

## ANTAGONISTS AND THE RISK-SENSITIVITY IN HUMMINGBIRDS: EXPERIMENTAL TESTS

Carlos Lara

Laboratorio de Ecología del Comportamiento, Centro Tlaxcala de Biología de la Conducta UAT-UNAM, Carretera Tlaxcala-Puebla Km 1.5, Colonia Xicohténcatl S/N, C.P. 90070, Tlaxcala, México. *E-mail*: laracar@garza.uatx.mx

**Resumen.** – Antagonistas y la sensibilidad al riesgo en colibríes: pruebas experimentales. – En las plantas polinizadas por colibríes, la variación en el contenido y la calidad del néctar dentro de las flores, debido a la variación en la producción del néctar por la planta y la explotación previa de otros visitantes, significa que el néctar es un recurso riesgoso. Esta variación en la disponibilidad y la calidad del néctar puede incrementarse por antagonistas tales como los robadores de néctar. Aquí, a través de la simulación de distintas intensidades de robo en el arbusto distílico *Bowardia ternifolia*, afectando el volumen y la concentración del néctar, evalué sus efectos en el forrajeo del Colibrí-orejiblanco (*Hylocharis leucotis*). Mis resultados mostraron que incrementar la varianza en el volumen del néctar (pero no en concentración) por flor, simulando la acción de los robadores de néctar, resulta en una disminución en el número de flores de *Bowardia* visitadas por colibríes, sugiriendo aversión al riesgo en estas aves.

**Abstract.** – In hummingbird-pollinated plants, the variation in the nectar quantity and quality within the flowers of a single species, due to variation in nectar production by the plant and previous exploitation of other foragers, means that the nectar is a naturally risky resource. This variation in the nectar availability and quality can commonly be increased by antagonists such as nectar robbers. Here, simulating different intensities of nectar theft in the distylous shrub *Bowardia ternifolia*, and manipulating nectar volume and concentration, I evaluated its effects on the foraging patterns of the White-eared Hummingbird (*Hylocharis leucotis*). My results showed that increasing only the variance in nectar volume per flower (but not in nectar concentration), simulating the action of nectar robbers, results in fewer *Bowardia* flowers visited by hummingbirds, suggesting risk-aversion in this bird species. *Accepted 5 December 2007.*

**Keys words:** Antagonists, hummingbirds, White-eared Hummingbird, *Hylocharis leucotis*, risk-aversion, risk-prone.

### INTRODUCTION

Animals presenting foraging options that offer the same average rate of gain but differ in variance generally show strong preferences (Bateson & Kacelnik 1998). This widespread behavioral phenomenon is known as risk sensitivity. There are aspects of the ecology of hummingbirds that make them ideal candidates for the study of risk sensitivity. For

example, for these birds, food intake comes mainly from nectar obtained from flowers, and a hummingbird foraging in nature faces a patchily distributed resource of variable quality (Gass *et al.* 1976). Likewise, the nectar is hidden within the flowers, and it is impossible for a bird to determine how much nectar will be available before visiting the flower, and this mean that nectar is a naturally risky resource (Biernaskie *et al.* 2002). Thus, a hum-

mingbird finds a resource variability at different spatial scales (e.g., Arregui 2004, Mauricio-López 2005, Ortíz-Pulido & Vargas-Licona 2008), and must make foraging decisions about which patches of flowers to visit and how to behave within each patch, partly as a function of the nectar reward (Pyke 1978, Gass & Montgomerie 1981).

It has been shown that some antagonistic organisms such as the nectar robbers act as important environmental factors affecting nectar reward for hummingbirds (McDade & Kinsman 1980, Irwin & Brody 2000). These antagonists can alter the standing crop of nectar and the sugar concentration of nectar available to hummingbird pollinators, affecting in a direct or indirect way the pollen flow among their visited plants and, in consequence, altering the reproduction of the plants (Irwin & Brody 1998, Lara & Ornelas 2002a, Irwin & Maloof 2002). In this work, I show that, by simulating the presence of nectar robbers on the distylous herb *Bowardia ternifolia* (Rubiaceae), the foraging decisions of the White-eared Hummingbird (*Hylocharis leucotis*) can be altered, because variation in nectar rewards was negatively affected.

## METHODS

*Study area and species.* From May to August 2006, hummingbirds were studied in the National Park “La Malinche”, Tlaxcala, México (19°14'N, 98°58'W, 2900 m a.s.l.). In this study site, *Bowardia ternifolia* (Rubiaceae) (hereafter *Bowardia*) produces flowers from May to August. Flowers have red tubular corollas in groups of 15–20 flowers at terminal cymes. White-eared Hummingbirds (*Hylocharis leucotis*) are their main pollinators (Lara 2006). *Bowardia* flowers produce nectar at a constant rate of 1–6  $\mu\text{l}$  nectar flower<sup>-1</sup> day<sup>-1</sup> with a concentration of 20–25% sucrose equivalents. The average standing crop of

nectar in individual flowers is approximately 1–2  $\mu\text{l}$  (Tórres *et al.* 2008). *Tropicoseius* sp. is a flower mite that lives in their flowers, and it has been shown that they can consume up to 50% of the nectar produced by the flowers (Lara & Ornelas, 2002b).

A total of 20 adult hummingbirds were captured in the field for this study (10 hummingbirds per experiment). Before trials, hummingbirds were housed individually for 1–2 h in field-collapsible cages (61×61×61 cm). These cages were placed at ambient light and temperature. Hummingbirds had free access to 20% (by mass) sugar solution. I found no effect on pre-trial housing on the hummingbird's performance.

*Experimental protocol.* I potted 2 flowering *Bowardia* from a natural population growing 3 km north of the field station La Malinche. Plants were chosen based on similarities in plant architecture, flowering phenology, and number of buds and flowers. I placed plants in a greenhouse for 2 day and watered and fertilized them daily to allow recovery from transplant shock. Plants were always maintained with 12 open flowers for the experiments.

To investigate the effects of nectar theft on the risk-sensitivity of hummingbird visiting *Bowardia*, I conducted the following experiment.

*Experiment 1.* Individual hummingbirds were presented with the two plants spaced 50 cm apart. One of the plants contained 1.5  $\mu\text{l}$  of 20% sucrose solution in all 12 flowers (without nectar robbing treatment). The other plant provided the same mean volume of nectar per flower (1.5  $\mu\text{l}$ ), but in a random distribution of six flowers containing 3  $\mu\text{l}$  at 20% concentration and six flowers with no nectar reward (with nectar robbing treatment). Before setting the treatments, I removed all the nectar from all the flowers on each plant

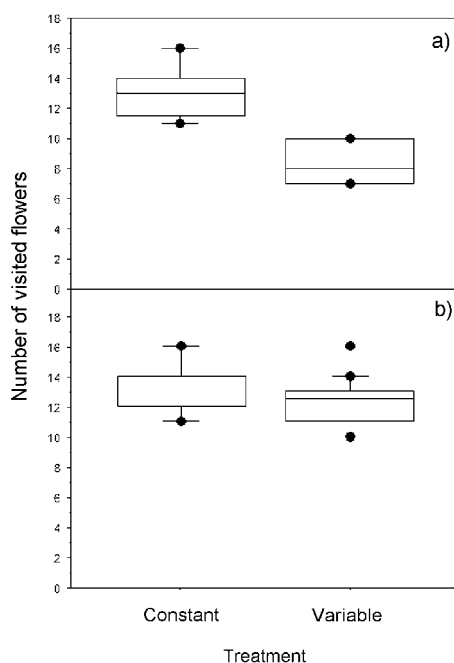


FIG. 1. Mean ( $\pm$  SE) number of flowers visited according to the plant treatment (constant or variable) when (a) nectar volume and (b) concentration were manipulated.

using a 10  $\mu$ l micro-capillary tube inserted through the front of the flowers. Empty flowers were included in an effort to increase the level of variation, since the coefficient of variation is the best predictor in risk-sensitivity (Shafir 2000), and this method of robbing flowers adequately mimics nectar robbing by flower mites in *Bomvardia* (Lara & Ornelas 2002b). For treatments with nectar, I injected 1.5  $\mu$ l or 3  $\mu$ l of 20% sucrose solution into the flowers using a 10  $\mu$ l glass syringe. A nectar volume at 20% concentration approximates the average concentration in field plants (Tórres *et al.* 2008). By adding the same concentration of nectar into each flower in the nectar-present treatments, I removed the between-plant differences in nectar concentration. In addition, immediately after setting

the treatments, I placed the experimental plants inside the aviary for 1 h only; therefore additional nectar production by plants did not significantly affect the nectar manipulation. A foraging bout was a visit to the plant at which the animal fed and left voluntarily. Hummingbirds were released one by one in to the aviary to visit the plants for 1 h and then were removed. During each foraging bout, time between visits and the number of flowers probed on each plant were recorded. Observations were conducted from 08:00 to 12:00 h and we used hummingbirds as we netted them.

*Experiment 2.* To determine if birds are risk-sensitive between plants with increased variation of nectar concentration, I used an experimental design identical to that of the experiment above but here I manipulated nectar concentration. Therefore, a plant had flowers with 1.5  $\mu$ l of 20% sucrose solution in all 12 flowers (without nectar robbing treatment) and the other plant provided the same mean concentration of nectar per flower (20%), but in a random distribution of six flowers containing 1.5  $\mu$ l at 25% concentration and six flowers with 1.5  $\mu$ l at 15% concentration (with nectar robbing treatment). Again, all flowers on the same plant received the same treatment, as indicated above. Flowers with 25% and 15% nectar concentration represent the nectar concentration found in unrobbed and robbed flowers, respectively, in the naturally growing plants (Tórres *et al.* 2008). Before setting treatments, I removed all nectar from all flowers with a 5  $\mu$ l micro-capillary tube. I added 1.5  $\mu$ l of 25% or 15% sucrose solution through the front of flowers using a 10  $\mu$ l glass syringe.

*Statistical analysis.* To determine the effect of treatment (constant vs variable nectar volume and concentration) on the flower visiting behavior of hummingbirds during the trials,

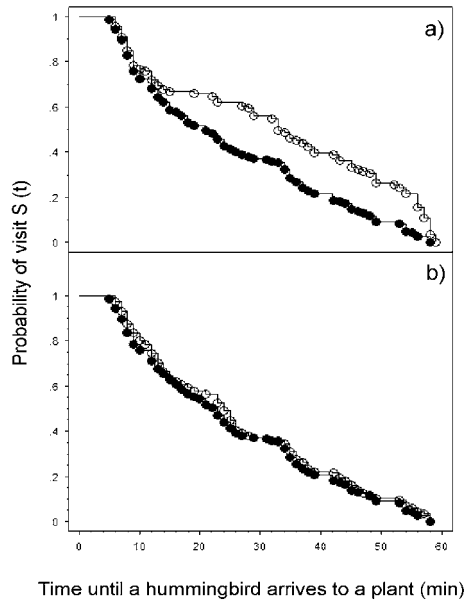


FIG. 2. Comparison between plants with different levels of (a) nectar volume and (b) concentration, in their probability to be visited by hummingbirds during one observation period. White dots represent the nectar variable treatment and black dots the nectar constant treatment.  $S(t)$  is the probability that a hummingbird has not yet visited a plant. Time until a hummingbird arrived refers to the time elapsed since the start of the observation period.

data were analyzed by using t-paired tests (Zar 1999).

I used survival analysis (Muenchow 1986) to analyze hummingbird visitation. For these data, the actual time of occurrence is not known but only a minimum length of time during which the event did not occur (censored data). If an event occurred for a given plant, then it became uncensored data, and if it never occurred, then it became censored data. I used the Kaplan-Meier product-limit non-parametric method for the computation of the probability that hummingbirds had not yet visited a flower in a plant 60 min after the start of observation, and the logrank (Mantel-

Cox) statistic to test for differences between treatments.

All statistical analyses were done using StatView and SuperANOVA (Abacus Concepts 1989, 1996).

## RESULTS

I recorded 250 foraging bouts in over 20 h of observations. Nectar variation had a significant effect on hummingbird visitation ( $t = 4.45$ ,  $df = 9$ ,  $P = 0.001$ , Fig. 1a), but no differences were found when concentration of nectar was manipulated ( $t = 0.19$ ,  $df = 9$ ,  $P = 0.845$ , Fig. 1b). Thus, birds avoided plants with variable quantity of nectar and made more visits to flowers with constant quantity of nectar.

I found significant differences among probability curves of hummingbirds visiting both plants when volume was manipulated to mimic nectar robbing (Logrank-Mantel Cox:  $\chi^2 = 3.42$ ,  $df = 1$ ,  $P = 0.019$ , Fig. 2a) but not when concentration was manipulated (Logrank-Mantel Cox:  $\chi^2 = 0.08$ ,  $df = 1$ ,  $P = 0.761$ , Fig. 2b). Hummingbirds first visited those plants with constant nectar volume (without nectar robbing) and the arrival time was longer when the variation of nectar volume was higher.

## DISCUSSION

Results obtained in this study suggest that by altering the variance in nectar volume, but not the concentration, we can affect the plant visitation patterns in hummingbirds. Previous studies have predicted that risk-averse pollinators should made fewer visits to inflorescences with variable nectar volumes (Pleasant 1983, Ratchcke 1992). Our data support these ideas. Hummingbirds observed showed shortened arrival times when flowers had constant nectar volume, compared to those with variable nectar volume. The risk aver-

sion-preference behavior is consistent with previous studies using artificial flowers (Waser & McRoberts 1998, Biernaskie *et al.* 2002). However, the present study is different, because I used natural flowers as a way to confront hummingbirds to a more realistic condition.

Hummingbirds visited more flowers in the constant nectar volume plants, when nectar volumes were manipulated. However, nectar concentration did not affect hummingbird visitation to flowers. Thus, hummingbirds probe fewer flowers on plants with the nectar variable treatments, and increase their visits when the nectar available is not variable. I interpret this risk-aversion behavior as a way to minimize the probability of falling below a long-term energy threshold as suggested by Stephens (1981) for foraging animals. This means that hummingbirds can perceive robbed flowers (variable) in nature as “risky” options, that is, they represent a lower energy gain and should be avoided in future visits. Likewise, as suggested by Biernaskie *et al.* (2002), hummingbirds left inflorescences early and increase their time to return, after sampling a number of flowers and recognizing unfavorable variation in the reward distribution.

Although hummingbirds avoided flowers without nectar reward more often, they did make errors in foraging decisions and many times foraged on plants and flowers without nectar. This behavior can be related with memory failure or the lack of visual and location cues for hummingbirds. It has been shown that usually hummingbirds make exploratory foraging visits to non-rewarding sites even after making frequent visits to a rewarding site (Miller & Miller 1971, Brown & Gass 1993, Hurly 1996). Thus, the incorrect foraging decisions recorded in this study may represent a deliberate mechanism to provide additional information about local patch quality without significantly reducing

energy intake, because plants were closer together.

In summary, I have shown that hummingbirds will visit fewer flowers on a plant when the variation in nectar volumes is increased and suggest that arrival times can be delayed in flowers with a higher variance of nectar, such as robbed plants.

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#### REFERENCES

- Abacus Concepts. 1989. SuperANOVA. Abacus Concepts Inc., Berkeley, California.
- Abacus Concepts. 1996. Statview reference. Abacus Concepts, Inc., Berkeley, California.
- Arregui, L. 2004. ¿A que nivel de la escala espacial seleccionan los colibríes su área de forrajeo? Tesis de DEA, Univ. Autónoma de Madrid, Madrid, España.
- Bateson, M., & A. Kacelnik. 1998. Risk-sensitive foraging: decision making in variable elements. Pp. 297–341 *in* Dukas, R. (ed.). Cognitive ecology: the evolutionary ecology of information, processing and decision making. Univ. of Chicago Press, London, UK.
- Biernaskie, J. M., R. V. Cartar, & A. Hurly. 2002. Risk-averse inflorescences departure in hummingbirds and bumble bees: could plants benefit from variable nectar volumes? *Oikos* 98: 98–104.
- Brown, G. S. & C. L. Gass. 1993. Spatial association learning in hummingbirds. *Anim. Behav.* 46:487–497.
- Gass, C. L., G. Angehr, & J. Centa. 1976. Regulation of food supply by feeding territoriality in the Rufous Hummingbird. *Can. J. Zool.* 54: 2046–2054.
- Gass, C. L., & R. D. Montgomerie. 1981. Hummingbird foraging behavior: decision making

- and energy regulation. Pp. 159–164 in Kamil, A. C., & T. D. Sargent (eds.). Foraging behavior: ecological, ethological, and psychological approaches. Garland Press, New York, New York.
- Hurly, T. A. 1996. Spatial memory in Rufous Hummingbirds: memory for rewarded and non-rewarded sites. *Anim. Behav.* 51: 177–183.
- Irwin, R. E., & A. K. Brody. 1998. Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. *Oecologia* 116: 519–527.
- Irwin, R. E., & A. K. Brody. 2000. Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. *Ecology* 81: 2637–2643.
- Irwin, R. E., & J. E. Maloof. 2002. Variation in nectar robbing over time, space and species. *Oecologia* 133: 525–533.
- Lara, C., & J. F. Ornelas. 2002a. Effects of nectar theft by flower mites on hummingbird behavior and the reproductive success of their host plant, *Moussonia deppeana* (Gesneriaceae). *Oikos* 96: 470–480.
- Lara, C., & J. F. Ornelas. 2002b. Flower mites and nectar production in six hummingbird-pollinated plants with contrasting flower longevities. *Can. J. Bot.* 80: 1216–1229.
- Lara, C. 2006. Temporal dynamics of flower use by hummingbirds in a highland temperate forest in Mexico. *Ecoscience* 13: 23–29.
- Mauricio-López, E. 2005. Interacción colibrí-planta: variación espacial en un matorral xerófilo de Hidalgo, México. Tesis de Licenciatura, Univ. Autónoma del Estado de Hidalgo, Pachuca, Hidalgo, México.
- McDade, L. A., & S. Kinsman, 1980. The impact of floral parasitism in two Neotropical hummingbird-pollinated plant species. *Evolution* 34: 954–958.
- Miller, R. S., & R. E. Miller. 1971. Feeding activity and colour preferences of Ruby-throated Hummingbirds. *Condor* 73: 309–313.
- Muenchow, G. 1986. Ecological use of failure time analysis. *Ecology* 67: 246–250.
- Ortíz-Pulido, R., & G. Vargas-Licona. 2008. Explorando la relación entre registros de colibríes y abundancia de flores con escalamiento espacio-temporal. *Ornitol. Neotrop.* 19 (Suppl.): 473–483.
- Pleasants, J. M. 1983. Nectar production in *Ipomopsis aggregata* (Polemoniaceae). *Am. J. Bot.* 70: 1468–1475.
- Pyke, G. H. 1978. Optimal foraging in hummingbirds: testing the marginal value theorem. *Am. Zool.* 18: 739–752.
- Rathcke, B. J. 1992. Nectar distributions, pollinator behavior and plant reproductive success. Pp. 113–138 in Hunter, M. D., T. Ohgushi, & P. W. Price (eds.). Effects of resource distribution on animal/plant interactions. Academic Press, San Diego, California.
- Shafir, S. 2000. Risk-sensitive foraging: the effect of relative variability. *Oikos* 88: 663–669.
- Stephens, D. W. 1981. The logic of risk-sensitive foraging preferences. *Anim. Behav.* 29: 628–629.
- Tórres, I., L. Salinas, C. Lara, & C. Castillo-Guevara. 2008. Antagonists and their effects in a hummingbird-plant interaction: field experiments. *EcoScience*.
- Waser, N. M., & J. A. McRoberts. 1998. Hummingbird foraging at experimental patches of flowers: evidence for weak risk-version. *J. Avian Biol.* 29: 305–313.
- Zar, J. H., 1999. *Biostatistical Analysis*. 4<sup>th</sup> ed. Prentice Hall Inc., Englewood Cliffs, New Jersey.