = 4.2$, *d.f.* = 1, $P = 0.04$). Males had higher values than females (Table 1).

No site effect was detected on $\partial^{13}C$ ($F = 0.68$, *d.f.* = 2, $P = 0.51$; Table 1). Assuming plant material as the primary food source, diet of *L. curasoae* was mostly based on CAM products (93–100%) at the 3 localities. *G. longirostris* was also heavily dependent on CAM products (83–87%) at the 3 sites, but these bats incorporated relatively more C_3 products (plant or insect derived) in their diet (13–17%) than did *Leptonycteris*. The effect of site was highly significant for $\partial^{15}N$ ($F = 159.2$, *d.f.* = 2, $P < 0.0001$; Table 1). This effect appeared to arise from the Margarita Island location, where the 2 bats had the highest $\partial^{15}N$ recorded in the study.

A significant time effect was detected for $\partial^{13}C$ ($F = 6.92$, *d.f.* = 3, $P < 0.0002$), as well as a significant interaction between species and time ($F = 3.38$, *d.f.* = 3, $P < 0.02$). Bat species had slightly different temporal patterns of $\partial^{13}C$ (Fig. 2). In *L. curasoae*, $\partial^{13}C$ varied little over time at any site. Assuming plant material as the primary source of carbon, these results indicate that *L. curasoae* relied mostly on CAM

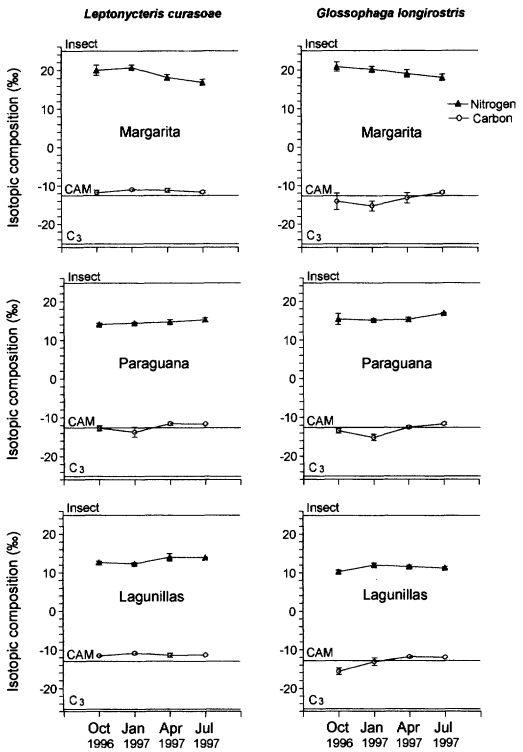

FIG. 2.—Estimates of carbon ($\partial^{13}C$) and nitrogen ($\partial^{15}N$) isotopic composition (‰) for muscle tissue of *Leptonycteris curasoae* and *Glossophaga longirostris* sampled from 3 arid locations (Margarita, Paraguana, and Lagunillas) in Venezuela, between October 1996 and July 1997. Data are means ± 1 *SE*. Horizontal lines indicate mean values of $\partial^{13}C$ for crassulacean acid metabolism photosynthetic pathway plants, C_3 (Calvin photosynthetic pathway) plants, and insects potentially used by bats in the localities studied.

plants year-round (81–100%) at all locations. Only bats from the Paraguaná Peninsula showed a slight increase (18%) in use of C_3 products (plant or insect derived) early in 1997. *G. longirostris* had temporal changes in $\partial^{13}C$, consistent with a substantial increase in use of CAM plants from the last quarter of 1996 (rainy season) to mid 1997 at the 3 localities. This increase was from 69% to 97% at Margarita Island, from 70% to 99% at the Paraguaná Peninsula, and from 67% to 97% at Lagunillas. Overall, C_3 plants or nocturnal insects (or both) represented 33% of the diet of *G. longirostris* during part of the year. However, neither a time effect nor a species by time interaction was detected for $\partial^{15}N$ (time: $F = 1.04$, $d.f. = 3$, $P = 0.38$; interaction: $F = 0.64$, $d.f. = 3$, $P = 0.59$).

DISCUSSION

Our results support the hypothesis that both sexes of *L. curasoae* and *G. longirostris* have a strong dependence on CAM plants throughout Venezuelan arid zones and year-round. This study included 4 sampling periods equally spaced during the year. Because each sampling period represents 30–45 days of the diet of the bats (Tieszen et al. 1983), overall, our data covered between 4 and 6 months of the year. This means that there were time intervals between sampling periods for which we lack information on the bats' diet. However, the small variation observed in values of $\partial^{13}C$ and $\partial^{15}N$ between sampling periods suggests that only minor changes occurred during the time gaps.

To our knowledge, none of the C_4 plants present in our study sites (several grasses) produce food items that could be used by these bats. The carbon signatures ($\partial^{13}C$) obtained from nocturnal insects (−24.82‰; Table 1) that could be potential food items do not correspond with $\partial^{13}C$ measures characteristic of CAM plants. Based on this observation, the CAM-like carbon isotopic composition estimates obtained from the bat samples can be attributed to the use of CAM plants and not to insects that fed on these plants and were then eaten by the bats.

Most plant species in the diet of these bats in Venezuela, Colombia, and Curaçao are columnar cacti (Nassar 1991; Nassar et al. 1997; Petit 1997; Ruiz et al. 1997; Soriano et al. 1991; Sosa and Soriano 1993, 1996). The great spatiotemporal importance of cacti in bat diets, at least in tropical arid zones, corroborates the designation of *L. curasoae* and *G. longirostris* as "obligate cactophiles" (Simmons and Wetterer 2002). Plants in the Agavaceae also play a signif-

icant role in the nutrition of these bats in southwestern United States, Mexico, Venezuela, and the Caribbean (Arizaga et al. 2000; Fleming et al. 1993; Howell 1979; Howell and Roth 1981; Petit 1997; Slauson 2000; Sosa and Soriano 1993). Thus, although linguistically unorthodox, the term "CAMphilic" might best describe the dietary habits of *L. curasoae* and *G. longirostris*.

Although both species have a strong dependence on CAM plants in Venezuela, *L. curasoae* seems to be more specialized on CAM plants than is *G. longirostris*. The latter appeared to consume relatively more C_3 plant products (or insects that feed on C_3 plants) than did *L. curasoae* at our 3 sites. Greater dependence on CAM plants in *G. longirostris* corresponded with periods of high production of flowers and fruits by columnar cacti at these localities (J. M. Nassar, in litt.). However, at times when cactus-derived resources are less available, dependence on alternative sources of food appears to increase. For example, in the Andean xeric patch at Lagunillas, up to 33% of the diet of *G. longirostris* but only 0.4% of the diet of *L. curasoae* may have consisted of C_3 plants or insects (or both) in October 1996. Soriano et al. (1991) found that between October and December, fruits of *Chlorophora tinctoria* (a C_3 species of Moraceae) become abundant in the Lagunillas area and that the diet of *G. longirostris* was composed of up to 11% of this resource at that time. At the same locality, *L. curasoae* ate only plant products from Agavaceae and Cactaceae year-round (Sosa and Soriano 1993). The diet of *G. longirostris* also contained an important proportion of C_3 products in the arid valley of La Tatacoa in Colombia (*Muntingia calabura*—Ruiz et al. 1997) and on Curaçao (e.g., *Ceiba pentandra*, *C. tinctoria*—Petit 1997).

Additional evidence supporting the hypothesis that *G. longirostris* is less dependent on CAM plants than *L. curasoae* comes from differences in their geographic distributions. In northern South America and the Dutch Antilles, *L. curasoae* is restricted to arid and semiarid environments (Linares 1998). In these zones, columnar cacti and agaves are dominant elements of plant communities. *G. longirostris* is more widely distributed in northern South America and occurs in habitats as diverse as dry forests, evergreen seasonal forests, lower montane forests, savannas, pastures, and disturbed areas (Eisenberg 1989; Linares 1998). In nonarid zones, cacti and agaves become less important elements of the vegetation or disappear completely (Huber and Alarcón 1988; M. Ponce, in litt.).

The level of dependence of *L. curasoae* on CAM plants varies considerably over its geographic range. Using carbon stable-isotope techniques, Fleming et al. (1993) found that *L. curasoae* in northern Mexico and southwestern United States feeds extensively or exclusively on CAM plants during its migration period along cactus and agave corridors. In Baja California, where this species appears to be a year-round resident and where cacti and agaves are abundant, populations behave as CAM specialists throughout the year. The same species feeds heavily on C_3 plants in southern Mexico (Ceballos et al. 1997), where agaves and columnar cacti are found in relatively low densities. Thus, even for the CAMphilic *L. curasaoe*, degree of dependence on cacti and agaves appears to vary geographically as a function of spatiotemporal availability of resources. In arid zones of northern South America, where CAM resources for nectar-feeding bats are abundant and widespread over the year (Petit 1997; Ruiz et al. 1997; Sosa and Soriano 1996), bat dependence on CAM plants again is high. Geographic variation in the outcomes of interspecific interactions seems to be a common phenomenon in nature (Bronstein 1994; Thompson 1988, 1994, 1997), and the bat–cactus mutualism is another example of this phenomenon.

Estimates of $\partial^{15}N$ found in *L. curasoae*, *G. longirostris*, and in some of the plants

and insects examined in this study were relatively high when compared with values recently reported for bats, bat plants, and insects (Fleming 1995; Herrera et al. 1998, 2001a, 2001b). Sealy et al. (1987) found that the highest $\partial^{15}N$ measures in South African herbivores occurred in arid zones (<400 mm of precipitation per year). They attributed this partly to the need to reduce urea excretion in response to water stress. The 2 species examined in our study are associated with arid zones. Besides the potential effect of water stress on the nitrogen isotopic composition in these animals, it is important to note the high $\partial^{15}N$ values found in some of the plants bats feed on in these habitats.

In general, in animal communities there is an enrichment of 2–4‰ in ^{15}N from 1 trophic level to the next (Ehleringer et al. 1986). Delta ^{15}N estimates in the 2 species of bats were quite similar, which suggests that *L. curasoae* and *G. longirostris* may occupy the same trophic position. However, our $\partial^{15}N$ estimates of plant and insect origin do not help support this conclusion. Plant $\partial^{15}N$ measures observed were highly variable, and some of them were even higher than $\partial^{15}N$ values obtained for insects. This means that if 1 bat species was ingesting more insects than the other, we would not be able to detect it by examining $\partial^{15}N$ estimates. To detect differential use of insects by these bats, additional detailed analyses of potential food items should be conducted together with a stable-isotope analysis using an element other than nitrogen, with low variation in isotopic composition among plant species.

Previous dietary studies using fecal samples indicate that the 2 species of bats seem to function primarily as herbivores in arid areas of Venezuela and Curaçao. Petit (1997) and Sosa and Soriano (1993) found insect parts and scales in <2% of fecal samples of these bats. This low dependence on insects suggests that the carbon and nitrogen compositions were due to diets based on plant products. In the case of *G. longirostris*, however, we cannot determine whether the low $\partial^{13}C$ values recorded during part of the year were the product of increased consumption of C_3 plants or insects that feed on C_3 plants. Interestingly, the 2 species had higher $\partial^{15}N$ values on Margarita Island than in the other 2 localities. It is possible that some of the plant material eaten by these bats on this island is rich in nitrogen compared with food available elsewhere.

Most bat–plant interactions are flexible in the sense that 1 species of plant receives pollination and seed dispersal services from several species of bats and 1 species of nectar- or fruit-eating bat may feed on several plant taxa. As a consequence of this, bats rarely evolve parallel to 1 or even a few plant species (Heithaus 1982). The interdependence between nectar-feeding bats and CAM plants in tropical arid regions supports the hypothesis that populations of *L. curasoae* and *G. longirostris* in these environments could be coevolving with columnar cacti and agaves. Outside the arid zones the picture might be different. For example, *Leptonycteris* populations studied in the dry forest of Chamela, Mexico, were apparently feeding on C_3 plants year-round in 1996.

Simmons and Wetterer (2002) presented morphological evidence consistent with the hypothesis of bat–cactus coevolution. Cactus-visiting glossophagine bats tend to differ from other members of their clades in being larger and having relatively longer jaws (Simmons and Wetterer 2002). *L. curasoae* is one of the largest nectar-feeding phyllostomid bats. Within *Glossophaga*, *G. longirostris* is the largest species and has the longest jaws. These features may allow CAMphilic bats to fly over long distances among patchily distributed plants and to extract nectar from large cactus flowers.

The strong interdependence between these bats and CAM plants in tropical arid zones suggests that these key mutualisms can be particularly vulnerable to distur-

bances affecting either the plants or the animals.

Resumen

Usamos análisis de isótopos estables de carbono y nitrógeno para probar la hipótesis de que los murciélagos nectarívoros *Leptonycteris curasoae* y *Glossophaga longirostris* dependen de cactus y agaves en las zonas áridas de Venezuela, y para comparar sus posiciones tróficas. Medimos las composición isotópica de tejido muscular en las 2 especies durante 1 año, en 3 localidades áridas. Las composiciones isotópicas de carbono ($\partial^{13}C$) en *L. curasoae* (−11.76‰) y *G. longirostris* (−13.28‰) asemejaron los valores isotópicos característicos de los cactus columnares y agaves (−12.47‰), los cuales tienen en común el uso de la ruta de fijación de CO_2 CAM (metabolismo ácido de crassulaceas). *L. curasoae* parece ser más dependiente de cactus y agaves (98%) que *G. longirostris* (85%). CAM-dependencia, como nosotros designamos la dependencia por cactus y agaves, fue evidenciada en todas las localidades muestreadas. Sólo en *G. longirostris* los niveles de CAM-dependencia variaron a lo largo del año. En conjunto, nuestros resultados indican que los dos murciélagos presentan una fuerte dependencia por plantas CAM en zonas áridas de Venezuela. La composición isotópica de nitrógeno ($\partial^{15}N$) no difirió entre *L. curasoae* (15.87‰) y *G. longirostris* (15.37‰). Aunque esta similitud de valores sugiere la misma posición trófica para las dos especies, nuestros resultados no rindieron evidencia conclusiva a este respecto. La fuerte interdependencia evidenciada entre estos murciélagos y sus fuentes de alimento CAM en el norte de Sur América, sugiere que cualquier alteración que afecte a 1 componente de la interacción tendría un fuerte impacto sobre el otro.

Acknowledgments

We thank J. Castro, J. F. Penoth, D. González, M. Muñoz, and J. Molinari for assistance in the field and A. Tejedor for assistance with isotope analyses. Prof. R. Pérez-Hernández, Instituto de Zoología Tropical, provided institutional support. K. Rodríguez-Clark gave useful advice on the statistical analysis of the data and valuable input on an earlier version of the manuscript. G. Herrera and G. Tellez contributed with helpful comments and suggestions on the 1st draft. The NGO "Provita" provided logistic support on Margarita Island. The Venezuelan Wildlife Service (Profauna) granted permission to collect bat tissues. The Venezuelan Ministry of Agriculture granted permission to export bat tissues to the United States. This research was partially supported by a Tropical Biology Fellowship from University of Miami, a Sigma-Xi Grant-in-Aid of Research, and a small Grant-in-Aid of Research from the American Society of Mammalogists to J. M. Nassar.

Literature Cited

Alcorn, S. M., S. E. McGregor, G. D. Buttler, and E. B. Kurtz. 1959. Pollination requirements of the saguaro (*Carnegiea gigantea*). Cactus and Succulent Journal 31:39–41.

Alcorn, S. M., S. E. McGregor, and G. Olin. 1962. Pollination requirements of the organpipe cactus. Cactus and Succulent Journal 34:134–138.

Arita, H. T., and S. R. Humphrey. 1988. Revisión taxonómica de los murciélagos magueyeros del género *Leptonycteris* (Chiroptera: Phyllostomidae). Acta Zoológica Mexicana 29:1–59.

Arizaga, S., E. Ezcurra, E. Peters, F. Ramírez de Arellano, and E. Vega. 2000. Pollination ecology of *Agave macroacantha* (Agavaceae) in a Mexican tropical desert. I. Floral biology and pollination mechanisms. American Journal of Botany 87:1004–1010.

Bender, M. M. 1971. Variations in ^{13}C:^{12}C ratios of plants in relation to the pathway of carbon dioxide fixation. Phytochemistry 10:1239–1244.

Bronstein, J. L. 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution 9:214–217.

Casas, A., A. Valiente-Banuet, A. Rojas-Martínez, and P. Dávila. 1999. Reproductive biology and the process of domestication of the columnar cactus *Stenocereus stellatus* in Central Mexico. American Journal of Botany 86:534–542.

Ceballos, G., T. H. Fleming, C. Chávez, and J. Nassar. 1997. Population dynamics of *Leptonycteris curasoae* (Chiroptera: Phyllostomidae) in Jalisco, Mexico. Journal of Mammalogy 78:1220–1230.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341–351.

Diaz, M., and E. Medina. 1984. Actividad CAM de cactáceas en condiciones naturals. Pp. 98–113 in

Physiological ecology of CAM plants (E. Medina, ed.). Centro de Ecología–IVIC, Caracas, Venezuela.

EHLERINGER, J. R., P. W. RUNDEL, AND K. A. NAGY. 1986. Stable isotopes in physiological ecology and food web research. Trends in Ecology and Evolution 1:42–45.

EISENBERG, J. F. 1989. Mammals of the Neotropics. The northern Neotropics. University of Chicago Press, Chicago, Illinois 1:1–449.

FLEMING, T. H. 1995. The use of stable isotopes to study the diets of plant-visiting bats. Pp. 99–110 in Ecology, evolution and behavior of bats (P. A. Racey and S. M. Swift, eds.). Clarendon Press, Oxford, United Kingdom.

FLEMING, T. H., AND J. M. NASSAR. 2002. Population biology of the lesser long-nosed bat *Leptonycteris curasoae*, in Mexico and northern South America. Pp. 283–305 in Columnar cacti and their mutualists: evolution, ecology and conservation (T. H. Fleming and A. Valiente-Banuet, eds.). University of Arizona Press, Tucson.

FLEMING, T. H., R. A. NUÑEZ, AND L. DA S. L. STERNBERG. 1993. Seasonal changes in the diets of migrant and non-migrant nectarivorous bats as revealed by carbon stable isotope analysis. Oecologia 94:72–75.

FLEMING, T. H., C. T. SAHLEY, J. N. HOLLAND, J. D. NASON, AND J. L. HAMRICK. 2001. Sonoran Desert columnar cacti and the evolution of generalized pollination systems. Ecological Monographs 71:511–530.

FLEMING, T. H., M. D. TUTTLE, AND M. A. HORNER. 1996. Pollination biology and the relative importance of nocturnal and diurnal pollinators in 3 species of Sonoran Desert columnar cacti. Southwestern Naturalist 41:257–269.

HEITHAUS, E. R. 1982. Coevolution between bats and plants. Pp. 327–367 in Ecology of bats (T. H. Kunz, ed.). Plenum Press, New York.

HERRERA, L. G., T. H. FLEMING, AND L. S. STERNBERG. 1998. Trophic relationships in a neotropical bat community: a preliminary study using carbon and nitrogen isotopic signatures. Tropical Ecology 39:23–29.

HERRERA, L. G., K. A. HOBSON, A. MANZO A., D. ESTRADA B., V. SÁNCHEZ-CORDERO, AND G. MÉNDEZ C. 2001a. The role of fruits and insects in the nutrition of frugivorous bats: evaluating the use of stable isotope models. Biotropica 33:520–528.

HERRERA, L. G., K. A. HOBSON, L. MIRÓN M., N. RAMÍREZ P., G. MÉNDEZ C., AND V. SÁNCHEZ-CORDERO. 2001b. Sources of protein in two species of phytophagous bats in a seasonal dry forest: evidence from stable isotope analysis. Journal of Mammalogy 82:352–361.

HOWELL, D. J. 1979. Flock foraging in nectar-feeding bats: advantages to the bats and to the host plants. American Naturalist 114:23–49.

HOWELL, D. J., AND B. S. ROTH. 1981. Sexual reproduction in agaves: the benefits of bats, the cost of semelparous advertising. Ecology 62:1–7.

HUBER, O., AND C. ALARCÓN. 1988. Mapa de vegetación. Ministerio del Ambiente y de los Recursos Naturales Renovables, Caracas, Venezuela.

LAMBERS, H., F. S. CHAPIN III, AND T. L. PONS. 2000. Plant physiological ecology. Springer, Berlin, Germany.

LAWLOR, D. W. 2001. Photosynthesis. 3rd ed. Springer-Verlag, New York.

LINARES, O. 1998. Mamíferos de Venezuela. Sociedad Conservacionista Audubon de Venezuela, Caracas, Venezuela.

MANLY, B. F. J. 1997. Randomization, bootstrap, and Monte Carlo methods in biology. Chapman and Hall, London, United Kingdom.

MINAGAWA, M., AND E. WADA. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\partial^{15}N$ and animal age. Geochimica Cosmochimica Acta 48:1135–1140.

NASSAR, J. M. 1991. Biología reproductiva de cuatro cactáceas quiropterófilas Venezolanas (*Stenocereus griseus*, *Pilosocereus moritzianus*, *Subpilocereus repandus* y *S. horrispinus*), y estrategias de visita de los murciélagos asociados a éstas. B.S. thesis, Universidad Central de Venezuela, Caracas, Venezuela.

NASSAR, J. M., N. RAMÍREZ, AND O. LINARES. 1997. Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. American Journal of Botany 84:918–927.

OLIVARES, E. 1984. Metabolismo de carbohidratos y fijación de CO_2 en Agavaceae. M.Sc. thesis, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

OSMOND, C. B., ET AL. 1973. Carbon isotope discrimination in photosynthesis of CAM plants. Nature 246:41–42.

PETERSON, B. J., AND B. FRY. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

PETIT, S. 1995. The pollinators of two species of columnar cacti in Curaçao, Netherlands Antilles. Biotropica 27:538–541.

PETIT, S. 1997. The diet and reproductive schedules of *Leptonycteris curasoae curasoae* and *Glossophaga longirostris elongata* (Chiroptera: Glossophaginae) on Curacao. Biotropica 29:214–223.

RICO, R., L. E. RODRÍGUEZ, R. PÉREZ, AND A. VALERO. 1996. Mapa y análisis de la vegetación xerófila de las lagunas de Caparú, cuenca media del río Chama, Estado Mérida. Plantula 1:83–94.

RUIZ, A., M. SANTOS, P. J. SORIANO, J. CAVELIER, AND A. CADENA. 1997. Relaciones mutualísticas entre el murciélago *Glossophaga longirostris* y las cactáceas columnares en la zona árida de La Tatacoa, Colombia. Biotropica 29:469–479.

SAHLEY, C. T. 1996. Bat and hummingbird pollination of an autotetraploid columnar cactus, *Weberbauerocereus weberbaueri* (Cactaceae). American Journal of Botany 83:1329–1336.

SARMIENTO, G. 1976. Evolution of arid vegetation in tropical America. Pp. 65–100 in Evolution of desert biota (D. W. Goodall, ed.). University of Texas Press, Austin.

SAS INSTITUTE INC. 2001. Statistical analysis systems, 8th ed. for Windows. SAS Institute Inc., Cary, North Carolina.

SEALY, J. C., N. J. VAN DER MERWE, J. A. L. THORP, AND J. L. LANHAM. 1987. Nitrogen isotopic ecology in southern Africa: implications for environmental and dietary tracing. Geochimica et Cosmochimica Acta 51:2707–2717.

SIMMONS, N. B., AND A. L. WETTERER. 2002. Phylogeny and convergence in cactophilic bats. Pp. 87–121 in Columnar cacti and their mutualists: evolution, ecology and conservation (T. H. Fleming and A. Valiente-Banuet, eds.). University of Arizona Press, Tucson.

SLAUSON, L. 2000. Pollination biology of two chiropterophilous agaves in Arizona. American Journal of Botany 87:825–836.

SMITH, B. N., AND S. EPSTEIN. 1971. Two categories of ^{13}C:^{12}C ratios for higher plants. Plant Physiology 47: 380–384.

SMITH, R. F., AND M. SALAZAR. 1991. Vegetación del Estado Lara. Pp. 9–13 in Ecología del Estado Lara (R. F. Smith, A. Rivero, F. Ortega, and J. A. Catalá, eds.). Biollania, Museo de Ciencias Naturales de la UNELLEZ, Guanare, Portuguesa, Venezuela.

SORIANO, P. J., M. SOSA, AND O. ROSSELL. 1991. Hábitos alimentarios de *Glossophaga longirostris* Miller (Chiroptera: Phyllostomidae) en una zona árida de los Andes venezolanos. Revista de Biología Tropical 39:267–272.

SOSA, M., AND P. J. SORIANO. 1993. Solapamiento de dieta entre *Leptonycteris curasoae* y *Glossophaga longirostris* (Mammalia: Chiroptera). Revista de Biología Tropical 41:529–532.

SOSA, M., AND P. J. SORIANO. 1996. Resource availability, diet and reproduction in *Glossophaga longirostris* (Mammalia: Chiroptera) in an arid zone of the Venezuelan Andes. Journal of Tropical Ecology 12:805–818.

SWANEPOEL, P., AND H. H. GENOWAYS. 1979. Morphometrics. Pp. 13–106 in Biology of bats of the family Phyllostomatidae, Part III (R. J. Baker, J. K. Jones, Jr., and D. C. Carter, eds.). Special Publications, The Museum, Texas Tech University, Lubbock.

THOMPSON, J. N. 1988. Variation in interspecific interactions. Annual Review of Ecology and Systematics 19:65–87.

THOMPSON, J. N. 1994. The coevolutionary process. University of Chicago Press, Chicago, Illinois.

THOMPSON, J. N. 1997. Evaluating the dynamics of coevolution among geographically structured populations. Ecology 78:1619–1623.

TIESZEN, L. L., T. W. BOUTTON, K. G. TESDAHL, AND N. A. SLADE. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\partial^{13}C$ analysis of diet. Oecologia 57:32–37.

TSCHAPKA, M., O. VON HELVERSEN, AND W. BARTHLOTT. 1999. Bat pollination of *Weberocereus tunilla*, an epiphytic rain forest cactus with functional flagelliflory. Plant Biology 1:554–559.

VALIENTE-BANUET, A., M. C. ARIZMENDI, A. ROJAS-MARTÍNEZ, AND L. DOMINGUEZ-CANSECO. 1996. Ecological relationships between columnar cacti and nectar-feeding bats in México. Journal of Tropical Ecology 12:103–119.

VALIENTE-BANUET, A., A. ROJAS-MARTÍNEZ, M. C. ARIZMENDI, AND P. DÁVILA. 1997a. Pollination biology of two columnar cacti (*Neobuxbaumia mezcalensis* and *Neobuxbaumia macrocephala*) in the Tehuacán Valley, Central Mexico. American Journal of Botany 84:452–455.

VALIENTE-BANUET, A., A. ROJAS-MARTÍNEZ, A. CASAS, M. C. ARIZMENDI, AND P. DÁVILA. 1997b. Pollination biology of two winter-blooming giant columnar cacti in the Tehuacán Valley, Mexico. Journal of Arid Environments 37:331–342.

WEBSTER, W. D., AND C. O. HANDLEY, JR. 1986. Systematics of Miller's long-tongued bat, *Glossophaga longirostris*, with description of two new subspecies. Occasional Papers, The Museum, Texas Tech University 100:1–22.

Submitted 3 May 2002. Accepted 8 July 2002.

Associate Editor was Thomas E. Tomasi.