UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

CENTRO DE INVESTIGACIONES EN ECOSISTEMAS

FACTORES QUE DEFINEN LA CALIDAD DEL NÉCTAR DE FLORES QUIROPTEROFÍLICAS EN EL BOSQUE CADUCIFOLIO DE JALISCO, MÉXICO: LA PLANTA O LAS PREFERENCIAS DEL POLINIZADOR?

TESIS

QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTORA EN CIENCIAS

PRESENTA

OLGA NELLY RODRÍGUEZ PEÑA

TUTORA PRINCIPAL DE TESIS: DRA. KATHRYN E. STONER SMITH
COMITÉ TUTOR: DR. LUIS ENRIQUE EGUIARTE FRUNS
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UNAM
POSGRADO
Ciencias Biológicas

Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
Presente

Por medio de la presente me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 17 de enero del 2011, se acordó poner a su consideración el siguiente jurado para el examen de DOCTORA EN CIENCIAS de la alumna **OLGA NELLY RODRÍGUEZ PEÑA** con número de cuenta **97563273,** con la tesis titulada: **"Factores que definen la calidad del néctar de flores quiropterofílicas en el bosque caducifolio de Jalisco, México: la planta o las preferencias del polinizador"**, bajo la dirección de la **Dra. Kathryn E. Stoner.**

Presidente:	Dr. Luis Enrique Eguiarte Fruns
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De acuerdo con lo anterior, la alumna se acogió a la nueva normatividad, con base en el artículo QUINTO TRANSITORIO en apegó a lo establecido en el Artículo 31 del Reglamento General de Estudios de Posgrado.

Sin otro particular, quedo de usted.

Atentamente
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F., a 24 de marzo del 2011.

Mª del Coro Arizmendi

Dra. María del Coro Arizmendi Arriaga
Coordinadora del Programa

c.c.p. Expediente de la interesada

AGRADECIMIENTOS

Primeramente al Posgrado en Ciencias Biológicas, UNAM.

A CONACYT, por la beca otorgada (No. De registro: 97434).

Al Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica PAPITT a KES (IN2251033, IN226007) y JES (IN 226710), Universidad Nacional Autónoma de México.

Al Programa de Apoyo a los Estudios de Posgrado PAEP por los apoyos recibidos para estancias y congresos.

A mi asesora Kathryn E. Stoner Smith y a los miembros del Comité Tutor: Dr. Luis Enrique Eguiarte Fruns, Dr. Juan Francisco Ornelas Rodríguez, Dr. Luis Gerardo Herrera Montalvo, Dr. Erick De La Barrera Montppellier y Dr. Jorge E. Schondube Friedewold.

AGRADECIMIENTOS A TÍTULO PERSONAL

A Kathy Stoner y Jorge Chon por haberme dado sus mejores consejos y apoyo incondicional y sobre todo por su amistad y cariño.

A Erick de la Barrera y Gerardo Herrera por haber estado conmigo y mi proyecto cada semestre.

A Carlos Martínez del Río y su familia por haber compartido sus conocimientos y amistad conmigo en una etapa que considero muy importante en mi formación académica y personal.

A César Flores, Luis Barbo y la maestra Josefina Vázquez Mota por su dedicación, apoyo y amistad durante mis estancias en el Laboratorio de Fisiología Vegetal (UBIPRO) de la FES Iztacala.

A Cristian Voigt, Ana Popa y a todos los integrantes del laboratorio en el Leibniz Institute for Zoo and Wildlife Research (IZW), de Berlín, Alemania por su hospitalidad, apoyo y amistad.

Al personal de la Estación de Biología Chamela por su compañía y amistad.

A mis colegas y amigos de laboratorio, Karla Oce, Selene, Mariana, Erika, Hesajim, Bernardo, Cristian, Manu, y de mi otro labo, Lore, Leti, Elisa, Hector, Ian y Alicia, Romeo, More, Carlitos, Javi y Pollo…y los que se me olviden, gracias especialmente por su amistad y en el camino nos volvemos a topar…

A las personas más allegadas a mi por haber marcado esta etapa con sus consejos, apoyo, sugerencias, regaños y amor gracias!

A toda mi super familia, a mi mamá y a mi hermana por siempre estar… y especialmente a Ney por su apoyo y amor, lo logramos bebé bat!, y recuerda que eres el motor k empuja mi corazón!

RESÚMEN GENERAL DE TESIS

Factores que definen la calidad del néctar de flores quiropterofílicas en el bosque caducifolio de Jalisco, México: ¿la planta o las preferencias del polinizador?

Para entender si el néctar de flores quiropterofílicas: i) es una característica constante y relativamente conservada entre especies de plantas relacionadas filogenéticamente, o si ii) sus características coinciden con las preferencias de un tipo particular de polinizadores, (i.e. los murciélagos), es necesario describir las variaciones naturales en las características del néctar de las plantas quiropterofílicas para poder inferir sobre el efecto que la variación natural en estas características tiene sobre la selección de recursos de los murciélagos nectarívoros en campo.

El hecho de que las flores quiropterofílicas comparten características en común y secretan néctares con una composición similar de azúcares, sugiere a la vez que, los murciélagos preferirán las características dominantes en su dieta natural (***CAPÍTULO 2***), para lo que se determinó el papel que ejerce la composición de azúcares en el néctar sobre las decisiones de forrajeo en dos especies de murciélagos nectarívoros con diferentes estrategias de uso de néctar: *Leptonycteris yerbabuenae* y *Glossophaga soricina*. Los murciélagos no mostraron ninguna preferencia entre los tipos de azúcar cuando las soluciones tenían la misma concentración. Sin embargo, *L. yerbabuenae* prefirió soluciones concentradas sobre diluidas independientemente del tipo de azúcar. Ambas especies de murciélagos perciben a las hexosas y sacarosa como energéticamente equivalentes. Los resultados sugieren que la evolución de la composición de azúcares en el néctar de flores polinizadas por murciélagos, no está determinada por las preferencias de los murciélagos, y que existen otros factores intrínsecos a la planta que pueden estar moldeando la composición de azúcares en las flores polinizadas por murciélagos.

Debido a que los aminoácidos son componentes frecuentes del néctar, se ha sugerido que pueden tener un papel importante en la atracción y nutrición para algunos de sus polinizadores. El efecto de los aminoácidos sobre la selección de recursos alimentarios en murciélagos aún no ha sido estudiado, por lo que la segunda meta de este trabajo (***CAPÍTULO 3***) consistió en determinar el papel que ejercen los aminoácidos del néctar sobre las decisiones de forrajeo en dos especies de murciélagos nectarívoros con diferentes estrategias de uso de néctar: *Leptonycteris yerbabuenae* y *Glossophaga soricina*. En este capítulo evalué: 1) el papel del néctar como fuente de nitrógeno, 2) el papel de la concentración de aminoácidos en relación a la concentración de azúcares, y 3) el papel del sabor que los aminoácidos proporcionan al néctar sobre la selección del alimento de murciélagos nectarívoros. Los resultados mostraron que: 1) los murciélagos fueron indiferentes a néctares con concentraciones contrastantes de nitrógeno simulando las concentraciones encontradas en plantas quiropterofílicas en su sitio de estudio, lo que sugiere que el néctar no es una fuente de nitrógeno importante para los murciélagos nectarívoros. 2) los murciélagos fueron indiferentes a néctares suplementados con nitrógeno, a concentraciones contrastantes de azúcares. Sin embargo, Leptonycteris yerbabuenae prefirió el néctar más concentrado cuando éstos no fueron adicionados con nitrógeno, lo que sugiere que la presencia de nitrógeno en el néctar podría reducir su capacidad para diferenciar entre las concentraciones totales de néctar (18 vs. 27%); 3) *Leptonycteris yerbabuenae* prefirió el sabor de los aminoácidos presentes en el néctar de *Pachycereus pecten* (Cactaceae) sobre el de *Ceiba aesculifolia* (Malvaceae). Los resultados sugieren que los aminoácidos del néctar pueden afectar las decisiones de forrajeo en los murciélagos nectarívoros, independientemente de las bajas concentraciones en las que están presentes en el néctar floral.

El néctar floral es la recompensa más importante para los polinizadores. La composición de azúcares del néctar puede verse afectada por factores bioquímicos, ecológicos y evolutivos; sin embargo, su influencia sobre la composición final de azúcares del néctar de plantas quiropterofilícas se desconoce. La tercera meta de este trabajo (***CAPÍTULO 4***), consistió en determinar: a) la composición de azúcares, el volumen y la concentración total en el néctar de plantas quiropterofílicas; b) si la composición de azúcares está relacionada con el volumen y la concentración del néctar; y c) cómo las estrategias de floración de las plantas afectan las características del néctar. Los resultados mostraron que las plantas quiropterofílicas producen néctares dominados por sacarosa (1 especie), ricos en sacarosa (2 especies), ricos en hexosas (8 especies), y dominados por hexosas (1 especie). Los porcentajes de fructosa obtenidos en este estudio son más altos que los de glucosa en casi todas las especies (11 de un total de 12). Las relaciones entre los porcentajes de azúcares y el volumen y concentración del néctar fueron no significativas. Por último, especies de plantas con floración masiva por noche producen néctares diluidos de hexosas mientras que plantas que ofrecen pocas flores por noche tienen néctares más concentrados con mayores proporciones de sacarosa. Los resultados sugieren la existencia de otros mecanismos, además de la digestión de sacarosa en el nectario, que pueden estar involucrados al momento de determinar la composición final de azúcares en el néctar en las especies de plantas quiropterofílicas analizadas. Entre la comunidad de plantas quiropterofílicas hay dos estrategias en la producción de néctar que podrían estar relacionados con los costos de energía asociada a la producción de flores y néctar.

En conclusión, los resultados obtenidos en este estudio rechazan la hipótesis de que los murciélagos nectarívoros tienen preferencia por los azúcares más comunes en su dieta natural. El hecho de que la concentración de aminoácidos en el néctar tiene la capacidad de confundir a los murciélagos para distinguir entre concentraciones de azúcares y, que algunos murciélagos tienen preferencias por el sabor que los aminoácidos proporcionan al néctar de especies de plantas específicas, sugiere que las plantas pueden estar utilizando la variación en esta característica para atraer a los animales nectarívoros. Finalmente, las características del néctar de plantas quiropterofílicas no parecen ser el resultado de las preferencias de los murciélagos, sino de los factores bioquímicos, ecológicos y evolutivos de la planta.

Factors that determine nectar quality of chiropterophylic flowers in the Tropical Dry Forest of Jalisco, Mexico: the plant or pollinator preferences?

To understand whether the nectar of chiropterophylic plants: i) is constant and relatively conserved among phylogenetically related plant species, or ii) if their actual traits match the preferences of a particular pollinator type (bats), it is necessary to describe the natural variations in the nectar traits of chiropterophylic plants to infer about the effect that natural variation in these traits has on resource selection of nectar by bats in the field.

The fact that chiropterophylic flowers share common characteristics and secrete nectars with similar sugar composition, suggests that the bats prefer the dominant traits present in their natural diet (Chapter 2), for which we determined the role of the nectar sugar composition on the foraging decisions on two species of nectar bats with different strategies of nectar use *Leptonycteris yerbabuenae* and *Glossophaga soricina*. The bats showed no preference between sugar types when test solutions had the same concentration. However, *L. yerbabuenae* preferred concentrated over diluted solutions regardless the sugar type. Both bat species perceive hexoses and sucrose as energy equivalent. The results suggest that changes in the sugar composition of nectar in chiropterophylic flowers is not determined by the preferences of bats, and other factors intrinsic to the plant that may be shaping the sugar composition in chiropterophylic flowers.

Since amino acids are common components of nectar, it has been suggested that they may have an important role in the attraction and nutrition for some of its pollinators. The effect of the amino acids on the food resources selection in bats has not been studied, so the second goal of this work (Chapter 3) was to determine the role of the nectar amino acids on foraging decisions in two nectar-feeding bat species with different strategies of nectar use *Leptonycteris yerababuenae* and *Glossophaga soricina*. In this chapter I evaluated: 1) the role of nectar as nitrogen source, 2) the role of the amino acid concentration in relation to the total nectar concentration, and 3) the role of taste that nectar amino acids provide to nectar on the foraging decisions of nectar feeding bats. The results showed that: 1) the bats were indifferent to nectars with contrasting nitrogen concentrations as found in field, suggesting that nectar is not an important nitrogen source for nectar bats. 2) the bats were indifferent to contrasting nectar concentrations (18 vs. 27%) when they were added with nitrogen, suggesting that the presence of nitrogen in nectar may reduce their ability to differentiate between the total nectar concentration and, 3) *Leptonycteris yerbabuenae* consistently preferred the taste of amino acids present in the nectar of *Pachycereus pecten* (Cactaceae) over *Ceiba aesculifolia* (Malvaceae). The results suggest that nectar amino acids may affect the foraging decisions of nectar bats, regardless of the low concentrations which are present in floral nectar of chiropterophylic plants.

The floral nectar is the most important reward for pollinators. Sugar composition of nectar may be affected by biochemical, ecological and evolutionary factors, but its influence on the final sugar composition of chiropterophylic plants is unknown. The third goal of this work (Chapter 4) was to determine: a) the nectar sugar composition, volume and total concentration of nectar from chiropterophylic plants b) if the sugar composition is related to the volume and nectar concentration, and c) how the flowering strategies of plants affect nectar traits. The results showed that chiropterophylic plants produce sucrose dominated nectar (1 species), sucrose rich nectar (2 species), hexoses rich nectar (8 species) and hexoses dominated nectar (1 species). The percentages of glucose in nectar, different those of fructose, may be explained by the sucrose digestion in the nectary by the activity of the invertase enzyme. There was a negative relationship between the sucrose percentage and the nectar volume. Finally, species of plants that produce massive flowers per night offer hexoses and diluted nectar while plants that produce few flowers per night have more concentrated nectar with higher proportions of sucrose. The results suggest the existence of other mechanisms in addition to the sucrose digestion in the nectary, which may be involved in determining the final sugar composition in the nectar of chiropterophylic plants. Among the chiropterophylic plant community, two strategies in nectar production were found, that could be related to energy costs associated with the flower and nectar production of flowers and nectar.

In conclusion, the results obtained in this study reject the hypothesis that nectar bats have a preference for the dominant sugars in their natural diet. The fact that the amino acids concentration in nectar has the ability to confuse the bats to distinguish between sugar concentrations, and that some bats have a preference for the taste that nectar amino acids provide to specific plant species, suggests that plants may be using the variation in this feature in nectar to attract animals. Finally, the nectar traits of chiropterophylic plants seem to be the result of the biochemical, evolutionary and ecological factors related with the plant and not the result of the food preferences of bats.

ÍNDICE

CAPÍTULO 1.

INTRODUCCIÓN GENERAL

Síndromes de polinización

Los distintos tipos de polinizadores ejercen diferentes presiones de selección en los caracteres florales (Fenster et al 1991). Stebbins (1970) sugiere que las características de las flores deben ser moldeadas por aquellos polinizadores que las visiten más frecuente y más efectivamente en la región donde están evolucionando. Se ha planteado que las preferencias de los polinizadores por ciertas características de las plantas de las que se alimentan han actuado como presiones selectivas que han moldeado estos elementos florales (Faegri & Van der Pijl 1979). Esto da como resultado, por un lado, grupos de plantas con características florales similares y por el otro, que los polinizadores se vean obligados a evolucionar características morfológicas y fisiológicas comunes. Por lo que se asume que estas innovaciones compartidas son el resultado de una evolución convergente, lo que ha sido definido como síndromes de polinización (Faegri & Van der Pijl 1979, Baker et al 1998). Bajo este contexto, las plantas cuyas flores son visitadas por los miembros de un mismo gremio de polinizadores tienden a tener una composición floral similar (forma, color, y esencias) y a secretar néctares con una composición similar de azúcares con concentraciones y volúmenes de néctares similares (Baker et al 1998), que sirven para atraer a un tipo particular de polinizador. Por lo tanto, los síndromes de polinización son de gran utilidad para entender los mecanismos de la diversificación floral. Sin embargo, el concepto de síndromes de polinización, implica que los sistemas de polinización sean del tipo especialista (Waser et al 1996, Johnson & Steiner 2000).

En general, las flores polinizadas por aves son de color amarillo y en algunos casos tienden al rojo brillante, con corolas alargadas y angostas, y producen néctar diluido y abundante (Faegri & van der Pijl 1979). Las flores polinizadas por abejas son de colores amarillos o azules, poseen pistas para el aterrizaje, y producen néctares concentrados dominados por sacarosa (Wilson et al 2004). Las flores polinizadas por esfíngidos son de color blanco o de tonalidades pálidas, de tamaños grandes, sus formas son tubulares y tienen una gran cámara nectarial. Las flores polinizadas por murciélagos presentan corolas blancas, amarillentas o pálidas, con pétalos resistentes y muchas anteras con grandes cantidades de polen. Generalmente se encuentran en lugares expuestos. La apertura floral es nocturna, la flor permanece abierta sólo por una noche y en algunos casos, por unas cuantas horas de la mañana (Grant & Grant 1979, Gibson & Nobel 1986, Howe & Westley 1988). La producción de néctar es elevada, se inicia poco antes de la apertura de la flor y se va incrementando a medida que avanza la noche y desciende al amanecer (Faegri & van der Pijl 1979, Howe & Westley 1988, Valiente-Banuet et al 1996, Petit & Freeman 1997). Las flores polinizadas por murciélagos en el Neotrópico ofrecen en su mayoría néctares ricos en hexosas (Baker & Baker 1983).

Sistemas especialistas y generalistas

En general, una planta es considerada como un sistema de polinización especialista si es polinizada exitosamente por un tipo o por un grupo funcional reducido de polinizadores (taxonómicamente relacionados o no). Estos polinizadores se comportan de manera similar en la flor, ejerciendo una presión de selección convergente sobre diversas características florales (Armbruster et al 1993, Fenster et al 2004). La ventaja de este tipo de polinización es la reducción de competencia por polinizadores (excluyendo otros tipos de polinizadores), y la disminución de la transferencia de polen interespecífico. Sin embargo, la polinización especialista ha sido considerada crítica para la especiación y radiación de las Angiospermas (Armbruster 1993, Johnson et al 1998). En este contexto, las plantas cuyas flores son visitadas por los miembros de un mismo gremio de polinizadores tienden a tener una composición floral similar (forma, color, y esencias; Faegry & van der Pijl 1979) y a secretar néctares con atributos (calidad y cantidad) similares para atraer a un tipo particular de polinizador (Baker & Baker 1982, 1983, Baker et al 1998).

En la biología de la polinización, el carácter longitud de corola, ha sido considerado como uno de los principales rasgos florales que determinan una estrecha interacción con sus polinizadores (Faegri & van der Pijl 1966, Fenster 1991, Wilson & Thompson 1996). Para ser polinizadas, las flores con largas corolas requieren los servicios de animales con adaptaciones morfológicas particulares. Estas características a su vez, restringirían sus posibilidades de obtener la recompensa floral, limitándolos a los organismos realizar visitas a flores con características morfológicas similares y, como consecuencia, estimulando la especialización conjunta. Las especies con largas corolas producen altos volúmenes de néctar y tienen grandes cantidades de azúcar (Dafni & Neal 1997). Entonces, también se puede afirmar que las flores que presentan alto volumen de néctar dominado por sacarosa, son especializadas. Según Baker & Baker (1998), las diferencias en la composición química del néctar de las plantas representan adaptaciones a tipos particulares de polinizadores. Por ejemplo las especies con largas longitudes de corola, presentan néctares ricos o dominantes en sacarosa. Incluso, se ha encontrado una relación entre el contenido promedio de aminoácidos en el néctar y el tipo de polinizador, desde 0.3mM en el caso de flores polinizadas por murciélagos y aves en el Viejo Mundo hasta 12-15 mM en flores polinizadas por moscas (Baker y Baker 1982). Por ejemplo, las flores polinizadas por esfíngidos son de colores pálidos, poseen largas y profundas corolas que permiten la deposición del néctar en la base y producen néctares concentrados a bajos volúmenes y dominados por sacarosa.

La incrementada especialización, sin embargo, en ocasiones provoca que los organismos sean más susceptibles a las variaciones en la disponibilidad de los recursos en un tiempo determinado. Los murciélagos que son extremamente nectarívoros especializados (como *Choeronycteris*), están obligados a hacer largas migraciones para asegurar un suplemento alimenticio adecuado o en dado momento a cambiar su dieta hasta donde su especialización se los permita, a los recursos que en ese momento estén disponibles como frutos.

Los patrones fenológicos de una planta dependen de la coevolución con sus polinizadores. En la polinización especialista, las plantas presentan periodos de floración largos con pocas flores disponibles por noche durante todo el periodo de floración. Este fenómeno ocasiona que los visitantes aprendan la ubicación y hagan visitas repetidamente. Además los visitantes se mueven entre plantas de la misma especie más constantemente. Un pequeño número de flores no atrae a animales oportunistas, por lo que la polinización es por un tipo de animal, por ejemplo murciélagos nectarívoros que se alimentan básicamente del néctar. Las flores de estructura campanulada y la hora de secreción de néctar restringe la diversidad de visitantes y la posibilidad de transferir polen a otras especies de plantas, lo que a su vez, reduce impacto de baja constancia floral entre polinizadores (Heithaus 1982). Este patrón de floración se conoce con el término de “steady-state” y promueve el trap-linning (tipo de forrajeo en donde se siguen rutas específicas. ie. en murciélagos).

En otro escenario, existe una gran cantidad de evidencia que muestra que una amplia variedad de plantas pueden ser polinizadas exitosamente por un diverso espectro de visitantes. Estos visitantes son usualmente de diverso origen taxonómico (Renner 1998), y no dependen caracteres florales (Gómez & Zamora 1999, Herrera 1988, 1996). El tipo de polinización generalista es favorecido cuando la disponibilidad del polinizador más efectivo no es predecible temporalmente (Steven & Steiner 2000). Las características generalistas de las plantas promueven el reemplazo espacio temporal de polinizadores, y permiten mantener una interacción ecológica sin consecuencias especie-específicas coevolutivas aparentes. En este tipo de polinización se presenta mayor variación fenotípica de las características florales que en las especies especialistas (Herrera 1996, Waser et al 1996). Además se sabe que este tipo de polinización es menos frecuente. Las plantas bajo este sistema presentan fuerte selección direccional en características florales (Steven & Steiner 2000). Esta variación es importante ya que es la base de la flexibilidad evolutiva. Adicionalmente, las especies que son polinizadores efectivos o agentes de dispersión en otros sistemas, pueden sostenerse de alimentos diversos en periodos de escasez. Más importante, esta variación reduce la dependencia de un animal a una planta dada que pueda variar en densidad o en disponibilidad, afectando así a la especie animal. Al mismo tiempo, si las plantas reciben servicios de polinizadores con diferentes características pueden limitar la habilidad de las plantas a responder a la coevolución a las características específicas de una o más especies (Heithaus 1982). Contrario a lo propuesto para flores especialistas, en este tipo de flores la longitud de la corola es menos profunda o son flores peniceladas o muy largas si son campanuladas, en donde los estambres, el pistilo y el néctar están completamente expuestos sin restringir el acceso a polinizadores (Baker et al 1998).

Entre las plantas que son visitadas por más de un taxón esta el caso del Saguaro. Esta planta es polinizada por murciélagos, pero también puede ser polinizada por abejas y palomillas con pérdidas de 16% y 28% de tasa de producción del semillas respectivamente, comparado con plantas polinizadas por *Leptonycteris sanborni* (Alcorn 1961). En la polinización generalista, el patrón de floración se conoce con el término de “big-bang”. Las plantas presentan periodos de floración cortos, lo que reduce la posibilidad de coincidir en tiempo de floración con otras especies. Producen un gran número de flores por noche. Este tipo de floración atrae a animales oportunistas, incluso a animales que generalmente no incluyen néctar en su alimentación pero que pueden promover el polen entre conespecíficos (Heithaus 1982).

Murciélagos nectarívoros como polinizadores

Mientras que las respuestas fisiológicas y morfológicas de las características de las plantas han incrementado el potencial en que los murciélagos explotan los recursos, el comportamiento de los murciélagos determina los beneficios recibidos por ambos grupos (plantas y murciélagos). Lo que los murciélagos comen, como tratan a su alimento, y en donde se mueven son los componentes críticos de las interacciones entre plantas y animales (Fleming *et al.* 2009). La efectividad de la polinización por las diferentes especies de murciélagos varía dependiendo del contacto con los estambres y pistilos en plantas conspecíficas (Heithaus 1982).

Se ha estimado que aproximadamente 250 de las 850 especies de murciélagos dependen total o parcialmente de las plantas como recurso de alimento ya sea néctar, polen o frutos (Hutcheon et al 2002). Los murciélagos nectarívoros pertenecen a dos familias (Simmons 2005, Fleming et al 2009): Pteropodidae y Phyllostomidae. Los miembros de la familia Pteropodidae (suborden Yinpterochiroptera; Fleming et al 2009), son comúnmente conocidos como mega-chirópteros o zorros voladores. Se distribuyen en regiones tropicales y subtropicales de África (incluyendo Madagascar), Asia (incluyendo Indonesia), Australia, Papua, Nueva Guinea y las islas del Pacífico (Fleming et al 2009). Los miembros de la familia Phyllostomidae (suborden Yangochiropthera), son comúnmente denominados murciélagos de hoja nasal. Habitan en regiones tropicales y subtropicales de América (Fleming *et al.* 2009). El uso de las plantas, ya sea por frugivoría o nectarivoría, se desarrolló convergentemente en estas dos familias (Hutcheon et al 2002). Las plantas polinizadas por murciélagos se encuentran distribuidas en 67 familias y 28 órdenes de angiospermas, de las cuales, 23 son exclusivamente visitadas por pteropódidos, 26 por filostómidos y 18 por ambos (Fleming et al 2009). Los murciélagos de ambas familias juegan un papel importante en la polinización de muchas especies de plantas. Por ejemplo, los pteropódidos visitan 168 especies de plantas de 100 géneros y 41 familias, y los filostómidos visitan 360 especies de plantas de 159 géneros y 44 familias.

El tamaño relativo del cráneo es una característica que coloca a los murciélagos nectarívoros aparte de los otros gremios de murciélagos, ya que es relativamente largo debido en gran parte a la expansión de las regiones olfatoria, visual, neocortical y cerebelar (Hutcheon et al 2002). Presentan una reducción de dientes incisivos, especialmente si poseen lenguas largas y la porción distal de la lengua está cubierta por papilas filiformes que facilitan la extracción de néctar (Freeman 1995). La familia Phyllostomidae está conformada por las subfamilias Glossophaginae, Phyllonycterinae y Brachyphyllinae, denominadas comúnmente como clado Glossophaginae, que contiene 16 géneros y 38 especies (Simmons 2005). Los murciélagos filostómidos son de tamaño pequeño a mediano con un peso promedio de 14 g (7.5 – 30 g; Fleming & Muchala 2008). Sus alas son morfológicamente más diversas y ligeras comparadas con las de los murciélagos pteropódidos, y parecen estar diseñadas para un vuelo lento y maniobrable (Fleming 1982). Con respecto a la orientación, los filostómidos se basan en la audición y ecolocación laríngea (Freeman 1995, Hutcheon et al 2002). La ecolocación está presente en todos los miembros de la familia Phyllostomidae (Fleming 1982).

Los murciélagos filostómidos utilizan la audición, el olfato y la ecolocación para identificar los sitios donde se encuentran flores disponibles. Usualmente hacen múltiples visitas rápidas revoloteando (hovering) en frente de las flores y generalmente tienen una duración de menos de un segundo (Horner et al1998, Quesada *et al* 2003, von Helversen & Winter 2003). En menor porcentaje, los murciélagos se cuelgan de las flores para obtener el néctar (Arias-Cóyotl et al 2006). Los filostómidos generalmente hacen visitas cortas en cada una de las flores, visitan varias flores en una planta y luego visitan otra planta para posteriormente regresar a la primera. Dependiendo de la especie de murciélago, forrajean en grupo (*Leptonycteris yerbabuenae*; Horner et al 1998) o prefieren el forrajeo solitario (*Glossophaga soricina*; Lemke 1984). El pico en alimentación coincide con la máxima secreción de néctar (Nassar et al 1997). Los patrones de forrajeo de estos organismos están determinados tanto por los patrones fenológicos de las especies que visitan (Stoner et al 2003), así como por los mecanismos fisiológicos que afectan su comportamiento limitando la manera en que pueden utilizar diversos recursos presentes en el néctar (Hernández & Martínez del Río 1992, Ayala-Berdón et al 2008, Mancina et al 2008.

El néctar floral

Las plantas que dependen de animales como vectores bióticos para ser polinizadas, tienden a desarrollar mecanismos que atraen y recompensan a sus polinizadores para asegurar un mayor número de visitas (Simpson & Neff 1983). Este tipo de recompensas son particularmente importantes cuando los polinizadores son recursos limitantes y las plantas compiten por su visita para asegurar su éxito reproductivo. El néctar además del polen, constituye una de las recompensas florales más importantes ofrecidas a los polinizadores (Baker & Baker 1982, Simpson & Neff 1983).

El néctar floral es una solución simple de azúcares disueltos en agua (Baker et al1998). La energía contenida en el néctar es la recompensa más preciada para el polinizador. Los azúcares principales son fructosa, glucosa (hexosas o azúcares simples), y la sacarosa (disacárido; Baker et al 1998). El néctar contiene con gran frecuencia, además de azúcares, otras sustancias, como aminoácidos, lípidos, proteínas, ácido ascórbico, iones e incluso alcaloides (Baker & Baker 1977). Debido a que los aminoácidos después de los azúcares son componentes frecuentes del néctar, se ha sugerido, que pueden tener un papel importante en la atracción y nutrición para algunos de sus polinizadores (Baker 1977, Baker & Baker 1973,1982, 1986, Baker et al. 1978, 1998,Gardener & Gillman 2002).

La composición de azúcares del néctar puede ser afectada tanto por factores bioquímicos, como por factores ecológicos y evolutivos (Nicolson 1998, Ornelas et al 2007).

Factores bioquímicos

La composición de azúcares en el néctar puede variar debido a cambios en la actividad de las enzimas en el nectario y a los procesos de osmorregulación causados por la presión osmótica generada por los diferentes azúcares (Nicolson, 1998; Nicolson & Fleming 2003). Los azúcares del néctar se originan del floema, rico en forma de sacarosa (De la Barrera & Nobel 2004). El néctar de sacarosa puede ser hidrolizado por la actividad de las enzimas invertasas en glucosa y fructosa, o puede ser secretado sin previa hidrólisis en forma de sacarosa (Nicolson 2002; De la Barrera & Nobel 2004). La composición final del néctar es determinada tanto por la actividad y la cantidad de enzimas invertasa en el nectario (Woodson & Wang 1987, Wenzler et al 2008). Cuando la sacarosa es hidrólizada en hexosas, la osmolalidad del néctar aumenta, haciendo que el agua se mueva de las paredes del nectario al néctar, por lo que resulta en néctares más diluidos (Nicolson, 1998; 2002). Como consecuencia, la composición de azúcares en el néctar puede afectar otras características del néctar, como el volumen y la concentración. Por ejemplo, en plantas polinizadas por aves se ha observado una dicotomía entre la composición de azúcares y el volumen y la concentración del néctar. En donde, los néctares dominados por hexosas son diluídos y abundantes, mientras que los néctares dominados por sacarosa son concentrados y menos abundantes (Baker & Baker 1982, Martínez del Río Baker & Baker 1992, Nicolson 1998, Nicolson & Fleming 2003, Lotz & Schondube 2006). Sin embargo, este patrón no ha sido evaluado en plantas chiropterofilicas.

Factores Ecológicos y Evolutivos

La variación en la composición de azúcares, el volumen y la concentración del néctar también se ven afectados a niveles ecológicos y evolutivos. Por una parte, desde el punto de vista de la planta, la inercia filogenética (Ornelas et al 2007), y los costos de producción de néctar y, por el otro, debido a la fisiología sus polinizadores. Algunos autores han propuesto que la producción de néctar es costoso para la planta en términos de la inversión en energía necesaria para producirlo (Pleasants & Chaplin 1983, Pyke 1991, Ashman & Shoen 1997; pero ver Harder & Barrett 1992 y Golubov et al 2004).

Por otra parte, las características presentes en el néctar han tratado de explicarse como adaptaciones de la planta para aumentar la frecuencia de visitas de los polinizadores potenciales como parte de un sistema de coevolución con los polinizadores (Simpson & Neff 1983). Características como volumen y concentración total del néctar, composición de azúcares, proporciones sacarosa-hexosas, y tiempo de secreción del néctar han sido frecuentemente relacionados con la interacción entre flores y polinizadores (Baker & Baker 1983, Freeman et al 1984, Bertin 1989, Stiles & Freeman 1993, Galetto et al 1998, Perret et al 2001, Pacini et al 2003, Wolff et al 2003). La existencia de los patrones florales arriba mencionados, sugiere a la vez que los distintos grupos de polinizadores tienden a preferir las características dominantes en su dieta. La composición de azúcares en los néctares de plantas polinizadas por diferentes grupos de animales es un buen ejemplo de la diversidad que se supone, es moldeada por las preferencias de los polinizadores (Baker & Baker 1983). Diversos estudios han llegado a la conclusión de que las preferencias de los polinizadores han actuado como presión de selección, moldeando las características del néctar (Heinrich & Raven 1972, Baker & Baker 1982, Martínez del Río et al 1989,1990, 1992, Erhardt 1991, Baker & Baker 1992, Baker et al 1998).

Animales vertebrados nectarívoros y sus preferencias por el alimento

Varios estudios muestran una relación positiva entre las preferencias de animales nectarívoros y la composición de su alimento (Stiles 1976, Martínez del Río 1990, Erhardt 1991, Martínez del Río et al 1992). Para algunos grupos de polinizadores la composición de azucares en el néctar es una limitante. Por ejemplo, se ha demostrado que aves Passerinas carecen de la sacarasa (enzima digestiva que de manera natural hidroliza la sacarosa en sus dos principales monómeros, glucosa y fructosa), razón por la cual, presentan aversión por los néctares que poseen sacarosa, y muestran preferencia por néctares dominados por glucosa y fructosa (Martínez del

Río et al 1989, 1990). Estudios con plantas polinizadas por este tipo de aves demuestran que el néctar de estas especies es dominado por las hexosas glucosa y fructosa sobre sacarosa, lo que sugiere que los polinizadores han ejercido una fuerte presión de selección sobre las características del néctar (Dupont et al 2004).

En contraste con los estudios que muestran una relación positiva entre las preferencias de aves nectarívoras y la composición de su alimento, en condiciones de laboratorio con murciélagos en condiciones de laboratorio con murciélagos filostómidos, en el único estudio realizado hasta el momento (Herrera 1999 a,), los murciélagos tienen preferencias por néctares ricos en sacarosa, a pesar de que los néctares secretados por las plantas polinizadas por murciélagos en el Neotrópico tienden a estar dominados por hexosas (glucosa y fructosa, Baker et al 1998). En el estudio de Herrera (1999) se utilizaron soluciones de fructosa-glucosa en relación 1:1 contra soluciones de sacarosa y soluciones de una sola azúcar. Aunque los resultados muestran que los murciélagos prefieren un azúcar que es diferente a los azúcares que dominan la dieta de estos animales, en este estudio se ofrecieron soluciones de azúcares particulares (e.g. soluciones de sacarosa vs. soluciones de glucosa o fructosa), las cuales son diferentes a las que se encuentran en la naturaleza. Además, en este estudio se utilizaron como modelo experimental dos murciélagos frugívoros (*Artibeus jamaicencis* y *Sturnira lilium*) y uno nectarívoro (*Anoura geoffroyi*). Ayala Berdón et al (2008) y Herrera y Mancina et al (2008), encontraron que en murciélagos que se alimentan de néctar, el consumo de los diferentes tipos de azúcares no parece estar limitado por la capacidad del intestino para procesarlos. Además, la actividad de la enzima sacarosa en el intestino de murciélagos filostómidos que se alimentan de néctar y frutas se ha encontrado en cantidades suficientes para asimilar la sacarosa presente en el néctar y frutas (Hernández & Martínez del Río 1992, Schondube et al 2001). Lotz & Schondube (2006) y Herrera (1999 b) proponen que las aves y los murciélagos con una gran capacidad para digerir la sacarosa deberían de ser capaces de consumir todos los tipos de néctar, independientemente de la composición de azúcares.

Justificación del estudio

En general, los estudios que evalúan la relación entre las preferencias de los polinizadores y las características dominantes en su dieta natural, se han llevado a cabo utilizando información de la literatura acerca de la composición del alimento. Esto genera dos problemas graves para la interpretación de los resultados. Primero, en muchos casos el contenido de azúcares de la "dieta" se ha determinado utilizando información sobre especies de plantas que no son consumidas por los animales estudiados, sino por especies cercanas taxonómicamente a ellos. Y segundo, cuando se han utilizado las especies de plantas presentes en su dieta, la información sobre contenido de azúcar en el néctar proviene de localidades distintas, lo que impide determinar si existe variación regional en la composición del néctar, y el efecto que esto pudiera tener sobre los patrones de selección de azúcares de los polinizadores.

En este contexto, para entender si el néctar de flores quiropterofílicas: i) es una característica constante y relativamente conservada entre especies de plantas relacionadas filogenéticamente, o si ii) sus características coinciden con las preferencias de un tipo particular de polinizadores, (i.e. los murciélagos), es necesario describir las variaciones naturales en las características del néctar de las plantas quiropterofílicas para poder inferir sobre el efecto que la variación natural en estas características tiene sobre la selección de recursos de los murciélagos nectarívoros en campo.

Estructura de la tesis

Este estudio se dividió en tres capítulos. El segundo capítulo, "**Efecto de la composición y concentración de azúcares en el néctar sobre la selección de los recursos"**, consistió en determinar el papel que ejercen la composición y concentración de azúcares en el néctar sobre las decisiones de forrajeo en dos especies de murciélagos nectarívoros con diferentes estrategias de uso de néctar: *Leptonycteris yerbabuenae* y *Glossophaga soricina*. El tercer capítulo, "**Los aminoácidos del néctar modifican la selección del alimento en murciélagos nectarívoros"**, consistió en determinar el papel que ejercen los aminoácidos del néctar sobre las decisiones de forrajeo en dos especies de murciélagos nectarívoros con diferentes estrategias de uso de néctar: *Leptonycteris yerbabuenae* y *Glossophaga soricina*. En este capítulo evalué: 1) la concentración de proteínas y aminoácidos, y la abundancia relativa de 17 aminoácidos en el néctar de 8 especies de plantas quiropterofílicas; 2) el papel del néctar como fuente de nitrógeno, 3) el papel de la concentración de aminoácidos en relación a la concentración de azúcares, y 4) el papel del sabor que los aminoácidos proporcionan al néctar sobre la selección del alimento de murciélagos nectarívoros. Finalmente el cuarto capítulo, "**Factores que afectan la composición de azúcares en plantas quiropterofílicas"** consistió en determinar: a) la composición de azúcares, el volumen y la concentración total en el néctar de plantas quiropterofílicas; b) si la composición de azúcares está relacionada con el volumen y la concentración del néctar; y c) cómo las estrategias de floración de las plantas afectan las características del néctar.

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CAPÍTULO 2.

EFECTO DE LA COMPOSICIÓN Y CONCENTRACIÓN DE AZÚCARES EN EL NÉCTAR SOBRE LA SELECCIÓN DE LOS RECURSOS

Effects of Sugar Composition and Concentration on Food Selection by Saussure's Long-nosed Bat (*Leptonycteris curasoae*) and the Long-tongued Bat (*Glossophaga soricina*)

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Source: Journal of Mammalogy, 88(6):1466-1474. 2007.

Published By: American Society of Mammalogists

DOI: 10.1644/06-MAMM-A-353R1.1

URL: http://www.bioone.org/doi/full/10.1644/06-MAMM-A-353R1.1

Journal of Mammalogy, 88(6):1466–1474, 2007

EFFECTS OF SUGAR COMPOSITION AND CONCENTRATION ON FOOD SELECTION BY SAUSSURE'S LONG-NOSED BAT (*LEPTONYCTERIS CURASOAE*) AND THE LONG-TONGUED BAT (*GLOSSOPHAGA SORICINA*)

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In the Neotropics, bat-pollinated plants secrete relatively dilute nectars dominated by hexoses (glucose and fructose) with only small amounts of sucrose. We investigated the concentration and sugar composition preferences of Saussure's long-nosed bat (*Leptonycteris curasoae*) and the long-tongued bat (*Glossophaga soricina*) to test the hypothesis that bats prefer the predominant characteristics (sugar composition and concentration) found in their natural diets. We offered bats pairs of test diets in large outdoor enclosures that allowed free flying. We used artificial nectars that simulated compositions and concentrations found in flowers visited by these 2 species at the study site. Contrary to our predictions, bats showed no preference between sugar types when test solutions had the same concentration. However, *L. curasoae* preferred concentrated over dilute solutions independent of sugar type. Only 1 preference for concentrated over dilute solutions was recorded for *G. soricina*. Both species of bat appeared to perceive sugar types as energetically equivalent in most trials. Our study rejects the hypothesis that nectar feeding neotropical bats act as a selective pressure on nectar composition in chiropterophilous plants. Other possible explanations for the predominance of hexose in chiropterophilous flowers need to be evaluated.

Key words: bats, Chiroptera, chiropterophilous flowers, concentration preferences, dry forest, energetic equivalence, Mexico, pollination ecology, sugar preferences

Floral nectars are sugar solutions that also contain small amounts of other substances such as amino acids, proteins, lipids, ascorbic acid, and alkaloids (Baker and Baker 1983). Floral nectars vary in both sugar concentration and composition (Baker et al. 1998; Galleto and Bernardello 2004; Nicolson 2002). The composition of nectar depends on several factors such as the flowering season (Torres and Galleto 1998), the floral stage (Pleasants 1983), and the structure of the floral nectary (Davis et al. 1998). Nectar composition and concentration also depend upon environmental factors such as temperature and humidity (Vezza et al. 2006). Additionally, large intraplant variations in nectar production have been reported for several species (Bernardello et al. 2004; Freeman and Wilken 1987; Reid et al. 1985; Severson and Erickson 1984).

Nectar characteristics are seen as adaptations to increase the frequency of visits by potential pollinators as part of a co-evolving system (Simpson and Neff 1983). Several studies conclude that preferences exhibited by nectarivorous foragers should select for the evolution of nectar characteristics (Heinrich and Raven 1972) and that nectarivores should prefer nectars with sugar composition similar to those found in their natural diet (Baker and Baker 1983; Baker et al. 1998; Erhardt 1991; Martínez del Río et al. 1988, 1992; Wykes 1952). Indeed, the variable distribution of sugars in the nectars of plants pollinated by different groups of animals is a good example of the diversity of rewards assumed to be molded by the preferences of pollinators (Baker and Baker 1983). Under experimental conditions, there is evidence that birds prefer

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TABLE 1.—Experimental design, sugar composition of test diets (%), and pairwise comparisons of concentrations.

Equivalent species	Diet	Fructose : glucose : sucrose
Acanthocereus occidentalis	Sucrose dominated (SD)	28.97%:17.06%:53.98%
Ceiba grandiflora	Hexose dominated 1 (HD1)	46.82%:39.43%:13.75%
Pseudobombax ellipticum	Hexose dominated 2 (HD2)	41.58%:23.05%:35.37%
Trials	Experiment 1 (SD:HD1)	Experiment 2 (HD1:HD2)
1	20%:20%	20%:20%
2	27%:18%	18%:15%
3	18%:27%	15%:18%

sugar concentrations and compositions similar to those found in their natural diet (Blem et al. 2000; Fleming et al. 2004; Lotz and Schondube 2006; Martínez del Río et al. 1992). In birds, preferences for sugar concentration and composition seem to be linked to their capacities for assimilation of sugar, and their abilities to differentiate among sugar concentrations (Lotz and Schondube 2006). Nevertheless, a laboratory study of bats found no relationship between sugar composition of chiropterophilous flowers in the wild and sugar preferences in the laboratory (Herrera 1999). However, it is noteworthy that this study was conducted under laboratory conditions with artificial nectar concentrations.

To further test the conjecture that sugar preferences of bats coincide with the composition of flower nectars in natural diets we examined the preferences of the New World nectar-feeding bats Saussure's long-nosed bat (*Leptonycteris curasoae*) and the long-tongued bat (*Glossophaga soricina*; Phyllostomidae: Glossophaginae). We used nectars with ecologically realistic composition and concentrations, simulating nectar of flowers consumed by these nectarivorous bats in the region. We predicted that bats would prefer hexose-dominated relative to sucrose-dominated nectars and concentrations similar to the average concentration of flowers found in natural diets (18%). Finally, we assessed whether bats perceived different sugar compositions as energetically equivalent. We expected that bats would perceive different nectar diets as energetically equivalent, independently of whether they discriminate between nectar compositions.

MATERIALS AND METHODS

Study site.—Our study was conducted at the Chamela-Cuixmala Biosphere Reserve on the central Pacific coast of Mexico (approximately 19°22′–19°35′N, 104°56′–105°03′W). The predominant vegetation type is tropical lowland deciduous forest (Lott 1993). There are 1,100 plant species recorded at this site, and the flowers of at least 22 species are visited by bats (Stoner et al. 2003). *L. curasoae* is a flower specialist (Stoner et al. 2003), whereas *G. soricina* is a nectarivore that also feeds on fruit and insects when flowers are unavailable (Alvarez et al. 1999; Herrera 1999).

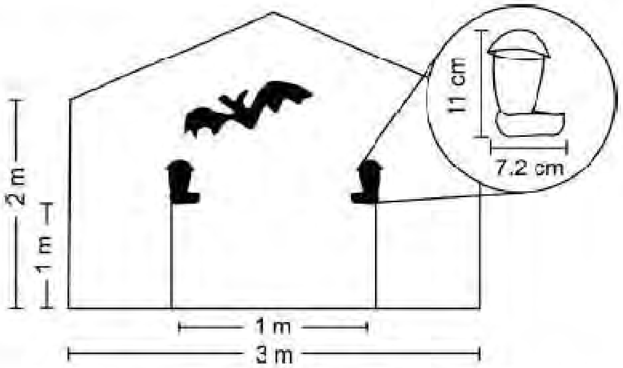

FIG. 1.—Preference experiments were conducted within enclosures (2 × 3 × 3 m) allowing individual bats to fly freely. These enclosures were placed within the natural environment of tropical dry forest where the bats were captured. Feeders faced each other.

Nectar.—To evaluate the sugar and concentration preferences of bats, we prepared 3 artificial nectars equivalent in sugar composition and concentration to nectar of bat-pollinated flowers consumed by nectarivorous bats in the Chamela region (Table 1). Nectar concentration and sugar composition were measured for 3 of the most common species in the diet of bats (Stoner et al. 2003): *Ceiba grandiflora* (Bombacaceae; $n = 35$ flowers from 21 individuals), *Pseudobombax ellipticum* (Bombacaceae; $n = 20$ flowers from 5 individuals), and *Acanthocereus occidentalis* (Cactaceae; $n = 9$ flowers from 5 individuals). The number of flowers sampled per plant varied because of differences in flowering strategies among species and the differences in the number of open flowers among individuals. Mature flower buds were covered with mesh bags 1 h before sunset. One hour after anthesis, nectar was extracted with capillary glass tubes and placed on Whatman No. 1 filter paper (Sigma Chemical Company, St. Louis, Missouri) for analysis of sugar composition. One drop of nectar was placed on a hand refractometer and concentration was measured in sucrose equivalents (% sugar = (sugar mass/total mass) × 100). Sugar composition was analyzed using the medium infrared Rreflectance method (Flores et al. 2003).

Care and housing of bats.—Bats were captured and humanely handled under the guidelines from the Oficina de Fauna Silvestre, Mexico, to JES (permit FAUT-0193), and met guidelines approved by the American Society of Mammalogists (Gannon et al. 2007). We used mist nets to capture adult nonreproductive males of the 2 species. After capture, bats were transferred to the laboratory and maintained in colonies of 8 individuals in aluminum cages (60 × 60 × 60 cm). Bats were fed the diet described by Mirón (2005) supplemented with a vitamin and mineral mix (NEKTON-Plus, Pforzheim, Germany) and fructose and sucrose (50%:50%). Body mass, wing membrane elasticity, and hair condition of all bats was monitored daily. All bats maintained constant body mass and appeared healthy while in captivity. At the conclusion of our experiments, bats were released at the capture site.

Preference tests.—We offered pairs of test diets to individual bats in large flight cages (2 × 3 × 3 m) immersed within their natural habitat in the forest (Fig. 1). Feeders were placed 1 m apart at a height of 1 m. Trials lasted 10 h and were conducted from 1900 to 0500 h. Feeders were filled and placed at 1900 h. The amount consumed was measured (g) and feeder position was switched at the middle of the experiment at 0000 h to evaluate potential positional biases (Jackson et al. 1998). The amount consumed was remeasured at 0500 h. A control feeder of each test diet was placed outside the flight cages to assess evaporation. These feeders were covered with mesh bags to prevent visits from insects or bats. No differences were observed in any of the control feeders indicating that evaporation did not account for any liquid lost during the experiment.

Experiment 1.—To test for sugar and concentration preference we offered bats 2 experimental diets that differed in sugar composition and concentration. One of the test diets was sucrose dominated (SD: composition: fructose [F]: 28.97%, glucose [G]: 17.06%, sucrose [S]: 53.98%) and the other was hexose dominated (HD1: composition: F: 46.82%, G: 39.43%, S: 13.75%). Three trials were conducted with these 2 test diets. In the 1st trial, both diets had the same total sugar concentration (20% weight : volume [w/v]). Thus, in this trial we examined the preferences of bats for sugars with different compositions. Although the concentration of these test solutions was the same, the solutions differed slightly in energetic content. The HD diet contained approximately 2% less energy per unit volume than the SD diet (see Fleming et al. 2004). In trial 2, we tested diets that simulated the natural concentrations found in flowers in the field. The sucrose diet (SD) simulated *A. occidentalis* and contained 27% (w/v) sugar, whereas the hexose diet (HD1) simulated *C. grandiflora* and contained 18% (w/v) sugar. In trial 3 we inverted the concentrations of trial 2. Thus, the sucrose diet (SD) contained 18% sugar whereas the hexose diet (HD1) contained 27% (w/v) sugar. Trials 2 and 3 examined the effect of sugar concentration on the preferences of the bats. The 3 trials with each of the 2 test diets were conducted with 6 different individuals of both species on 3 consecutive nights.

Experiment 2.—A 2nd experimental series was performed using diets that consisted primarily of hexoses but with differing content of sucrose. We offered bats 2 hexose-dominated diets (HD1: F: 46.82%, G: 39.43%, S: 13.75%; and HD2: F: 41.58%, G: 23.05%, S: 35.37%). As in experiment 1, this experiment consisted of 3 trials. In the 1st trial, test diets had the same concentration (20%). Again, although the concentration of these test solutions was the same, the solutions differed slightly in energetic content. In trial 2 the test diets had the concentrations found in the field (HD1: 18%; HD2: 15%) and in trial 3 we inverted the concentrations of trial 2 (HD1: 15%, HD2: 18%; Table 1). Each trial was conducted using 6 bats of both species on 3 consecutive nights.

Data analysis.—To estimate preference we used the ratio of the nightly ingestion of 1 of the test diets divided by total nightly ingestion: preference = (ingestion of diet A)/(total ingestion). A preference value of 0.5 indicates bats consume both solutions in equal amounts (Martínez del Río 1990). We arcsine square-root transformed the data to normalize preference values (Zar 1996) and used 1-sample *t*-tests to test the null hypothesis that preference was not significantly different from 0.5.

Positional biases.—Positional biases in nectar-feeding animals have been documented (Jackson et al. 1998). A positional bias is present when an animal tends to visit 1 of the feeders independently of the identity of the solution in it. We used a sign test in each trial to assess whether bats changed sides more frequently than expected by chance assuming a 0.5 probability of side change. We expected that when bats had a significant preference for a given solution, this preference would result in a side change upon moving the position of the feeder. Conversely, when bats showed no preference, we expected that visits would be random and each feeder would receive a similar amount of visits (i.e., with a similar amount of nectar withdrawn). The side the bats 1st prefer is defined as the side from which they drink the greatest amount of nectar during the 1st part of the night. A side change is documented when they drink more nectar from the other feeder during the 2nd half of the night.

Energetic equivalence.—To determine whether bats perceived the sugars in the offered solutions as energetically equivalent, we determined whether an increase in consumption of 1 solution resulted in a significant decrease in consumption of the other, and whether the magnitude of this decrease was consistent with the ratio in energy content of the 2 solutions. For example, if the concentrations of the 2 test solutions were equal, then the expected slope of the line relating the intake of 1 solution against that of another should be −1. This implies that ingesting 1 g of 1 solution would decrease the intake of the alternative solution by 1 g. When the 2 solutions had different concentrations, the slope of this regression line should equal the ratio of the 2 concentrations of the 2 test solutions. For example, if the solutions had sugar concentrations of 18% and 27%, respectively, then the expected slope of the line relating intake of the 18% solution on the y axis with that of the 27% solution on the x axis should be equal to $-27/18 = -1.5$. In this situation, an increase in the ingestion of 1 g of the 27% solution implies a decrease of 1.5 g of the 18% solution. The observed slope for each bat for each trial was estimated (SAS Institute Inc. 2003) by relating intake of the 2 test solutions using the following linear model:

$$y = \beta_0 + \beta_1 x1 + \sum_{i=1}^{6} \beta_i xi + \varepsilon.$$

In this model y is the consumption of solution 1, x1 is the consumption of solution 2, β_1 is the estimate of the common slope relating y and β_1, and β_i is the "effect" of bat i on the intercept (xi equals 0 if the bat is other than i and 1 if it is bat i). We did not include interaction terms because we would have ended up with 8 possible interactions, which are very difficult to interpret. Our approach recognizes that some bats may have different slopes, but estimates the average slope and tests whether it differs from that expected. Because we found that animals sometimes ate preferentially of alternate solutions in the 2 periods of the night (see "Results"), we included data for both the 1st and 2nd half of the night. Including these data

Table 2.—Preferences for diets of different sugar composition and concentration in 2 species of nectarivorous bats. Preference values were calculated as the ratio of consumption of 1 of the test diets divided by the total nightly ingestion. A preference was deemed significant when this preference was significantly different from 0.5 after a 1-sample *t*-test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Experiment 1 (SD:HD1)		Experiment 2 (HD1:HD2)	
Concentration	Preference ± *SE*	Concentration	Preference ± *SE*
	Leptonycteris curasoae		
20%:20%	0.44 ± 0.04	20%:20%	0.42 ± 0.05
27%:18%	0.17 ± 0.03***	18%:15%	0.83 ± 0.02***
18%:27%	0.77 ± 0.03***	15%:18%	0.26 ± 0.03***
	Glossophaga soricina		
20%:20%	0.48 ± 0.05	20%:20%	0.43 ± 0.03
27%:18%	0.37 ± 0.05	18%:15%	0.55 ± 0.05
18%:27%	0.65 ± 0.05*	15%:18%	0.41 ± 0.03

Table 3.—Number of side changes observed during each trial for each bat species. A sign test assuming a 0.5 probability of side change as a null hypothesis was used to test for significant differences. The critical 0.05 value for the sign test is 14 or more changes in 18 trials (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Experiment 1 (SD:HD1)		Experiment 2 (HD1:HD2)	
Trial	Side change/total	Trial	Side change/total
	Leptonycteris curasoae		
20%:20%	5/18	20%:20%	11/18
27%:18%	16/18*	18%:15%	18/18*
18%:27%	13/18	15%:18%	16/18*
	Glossophaga soricina		
20%:20%	11/18	20%:20%	7/18
27%:18%	9/18	18%:15%	8/18
18%:27%	12/18	15%:18%	7/18

points increased the range of x1 in our analyses and thus increased the accuracy for estimating the slopes. Using the 36 data points for each trial (6 bats × 3 nights × 2 foraging bouts per night), a *t*-test was used to compare the expected slope with the observed slope (Zar 1996). Significant differences indicate that bats do not perceive the 2 solutions as energetically equivalent.

Results

Sugar composition and concentration preferences.—The specialist nectarivore *L. curasoae* showed no preferences for 1 test solution over the other when concentrations of the test solutions were equal (Table 2, trial 1); however, in all cases, *L. curasoae* preferred concentrated over dilute solutions when offered test diets with different concentrations (Table 2, trials 2 and 3). This species always preferred the more concentrated solution. The more generalist omnivore, *G. soricina*, exhibited no significant preferences for any of the test solutions (Table 2, trials 1 and 2) except for the 27% hexose-dominated solution (HD1) over the 18% sucrose-dominated solution (Table 2, trial 3).

Positional biases.—No positional biases were observed for *L. curasoae* (Table 3). In 3 out of the 4 trials in which *L. curasoae* significantly preferred a test solution, individuals changed the side at which they fed more frequently than expected by chance (sign test $P < 0.05$), changing sides when test solutions were moved in the middle of the night. This behavior shows that they continue feeding on the preferred solution regardless of the position. In spite of the fact that the difference was not significant for 1 trial in which *L. curasoae* showed preferences, the pattern was in the same direction (13/18), and indeed, would have been significant with only 1 more side change. In addition, in 2 out of 2 experiments in which individual *L. curasoae* did not prefer a test solution (i.e., because they were the same concentration), they changed sides at frequencies that did not differ significantly from those expected by chance, showing that the position of test solutions did not affect the side from which they fed.

Similarly, no positional biases were observed for *G. soricina* (Table 3). In all 5 trials that *G. soricina* showed no preference for test solutions, the frequency with which *G. soricina* changed sides did not differ significantly from the value expected by chance (sign test $P > 0.05$). This shows that they randomly drank from both feeders regardless of the position. In the 1 trial that they showed preferences for 1 test solution over the other, the side change was greater than in all of the other trials (12/18); however this difference was not significant (sign test $P > 0.05$).

Energetic equivalence.—In *L. curasoae*, the consumption of 1 test solution significantly affected the consumption of the other solution in all trials (r^2 ranged from 0.45 to 0.71, $P < 0.001$ for 5 trials; $r^2 = 0.31$, $P < 0.06$ for 1 trial; $n = 36$ data points per trial; Fig. 2). Furthermore, in 3 of 6 trials the observed slopes were not significantly different from the expected slopes, indicating that sugars are perceived as energetically equivalent (Table 4). In the 3 other trials these slopes were negative, but significantly lower than those expected (Table 4).

Similarly, in *G. soricina*, the consumption of 1 test solution significantly affected the consumption of the other solution in all trials (r^2 ranged from 0.74 to 0.86 in the 6 trials, $P < 0.005$ for all trials, $n = 36$ data points per trial; Fig. 3). The estimated slopes were not significantly different from those predicted in 5 of 6 trials (Table 4), indicating that sugars are perceived as energetically equivalent. In only 1 trial these slopes were significantly lower than those expected (Table 4).

Discussion

Our results did not support all of our expectations and predictions. First, when solutions had equal concentrations, bats showed no significant preference for solutions with different sugar compositions; therefore, they did not prefer hexose-dominated over sucrose-dominated solutions as we had predicted. Second, *L. curasoae* significantly preferred concentrated over dilute solutions. We had originally predicted that these bats would prefer what is most common in their natural diet (18% concentration). Preference for concentrated solutions was not common in *G. soricina*. We observed such a preference

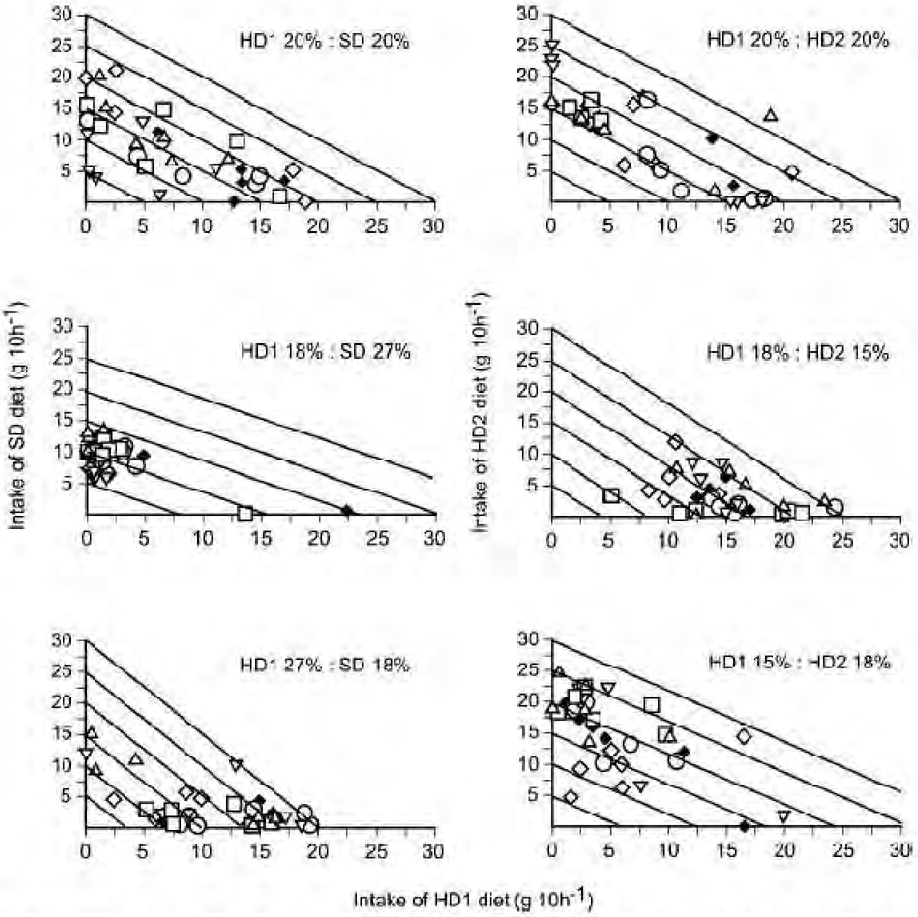

FIG. 2.—Energetic equivalence of test diets for *Leptonycteris curasoae*. The panels on the left represent experiment 1, whereas those on the right represent experiment 2. See Table 4 for observed and expected slopes.

only in 1 of 4 trials in which solutions differed in sugar concentration by 9%. As predicted, in some of the trials bats perceived hexose-rich and sucrose-rich diets as energetically equivalent.

Sugar preferences in bats and their implications.—Several authors have suggested that the preferences of pollinators act as selective pressures that maintain sugar compositions in nectar (Baker and Baker 1983; Baker et al. 1998; Martínez del Río et al. 1992). Furthermore, some studies have hypothesized that the preferences of pollinators differ as a result of variation in digestive traits (Hernández and Martínez del Río 1992). For example, the notion that nectar-feeding animals discriminate among different sugar types is based on the observation that several bird species are either incapable of or poor at digesting sucrose (Martínez del Río and Stevens 1989). Our results suggest that nectar-feeding bats are equally capable of assimilating sucrose, glucose, and fructose. Indeed, Herrera (1999) reported no differences in the efficiency with which bats assimilated sucrose, glucose, and fructose (see also Winter 1998).

Our results do not support the hypothesis that pollinator preferences act as selective pressures on nectar composition, at least in bat-pollinated plants. In particular, we found no evidence that bats preferred one sugar combination over another when they had a choice between solutions of equal concentration. These results are similar to those reported by Law (1993), who found no significant preferences for Old World blossom bats (*Syconycteris australis*) when offered pure sucrose, pure glucose, or an equal volume mixture of sucrose, glucose, and fructose. Nevertheless, Herrera (1999) reported that 2 frugivorous phyllostomid bats (*Artibeus jamaicensis* and *Sturnira lilium*) and a nectar-feeder (*Anoura geoffroyi*) preferred sucrose over solutions of a single hexose (either fructose or glucose). He concluded that these bats preferred sucrose. The discrepancy between our study and that of Herrera (1999) could result from at least 2 factors. First, we allowed bats to choose between solutions containing mixtures of sugars rather than single sugars. This situation is what bats are most likely to encounter in nature, because few or no plants secrete nectars with only 1 sugar (Baker and Baker 1983). Second, we studied 2 species, both of which are nectarivores, 1 a specialized

TABLE 4.—Results of t-test comparing observed and expected slopes (i.e., caloric ratio values) for each species for each trial.

Concentration	Expected slope	Observed ± *SE*	t	P
		Leptonycteris curasoae		
SD:HD1				
20%:20%	−1.00	−0.74 ± 0.10	2.60	0.01
27%:18%	−0.66	−0.58 ± 0.07	1.14	0.26
18%:27%	−1.50	−0.35 ± 0.08	14.4	<0.0001
HD2:HD1				
20%:20%	−1.00	−0.84 ± 0.13	1.2	0.24
15%:18%	−1.20	−0.27 ± 0.12	7.75	<0.0001
18%:15%	−0.83	−0.72 ± 0.16	0.68	0.50
		Glossophaga soricina		
SD:HD1				
20%:20%	−1.00	−0.72 ± 0.09	2.89	0.007
27%:18%	−0.66	−0.78 ± 0.10	1.20	0.24
18%:27%	−1.50	−1.40 ± 0.11	0.89	0.38
HD2:HD1				
20%:20%	−1.00	−0.87 ± 0.09	1.34	0.19
15%:18%	−1.20	−1.17 ± 0.08	0.34	0.73
18%:15%	−0.83	−0.67 ± 0.09	1.7	0.09

nectarivore (*L. curasoae*) and the other a generalist nectarivore (*G. soricina*). It is possible that differences exist between nectar- and fruit-eating bats in sugar preferences and because the study of Herrera (1999) used 2 frugivores and 1 nectarivore, this may have resulted in different conclusions. These potential differences remain to be investigated. In spite of these differences, our results contradict the hypothesis that nectarivores will prefer sugars most common in their natural diet (Martínez del Río et al. 1992). Bats did not prefer hexose-dominated solutions over sucrose-dominated solutions. The prevalence of hexose-dominated nectars among bat-pollinated plants in the New World does not appear to be a result of bats preferring hexose-dominated nectar. The predominance of hexose-dominated chiropterophilous flowers is an ecological pattern that remains to be explained.

Concentration preferences in bats and their implications.—Although bats did not exhibit preferences when offered nectars with different sugar compositions, *L. curasoae* preferred concentrated over dilute nectars in all trials. The nectar specialist, *L. curasoae*, appeared to be able to distinguish concentration differences even when the difference between 2 solutions was small (3%). The preference for more concentrated nectars also has been reported for the megachiropteran nectar specialist *S. australis* (Law 1993). It appears as if the more generalist nectarivore, *G. soricina*, only can distinguish between concentrations when the difference is large (9%). These results concur with those of Roces et al. (1993), who found that *G. soricina* discriminated between sugar solutions with a large difference in concentration (20%). In our experiments, test solutions differed in concentration by only 3% and 9%.

Leptonycteris curasoae and *G. soricina* differed in their ability to discriminate between solutions with contrasting sugar concentrations. *L. curasoae* is more dependent on nectar than the more omnivorous *G. soricina*, and it seems that *L. curasoae* has a better capacity to discriminate between solutions with different concentrations. We speculate that among bats, more-specialized nectarivores will be able to discriminate smaller differences in sugar concentration. Levey (1987) proposed a similar hypothesis for fruit-eating birds. He speculated that frugivores had finer discrimination abilities than insectivores (see also Schaefer et al. 2003). The potential correlation between the ability to discriminate small differences in sugar concentration and specialization to a nectar or fruit diet remains to be tested thoroughly in both bats and birds.

In the Neotropics, the nectar secreted by bat-pollinated plants is relatively dilute, ranging in concentration from 5% to 29% (w/v—Helversen 1993). At our study site the flowers visited by bats secrete nectars that range from 3% to 33% with species averages ranging from 15% to 27%. (w/v—N. Rodríguez-Peña and K. E. Stoner, in litt.). If *L. curasoae* prefers more concentrated nectars, why is it that bat-pollinated plants secrete relatively dilute nectars? Nicolson (2002) reviewed the factors that might determine the prevalence of dilute nectars among flowers pollinated by passerines. She concluded that this prevalence was the result of 4 factors: hexose dominance, an open floral morphology, the secretion of a relatively dilute nectar, and production of copious nectar. In other words, the presence of hexoses in nectar may facilitate the secretion of a large volume at the expense of nectar concentration and may reduce evaporative water losses in open flowers (Nicolson 2002). These 4 factors also are present in bat-pollinated flowers, and may override the importance of the preferences of specialized pollinators.

Do bats perceive different sugars as energetically equivalent?—The results for energetic equivalence were variable both between experiments and bat species; nevertheless, in 68% of the trials bats perceived sugars as energetically equivalent. Given the result that bats showed no significant preferences between solutions with contrasting sugar compositions (previous section) how can we explain the discrepancy found in some of the trials if bats are perceiving sugars as energetically equivalent? One possibility is that the difference is a statistical artifact that results from points concentrated in a narrow range of one of the axes (Fig. 2; trial 3 in experiment 1 and trial 2 in experiment 2 for *L. curasoae*), or from using standard linear least squares regression in a situation in which there is error of the same magnitude in both the x and the y variable. Whenever the 2nd situation is encountered, statisticians advise using a reduced major axis regression (Bohonak and Van Derlinde 2004). Nevertheless, we opted not to use major axis regression estimates because they are derived assuming that all points in the regression are independent, whereas in our study repeated measurements on a single individual were used. For these reasons, our rejection of the null hypothesis of energetic equivalence must be tempered by the observation that the value of our estimates of slopes may be biased by an uneven distribution of points in our scatter grams, and by the biases introduced by standard linear least squares on data sets in which both the x and the y variable are measured with error. Further experiments conducted to assess whether different sugars are perceived as energetically equivalent by bats must control for these potentially confounding effects.

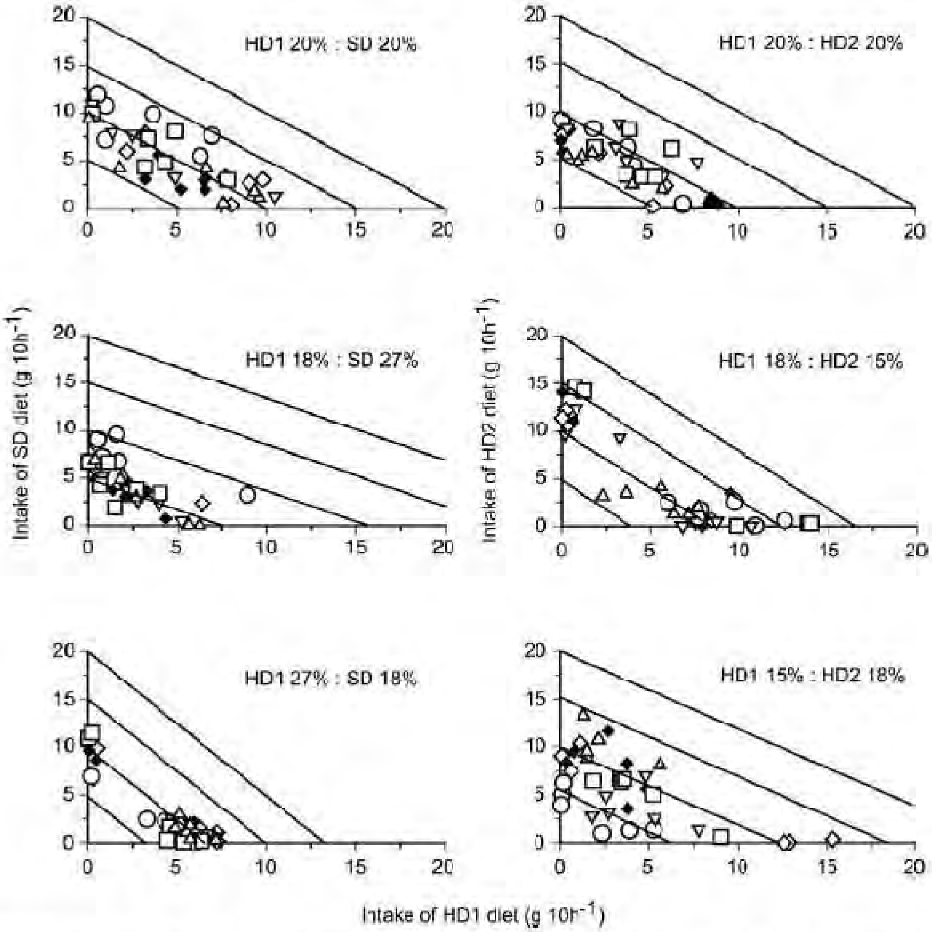

FIG. 3.—Energetic equivalence of test diets for *Glossophaga soricina*. The panels on the left represent experiment 1, whereas those on the right represent experiment 2. See Table 4 for observed and expected slopes.

In conclusion, our study rejects the hypothesis that nectar-feeding neotropical bats act as a selective pressure on nectar composition in chiropterophilous plants. Other possible explanation for the predominance of hexose in chiropterophilous flowers need to be evaluated. The result that bats prefer more concentrated nectar suggests that plants can use variation in this trait to attract bat pollinators, and possibly influence pollinator movements in a way that benefits the plant. Future studies should document the variation in nightly nectar production and relate this to bat pollinator visits to further evaluate this possibility. Finally, our results suggest that other factors than sugar composition influence preference within diets of neotropical bats.

RESUMEN

En el Neotrópico, las plantas polinizadas por murciélagos producen néctares relativamente diluidos y dominados por las hexosas (glucosa y fructosa), con pequeñas cantidades de sacarosa. Investigamos las preferencias en composición y concentración de azúcares en el néctar de dos murciélagos neotropicales nectarívoros (*Leptonycteris curasoae* y *Glossophaga soricina*; Phyllostomidae) con el fin de probar la hipótesis de que los murciélagos prefieren las características que de manera natural predominan en el néctar que suelen consumir. Ofrecimos individualmente a los murciélagos pares de dietas en grandes encierros artificiales inmersos en condiciones naturales, que permitían el libre forrajeo de los organismos. Preparamos néctares artificiales simulando la composición y concentración de azúcares de los néctares de flores visitadas por ambas especies de murciélagos en la zona de estudio. Contrario a lo esperado, los murciélagos no presentaron preferencias por ninguna combinación de azúcares, cuando éstas fueron ofrecidas a una misma concentración. Sin embargo, *L. curasoae* (nectarívoro especialista) consistentemente mostró preferencia por néctares concentrados sobre diluidos independientemente de la composición de los mismos, este mismo patrón no fue encontrado en el caso de *G. soricina* (omnívoro). Ambas especies de murciélagos percibieron las diferentes combinaciones de azúcares como energéticamente

equivalentes. Nuestros resultados contradicen la hipótesis de que los murciélagos nectarívoros neotropicales actúan como presiones selectivas que han determinado la composición del néctar en plantas quiropterofílicas. Se necesita evaluar otras posibles explicaciones para la predominancia de hexosas en las flores quiropterofílicas.

ACKNOWLEDGMENTS

We acknowledge support by a grant to KES and JES from the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica PAPITT (IN2251033), Universidad Nacional Autónoma de México. Scholarship support to NR-P was provided by the Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico. We thank C. García for assistance in the field; L. Barbo for help in the laboratory; G. Sánchez Montoya, H. Ferreira, and A. Valencia García for technical assistance; and the Estación de Biología Chamela for logistical support.

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Submitted 13 October 2006. Accepted 27 March 2007.

Associate Editor was R. Mark Brigham.

CAPÍTULO 3.

LOS AMINOÁCIDOS DEL NÉCTAR MODIFICAN LA SELECCIÓN DEL ALIMENTO EN MURCIÉLAGOS NECTARÍVOROS

Nectar amino acids modify food selection by nectarivorous bats

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Running headline: amino acid selection by nectar-eating bats.

Abstract

1. Chiropterophylic flowers secrete sugar nectar with small amounts of amino acids which may function to attract animals; nevertheless, their role in food selection by bats is unknown.
2. We investigate: a) the role of nectar as a nitrogean N source, b) the importance of amino acid concentration in relation to sugar concentration, and c) the role of the flavor provided by amino acids to nectar for food selection by nectarivorous bats.
3. Using individual flight cages, we offered bats (*Leptonycteris yerbabueanae* and *Glossophaga soricina*) pairs of experimental diets. We used artificial nectars mimicking the nitrogen concentration and the relative abundance of amino acids found in flowers from the main plant species visited by these bats in a tropical dry forest.
4. Our results showed that: a) bats did not discriminate between nectar diets at contrasting nitrogen concentrations—not when experiments were conducted on individuals previously maintained under conditions of a nitrogen-balanced diet, nor when they were maintained with a nitrogen-free diet; b) *Leptonycteris yerbabuenae* preferred the nitrogen-free concentrated nectar; however, bats did not discriminate between contrasting sugar concentrations (18 vs. 27%) when supplemented with nitrogen; and c) *Leptonycteris yerbabuenae* preferred the taste of amino acids present in the nectar of *Pachycereus pecten* (Cactaceae) over *Ceiba aesculifolia* (Malvaceaceae).
5. Our results suggest that nectar amino acids could affect the foraging decisions of nectar-eating bats, regardless of the low concentrations at which they are present in floral nectar.

Key-words: amino acid preferences, chiropterophylic flowers, nectar flavor, nectar nutrient composition, phyllostomid bats, pollination ecology.

Introduction

Understanding the factors that affect food selection by pollinators has critical ecological implications. Floral nectars are sugar solutions that also contain small amounts of other compounds like amino acids, lipids, proteins and alkaloids (Baker, Baker & Hodges 1998). Amino acids are the most abundant compounds in nectar after sugars. Nectar amino acids could affect pollinators in two ways: 1) acting as a nitrogen (N) source, and 2) providing nectar with specific flavors. Flowers whose pollinators´ mai N source of N is nectar tend to offer high amino acid concentrations (i.e. Lepidoptera), whereas those whose pollinators obtain N from other food items like pollen, fruits or insects (i.e. mammals and birds) have lower amino acid concentrations (Baker & Baker 1973; Baker, Opler & Baker 1978; Baker & Baker 1982; Baker & Baker 1986). However, because nectar amino acids are found at low quantities, sometimes only as traces, their potential role in food selection by this second group of nectarivores has been largely dismissed (Baker et al. 1978; Scogin 1980; Petanidou et al. 2006).

Baker et al. (1978) suggested that amino acid composition and concentration could determine nectar flavor for many pollinators, affecting their food selection patterns. Furthermore, it appears that the relative abundance of each amino acid type in nectar is important for providing a characteristic taste profile (Shiraishi and Kuwabra 1970; Gardener & Gillman 2001, 2002). Petanidou et al. (2006) found that phenylalanine, an essential amino acid, generates a strong phago-stimulatory effect on pollinators. Under experimental conditions there is evidence that butterflies and bees have preferences for solutions containing specific amino acids at certain concentration ranges (Inouye & Waller 1984; Alm et al. 1990). Studies on this topic in nectarivorous vertebrates are limited to birds. Hummingbirds and sunbirds do not respond to the nectar amino acid concentrations found in plants, and reject nectar with one, or a mixture of six amino acids at higher concentrations (Haisworth & Wolf 1976; Leisegneur Verburgt & Nicolson 2007). In the case of nectar-eating bats it is known that they cannot meet their Minimal Nitrogen Requirements (MNR) while feeding only on nectar. As a consequence nectarivorous bats need to supplement their diet using other items as protein as an N sources (i.e. pollen, insects and fruit; Carvalho 1961; Alvarez & Gonzalez 1970; Howell 1974; Heithaus 1975, Fleming & Opler 1975; Lemke 1984; Delorme & Thomas 1996; Herrera et al. 2001).

However, no studies about the role of nectar as an N source and the possible influence on nectar flavor caused by them in the foraging ecology of bats exist.

The goal of our study was to understand the role that nectar amino acids have on the foraging decisions of Neotropical nectarivorous bats (family Phyllostomidae). We focused our research on the following questions: 1) Do bats perceive nectar amino acids as a possible N source under conditions of both, N-balanced and N-free maintenance diets. 2) How important is amino acid concentration in relation to sugar concentration for food selection? and 3) Does the flavor provided by amino acids influence nectar selection by phyllostomid bats? To answer these questions we used artificial nectars simulating the nectar N concentration and the relative abundance of amino acids from the most important plant species visited by nectarivorous bats in the wild at our study site. We hypothesized that because nectarivorous bats supplement their nectar diet using other items as protei as an N source (Carvalho 1961; Alvarez & Gonzalez 1970; Howell 1974; Heithaus 1975, Fleming & Opler 1975; Lemke 1984; Delorme & Thomas 1996; Herrera et al. 2001) they would be indifferent to the N present in nectar when experiments were done after a period under an N-balanced maintenance diet, but would change to prefer nectar with high N concentrations when experiments were done after a period under an N-free maintenance diet. Second, because nectar feeding bats are sensitive to changes in the sugar concentration of their diets (Rodriguez-Peña et al. 2007; Ayala-Berdón et al. 2008) we expected bats to prefer sugar concentration over nitrogen concentration. Finally, for the third question we had two predictions: (1) bats would prefer amino acid-free nectar, because feeding on an amino acid unbalanced diet could affect their protein metabolism (Harper 1967; Leung, Rogers & Harper 1968); and (2) bats, when offered nectars that mimic the amino acid composition and total nectar concentration as found in the most common plant species in their natural diet, would prefer the flavor associated with the nectar that contains more energy.

Materials and methods

STUDY SITE

Bat species and chiropterophylic flowers were collected in the Chamela region in the central Pacific coast of Mexico (approximately (19°22´- 19°35´ N, 104°56´- 105°03´ W). The predominant vegetation type is tropical lowland deciduous forest (Lott 1993). Nectarivorous bats consume nectar and presumably pollinate the flowers of 22 plant species at this site (Stoner et al. 2003). We conducted our experiments using two nectar eating bat species. The Saussure´s long-nosed bat (*Leptonycteris yerbabuenae*; Martínez and Villa-R 1940) and the long-tongued bat (*Glossophaga soricina*; Pallas 1976). Although both bat species consume floral nectar, they also feed on fruit and insects when flowers are unavailable (Alvarez, Willig and Webster 1999; Herrera 1999). *Leptonycteris yerbabuenae*, (before curasoae) is considered a derived nectarivorous species (Koopman 1981); its diet includes pollen, nectar, fruit, and a small amount of insects (Gardner 1977). Pollen constitutes an important part of the diet of this species throughout the year (Alvarez and Gonzalez 1970, Fleming 1995, Valiente-Banuet et al. 1996). However, *Glossophaga* is more insectivorous and frugivorous than *Leptonycteris* (Nassar et al. 2003 Mirón et al. 2006).

NECTAR BACKGROUND

A total of 40 flowers distributed in 8 species and 5 families regularly consumed by nectarivorous bats in this region (Stoner et al*.* 2003) were investigated. Nectar collections were made opportunistically depending on the flowering period of the plant species. Mature flower buds were covered with mesh bags 1 hour before sunset. Nectar was extracted with capillary glass tubes one hour after anthesis. To prevent possible contamination with pollen amino acids, we emasculated the flowers. Nectar samples were frozen before analysis.

Total concentration of amino acids was determined by the ninhidrine method (Moore & Stein 1954; Hirs 1967), and total concentration of soluble proteins was determined by the Bradford method (Bradford 1976). Total N concentration was obtained considering N contained in both amino acid and protein content. The results of this section were used to mimic N nectar concentrations as found in plants in Experiments 1 and 2 (Described below).

To determine the relative abundance of amino acid in nectar, 17 specific free nectar amino acids were measured by high performance liquid chromatography (HPLC) in an Agilent 1100 Series equipment (Hewlett Packard), following the Agilent 1090 series method. We used a two step precolumn derivatization, with ortho-phthalaldehyde (OPA) for primary amino acids and 9-fluorenylmethyl chloroformate (FMOC) for the secondary amino acid. A 0.4 N borate buffer was used with pH 10.4. Separation was performed by using a Hypersil AA-ODS 2.1 x 200 mm Agilent column. We used a solvent gradient system with two mobile phases: (A) sodium acetate, trietilamine and tetrahydrofuran water mix, and (B) sodium acetate, acetonitrile and methanol water mix buffers (pH 7.20). The gradient was started with 100% A, at 17 minutes 60% B, at 18.1 minutes flow 0.45, at 18.5 minutes flow 0.8, at 23.9 minutes flow 0.8, at 24 minutes 100% B and flow 0.45, at 25 minutes 0% B. Detection was via a Perkin Elmer (LS50B) Luminescence Spectrometer (excitation at 340 nm and emission at 450 nm for primary amino acids and excitation at 266 nm and emission at 305 nm for the secondary amino acid). Data collection was done with FL Win Lab Perkin Elmer software.

Chromatograms were compared with standards for identification of individual amino acids. Total concentration and percentage of each amino acid was calculated for each sample. The results of this section were used to mimic the relative abundance of amino acids as found in plants in Experiment 3 (Described below).

BAT CARE AND HOUSING

Bats were captured and handled under permission from the Oficina de Fauna Silvestre, Mexico, to KES (permit SGPA/DGVS/03644/08), and met humane handling guidelines approved by the American Society of Mammalogists (Gannon, Sikes & The animal care and use Committee of the American Society of Mammalogists 2007). We used mist nets to capture adult non-reproductive males of the two bat species. After capture, bats were transferred to a laboratory with a controlled environment of 27 °C and 50% relative humidity. Bats were maintained in groups of seven individuals in aluminium cages (60 x 60 x 60 cm) and were fed on the diet described by Mirón et al. (2006). Body mass, wing membrane elasticity and hair condition of all bats was monitored daily. All bats maintained constant body mass and were healthy during captivity. Bats lost weight under some experimental conditions; however, they regained body mass after the experiments were over.

PREFERENCE TESTS

We offered pairs of test diets to individual bats in small flight cages (50 x 50 x 50 cm) located under laboratory conditions. Feeders were placed 50 cm apart and at a height of 30 cm. Trials lasted 10 h and were conducted from 1900 to 0500 h. Feeders were filled and placed at 1900 h. The amount of food consumed per feeder was measured (g) and their position switched at the middle of the experiment at 0000 h to control for potential positional biases (following Jackson, Nicolson & Lotz 1998). The amount of food consumed was again measured at 0500 h. An additional feeder of each test diet was placed outside the flight cages to control for evaporation. To determine the sample size we computed *a priori* power analysis (G power® software). We obtained values over 0.8 of statistical power with a sample size of 4 to 9 bats per trial. Since one of the bat species, *Leptonycteris yerbabuenae* is included in the NOM-059SEMARNAT 2001 as a protected species we used 7 different experimental bats for each trial in each experiment.

EXPERIMENT 1— DO BATS PERCEIVE NECTAR AS AN N SOURCE?

To test if bats perceive nectar as an N source, we offered three experimental diets that differed in N concentration. Two of them mimic those present in the flowers bats visit in the Chamela region (*Crescentia alata,* Bignoniaceae, with the lowest N concentration of 320 µg/ml—hereafter LN; and *Pachycereus pecten*, Cactaceae, with the highest N concentration of 1505 µg/ml—hereafter HN; Table 1). Experimental diets were based on sugar solutions at 20% (weight / volume of sucrose). The first diet was supplemented with the LN (320 µg/ml; LN). The second diet contained the HN (1505 µg/ml; HN). The third diet had only sugars (20% v/w) and contained no nitrogen (0 µg/ml; NN). We used a soy protein based product (PRONAT ProWinner®; www.prowinner.com.mx) as an N source. This product contains a balanced mix of amino acids that will not cause a nutritional imbalance in the bats (Harper 1967; Leung, Rogers & Harper 1968). Each bat faced 2 solutions per trial. Six trials were conducted with these test diets. In the first trial, we offered bats the N-free diet (NN) vs. the low N diet (LN). In the second trial we offered them the NN and the high N diet (HN). Thus, trials 1 and 2 examined if bats showed a preference for the lowest (Trial 1) or the highest amino acid concentration found (Trial 2) in their natural diets. In the third trial we compared the LN with the HN diet. This trial examined N concentration preference by bats. These three trials were were done after bats were maintained under an N-balanced maintenance diet N-balanced(Mirón et al. 2006). Trials 4, 5 and 6 were the same as 1, 2 and 3 but this time experiments were done after bats were maintained for 4 days under an N-free maintenance diet (only-sugar diet). Each trial was conducted using 7 different individuals of each of the two bat species.

EXPERIMENT 2—IS SUGAR OR NITROGEN CONCENTRATION MORE IMPORTANT FOR FOOD SELECTION?

A second series of trials were performed to test if bats preferred total sugar or N concentration. This time experimental diets differed in their total sugar concentration (w/v). In trial 1, we tested diets that simulated the natural sugar and N concentrations found in flowers in the field (Rodríguez-Peña et al. 2007). The high N diet with 1505 µg/ml simulated *P. pecten* and contained 27% (w/v) sugar, whereas the low N diet with 320 µg/ml simulated *C. alata*, and contained 18% (w/v) sugar. In trial 2, we inverted the sugar concentrations used in trial 1. Thus, the high N diet contained 18% sugar, whereas the low N diet contained 27% (w/v) sugar. Finally, in trial three we offered bats the sugar concentrations as found in the field, (*P. pecten* contained 27% and *C. alata* 18% w/v sugar) but this time test solutions were N-free. In trial 3 we examined if bats showed a preference for the total nectar concentration as found in their natural diets without the N presence influence. Each trial was conducted using 7 different individuals of each of the two bat species.

EXPERIMENT 3—DOES THE FLAVOR PROVIDED BY AMINO ACIDS INFLUENCE NECTAR SELECTION?

A third set of trials were performed to test if bats showed preferences for the flavor that the presence of amino acids provide to nectar as found in the field. This time we offered bats experimental diets that differed in the relative abundance of 17 amino acids mimicking those of two of the most important species they visit in the field. One of the test diets simulated the natural composition of nectar amino acids of *C. aesculifolia* (hereafter Ca; Appendix 1), while the second diet simulated those present in the nectar of *P. pecten* (hereafter Pp; Appendix 1). Both experimental diets consisted of sugar solutions supplemented with a total 7mM concentration of amino acids. We chose this total amino acid concentration because it was the highest concentration we found in the field. While the total concentration (7 mM) and types of amino acids did not vary between diets, the concentration of the different individual amino acids in solutions varied following those of the selected plant species (Appendix 1). To prepare the experimental diets we used 100% pure individual amino acids (SIGMA®). The third experimental diet was amino acid-free (NA). Each bat faced 2 solutions per trial. In the first trial we offered bats the amino acid-free sugar solution vs. *C. aesculifolia*. In the second trial we offered the amino acid-free sugar solution vs. *P. pecten*. Thus, in trials 1 and 2 we examined if bats could detect the amino acids present in their natural diets, and if they preferred nectars with or without amino acids. In the third trial we offered the two amino acid-supplemented diets. Thus, trial 3 examined the preference of bats for nectar that had the same amino acids in different proportions. In trials 1, 2 and 3 both experimental diets consisted of sugar solutions (20%, weight / volume of sucrose). In the fourth trial we offered the two diets that differed in amino acid composition (Ca vs. Pp), but this time at the natural sugar concentration present in each of these plant species, thus Ca (18% weight / volume of sucrose) and Pp (27% weight / volume of sucrose). Finally, in the fifth trial, we offered bats the two diets that differed in amino acid composition (Ca vs. Pp), but this time we inverted the sugar concentrations, thus Ca (27% weight / volume of sucrose) and Pp (18% weight / volume of sucrose). In trials 4 and 5 we examined the effect of sugar concentration on bat preferences for amino acid composition. Each trial was conducted using 7 different individuals of each of the two species.

DATA ANALYSIS

To estimate preference we used the ratio of the nightly ingestion of one of the test diets divided by total nightly ingestion:

Preference = Ingestion of diet A / Total ingestion.

A preference value of 0.5 indicates that bats consumed both solutions in equal amounts. One sample t-tests on arcsin √ transformed preference values were used to test the null hypothesis that preference was not significantly different from 0.5 (following Martínez del Río 1990). Because several simultaneous tests were conducted to test single hypotheses, the significance level was adjusted using the standard Bonferroni method (Rice 1989). We adjusted α separately for the number of trials involved in each hypothesis (experiment 1 = 6; experiment 2 = 3; experiment 3 = 5).

Results

NECTAR BACKGROUND

We calculated total amino acid and protein concentration for each of the eight plant species (n= 5; Table 1). Average amino acid concentration was 2.89 ± 2.04 (S. D.) mM and ranged from 1.04 ± 0.53 mM in *Bahuinia pauletia* (Bignoniaceae) to 6.83 ± 7.6 mM in *Pachycereus pecten* (Cactaceae; Table 1). Similarly, average protein concentration was 161.93 ± 120.83 μg/ml and ranged from 58.93 ± 101.03 μg/ml in *Bahuinia pauletia* (Bignoniaceae) to 439.18 ± 402.23 μg/ml in *Pachycereus pecten* (Cactaceae; Table 1).

We detected a total of 17 individual amino acids. All 17 amino acids were present in all nectars, and their relative abundance (%) in each of the eight plant species is summarized in Appendix 1.

DO BATS PERCEIVE NECTAR AS AN N SOURCE?

Both bat species showed no preferences in any of the trials either when trials were done under an N-balanced maintenance diet or when bats were under an N-free maintenance diet (Figure 1, Table 2).

IS SUGAR OR NITROGEN CONCENTRATION MORE IMPORTANT FOR DIET SELECTION?

Both bat species exhibited no preferences for diets at contrasting sugars and N concentrations (Figure 2, Table 2). However *L. yerbabuenae* showeda preference for the N-free most concentrated diet (Figure 2, Table 2).

DOES THE FLAVOR PROVIDED BY AMINO ACIDS INFLUENCE NECTAR SELECTION?

Leptonycteris yerbabuenae always preferred the sugar only diet (NA) over the two amino acid-supplemented options (Figure 3, trials 1 and 2, Table 2). This bat species preferred solutions containing *P. pecten* amino acid relative abundance over those present in *C. aesculifolia* when tested at field sugar concentrations (Figure 3, trial 4). However, when tested at equal (trial 3) and inverted (trial 5) sugar concentrations, the bats were indifferent. *Glossophaga soricina* showed no preferences in any of the trials (Figure 3, Table 2).

Discussion

The role of the amino acids in nectar, and the perception of their flavor by nectar-eating bats have been ignored in the past as a result of the low amino acid concentrations found in the nectar of bat pollinated flowers (Baker & Baker 1982; von Helversen 1993; Winter & von Helversen 2001). Our results showed that amino acids present in nectar influenced bats' food selection; however, our results did not support all of our predictions. First, as predicted, bats were indifferent to the N present in nectar when experiments were done after bats were under an N-balanced maintenance diet. However, bats were also indifferent to the N present in nectar when experiments were done after bats were maintained with an N-free maintenance diet. We had predicted that bats would change to prefer nectar with high N concentrations when experiments were done after an N-free maintenance diet. Second, as predicted *L. yerbabuenae* preferred the most concentrated N-free sugar diet. Although we predicted that bats would prefer sugar over N concentrations, both bat species showed no preferences for the sugar concentration when diets where N supplemented. Third, as predicted, *L. yerbabuenae* preferred the amino acid-free diet (NA) over the diet containing amino acids. However, *G. soricina* was indifferent in both trials. Finally, we predicted that both bat species would prefer the flavor of the amino acids found in the nectar with the highest sugar concentrations they use in the field (*P. pecten*). We found that *L. yerbabuenae* showed preference for the flavor of *P. pecten* in 1 of 3 trials, whereas *G. soricina* showed no preferences. In this discussion we first focus on the role of amino acids in nectar as an N source. We then consider the effect that nectar amino acids could have on the ability of bats to discriminate among sugar concentrations. Lastly, we discuss the relationship between the flavor generated by the presence of amino acids in nectar, and food selection patterns of our studied bat species.

ROLE OF AMINO ACIDS IN NECTAR AS AN N SOURCE FOR NECTARIVOROUS BATS

Our results showed that under N balanced and N depleted conditions, both bat species were indifferent to the presence of low or high nitrogen concentrations in nectar (Fig. 1). We believe that the general indifference that both bat species exhibited for N-containing nectars is the result of: 1) it is unnecessary for bats to select N supplemented nectars when maintained with an N supplemented diet, and 2) bats perceive both experimental diets as containing N, albeit low, under the N depleted conditions and therefore they do not discriminate between them. To test this hypothesis, we calculated how much N the bats ingested under experimental conditions and compare this data with their minimal N requirements (MNR) from the literature. While there is information on the MNR for *L. yerbabuenae* (13–17 mg nitrogen day^{-1}; Howell 1974; Voigt & Matt 2004), the only report for *G. soricina* (Herrera, Ramírez & Mirón 2006) used a different methodology and the data reported for this species is not comparable, thus we present the analysis only for *L. yerbabuenae*. Considering that this species ingested 23.9 and 16.5 ml day^{-1} from the nectar of *C. alata* and *P. pecten,* respectively (Ayala-Berdón et al. 2008), it can only obtain between 9.5 and 30.3 % of its MNR when feeding only on *C. alata* or *P. pecten* nectar (1.2 and 4 mg N day^{-1}). While we cannot calculate these values for *G. soricina*, we expect a similar result for this species.

These calculations suggest that nectar-eating bats cannot meet their MNR while feeding only on nectar. As a consequence nectarivorous bats need to supplement their diet using other items as protein as an N source (i.e. pollen, insects and fruit; Carvalho 1961; Alvarez & Gonzalez 1969; Howell 1974; Heithaus, Fleming & Opler 1975; Lemke 1984; Delorme & Thomas 1996; Herrera et al. 2001). Both bat species supplement their nectar diet with other sources. *Glossophaga soricina* includes fruits, pollen and insects in its diet (Fleming 1982), and uses primarily insects to satisfy its nitrogen requirements (~ 70%, Herrera et al. 2001). *Leptonycteris yerbabuenae* meets its N requirements from ingesting pollen (Howell 1974).

NECTAR AMINO ACIDS EFFECT ON THE ABILITY OF BATS TO DISCRIMINATE AMONG SUGAR CONCENTRATIONS

Our results showed that *L. yerbabuenae* preferred the most concentrated N-free sugar diet. The effect of nectar sugar concentration on bats´ sugar preferences has been studied (Roces, Winter & Helversen 1993; Rodríguez-Peña et al. 2007). Roces et al. (1993) found that *G. soricina* always preferred the higher of two nectar concentrations, when the differences in sugar concentration between the two solutions were > 10%. Rodríguez-Peña and collaborators (2007) found that *L. yerbabuenae* and *G. soricina* preferred the more concentrated sugar solution even when the difference in concentration between the two solutions was small (3% for *L. yerbabuenae* and 9% for *G. soricina*). However, bats had no preferences for diets with different sugar concentrations when amino acids were added to the artificial nectar solutions. While the two bats species have the ability to detect small differences in sugar concentration, they were indifferent while facing two sugar solutions differing in 9% when amino acids were added. This was a surprising result, and suggests that the presence of amino acids in nectar causes a confounding effect that modifies the bats' capacity to discriminate among sugar concentrations. If the flavor generated by nectar amino acids reduces the ability of bats to discriminate for sugar concentrations, as our results indicate, the presence of these substances in floral nectar could be the result of a plant strategy to confound floral visitors. Plants could produce nectars with lower sugar concentrations, and by adding small amounts of amino acids, they could trick their visitors to perceive different sugar solutions as energetically similar, saving sugars and maintaining visit rates. However, this hypothesis remains to be further investigated.

RELATIONSHIP BETWEEN THE FLAVOR GENERATED BY THE PRESENCE OF AMINO ACIDS IN NECTAR AND FOOD SELECTION PATTERNS OF BATS

Leptonycteris yerbabuenae responded to the flavor of nectar amino acids by changing their food selection patterns. While *Glossophaga soricina* was indifferent to the different sugar concentration and amino acid relative abundance used in our experiments, *Leptonycteris yerbabuenae* had preferences for sugar only diets (NA) when offered an amino acid supplemented counterpart, and in 1 out of 3 trials preferred the flavor of the amino acids of *P. pecten* (Cactaceae) over those found in *C. aesculifolia* (Malvaceae). Amino acid rejection also has been reported for bees (Inouye & Waller 1984), hummingbirds (Haisworth & Wolf 1976) and sunbirds (Leseigneur et al. 2007). Usually this rejection is associated with the presence of amino acid concentrations higher than those found naturally in the nectar ingested by these animals in the field. We speculate *L. yerbabuenae* rejection of amino acid supplemented solutions is due to: 1) a nutritional imbalance caused by the concentration/composition of amino acids we used in our trials, and 2) the flavor that amino acid relative abundance provides to the experimental solutions.

The preference of amino acid-free diet over amino acid-supplemented nectar by *L. yerbabuenae* could be due to an amino acid imbalance. An amino acid imbalance is defined as adverse effects caused by the ingestion of a surplus of an individual, or group of amino acids (Krehl et al. 1945; Harper et al. 1970). Adverse effects of amino acid imbalance have been well documented and go from moderate depression of food intake and reduced growth, to the development of pathological lesions and low survival rates (Harper 1967; Harper, Benevenga & Wohlheuter 1970; Harper 1974). Rodents and other mammals have been found to select protein-free diets, or a diet with a balanced amino acid pattern, over those presenting amino acid imbalance (Harper 1967; Leung et al. 1968). *Leptonycteris yerbabuenae* showed no preferences between sugar only and N supplemented diets when these diets where balanced in their amino acid composition (Experiments 1 and 2); however, they preferred the sugar only solutions when they were offered a specific amino acid composition as their other option (Experiment 3). Since we were offering the bats the specific amino acid composition found in the plants they eat in the field this “rejection” is surprising. Nevertheless, they are unable to find amino acid free nectars in the field, and therefore must feed on them to survive.

The fact that *L. yerbabuenae* in 1 out of 3 trials (Fig 3, experiment 3, trials 3 and 4) preferred the flavor of the amino acids present in the nectar of *P. pecten* over *C. aesculifolia* is evidence that this species perceives and recognizes the flavor that amino acid relative abundance provides to the nectar of each plant species. Gardener & Gillman (2001) suggested that amino acid composition has more influence than amino acid concentration on the taste profiles of nectar. Our results suggest that the relative abundance of the different amino acids in nectar contributes to provide a specific taste to nectar that some bats can use as information to select their food accordingly (Baker & Baker 1973, Baker 1977, Baker et al. 1998, Gardener & Gillman 2002).

The amino acid related flavor preferred by *L. yerbabuenae* is associated with the nectar with the natural highest sugar concentration (27%; *P. pecten*). We speculate that *L. yerbabuenae* is associating the flavor caused by the particular amino acid relative abundance in the nectar of this cactus over other nectar attributes like sugar concentration. *Leptonycteris yerbabuenae* preferred the nectar imitation of *P. pecten* over that of *C. aesculifolia*. This is not surprising if we consider that *P. pecten* has more sugar in its nectar. When we changed sugar concentration to 20% in both solutions, and when we inverted the sugar concentrations, so that the solution mimicking the nectar of *C. aesculifolia* had a higher sugar concentration, bats were indifferent. These results suggest that relating nectar quality with amino acid flavor could allow bats to use the presence of certain amino acids to influence foraging decisions under natural conditions in the field.

Leptonycteris yerbabuenae and *G. soricina* differed in their ability to discriminate between solutions with contrasting nectar amino acid relative abundances. *Leptonycteris yerbabuenae* needs more concentrated nectars to maintain a positive energy balance (Ayala et al. 2008). Additionally, *L. yerbabuenae* has a better capacity to discriminate between solutions with different sugar concentrations (Rodriguez-Peña et al. 2007). Although both bat species consume nectar, fruits and insects, our results suggest that among bats, ecologically more specialized nectarivores will be better at using amino acid flavor clues to select the best nectars available to them in the field; however, this hypothesis remains to be tested.

In conclusion, our study shows that nectar amino acids could affect the foraging decisions of nectar-eating bats, regardless of the low concentrations at which they are present in floral nectar. The fact that amino acid concentration in nectar confounds bats´ capacity to distinguish among sugar concentrations, and that some bats have preferences for the taste that some amino acids provide to nectar of specific plant species, suggests that plants can use variation in this trait to attract bat pollinators. Future studies should document the nutritional contribution of specific amino acids in nectar for bats, and the interaction between nectar amino acid and sugars types on flavor and on foraging decisions by bats.

Aknowledgements

The author's acknowledge support by a grant from the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica PAPITT (IN226007), Universidad Nacional Autónoma de México to KES. Scholarship support to Nelly Rodríguez-Peña was provided by Consejo Nacional de Ciencia y Tecnología CONACYT. We thank J. M. Lobato for assistance in the field, L. Barbo for help in the laboratory, and the Estación de Biología Chamela for logistical support.

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Tables

Table 1. Total nectar (w/w), amino acid (mM) protein concentration (µg/ml), and N content (µg/ml) in nectar of 8 chiropterphylic species from the Tropical Dry Forest, from the Chamela region in Jalisco, Mexico.

Table 2. Results from *t*-tests of preference values for foraging experiments on nectarivorous bats. Preference ratios > 0.5 significance levels (α) were adjusted for number of trials involved in each hypothesis using the Bonferroni technique (Rice 1989). Significant values are highlighted in bold.

Tables

Table 1.

FAMILY AND SPECIES	Total nectar concentration (w/w) (range) (N flowers / N individuals)	Total amino acid concentration (mean ± SD) mM	Total protein concentration (mean ± SD) µg/ml	Nitrogen content (µg/ml)
Bignoniaceae				
Crescentia alata	19.48±0.39 (12-27) (82/5)	1.12±0.41	148.47±96.47	322.8
Malvaceae				
Ceiba aesculifolia	16.85±0.10 (14-18.8) (83/5)	3.20±0.34	127.43±14.70	627.49
Ceiba grandiflora	18.83± 0.74 (5-25) (35/12)	4.13±0.27	82.93±5.62	726.46
Ceiba pentandra	16.66±0.18 (3-25) (229/11)	1.87±0.47	152.4±14.06	444.6

Pseudobombax ellipticum	15.44±0.35 (8-19) (38/7)	1.05±0.39	199.23±142.10	363.8
Cactaceae				
Pachycereus pecten	26.17±1.11 (18-31) (12/6)	6.83±7.6	439.18±402.23	1504.36
Convolvulaceae				
Ipomoea ampullacea	24.42±1.02 (11-30.8) (18/4)	3.90±1.12	86.9±58.39	695.67
Fabaceae				
Bahuinia pauletia	18.12±0.15 (14.8-20.5) (92/6)	1.04±0.53	58.93±101.03	221.83

For amino acids and protein concentration, and N content for all species N=3, except *P. pecten* (N=2).

Table 2.

Trial	Pairwise comparison	*Leptonycteris yerbabuenae* PI ± SE (t ; P)	*Glossophaga soricina* PI ± SE (t ; P)
EXPERIMENT 1: DO BATS PREFER NECTAR AS AN N SOURCE?			
α = 0.008			
UNDER N-SUPPLEMENTED MAINTENANCE CONDITIONS			
1	LN : NN	0.52 ± 0.07 (0.23 ; 0.82)	0.45 ± 0.05 (0.88 ; 0.39)
2	HN : NN	0.49 ± 0.09 (0.10 ; 0.92)	0.40 ± 0.06 (1.03 ; 0.32)
3	LN : HN	0.50 ± 0.05 (0.03 ; 0.98)	0.47 ± 0.03 (1.87 ; 0.08)
UNDER N-FREE MAINTENANCE CONDITIONS			
4	LN : NN	0.54 ± 0.08 (0.53 ; 0.60)	0.47 ± 0.04 (0.67 ; 0.51)
5	HN : NN	0.54 ± 0.04 (0.95 ; 0.36)	0.44 ± 5.28 (1.28 ; 0.23)
6	LN : HN	0.44 ± 0.07 (0.79 ; 0.44)	0.42 ± 0.03 (2.48 ; 0.03)
EXPERIMENT 2: IS SUGAR OR NITROGEN CONCENTRATION MORE IMPORTANT FOR DIET SELECTION?			
α = 0.016			
N-enriched trials			

1	LN : HN (18 : 27%)	0.46 ± 0.07 (0.59 ; 0.56)	0.44 ± 0.03 (2.02 ; 0.06)
2	LN : HN (27:18 %)	0.49 ± 0.08 (0.11 ; 0.90)	0.53 ± 0.02 (1.31 ; 0.22)
		N-free trial	
3	LN : HN (18 : 27%)	0.28 ± 0.06 (3.81 ; **0.002**)	0.44 ± 0.03 (1.49 ; 0.16)

EXPERIMENT 3: DOES THE TASTE PROVIDED BY AMINO ACIDS INFLUENCE NECTAR SELECTION?

α = 0.01

1	Ca : NA (20%)	0.33 ± 0.04 (4.92 ; **0.0004**)	0.50 ± 0.06 (0.02 ; 0.98)
2	Pp : NA (20%)	0.38 ± 0.04 (2.98 ; **0.01**)	0.43 ± 0.06 (1.07 ; 0.31)
3	Ca : Pp (20%)	0.41 ± 0.04 (2.05 ; 0.05)	0.47 ± 0.05 (0.64 ; 0.53)
4	Ca : Pp (18:27%)	0.31 ± 0.06 (3.01 ; **0.01**)	0.49 ± 0.06 (0.16 ; 0.87)
5	Ca : Pp	0.62 ± 0.07 (1.74 ; 0.11)	0.57 ± 0.05 (1.57 ; 0.14)

(27:18%)

Figure legends

FIG. 1 Preferences for nectar-feeding bats between low and high N concentrations and sugar only solutions after a diet under N-balanced (trials 1, 2 and 3) and N-free conditions (trials 4, 5 and 6). Values above 0.5 indicate preference for the diet on the left hand side of each trial, whereas values below 0.5 indicate preferences for the diet on the right hand side of each trial. Values of 0.5 indicate no preferences. Both bat species were indifferent to N presence and concentration. This indifference suggests that bats donot consider N in nectar as an important an N source. LN: low nitrogen diet (320 µg/ml); HN: high nitrogen diet (1505 µg/ml); NN: no nitrogen diet.

FIG. 2 Preferences of nectar-feeding bats for sugar or N concentrations. Value interpretations same as Fig. 1. Both bat species were indifferent in the N-supplemented trials, but *L. yerbabuenae* preferred the N-free most concentrated sugar diet, suggesting that the presence of N reduces their ability to discriminate among sugar concentrations. Trial 1. N-supplemented diets conducted at natural sugar concentrations (LN 18% : HN 27%); Trial 2. N-supplemented diets conducted at inverted sugar concentrations (LN 27% : HN 18%); and Trial 3 N-free diets conducted at natural sugar concentrations.

FIG. 3 Preferences of nectar-eating bats for the flavor provided by specific amino acid combinations of nectar. Value interpretations same as Fig. 1. The preference for *P. pecten* nectar by *L. yerbabuenae* suggests that these bats can detect and recognize the specific amino acid relative flavor of each plant species. This pattern was not found in *G. soricina*. Trials 1, 2 and 3 amino acid-supplemented diets conducted at inverted equal sugar concentrations (20 %); Trial 4 amino acid-supplemented diets conducted at natural sugar concentrations (Ca 18% : Pp 27%); Trial 5 amino acid-supplemented diets conducted at inverted sugar concentrations (Ca 27% : Pp 18%);Ca: *Ceiba aesculifolia* diet; Pp: *Pachycereus pecten* diet; NA: amino acid-free diet.

Figures

Figure 1

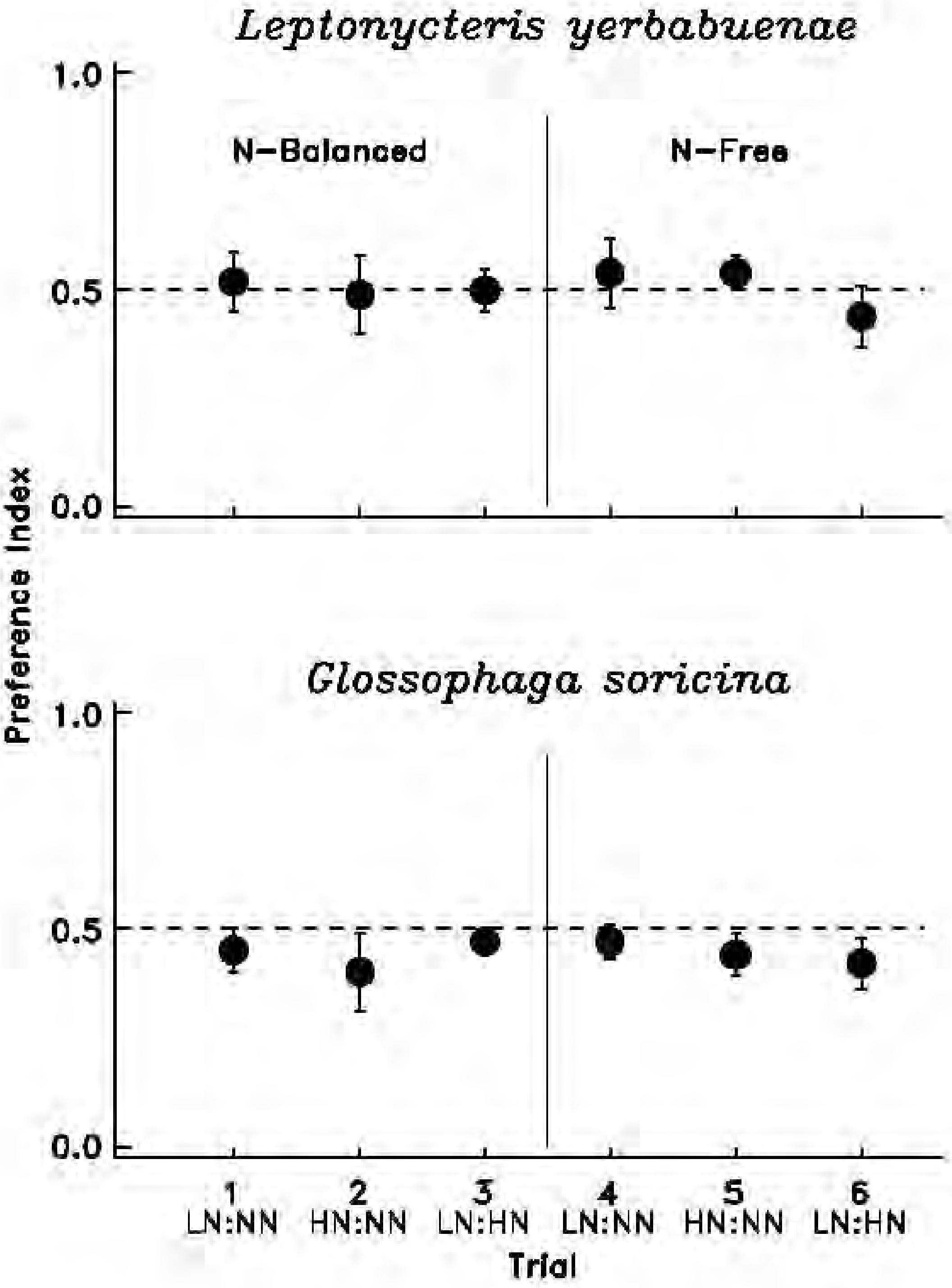


Figure 2

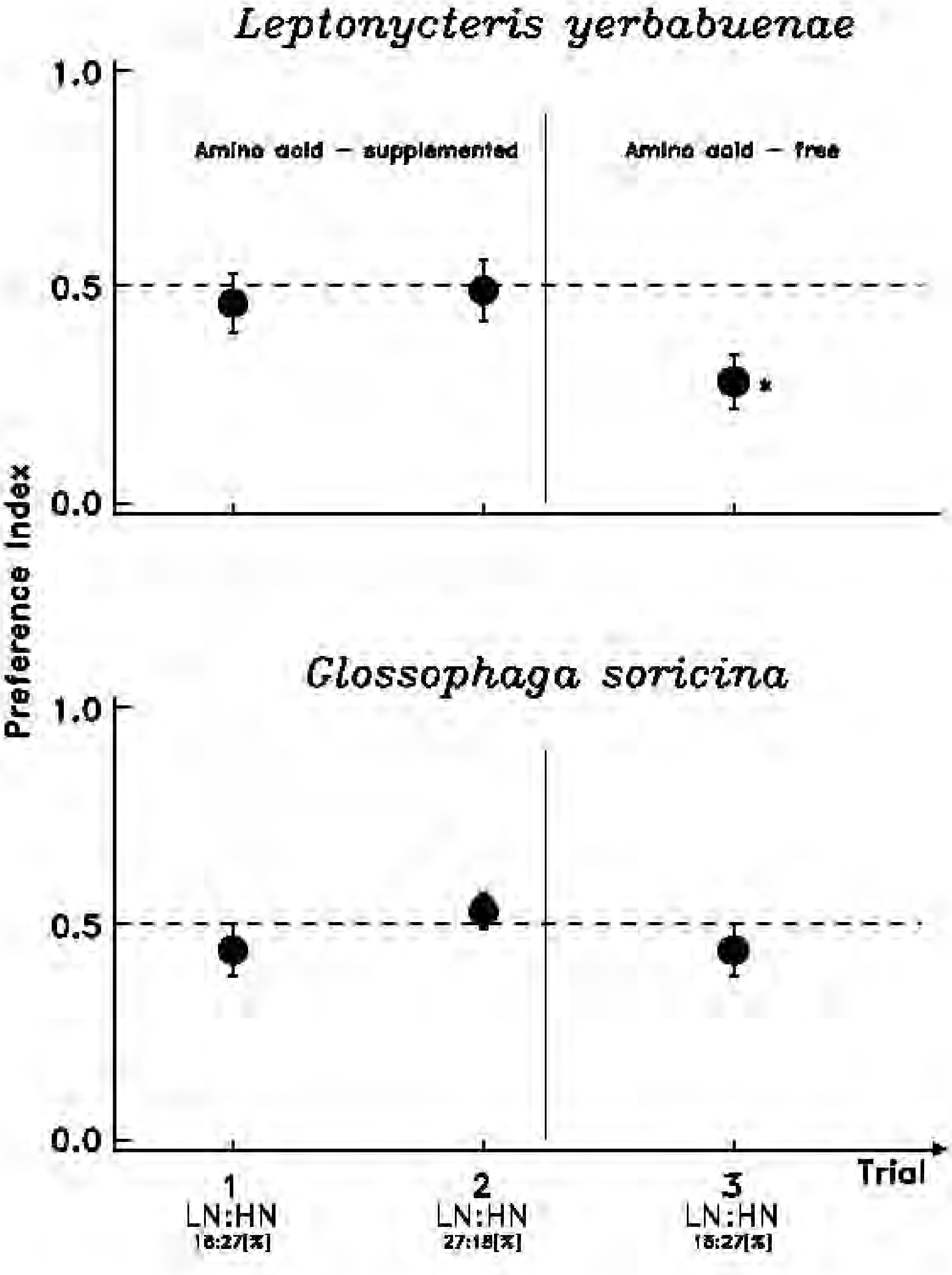
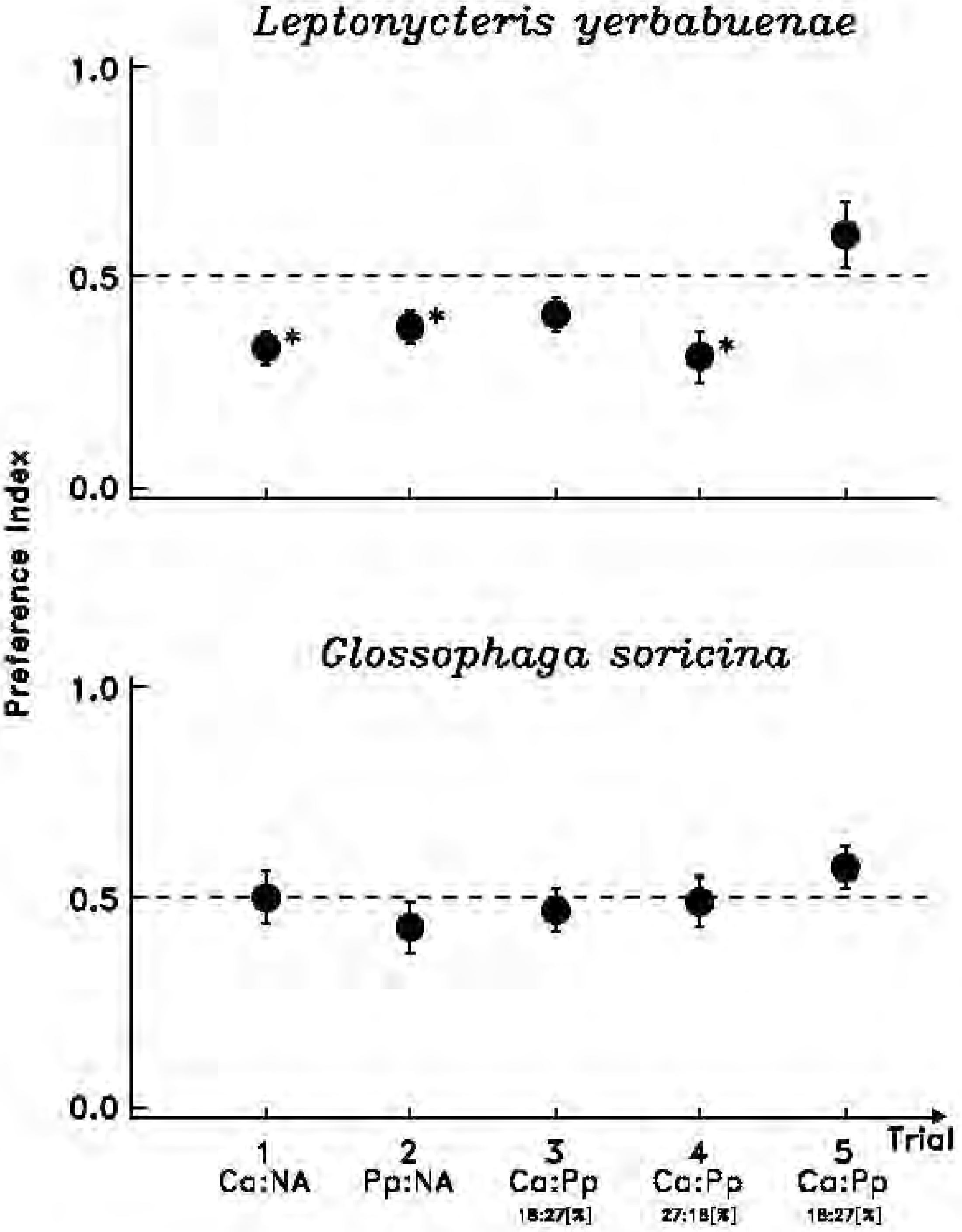

Leptonycteris yerbabuenae
Amino acid – supplemented
Amino acid – free
*
Glossophaga soricina
Preference Index
1.0
0.5
0.0
1.0
0.5
0.0
Trial
1
LN:HN
18:27[%]
2
LN:HN
27:18[%]
3
LN:HN
18:27[%]

FIGURE 3


Leptonycteris yerbabuenae
Glossophaga soricina
Preference Index
1.0
0.5
0.0
*
*
*
1.0
0.5
0.0
Trial
1
Ca:NA
2
Pp:NA
3
Ca:Pp
18:27[%]
4
Ca:Pp
27:18[%]
5
Ca:Pp
18:27[%]

Appendix 1. Relative abundance (%) of 17 amino acids in the nectar of 8 species of bat-pollinated plants in the Tropical Dry Forest in the region of Chamela, Jalisco.

Amino acid	**Bignoniaceae**	**Malvaceae**				**Cactaceae**	**Convolvulaceae**	**Fabaceae**
	Crescentia alata	*Ceiba aesculifolia*	*Ceiba grandiflora*	*Ceiba pentandra*	*Pseudobombax ellipticum*	*Pachycereus pecten*	*Ipomoea ampullacea*	*B. pauletia*
arg	5.96±3.10	4.08±6.69	2.22±0.33	1.69±1.20	3.51±4.51	1.65±1.65	4.81±5.16	4.41±3.84
his	14.48±6.65	6.59±5.51	4.54±2.98	2.41±1.22	5.38±5.96	10.89±7.61	5.42±7.48	3.10±5.25
ile	6.74±3.93	7.28±5.12	4.86±0.62	12.14±6.98	5.37±5.94	3.39±2.76	5.49±1.82	5.82±3.52
leu	7.50±10.88	11.85±8.43	1.60±1.62	8.76±4.65	11.36±15.57	7.78±1.45	26.73±29.98	14.94±20.78
lys	9.28±4.60	19.37±19.51	10.84±1.96	19.88±4.68	42.76±25.29	7.64±2.41	20.40±9.72	15.87±5.09
met	0.53±0.26	0.84±0.86	0.86±0.03	2.39±2.70	0.48±0.41	2.32±1.65	0.37±0.20	2.06±1.78
phe	4.76±7.07	0.45±0.33	0.06±0.05	0.89±1.01	0.74±0.85	1.07±1.52	0.64±0.61	0.92±0.72
thr	3.02±0.58	4.31±4.53	1.22±0.67	3.35±5.14	3.52±3.05	3.99±4.98	3.57±1.95	2.91±1.98
val	0.57±0.14	3.34±4.41	9.68±1.88	1.25±0.63	0.74±0.39	2.88±2.39	2.47±4.01	1.23±1.32
asp	7.89±2.69	2.73±1.45	19.30±3.12	5.64±1.55	5.10±3.42	6.75±6.07	1.78±1.38	2.11±2.77
glu	11.26±6.19	3.49±2.01	16.70±2.33	5.73±1.27	3.81±2.44	6.08±5.27	1.17±0.76	3.82±5.14
ser	4.89±10.80	18.15±17.40	9.61±2.21	5.52±4.57	3.51±4.83	8.85±8.04	0.75±0.77	3.29±1.87
gly	5.66±2.62	2.27±0.91	15.43±2.13	4.95±6.16	5.75±4.77	10.78±5.04	9.62±7.56	26.12±21.83
ala	4.85±7.06	1.27±1.61	0.80±0.09	2.54±1.50	3.52±4.53	12.24±20.27	1.57±1.71	2.49±3.22
tyr	1.12±1.15	0.20±0.18	0.08±0.04	1.32±1.69	0.85±0.79	2.91±4.20	2.12±1.45	0.55±0.41

cys	2.31±0.87	0.19±0.11	0.46±0.03	0.34±0.28	0.62±0.27	1.42±1.79	3.63±4.54	2.68±2.44
pro	9.16±6.24	13.57±13.18	1.72±1.14	21.20±18.87	2.96±2.02	9.33±10.65	9.43±14.24	7.65±4.72

CAPÍTULO 4.

FACTORES QUE AFECTAN LA COMPOSICIÓN DE AZÚCARES EN PLANTAS QUIROPTEROFÍLICAS

Factors affecting nectar sugar composition in chiropterophilic plants

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Summary

1. Floral nectar is the most important reward offered to pollinators. Nectar sugar composition may be affected by biochemical, ecological and evolutionary factors; nevertheless, their role in determining chiropterophilic nectar is unknown.

2. We investigated: a) nectar sugar composition, volume and concentration; b) whether sugar composition is related to nectar volume and concentration; and c) how the flowering strategies of plants affect their nectar traits.

3. We determine nectar traits of 12 species of chiroptherophylic plants distributed in 5 families. We assess phylogenetic and non-phylogenetic relationships between nectar sugar composition and the other nectar traits, and we use a Principal Component Analysis (PCA) to determine whether nectar traits are related to the flowering characteristics.

4. We found chiropterophilic plant species to produce sucrose-dominant nectar (1 species), sucrose-rich nectar (2 species), hexose-rich nectar (8 species), and hexose-dominant nectar (1 species). No significant relationships between sugar percentages (sucrose, glucose and fructose) and nectar volume and concentration were found. A general pattern within the chiropterophilic plant community was found in which species with large flower production offered copious hexose-rich nectars and, plants with low flower production offered concentrated nectar with higher sucrose content.

5. Our results suggest that: 1) ecological factors play an important role in determining nectar sugar composition; and 2) flowering and nectar production pattern could be related to different energy costs associated to producing flowers and nectar in chiropterophilic plant species.

Key-words: chiropterophilic plants, nectar, sugar composition.

Introduction

Nectar constitutes the most important floral reward that plants offer to pollinators (Simpson & Neff 1983). The three most common and abundant sugars in nectar are by far the hexose monosaccharides glucose and fructose, and the disaccharide sucrose (Baker & Baker 1983). Sugar composition and other characteristics of nectar vary with biochemical, ecological and evolutionary factors (Baker, Baker & Hodges 1998, Nicolson 1998, Ornelas *et al.* 2007).

Biochemically, sugar composition of nectar may vary due to changes in the activity of nectary enzymes and to osmoregulation processes caused by the osmotic pressure generated by the different sugars (Nicolson 1998; Nicolson & Fleming 2003). Nectar sugars are derived from sucrose translocated in phloem sap. This sucrose rich nectar is either hydrolyzed by the activity of the invertase enzymes into glucose and fructose or it is secreted without prior hydrolysis as sucrose (Nicolson 2002; De la Barrera and Nobel 2004). The final composition of nectar is determined by both the activity and amount of nectary invertase enzymes (Woodson & Wang 1987; Sturm & Guo-Qing 1999). Sucrose hydrolysis into hexoses increases nectar osmolality, causing water to move from the nectar walls into nectar and thus resulting in more dilute nectars (Nicolson 1998; 2002). As a consequence, nectar sugar composition could affect other nectar traits such as volume and concentration. For example, a dichotomy among volume and concentration with sugar composition has been observed in bird pollinated flowers in which dilute and copious nectar is generally hexose-rich, while concentrated and less copious nectar is usually sucrose-rich (Baker & Baker 1982; Martínez del Rio, Baker & Baker 1992, Nicolson 1998, Nicolson & Fleming 2003, Lotz & Schondube 2006). Nevertheless, this pattern has not been evaluated for chiropterophilic flowers.

Variation in sugar composition, nectar volume and concentration are also affected at ecological and evolutionary levels by plant mechanisms that are selected based on nectar production costs and, by their pollinators´ physiology. Nectar production is costly for the plant in terms of the amount of dry mass or energy investment (Pleasants & Chaplin 1983, Southwick 1984, Pyke 1991, Ashman & Shoen 1997). However, Harder & Barrett (1992) and Golubov *et al,* (2004) suggest small energetic costs of nectar production. Lanza *et al.* (1995) explained differences in nectar volume on the basis of a cost- benefit balance, in which plants are "fitter" when they produce the smallest possible nectar volume to attract their pollinators.

Additionally, preferences of nectarivore foragers based both on their morphology and physiology could have selective effects on the evolution of nectar characteristics (Heinrich & Raven 1972; Faegri & van der Pijl 1979; Baker & Baker 1982; Erhardt 1991; Martinez del Ro *et al.* 1988; 1992; Baker *et al.* 1998; Martinez del Rio, Schondube & McWhorter 2001, Ornelas *et al.* 2007). However, in the case of Neotropical nectarivorous bats Herrera (1999) and Rodríguez-Peña *et al.* (2007) found no evidence that bats prefer the dominant sugar composition in their natural food stuff (hexoses; Baker & Baker 1982, Baker *et al.* 1998). This finding cast doubt on the validity of the hypothesis that bats´ preferences act as selective pressures on the nectar composition of the plants they visit. The prevalence of hexose-dominated nectars among bat-pollinated plants in the Neotropics does not appear to be a result of bats preferring hexose-dominated nectar. The predominance of hexose-rich chiropterophilic flowers is an ecological pattern that remains to be explained.

The goal of our study was to understand the biochemical, ecological and evolutionary factors that influence sugar composition of chiropterophilic plant species. We collected the nectar of 12 plant species that previously have been recognized as principally visited and presumably pollinated by bats in the tropical dry forest of Jalisco, Mexico (Stoner *et al.* 2003), and determine: 1) their nectar sugar composition, volume and concentration; 2) how their sugar composition is related to other nectar traits (volume and concentration); and 3) if flowering characteristics of plants are connected with a suit of nectar traits. We hypothesized that because bats' sugar preferences (Rodríguez-Peña 2007) are important in determining sugar composition in nectar, a lack of a distinctive sugar composition would be associated with the chiropterophilous syndrome, and we expected to find a large variation in sugar composition among our study species. Second, because an increase in nectar osmolality in hexose-rich nectars causes water to move from the nectary walls into nectar (Nicolson 2002), we expected plants with hexose-dominate nectars to produce large volumes of diluted nectars, while plants with sucrose-dominate nectars should produce small quantities of concentrated nectar. Finally, because flowering characteristics and nectar traits are related to nectar production costs (Southwick 1984; Pyke 1991), we predicted that plant species with large flower production during a short period would produce dilute and abundant hexose-rich nectars, whereas plant species with low flower production during a long period should produce smaller volumes of more concentrated nectar dominated by sucrose.

Materials and methods

Study site and nectar samples

Nectar samples were collected at the Chamela-Cuixmala Biosphere Reserve on the central Pacific coast of Mexico (ca. 19º22'-19º35'N, 104º56'-105º03´W). The predominant vegetation type is tropical lowland deciduous forest with patches of riparian forest (Lott 1993). Twelve plant species constitute the main part of the annual diet of nectarivorous bats in this region (Stoner *et al.* 2003). The number of flowers sampled per plant was variable because of differences in flowering strategies among species and the differences in the number of open flowers among individuals. The average number of flowers collected per species was 57.0 ± 66.7 SD. The minimum number of flowers collected was 7 for *Acanthocereus occidentalis* and *Stenocereus chrysocarpus* (Cactaceae), while the maximum number of flowers collected was 240 for *Ceiba pentandra* (Malvaceae). For details on flowering characteristics of plant species see Stoner *et al.* (2003). To collect the nectar, we covered mature flower buds using mesh bags 1 hour before sunset. One hour after anthesis nectar was extracted and volume measured with glass capillary tubes (μl), and placed on Whatman No. 1 filter paper.

Nectar traits

Volume, concentration, and sugar composition were measured for each flower collected. One drop of nectar was placed on a Leica® hand refractometer and concentration was measured in sucrose equivalents [% sugar = (sugar mass/total mass) x 100].

Sugar composition was analyzed using the Medium Infrared Reflectance Method (MIR). Samples were analyzed in a Spectrum 2000 FT spectrophotometer (Perkin Elmer, Boston, MA, USA; following Flores *et al.* 2003). Multidimensional statistical analysis was performed with Quant software (Nicolet, Madison, WI, USA) using the Partial Least Squares PLS algorithm to determine sugar composition. The spectra modifications employed were normalized with a baseline correction using the second derivative, and suppression of the signals of water and carbon dioxide (Flores *et al.* 2003). Sugar ratios were obtained (sucrose to glucose plus fructose: S/G +F) to compare nectar sugar ratios between plant species. We recognized four classes of nectar: sucrose-dominant when sucrose / (glucose + fructose) ratio was greater than 0.999; sucrose-rich when ratios were between 0.5 and 0.999; hexose-rich when ratios were between 0.1 and 0.499; and hexose-dominant when ratios were less than 0.1, following Baker & Baker (1982). Although in this study we still use Baker´s terminology to facilitate comparisons within the literature, we also included the individual sugar percentages for all plant species as suggested by Nicolson & Thornburg (2007).

Phylogenetic and non-phylogenetic relationships between nectar sugar composition and other nectar traits

To identify interspecific relations among sugar composition and nectar volume and concentration, we performed both linear effect models and phylogenetically independent contrasts (PICs; Felsestein 1985, Garland et al. 1992). We used the percentage of glucose, fructose and sucrose as independent variables and nectar volume and concentration as the response variables in separate models. Additionally the flower nested in tree was included in the random part of the model. The models were fitted using the maximum likelihood method. For the PICs analysis, the response and explanatory variables were the same considered in the linear mixed effect models. We first built a preliminary phylogeny using the on-line phylogenetic tool “phylomatic” (http://www.phylodiversity.net) applying the maximum resolution for the seed plants phylogenetic tree. In order to get a fully dichotomous phylogeny we improved the preliminary phylogeny by randomly solving polytomies following Grafen (1989). Since some of the plant species we analyze in this study have not been considered previously in phylogenetic studies, branch lengths for our phylogeny are unknown. To conduct our analysis we assigned a length of one to all branches. We adjusted P values using the sequential Bonferroni correction. All analyses were performed using R®.

Relationships among flowering strategies and nectar traits

We performed a principal component analysis (PCA), using JMP® version 8 software, to determine whether sugar percentages, and nectar volume and concentration were associated with the flowering characteristics of the sampled plant species. Flowering patterns were categorized following Stoner *et al.* (2003). We measured: 1) the number of months that a plant species has flowers, and 2) the number of flowers produced per individual per month. This last value was categorized in three groups as follows: (a) 1–99 flowers per month, (b) 100–999 flowers per month, and (c) more than 1000 flowers per month.

Results

Nectar traits

We collected seven hundred and three flowers from 12 species in 5 families (Appendix 1). The flowers of the 12 plant species fall into the four distinct sugar composition groups using Baker´s terminology: 1 sucrose-dominant species, 2 sucrose-rich species, 8 hexose-rich species, and 1 hexose-dominant species. Nectar sucrose showed a right-skewed normal distribution, with an average of 25.46 ± 16.43%. Sugar in the form of hexoses showed a left-skewed normal distribution with an average of 74.54 ± 16.43 % (Fig. 1).

Total nectar concentration showed a normal distribution with an average value of 18.07 ± 3.75% (Fig. 1) and a range from 3% in *C. pentandra* (Malvaceae) to 33% in *A. occidentalis* (Cactaceae; Appendix 1). Nectar volume at anthesis showed a right-skewed non-normal distribution with an average of 151.37 ± 144.27μl (Fig. 1). Values ranged from 10 μl in *H. baruensis* (Malvaceae) to 1040 (μl) in *C. aesculifolia* (Malvaceae; Appendix 1).

Phylogenetic and non-phylogenetic relationships between nectar sugar composition and other nectar traits

Nectar concentration showed a statistically phylogenetic signal (P = 0.001) with a K-statistic value of 1.56 (Table 1). Relationships between the percentage of sugars in nectar and nectar concentration were statistically insignificant when corrected with the Bonferroni adjustment for both the linear effect models and PICs (Table 2).

Relationships between flowering strategies and nectar traits

The principal component analysis explained 75.87% of the overall variability of nectar traits and species' flowering characteristics among the chiropterophyllic plant community and synthesized this into two principal components (Fig. 2). The first principal component (PC1) explained 49.48% of the variation and was related to the sugar composition of nectar generating a gradient that segregated hexose-rich nectars (loadings = 0.49 and 0.46 for glucose and fructose, respectively) from sucrose-rich nectars (loading = -0.5). The second principal component (PC2) explained 26.40% and was related to the number of flowers produced per individual per month, nectar concentration, flowering duration and nectar volume (loadings = 0.6, -0.53,-0.37, and 0.34 respectively) and delineates two patterns of nectar production in relation to their flowering characteristics. On one side, plants that in general produce more flowers per night (*Crescentia alata*, *Bahunia pauletia*, and all Malvaceae sampled species 50-1000 flowers except *Ceiba grandiflora* and *Helicteres baruensis* which produced 10 and 5 flowers per individual per night, respectively), all of them with copious (202.02 ± 173.36 µl) and dilute (17.12 ± 1.81 w/w) hexose-rich nectars. On the other side, plants that offer fewer flowers per night (5 to 30) with less (103.17 ± 81.07 µl) and more concentrated (25.22 ± 1.99 w/w) nectars with higher sucrose proportions (*Ipomoea ampullaceae* and all the sampled Cactaceae species).

Discussion

The role of the biochemical, ecological, and evolutionary factors influencing nectar traits of chiropterophilic plants have been studied very little (Rodríguez-Peña et al 2007, Herrera1999 a, b, Baker et al. 1998). Our results showed that flowering characteristics of plants are related to differences in nectar sugar composition in chiropterophyllic plants. As predicted, we found a large variation in sugar composition among the studied plant species. Our results indicate that chiropterophyllic plant species can produce sucrose-dominant nectars (*Acanthocereus occidentalis*); sucrose-rich nectars (*Pseudobombax ellipticum* and *Bahuinia pauletia*); hexose-rich nectars (*Crescentia alata*, *Ceiba grandiflora*, *Ceiba pentandra, Helicteres baruensis, Pachycereus pecten, Stenocereus chrysocarpus, Stenocereus standleyi* and *Ipomoea* *ampullaceae*); and hexose-dominant nectars (*Ceiba aesculifolia*). Although we also predicted that plants with hexose-dominate nectars should produce larger volumes of diluted nectars, while flowers with sucrose-rich nectars should produce smaller quantities of concentrated nectar, we found no significant relationships between all three sugar percentages in nectar and volume and concentration. Finally, as predicted, plant species with large daily flower production had more dilute and abundant hexose-rich nectars, whereas plant species offering fewer flowers produced smaller volumes of more concentrated nectar with a higher proportion of sucrose. In this section we first comment on the large variation in sugar composition among the studied plant species. Then, we present the role that biochemical factors could have on nectar sugar composition.Finally, we discuss the relationship between nectar traits and flowering patterns and their ecological implications for bats.

Nectar sugar composition of chiropterophyllic plant species

Our results showed that nectar sugar percentages of 9 out of 12 chiropterophyllic plant species have greater proportions of hexoses over sucrose. These results concur with those previously reported for bat-pollinated plants (Baker *et al.*1998). However, the other three species were found to produce sucrose-rich nectars. Sucrose percentages found for two of these species, *P. ellipticum* and *B. pauletia,* where very close to the sucrose values that have been used to discriminate between nectar types (S= 33 % following Baker & Baker 1982). The highest sucrose value obtained for cchiropterophyllic nectars in this study was 53.98% in *Acanthocereus occidentalis* (Cactaceae; Appendix 1). The high sucrose values exhibited by *A. occidentalis* (S= 53.98%) compared to the other chiropterophyllic plant species suggest that effective pollination of this speices may occur by another pollinator type in this region. Indeed,Grant and Grant (1979) mention that this species has a hawkmoth pollinated flower, characterized by strongly scented night-blooming flowers with white or whitish long perianths and floral tubes.

Recently, Nicolson and Thornburg (2007) suggest that Baker´s terminology places too much value on the sucrose content of nectar because the transition value from “hexose-rich” to “sucrose-rich” is only 33% sucrose, when it should be 50% sucrose. These authors argue that a sugar ratio of 1.0 should be more appropriate to describe a "balanced" nectar (containing equal weights of sucrose and hexoses) instead of considering a balanced nectar as one with equal weights of the three sugars. These authors also argue that because the percentages of the three sugars are not independent, we should avoid the use of sugar ratios. However, this discussion on the best way to describe the dominance of different sugars in nectar needs to take into account the capacity of floral visitors to assimilate the different sugars present in nectar (Lotz & Schondube 2006). A ratio of 1 would be a good measurement of a balanced nectar when the limiting factor for sugar assimilation is sucrose digestion. However, if glucose and fructose have different assimilation efficiencies, evaluating nectar composition in a proportional way for the different sugars could be a better way to understand the interaction between the plant and its floral visitors.

Similar to results reported in previous studies (Baker *et al.* 1998, Chalcoff et al. 2006, Wolff 2006, Hölseher *et al.* 2008, Wenzler *et al.* 2008), the fructose percentages we obtained were higher than those of glucose in almost all of the plant species (10 out of 11; Appendix 1). It has been assumed that the similar amounts of glucose and fructose present in floral nectars are the result of enzymatic breakdown of sucrose by invertase activity in the nectar (Elias, Rozich & Newcombe 1975; Nicolson 2002; Nicolson & Fleming 2003, Lotz & Schondube 2006, Santos 2006). However, the nectar sugar percentages of chiropterophyllic plants in our study indicates that more mechanisms than sucrose digestion by the invertase enzymes in the nectary are involved in determining the final composition of sugars in nectar. These additional factors may inlucde the presence of micro organisms (Lüttge 1961), occurrence of oligosaccharides in nectar (Lüttge 1962), and other metabolic processes involved in nectar formation (Wenzler *et al.* 2008). The presence of additional amounts of fructose in the nectar of chiropterophyllic plants is not easy to explain, and seems to be independent of the presence of sucrose and glucose. This topic remains to be further studied.

The large gradient in sugar composition we found suggests that phyllostomid bats are not acting as a selective pressure to mold the sugar composition of the plants they visit. As suggested by Herrera (1999) and Rodriguez-Peña *et al.* (2007), this could be due to the lack of preference for different sugars shown by nectar-eating bats. This finding is also supported at a physiological level by the results of Ayala-Berdón *et al.* (2008) and Herrera and Mancina *et al.* (2008), who found that the consumption of the different sugar types does not appear to be limited by the gut capacity to process them in nectar-feeding bats. Additionally, the activity of the enzyme sucrose in the gut of nectar and fruit eating phyllostomid bats is high enough to assimilate all the sucrose present in nectar and fruit (Hernandez & Martinez del Rio 1992, Schondube et al. 2001). Because nectar-eating phyllostomid bats have large sucrase activity, and perceive both sucrose and hexoses as energetically equivalent, there is no digestive reason for them to be selective for the sugar composition of nectar when feeding in the field. This is similar to what Lotz & Schondube (2006) proposed for hummingbirds and what Herrera (1999 b) proposed for bats. They suggested that birds and bats with a large capacity to digest sucrose should be able to use all nectar sources regardless of their sugar composition, acting as generalist pollinators that could release selective pressures on the sugar composition of the plants they visit.

Phylogenetic and non-phylogenetic relationships between nectar sugar composition and other nectar traits

Our results showed no significant relationships between all three sugar percentages in nectar and volume and concentration. Our results did not concur with those reported for bird-pollinated plants, in which a dichotomy among volume and concentration related to sugar composition has been observed (Baker & Baker 1983; Baker *et al.* 1998; Nicolson & Fleming 2003). In this dichotomy dilute (20 – 25% w/w) and copious nectars are generally found to be hexose-rich (63.0 ± 61.7% sucrose mean ± S.D.) while concentrated and less copious nectars are usually sucrose-rich 64.4 ± 18.5% (mean ± S.D.; Nicolson 1998; Nicolson & Fleming 2003). Though, this was an unexpected result, we believe that the lack of a significant relation between sugar composition and nectar volume and concentration in this study could be due to the small sample size of chiropterophyllic plants (12 species) analyzed in this study. The relationship between sugar composition and nectar volume and concentration in chiropterophyllic plants is a topic that remains to be studied.

Our results showed the existence of a general pattern within the chiropterophyllic plant community in which species with large flower production offer copious hexose-rich nectars, and plants with low flower production offer concentrated nectar with higher sucrose content. We believe that this pattern could be related to different energy costs associated with producing flowers and nectar.

Energy is transported as sucrose in the plant (De la Barrera and Nobel 2004, Nicolson & Fleming 2003). This makes the secretion of sucrose-rich nectars less costly than the secretion of hexose-rich nectars. By secreting sucrose-rich nectars, plants could save the energy necessary to produce invertase enzymes needed to break down sucrose into hexoses in the nectary (De la Barrera & Nobel 2004). However the secretion of hexoses could also have some energetic advantages. Plants secreting hexose-rich nectars would produce more volume of nectar per amount of sugar invested, becoming more attractive to floral visitors (Nicolson & Fleming 2003).

In the case of a pollinator indifference to sucrose or hexose nectars (Lotz & Schondube 2006, Rodríguez-Peña *et al.* 2007), sucrose may be the dominant nectar sugar, but this was not the case in our data set, with 9 out of 12 of the chiropterophyllic plant species producing hexose-rich nectars. This result suggests that plant characteristics could be more important than floral visitors in determining nectar traits in chiropterophyllic plants. Among the different plant characteristics, we found a relationship between flowering patterns and nectar traits.

Baker & Baker (1983) mentioned that plant species pollinated by large animals such as birds and bats tend to produce large amounts of nectars based on a cost-benefit basis (Baker & Baker 1983). Flower visiting birds and bats need large amounts of nectar because they are larger than other pollinators, and have greater energy requirements associated with their metabolic expenses. Despite the potentially greater costs associated with attracting and rewarding these pollinators, they offer several advantages including: 1) they are highly mobile,

2) they have the capacity to move large pollen loads, and 3) their daily and seasonal tracking of nectar resources adds to their value as effective pollen vectors (Fleming 1982; Renner & Ricklefs 1995; Fleming & Muchhala 2008).

To attract nectar-feeding bats, chiropterophyllic plants do not have to offer a specific sugar composition or concentration, this allows them to exhibit different flowering patterns and nectar traits. One strategy is found inplants that invest the energy necessary to generate invertase enzymes and to produce hexose-rich nectar offering more volume per amount of sugar invested in a large number of flowers per night. A second stragegy is found in plants that save the energy necessary to produce the invertase enzymes needed to break down sucrose into hexoses, and produce concentrated nectars with a greater proportion of sucrose in less volume of water, offering a lower flower number per night. Plants using either of these two strategies will attract bats if they offer nectars with a sugar concentration higher than 15 % (w/w; Rodríguez-Peña *et al.* 2007, Ayala-Berdón et al. 2008; Ayala-Berdón, Schondube & Stoner 2009). The constraints that determine which strategy each plant species uses seems to be related to their abilities to store photosyntetases and water (Bazzaz & Carlson 1979; Southwick 1984).

Plants that produce few flowers per individual with concentrated sugar nectars tend to be visited only by a few pollinators of several species. These floral visitors spend short foraging periods in a plant and then move to another plant, and in most cases travel long distances between conspecific individuals (Heinrich & Raven 1972; Frankie *et al.* 1976). Plants that produce large number of flowers with more diluted sugar nectar tend to attract several individuals of the same pollinator species, which stay close to the plant for long periods of time (Sperr *et al.* 2010). Interestingly, it has been found that nectar-feeding bats present two contrasting foraging strategies when they are confronted with different nectar concentrations: 1) when feeding on concentrated nectars bats eat less food and spend large amounts of time flying, and 2) when feeding at intermediate or low sugar concentrations they ingest larger volumes of nectar, and reduce the time they spend flying (Ayala-Berdón *et al.* 2008, and unpublished data). These two foraging strategies allow the bats to respond effectively to the two different flowering strategies used by the plants they visit in the field. It is unclear how the concordance between the behavioral responses of bats to changes in sugar nectar concentration and the flowering strategies of chiropterophyllic plants evolved. This is a topic that requires further research.

Acknowledgements

The author's acknowledge support from grants from the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPITT Universidad Nacional Autónoma de México) to KES (IN 226007), and to JES (IN 226710). Scholarship support to NRP was provided by the Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico. We thank K. A. O.-Salazar for assistance in the field, L. Barbo for help in the laboratory, and the Estación de Biología Chamela for logistical support.

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Tables

Table 1. — Phylogenetic signal. K statistic indicating amount of phylogenetic signal and significance test for presence of phylogenetic signal (Blomberg *et al.* 2003).

Table 2. — Results of ordinary and phylogenetically independent contrasts (PIC) evaluating the effect of nectar attributes on specific sugars. Data were analyzed with linear regression models (Ordinary) and phylogenetically independent contrasts (PIC). The slopes (Coefficients) and standard error are displayed along with the F value and associated P value. Degrees of freedom are 1 and 10 in all cases. Significant values are presented in bold ($P < 0.5$). α values (corrected P) were adjusted by the Bonferroni method.

Table 1.

Nectar trait	K	P signal
Sucrose	0.54	0.62
Glucose	0.48	0.76
Fructose	0.62	0.33
Volume	0.58	0.19
Concentration	1.56	0.001

Table 2.

Sugar	Nectar attribute	Analysis	Coefficient ± SE	F Statistic	P	Adjusted P
Sucrose	Volume	Ordinary	-25.86 ± 11.34	2.28	**0.04**	0.14
		PIC	-29.39 ± 27.33	2.35	**0.04**	0.12
	Concentration	Ordinary	0.27 ± 0.97	0.78	0.78	2.35
		PIC	0.05 ± 1.77	0.001	0.97	2.93
Glucose	Volume	Ordinary	13.91 ± 7.76	1.79	0.11	0.33
		PIC	19.37 ± 8.27	2.34	**0.04**	0.12
	Concentration	Ordinary	0.05 ± 0.62	0.006	0.93	2.79
		PIC	0.03 ± 1.17	0.005	0.98	2.94
Fructose	Volume	Ordinary	11.95 ± 5.10	2.34	**0.04**	0.12
		PIC	10.01 ± 5.77	1.74	0.11	0.34
	Concentration	Ordinary	-0.32 ± 0.43	0.56	0.47	1.41
		PIC	-0.08 ± 0.75	0.01	0.92	2.75
Hexose	Volume	Ordinary	25.86 ± 11.34	2.79	**0.04**	0.14
		PIC	29.39 ± 12.50	2.35	**0.04**	0.12
	Concentration	Ordinary	-0.27 ± 0.96	0.08	0.78	2.35
		PIC	-0.05 ± 1.77	0.001	0.97	2.93

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Figures

Figure 1. Nectar concentration (% w/w), nectar volume (µl), and sugar composition (% total sugar as sucrose and hexoses) mean values per species of chiropterophyllic plant.

Figure 2. Principal component analysis showing how the flowering strategies of the study plants were related to nectar traits of chiropterophyllic plants. The first two axes explained 75.87 % of total inertia (49.48 %, and 26.40 %, respectively, for Axis 1 and Axis 2). Axis 1 represents sugar composition (capitalized bold text). Axis 2 represents number of flowers, nectar concentration, flowering duration and nectar volume at anthesis (italics text). Numbers indicate plant species: (1) *Crescentia alata* (Bignoniaceae), (2) *Ceiba aesculifolia* (Malvaceae), (3) *Ceiba grandiflora* (Malvaceae), (4) *Ceiba pentandra* (Malvaceae), (5) *Pseudobombax ellipticum* (Malvaceae), (6) *Helicteres Baruensis* (Malvaceae), (7) *Acanthocereus occidentalis* (Cactaceae), (8) *Pachycereus pecten* (Cactaceae), (9) *Stenocereus chrysocarpus* (Cactaceae), (10) *Stenocereus standleyi* (Cactaceae), (11) *Ipomoea ampullacea* (Convolvulaceae), (12) *Bauhinia pauletia* (Fabaceae), (12)

FIG 1.

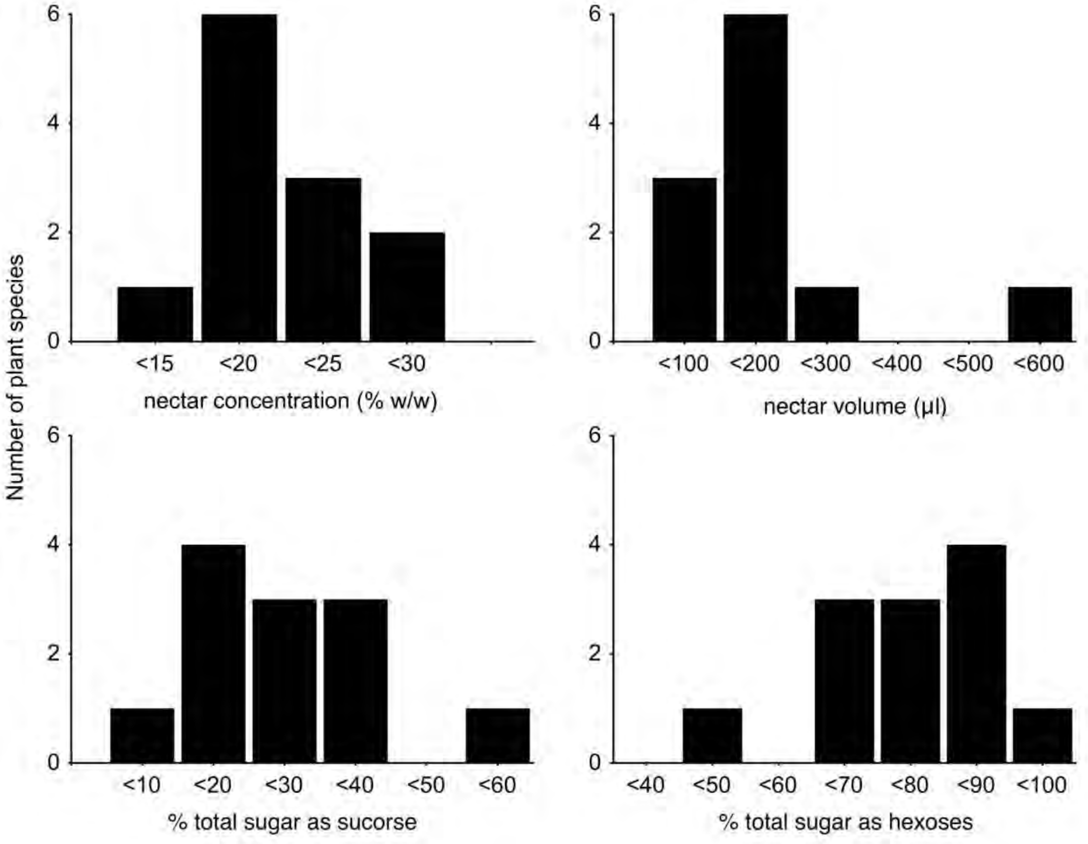
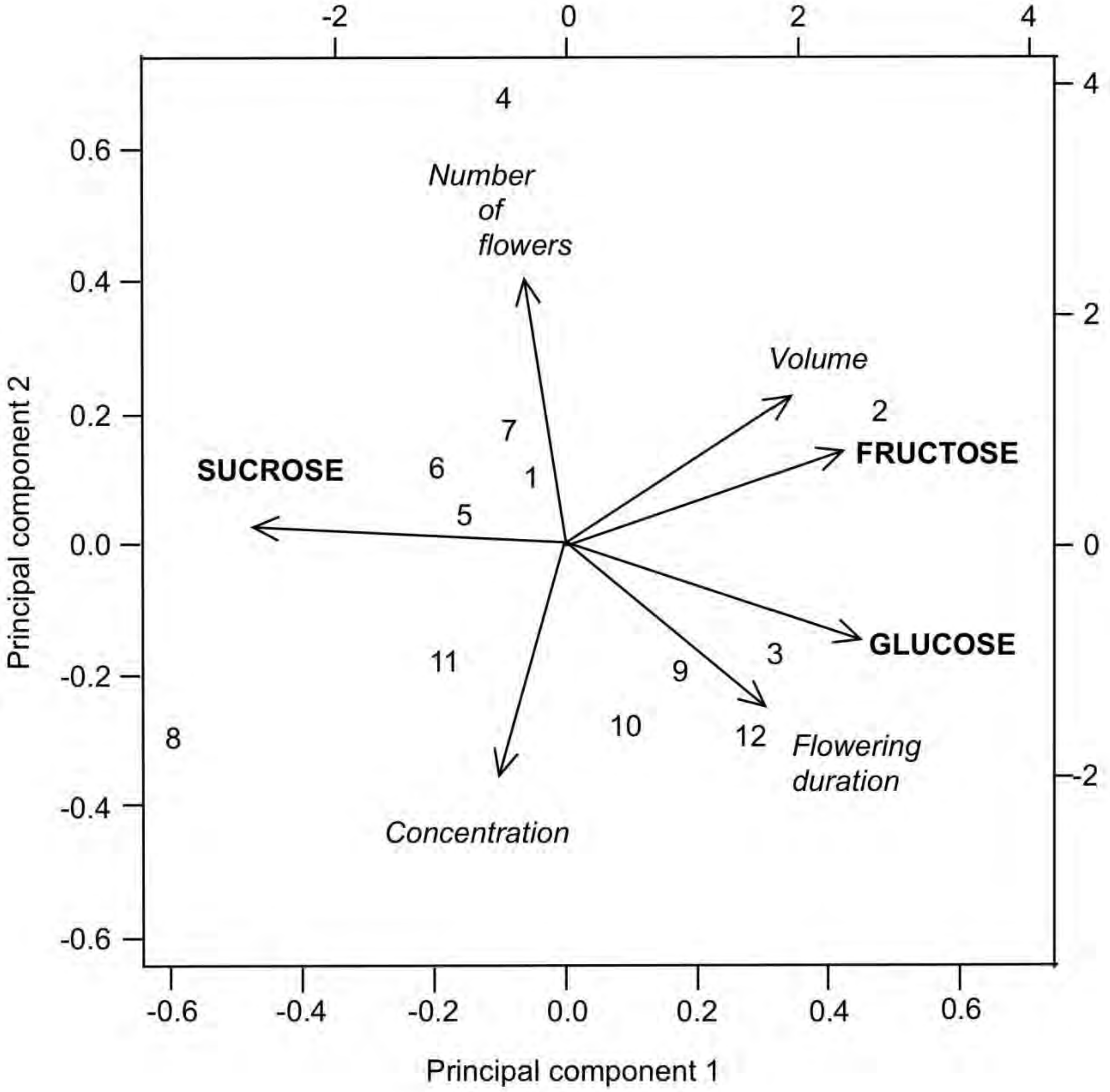

Number of plant species
6
4
2
0
<15
<20
<25
<30
nectar concentration (% w/w)
<100
<200
<300
<400
<500
<600
nectar volume (µl)
<10
<20
<30
<40
<50
<60
% total sugar as sucorse
<40
<50
<60
<70
<80
<90
<100
% total sugar as hexoses

FIG 2.


-2
0
2
4
4
0.6
Number of flowers
0.4
2
Volume
2
0.2
7
FRUCTOSE
SUCROSE
6
1
5
0.0
0
11
9
3
GLUCOSE
-0.2
8
10
12
Flowering duration
-2
-0.4
Concentration
-0.6
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
Principal component 1
Principal component 2

APPENDIX 1.— Nectar traits of 12 chiropterophyllic plant species in the Tropical Dry Forest at the Chamela-Cuixmala Biosphere Reserve. Volume: mean ± SD (range) (nb of flowers/nb individuals). Concentration: mean ± SD (range) (N flowers/N individuals). Sugar ratios and nectar type: sucrose-dominant (SD) = sucrose/(glucose + fructose) ratio is greater than 0.999; sucrose-rich (SR)= ratios between 0.5 and 0.999; hexose-rich (HR) = ratios between 0.1 and 0.499; and hexose-dominant (HD) = ratios less than 0.1).

Species	Nectar Volume (µl)	Nectar concentration (w/w)	SUGAR COMPOSITION			
			Fructose (%) Mean ± SD	Glucose (%) Mean ± SD	Sucrose (%) Mean ± SD	Sugar ratio (nectar type)
Bignoniaceae						
Crescentia alata	149.85±11.37 (15-580) (102/5)	19.48±0.39 (12-27) (82/5)	41.03±0.69	29.25±1.08	29.72±16.05	0.42 (HR)(104/5)
Malvaceae						
Ceiba aesculifolia	579.23±19.06 (220-1040) (83/5)	16.85±0.10 (14-18.8) (83/5)	49.82±0.63	47.18±0.90	2.99±0.96	0.03 (HD)(81/5)
Ceiba grandiflora	142±19.76 (24.3-584.2) (38/12)	18.83± 0.74 (5-25) (35/12)	46.82±0.82	39.43±1.54	13.75±1.83	0.16 (HR)(36/12)
Ceiba pentandra	127.44±3.25	16.66±0.18	48.51±0.42	23.37±0.53	28.13±0.67	0.39

	(19-300)	(3-25)				(HR)(232/11)
	(240/11)	(229/11)				
Helicteres baruensis	51.88±8.50	14.46±0.36	39.74±0.71	27.80±1.40	32.46±1.68	0.48 (HR)(16/6)
	(10-120)	(13-16.5)				
	(15/6)	(12/6)				
Pseudobombax	219.37±24.43	15.44±0.35	38.33±1.8	26.41±1.8	35.26±2.63	0.54 (SR)
ellipticum	(20-660)	(8-19)				(41/7)
	(40/7)	(38/7)				
Cactaceae						
Acanthocereus	37.2±7.54	28± 1.31	28.97±3.14	17.06±2.93	53.98±5.92	1.17
occidentalis	(10.8-68.7)	(24-33)				(SD) (8/5)
	(7/5)	(6/5)				
Pachycereus pecten	109.68±12.24	26.17±1.11	49.08±1.73	36.80±3.06	14.12±4.14	0.16
	(24.4-204.2)	(18-31)				(HR)(19/6)
	(19/6)	(12/6)				
Stenocereus	74±10	22.66±0.48	39.90±1.12	41.85±3.72	18.26±3.75	0.22
chrysocarpus	(53-114.3)	(21-24)				(HR) (7/2)

	(7/2)	(7/2)				
Stenocereus	54.98±8.3	25.01±0.37	38.42±0.99	32.66±1.39	28.93±1.65	0.40
standleyi	(10.6-196.6)	(22-29)				(HR)(24/5)
	(24/5)	(19/5)				
Convolvulaceae						
Ipomoea	240±10.51	24.42±1.02	46.58±1.68	40.93±2.62	12.49±2.50	0.14
ampullaceae	(65-240)	(11-30.8)				(HR)(18/4)
	(18/4)	(18/4)				
Fabaceae						
Bahuinia pauletia	144.40±8.40	18.12±0.15	35.60±0.70	25.63±0.96	38.77±1.06	0.63 (SR)(91/6)
	(30-340)	(14.8-20.5)				
	(92/6)	(92/6)				

CAPÍTULO 5.

DISCUSION Y CONCLUSIONES GENERALES

En el SEGUNDO capítulo de esta tesis titulado, "**Efecto de la composición y concentración de azúcares sobre la selección de los recursos"**, determiné el papel que ejercen la composición y concentración de azúcares en el néctar sobre las decisiones de forrajeo en dos especies de murciélagos nectarívoros con diferentes estrategias de uso de néctar: *Leptonycteris yerbabuenae*, y *Glossophaga soricina*. Los resultados indicaron que los murciélagos nectarívoros no tienen preferencia por soluciones de hexosas sobre soluciones de sacarosa.

Los murciélagos nectarívoros perciben tanto a las hexosas como a la sacarosa como energéticamente equivalentes. Los resultados sugieren que los murciélagos nectarívoros tienen la misma capacidad para asimilar las hexosas y la sacarosa.
Leptonycteris yerbabuenae y *Glossophaga soricina* difieren en su capacidad para discriminar entre soluciones con concentraciones de azúcares contrastantes. Este resultado sugiere que los murciélagos nectarívoros más especializados son capaces de discriminar entre diferencias más pequeñas en la concentración de azúcares en el alimento.

En el TERCERO capítulo titulado: "**Los aminoácidos del néctar modifican la selección del alimento en murciélagos nectarívoros"**, estudié el papel que ejercen los aminoácidos del néctar sobre las decisiones de forrajeo en dos especies de murciélagos nectarívoros con diferentes estrategias de uso de néctar: *Leptonycteris yerbabuenae*, y *Glossophaga soricina*. Ambas especies de murciélagos son indiferentes a la presencia de nitrógeno en néctar. Esta indiferencia sugiere que los murciélagos no consideran el néctar como una fuente importante de nitrógeno. Sin embargo, ambas especies de murciélagos son indiferentes a concentraciones contrastantes de azúcar cuando éstas fueron adicionadas con aminoácidos, y *Leptonycteris yerbabuenae* tuvo preferencia por la concentración de azúcar más alta cuando las dietas fueron preparadas sin aminoácidos. Lo que sugiere que el sabor generado por los aminoácidos en el néctar reduce su capacidad para discriminar entre las concentraciones de azúcar.

La preferencia consistente de *Leptonycteris yerbabuenae* por la abundancia relativa de los aminoácidos en el néctar de *Pachycereus pecten* (Cactaceae) sobre el de *Ceiba aesculifolia* (Malvaceae) sugieren que estos murciélagos pueden detectar y reconocer el sabor que la mezcla de aminoácidos proporciona al néctar. Este patrón no se encuentra en *Glossophaga soricina*. Este resultado sugiere que los murciélagos nectarívoros más especializados son capaces de discriminar entre la composición de aminoácidos en el néctar.

Este capítulo demuestra que a pesar de la baja concentración a la que los aminoácidos se encuentran en el néctar, éstos pueden afectar las decisiones de forrajeo de los murciélagos nectarívoros. Finalmente, el hecho de que la concentración de aminoácidos en el néctar tiene la capacidad de confundir a los murciélagos para distinguir entre concentraciones de azúcares y, que algunos murciélagos tienen preferencias por el sabor que algunos aminoácidos proporcionan al néctar de especies de plantas específicas, sugiere que las plantas pueden utilizar la variación en este característica para atraer a los murciélagos polinizadores.

Finalmente, en el CUARTO capítulo titulado “**Factores que afectan la composición de azúcares en plantas quiropterofílicas”**, determiné el efecto que los factores bioquímicos y ecológicos evolutivos de la planta tienen sobre la composición de azúcares, concentración y volumen en el néctar. El néctar de las 12 especies de plantas quiropterofílicas analizadas en este capítulo de acuerdo con la terminología propuesta por Baker y Baker (1982) es: dominado por sacarosa (1 especie), rico en sacarosa (2 especies), rico en hexosas (2 especies) y dominado por hexosas (1 especie).

Los resultados mostraron relaciones no significativas entre los porcentajes de azúcares (glucosa, fructosa y sacarosa) y el volumen y la concentración del néctar en las especies de plantas quiropterofílicas analizadas. Nuestros resultados no coinciden con lo reportado para plantas polinizadas por aves, en donde se ha encontrado una dicotomía, en la que los néctares dominados por hexosas son diluidos y copiosos, mientras que los néctares dominados por sacarosa son concentrados y con menos volumen (Baker & Baker, 1983;. Baker et al 1998; Nicolson & Fleming 2003).

Los porcentajes de fructosa obtenidos en este estudio son más altos que los de glucosa en casi todas las especies (11 de un total de 12) lo que concuerda con lo reportado por (Baker et al. 1998, Chalcoff et al. 2006, Wolff 2006, Hölseher et al. 2008, Wenzler et al. 2008). Esto significa, que la relación fructosa : glucosa no es de 1:1. La composición de azúcares en las especies de plantas quiropterofílicas analizadas en este estudio sugiere la existencia de otros mecanismos, además de la digestión de sacarosa en el nectario, que pueden estar involucrados al momento de determinar la composición final de azúcares en el néctar.

Nuestros resultados muestran que entre la comunidad de plantas quiropterofílicas hay dos estrategias en la producción de néctar: 1. La planta invierte en la energía necesaria para producir la enzima invertasa necesaria para hidrolizar la sacarosa en hexosas, y ofrecer néctares a altos volúmenes con menor cantidad de energía en un gran número de flores por noche y, 2. La planta invierte la energía necesaria para producir néctares concentrados con mayores proporciones de sacarosa, en un número menor de flores por noche. Este patrón podría estar relacionado con los costos de energía asociada a la producción de flores y néctar.

A manera de conclusión, la prevalencia de néctares dominados por hexosas en plantas polinizadas por murciélagos no parece ser el resultado de las preferencias de los murciélagos, sino de decisiones intrínsecas de la planta. Los resultados obtenidos en este estudio rechazan la hipótesis de que los animales nectarívoros tienen preferencia por los azúcares más comunes en su dieta natural. El hecho de que la concentración de aminoácidos en el néctar tiene la capacidad de confundir a los murciélagos para distinguir entre concentraciones de azúcares y, que algunos murciélagos tienen preferencias por el sabor que algunos aminoácidos proporcionan al néctar de especies de plantas específicas, sugiere que las plantas pueden estar utilizando la variación en esta característica para atraer a los animales nectarívoros En las especies de plantas quiropterofílicas los factores ecológicos, juegan un papel importante para determinar la composición de azúcares del néctar. En estudios futuros es importante determinar por una parte, la existencia de otros mecanismos, además de la digestión de sacarosa en el nectario, que pueden estar involucrados al momento de determinar la composición final de azúcares en el néctar. Por otra parte, aún no está claro cómo la concordancia entre las respuestas de comportamiento de los murciélagos a los cambios en la concentración de azúcar en el néctar y las estrategias de floración de las plantas quiropterofílicas han evolucionado.