Hummingbird foraging preferences under natural and experimental conditions

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Background. Different studies have assessed hummingbirds' preferences for feeding resources, mainly according to floral characteristics such as shape, color, and morphology, in addition to the nectar concentration, quantity, and sugar composition of flowers visited. Flower preferences can also depend on hummingbirds' life history with respect to flower use. Hence, latitudinal migrant hummingbirds likely differ from resident species as they are accustomed to using a wider range of resources. In this study, we assessed the flower preferences of a migrant and a resident species that are common during winter in northern Mexico using both observational and experimental methods. **Methods.** We assessed hummingbird preferences for the most common plant species in the study region. In particular, we compared the preferences of two common hummingbird species, one resident (*Amazilia beryllina*) and one latitudinal migrant (*Selasphorus rufus*), for the most regionally common plant species, *Cestrum thyrsoideum* and *Salvia iodantha*, which have different color flowers yet produce similar energetic rewards. We calculated the Jacob selectivity index from preference data obtained under natural field conditions and with a flight cage in order to evaluate specific interactions. **Results:** Both hummingbird species showed different visitation rates to the studied plants under natural conditions in the study site. *A. beryllina* visited the yellow flowers of *C. thyrsoideum* more frequently, while *S. rufus* visited the fuchsia flowers of *S. iodantha* with greater frequency. In the flight cages, both species preferred the fuchsia flowers of *S. iodantha* when presented in similar or lesser abundance than the yellow flowers. **Discussion**. Under natural conditions, *A. beryllina* visited *C. thyrsoideum* to a greater extent in comparison with *S. iodantha* yet preferred *S. iodantha* in the flight cage when other hummingbirds were absent and even when *S. iodantha* was not the most abundant species. This could confirm that competition is an important process that drives the niche displacement of *A. beryllina*. On the other hand, the latitudinal migratory species *S. rufus* maintained its preferences for flowers of a familiar color under both natural and experimental conditions. **Conclusions.** Our results

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showed that the feeding preferences of the studied hummingbird species depend on the life history of each species. Therefore, the response of these migratory hummingbird species to feeding resources and possible exclusion from their preferred resources depends on their type of migration, territoriality, and possible associations with local plants.

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ABSTRACT

Background. Different studies have assessed hummingbirds' preferences for feeding resources, mainly according to floral characteristics such as shape, color, and morphology, in addition to the nectar concentration, quantity, and sugar composition of flowers visited. Flower preferences can also depend on hummingbirds' life history with respect to flower use. Hence, latitudinal migrant hummingbirds likely differ from resident species as they are accustomed to using a wider range of resources. In this study, we assessed the flower preferences of a migrant and a resident species that are common during winter in northern Mexico using both observational and experimental methods.

Methods. We assessed hummingbird preferences for the most common plant species in the study region. In particular, we compared the preferences of two common hummingbird species, one resident (*Amazilia beryllina*) and one latitudinal migrant (*Selasphorus rufus*), for the most regionally common plant species, *Cestrum thyrsoideum* and *Salvia iodantha*, which have different color flowers yet produce similar energetic rewards. We calculated the Jacob selectivity index from preference data obtained under natural field conditions and with a flight cage in order to evaluate specific interactions.

Results: Both hummingbird species showed different visitation rates to the studied plants under natural conditions in the study site. *A. beryllina* visited the yellow flowers of *C. thyrsoideum* more frequently, while *S. rufus* visited the fuchsia flowers of *S. iodantha* with greater frequency. In the flight cages, both species preferred the fuchsia flowers of *S. iodantha* when presented in similar or lesser abundance than the yellow flowers.

Discussion. Under natural conditions, *A. beryllina* visited *C. thyrsoideum* to a greater extent in comparison with *S. iodantha* yet preferred *S. iodantha* in the flight cage when other hummingbirds were absent and even when *S. iodantha* was not the most abundant species. This could confirm that competition is an important process that drives the niche displacement of *A.*

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beryllina. On the other hand, the latitudinal migratory species *S. rufus* maintained its preferences for flowers of a familiar color under both natural and experimental conditions.

Conclusions. Our results showed that the feeding preferences of the studied hummingbird species depend on the life history of each species. Therefore, the response of these migratory hummingbird species to feeding resources and possible exclusion from their preferred resources depends on their type of migration, territoriality, and possible associations with local plants.

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INTRODUCTION

Hummingbirds are small birds with a high metabolic rate and, consequently, are highly dependent on feeding resources (*del Hoyo et al. 1999*). Feeding resources are selected based on a series of non-exclusive factors, mainly morphological (i.e., flower color, shape, position, and length; *Stiles 1976; Melendez-Ackerman et al. 1997; Lara & Ornelas 2001; Temeles et al. 2009; Handelman & Kohn 2014; Maglianesi et al. 2015*), environmental (i.e., abundance and distribution of hummingbirds and flowers; *Stiles 1976; Abrahamczyk & Kessler 2010*), physiological (i.e., energetic requirements of hummingbirds; *Martínez del Rio 1990; Schondube & del Rio 2003; Medina-Tapia et al. 2012*), demographic (i.e., abundance of other coexisting hummingbirds; *Maglianesi et al. 2015*), and behavioral (i.e., hummingbird foraging strategies and dominance; *Stiles 1976;Stiles & Wolf 1970; Rodríguez-Flores & Arizmendi 2016*) in addition to the quality and quantity of rewards offered by plants (*Temeles et al. 2009; Temeles et al. 2006; González-Gómez et al. 2011*).

In addition, flower color is another factor that has been proposed as an attractant that induces visitation of hummingbirds (*Stiles 1976; McDade 1983; Paige & Whitham 1985; Melendez-Ackerman et al. 1997; Lunau et al. 2011; Shrestha et al. 2013; Handelman & Kohn 2014*). Flower color provides a visual stimulus that can be detected at a distance to attract hummingbirds to energy-rich nectar sources (*Stiles 1976; Melendez-Ackerman et al. 1997*). In this respect, flower color has been regarded to have coevolved with the visual system of pollinators (*Shrestha et al. 2013*). Fenster (*2004*) stated that flower color is one the most important features in pollination systems, wherein red flowers were reported as being pollinated the most by hummingbirds (*Stiles 1976; Stiles 1981*). In particular, the color red has been shown to attract hummingbirds and repel bees (*Tadey & Aizen 2001; Lunau et al. 2011; Muchhala et al.*

2014). One explanation rests in the fact that hummingbirds have tetrachromatic vision with photoreceptors sensitive to UV, blue, green, and red wavelengths, while bees have photoreceptors sensitive to UV, blue, and green wavelengths (*Lunau et al. 2011*).

Hummingbirds inhabiting the USA and Canada mainly feed on red flowers (*Healy & Hurly 2001*). Brown and Kodric-Brown (*1979*) showed that hummingbirds in Arizona pollinated plants with flowers that converged in shape, color, quantity, and quality of nectar produced, which reduced hummingbird selectivity and promoted high visitation rates. However, during winter migration, hummingbirds often face a wider array of flower colors, including non-typical ones (*Arizmendi & Ornelas 1990; Arizmendi 2001*). Lara et al. (*2009*) proposed that migrant hummingbird species might learn how to use novel resources, such as yellow flowers, following the lead of resident hummingbird species in their foraging bouts.

Hummingbird preferences for flower attributes have also been assessed experimentally, showing that hummingbirds prefer concentrated nectars (*Roberts 1996; López-Calleja et al. 1997*), conspicuous flowers (*Henderson et al. 2006b; Handelman & Kohn 2014*), and/or flowers that morphologically match their bill morphology (*Stiles 1976; Maglianesi et al. 2015*). The combination of experimental and natural studies can contribute toward a greater understanding of the ecological, physiological, and behavioral patterns that have been observed in studies on the community ecology of hummingbirds (*Stiles 1976; Maglianesi et al. 2015*).

We evaluated two hummingbird species' use of the most abundant plant species in our study region in northwestern Mexico. Specifically, we considered the preferences of two hummingbird species with different migratory status (a resident with seasonal altitudinal migrations, *Amazilia beryllina*, and a latitudinal migrant, *Selasphorus rufus*) for the plant species *Salvia iodantha* or *Cestrum thyrsoideum*, which had flowers of contrasting color (fuchsia and yellow, respectively). These plants represent the most abundant nectar resources during winter in the study region (*López-Segoviano 2012*), and this region also coincides with one of the main migratory pathways of Mexico (*Newton 2007*). The main purpose of this research was to assess the rewards offered by two flowering plants of contrasting color in order to determine both under natural and experimental conditions whether hummingbirds differentially use flowers and or exhibit preferences for certain flowers under isolation experiments. Differential flower use was expected for the two hummingbird species. The latitudinal migrant hummingbird was expected to prefer flowers similar to the ones found near their breeding grounds, i.e., reddish with tubular corollas, while the altitudinal migrant was expected to visit flowers according to their abundance in the region.

METHODS

Study Area

This experimental portion of this study was conducted in the ejido forestal El Palmito Concordia (23°34'16" N; 105°50'15"W), which forms part of the Sierra Madre Occidental in northwestern Mexico and has an altitudinal range of 1800 to 2200 masl. The present vegetation types include mostly pine, pine-oak, and oak forest in addition to some cloud forest and riparian vegetation (*Díaz 2005*).

Fourteen hummingbird species have been registered for El Palmito, five of which are considered residents (*Hylocharis leucotis, Lampornis clemenciae, Eugenes fulgens, Selasphorus platycercus*, and *Atthis heloisa*), four residents with altitudinal migrations (*Amazilia violiceps, A. beryllina, Cynanthus latirostris*, and *Colibri thalassinus*), and five latitudinal migrants (*Selasphorus rufus, S. sasin, S. calliope, Calypte costae*, and *Archilochus colubris*; *Howell &*

Webb 1995; López-Segoviano 2012). *Hylocharis leucotis* is the most abundant resident species, followed by the migrant *S. rufus* (latitudinal) and A. *beryllina* (altitudinal) (*López-Segoviano 2012*). Observations under natural conditions were carried out from November 2010 to January 2011 and under experimental conditions from November 2013 to February 2014, November 2014 to February 2015 and November 2015 to February 2016 during the fall and winter seasons.

Studied species

Selasphorus rufus J. F. Gmelin, 1788. Breeds in southern Alaska, western Canada, and northwestern USA and migrates to Mexico during winter (*Arizmendi & Berlanga 2014*). Small size (8.5 cm total length and 2.9–3.9 g; *del Hoyo et al. 1999*). Migrates up to 4000 km from breeding to wintering sites (*Phillips 1875; Calder 2004; Schondube et al. 2004; Healy & Calder 2006*). The migratory pathway of *S. rufus* follows the flowering season of its preferred plants (*Healy & Calder 2006*). However, in Mexico, little is known about the plants and migratory routes that this species uses (*Schondube et al. 2004*).

Amazilia beryllina W. Deppe, 1830. Distributed from southern Arizona throughout mountainous western Mexico (Sierra Madre Occidental, Eje Neovolcánico, Sierra Madre del Sur, and Sierra Madre de Chiapas; *Arizmendi & Berlanga 2014*) from 500 to 1500 masl and up to 3000 masl (*del Hoyo et al. 1999*). An altitudinal migrant that follows the flowering of plant resources (*Des Ganges 1979; Arizmendi 2001*).

Salvia iodantha Fernald 1900. Perennial shrub (height = 50 cm to 3 m) of the Lamiaceae family bearing pink, purple, or fuchsia flowers (*Cornejo-Tenorio & Ibarra-Manríquez 2011*). This plant species has been studied in Manantlán where it is visited by hummingbirds and bumblebees; the latter are nectar robbers (*Méndez Solís 2012*).

Cestrum thyrsoideum Kunth 1818. Perennial shrub (height = 50 cm to 3 m) of the Solanaceae family bearing white or yellow flowers (*Biblioteca Digital de la Medicina Tradicional Mexicana 2009*). In Sinaloa, it is reportedly visited by hummingbirds, mainly *H. leucotis* and *A. beryllina* (*Bribiesca 2012; López-Segoviano 2012*).

Nectar evaluation

To assess nectar quantity and quality, we used the standing crop procedure in which 20 flowers per plant were taken at random during each assessment period between 7:30 and 16 hrs (*Kearns & Inouye 1993*). The standing crop was used to assess nectar availability under natural conditions. Nectar was extracted using microcapillary tubes. After measuring the amount of nectar produced, a drop of nectar was placed in a handheld refractometer (ATAGO model N-1EBX rage 0-32 brix) to measure its sugar concentration. Using nectar quantity and sugar concentration, we calculated the calories produced per flower using the procedure described by Stiles (*1975*). Nectar was extracted from 2235 flowers of *C. thyrsoideum* and 7940 flowers of *S. iodantha* during the winter season (2010-2011).

Natural flower use

To determine flower use by hummingbirds under natural conditions, foraging observations were performed from 21 November 2010 to 11 January 2011 in flower patches of *S. iodantha* and *C. thyrsoideum*, which have been recorded as the most abundant plant species in the study region and overlap their flowering (*López-Segoviano 2012*). Other foraging plants were also observed and recorded, including 135 distinct floral patches of 8 different plant species in the region (*Salvia elegans*, *Cuphea hookeriana*, *C. calcarata*, *Loeselia mexicana*, *Castilleja tenuiflora*, and

Agave inaequidens barrancensis). Observations were conducted for a period of 60 minutes from an approximate distance of 8 m from the floral patch (*Cotton 1998*). For each foraging bout, hummingbird species, sex, time of arrival, number of flowers visited, and duration of visit were recorded.

Hummingbird preferences

A flight cage (4.5–3.9 m) was placed over a flower patch of *S. iodantha* and *C. thyrsoideum*, each bearing the same number of flowers (e.g., 50 open flowers per flowering plant). Then, one individual of each hummingbird species was introduced into the flight cage and observed for 30 minutes. The number of flowers of each plant species visited by the hummingbird was recorded. This was repeated using different individuals of each hummingbird species (*A. beryllina*, N=21; *S. rufus*, N=18).

A second experiment was subsequently performed, noting that both hummingbird species concentrated their foraging activities on *S. iodantha* under isolation conditions. In this experiment, we covered flower patches bearing double the number of flowers of the non-preferred plant species to test if the abovementioned preference was maintained even in distinct abundance conditions (e.g., 50 *S. iodantha* flowers and 100 *C. thyrsoideum* flowers). Hummingbird visits were registered for different individuals of *A. beryllina* (N=19) and *S. rufus* (N=35), using the same protocol described above.

Preference experiments were performed in the study region during the winter seasons (November–February) of 2013–2014, 2014–2015 and 2015–2016.

Statistical Analysis

To evaluate differences in nectar quality and quantity, we used a Mann-Whitney test after considering the normality and the homogeneity of variance of the data (Kolmogorov-Smirnov tests).

We used Jacob's index of selectivity (D_i) to evaluate and to compare the feeding resources (plant species) used by hummingbirds under natural conditions, expressed as $D_i=(r_i-p_i)/(r_i+p_i-2\,r_ip_i)$, where r_i is the proportion of flowers visited by each hummingbird species with respect to all flowers visited by this species and p_i is the proportion of resources represented by this species (*Jacobs 1974; Manly et al. 2002*). The index value (D_i) varies from 1 to -1, where positive values indicate a preference for a feeding item, negative values avoidance of the item, and a near zero value a lack of selectivity (Janeček et al. 2012). To determine resource availability in the region (p_i), we used the proportion of observed flower patches of each plant species with respect to all observed patches (Janeček et al. 2012). From the experimental data, we also calculated Jacob's index (D_i) (Jacobs 1974; Manly et al. 2002). In this case, we used total flowers available in each patch (p_i), and the selectivity index was calculated for each hummingbird evaluated in the flight cage. The resulting values were then analyzed using a Mann-Whitney test after evaluating the normality and the homogeneity of variance of the data.

RESULTS

Nectar

The volume of nectar produced by the evaluated plant species of *S. iodantha* and *C. thrysoideum* did not differ statistically (*Mann-Whitney* U=8732000.00, p=0.250; Fig. 1), although the sugar concentration of the nectar of *S. iodantha* flowers was higher (*Mann-Whitney* U=172500.00, $p<0.001$; Fig. 1). The calories produced per flower did not differ statistically between the species (U=8649000.00, p=0.068; Fig. 1).

Natural flower use

In the 135 flower patches observed, *A. beryllina* visited flowers of four plant species (*S. iodantha*, *C. thyrsoideum*, *Loeselia mexicana*, and *Agave inaequidens barrancensis*) out of the eight plant species observed. *Amazilia beryllina* concentrated its foraging activities on *S. iodantha* (46.1%) and *C. thyrsoideum* (45.6%). Meanwhile, *S. rufus* only visited flowers of two plant species (*S. iodantha* and *C. thyrsoideum*), visiting *S. iodantha* (87.4%) more frequently in comparison with *C. thyrsoideum* (12.6%).

According to Jacob's index (D_i), *S. rufus* preferred to a greater extent flower patches with fuchsia *S. iodantha* flowers (0.66) and avoided those with *C. thyrsoideum* flowers (-0.47; Fig. 2). On the other hand, *A. beryllina* preferred flower patches with yellow *C. thyrsoideum* flowers (0.34) and avoided those with *S. iodantha* flowers (-0.24; Fig. 2).

Preference experiments

When both plant species were presented with the same number of flowers in the flight cages, *S. rufus* preferred the fuchsia flowers of *S. iodantha* and avoided the yellow flowers of *C. thyrsoideum* (Fig. 3)*,* and *A. beryllina* followed the same pattern, preferring *S. iodantha* and avoiding *C. thyrsoideum* (Fig. 3). The Mann-Whitney test showed that these preferences were statistically significant for *S. rufus* (*Mann-Whitney* U=40.00, p<0.0001; Fig. 3) as well as for *A. beryllina* (*Mann-Whitney* U=85.00, p=0.0007; Fig. 3). Under isolation conditions, both hummingbird species preferred the fuchsia flowers of *S. iodantha*.

In the second experiment, when *C. thyrsoideum* was presented with twice the number of flowers of *S. iodantha*, the hummingbirds behaved similarly to that of the prior experiment (same number of flowers of both species), preferring the fuchsia flowers of *S. iodantha*. The statistical analysis showed that both *S. rufus* and *A. beryllina* individuals preferred to feed on fuchsia flowers and to avoid yellow flowers (*S. rufus Mann-Whitney U*=97.00, *p*<0.0001; *A. beryllina Mann-Whitney U*=44.00, *p*<0.0001; Fig. 4). Thus, both hummingbird species preferred *S. iodantha* flowers even when they were half as abundant as *C. thyrsoideum* flowers.

DISCUSSION

Nectar quantity

Our results show that both plant species offered a statistically similar quantity of nectar and calories per flower. However, *C. thyrsoideum* flowers had a lower sugar concentration than those of *S. iodantha* (24% and 29%; Fig. 2). Flowers pollinated by hummingbirds have a sugar concentration of around 20%, differing from those pollinated by bees, which usually have a sugar concentration between 70% and 80% (*Baker 1975*). Heinemann (*1992*) proposed that the nectar of flowers visited by hummingbirds typically has a sugar concentration of around 26%, which is ideal for hummingbirds as it maximizes the energetic reward. In this case, nectar viscosity may be a factor and may be considered as a proxy of time dedicated to extraction. Similarly, Stiles (*1976*) stated that of the native and the exotic flowers of California, hummingbirds preferred plants with the highest nectar flow over concentration. Also, he proposed that unlike experiments with feeders, where the concentration of sugars is the best measure of the quality of nectar, in the field, the volume of nectar produced could be the best measure of flower quality for ornithophilous species.

Natural and experimental preferences of *A. beryllina*

In this study, we found that the altitudinal migratory hummingbird *A. beryllina* visited the most abundant flowering plants species in the region, *S. iodantha* and *C. thyrsoideum*, as determined by López-Segoviano (*2012*) and Bribiesca (*2012*). Under natural conditions, *A. beryllina* preferred the yellow flowers of *C. thyrsoideum* and avoided the fuchsia flowers of *S. iodantha*. At the study site, *A. beryllina* has been observed to establish its territory in patches of *C. thyrsoideum*, excluding species such as *H. leucotis* and *S. rufus* (*Bribiesca 2012; López-Segoviano 2012*). These flower patches also represent the ones with the most flowers and calories produced (*López-Segoviano 2012*). *Amazilia beryllina* has been considered a territorial species (*Rodríguez-Flores & Arizmendi 2016*) that gains access to the best resources (*Stiles & Wolf 1970*). In this context, the feeding behavior of one hummingbird species can be strongly influenced by the feeding behavior of other hummingbird species, depending on the relative dominance and abundance of different hummingbird species in the region (*Sandlin 2000a*).

However, when preferences were tested experimentally, *A. beryllina* individuals preferred to feed on the fuchsia flowers of *S. iodantha*. Maglianesi et al. (*2015*) stated that a hummingbird may change its natural feeding preference when subjected to experimental conditions and attributed this to the aggressive interactions that may occur with other hummingbirds under field conditions. In this sense, feeding preferences can be strongly influenced by the dominance strategies and the abundances of other hummingbird species (*Temeles et al. 2006*). Thus, hummingbirds are capable of adjusting their foraging niches depending on the abundance of flowers, the abundance of other hummingbirds, and their dominance strategies (*Rodríguez-Flores & Arizmendi 2016*).

Furthermore, the nectar of *C. thyrsoideum* could contain some substances, like alkaloids, that repel hummingbirds. Within the Solanaceae family, many plants contain alkaloids in their fruits, leaves, stems, and even in their flowers (*Scott et al. 1957*). In fact, three plant species of the genus *Cestrum* have been reported as having an alkaloid in their leaves (*C. parqui, C. albotomentosum*, and *C. nocturnum*) and *C. parqui* in its flowers (*Scott et al. 1957*). In *Nicotiana attenuata*, the gene that produces the nicotine alkaloid was isolated, and the variation in the presence of nicotine among plants was shown to possibly have consequences on the pollinating behavior of hummingbirds (*Kessler et al. 2012*). In another study in South Africa, nicotine was added to artificial nectar, and the tolerance of nectarivorous *Pycnonotus tricolor*, *Cinnyris talatala*, and *Zosterops virens* to the alkaloid depended on the amount of nicotine and the sugar concentration of the nectar (*Lerch-Henning & Nicolson 2013*). Similarly, when a small amount of nicotine (10–25 µM) was added to nectar in artificial flowers, hummingbirds did not differentiate between flowers with and without nicotine, but when the amount of nicotine increased (50 µM), hummingbirds clearly rejected the flowers (*Kessler et al. 2012*). Therefore, future studies should be carried out to determine the non-energetic characteristics of nectar since these can influence the foraging behavior of hummingbirds and their feeding preferences.

Natural and experimental preferences of *S. rufus*

The latitudinal migratory hummingbird *S. rufus* preferred the fuchsia flowers of *S. iodantha* and discriminated the yellow flowers of *C. thyrsoideum*, confirming that it prefers to feed on a more familiar color resource. In this case, reddish flowers are more common in *S. rufus* breeding sites (*Brown & Kodric-Brown 1979*). However, this result could be a consequence of the feeding behavior dynamics of the regional hummingbird community. In fact, competitive pressures could be affecting the foraging decisions of these hummingbirds (*Sandlin 2000a; González-Gómez et al. 2011*) since the presence of a dominant hummingbird may influence the apparent preferences of subordinate hummingbirds (*Sandlin 2000b; Muchhala et al. 2014*). Accordingly, a hummingbird of low dominance can present an apparent preference for places with poorer resources (*Stiles 1976; Sandlin 2000b*). This dynamic could lead the subordinate *S. rufus* to forage in places with less energetic rewards in terms of amount of nectar per flower and number of flowers (*Des Ganges 1979; López-Segoviano 2012; Rodríguez-Flores & Arizmendi 2016*).

However, during the flight cage experiment with *S. rufus*, a preference for the fuchsia flowers of *S. iodantha* was confirmed, while this hummingbird discriminated against the yellow flowers of *C. thyrsoideum*. Thus, *S. rufus* likely fed on flowers of familiar color (*Brown & Kodric-Brown 1979; Ornelas & Lara 2015*), even when more yellow flowers were available. A study with *Mimulus aurantiacus*, a plant species with different flower colorations, showed that hummingbirds prefer to feed on flowers with stronger colors (orange-red) and discriminate flowers of opaque colors (yellow; *Heinemann 1992*). Similarly, *S. rufus* and *S. platycercus* prefer to visit red flowers (*Ipomopsis aggregata*) rather than flowers of paler colors (*I. tubatenuis* and hybrids) when the morphology and rewards of these flowers are similar; however, this may change if pale flowers have a greater amount of nectar (*Melendez-Ackerman et al. 1997*). Healy and Hurly (*2001*) established that color plays a minor role in the flower preference of *S. rufus* but can influence the speed with which this hummingbird learns the location of flowers with greater rewards.

For a hummingbird with low dominance that requires energy to carry out its latitudinal migration, the ability to rapidly learn which resources are the best is essential. In fact, *S. rufus* has been proven to incorporate information learned from the environment in its feeding behavior (*Healy & Hurly 1995; Healy & Hurly 2001; Henderson et al. 2006a*). For example, *S. rufus* has been shown to remember the characteristics of a visited floral patch: its location, amount of rewards, and color of its flowers (*Healy & Hurly 1995; Healy & Hurly 2001; Hurly & Healy 2002; Healy & Hurly 2004; Henderson et al. 2006a*;). Hurly et al. (*2010*) suggested that *S. rufus* uses the intrinsic visual cues of a flower to confirm that they have arrived at the correct place and are able to relocate exactly where they fed the previous year. Remembering the location of a place with shrubs or groups of flowers from one season to another is of great importance for a long-lived animal in a habitat with short seasonal blooms (*Stiles 1976*). Thus, migratory birds incorporate information learned on their migratory route and are able to afterwards locate and remember the best places in terms of resources (*Newton 2007*). In the context of the present study, adult *S. rufus* individuals may be remembering that in the study region, *S. iodantha* flowers are the best feeding resource.

CONCLUSION

Our results showed that a hummingbird species changed its feeding preference when another hummingbird species was excluded from resources. Under natural conditions, foraging niches are shaped by preferences but are also balanced with the costs of dominance. Cage experiments using captive hummingbirds may not always be representative of preferences under natural conditions. Therefore, the combination of both experimental and observational studies allows us to better understand hummingbirds' feeding behavior (*Stiles 1976; Maglianesi et al. 2015*).

In particular, our study showed that *S. rufus* had a strong preference for feeding on *S. iodantha* flowers in both natural and experimental environments. *Salvia iodantha* has flowers of a more familiar color for this latitudinal migratory hummingbird. In addition, *S. iodantha* flowers could be the best food source for *S. rufus* for several reasons: 1) *Salvia iodantha* flowers are the most abundant resource in the region. 2) The phenology of *S. iodantha* correlates with the dates of *S. rufus* migration in the region (*López-Segoviano 2012*). Finally, 3) the largest and most dominant hummingbirds in the region were observed to mainly use floral patches of *A. inaequidens* and *C. thyrsoideum* (*López-Segoviano* 2012).

Our results showed that the feeding preference of the studied hummingbird species depended on the life history of each species. The response of these migratory hummingbird species to exclusion from preferred feeding resources will depend on the type of migration they perform, their territoriality, and their possible associations with local plants. Further studies are needed to better understand the feeding strategies of *Amazilia beryllina* and *Selasphorus rufus*.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

Gabriel López-Segoviano conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, and reviewed drafts of the paper.

María del Coro Arizmendi conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, and reviewed drafts of the paper.

REFERENCES

Abrahamczyk S, Kessler M. 2010. Hummingbird diversity, food niche characters, and assemblage composition along a latitudinal precipitation gradient in the Bolivian lowlands. *Journal of Ornithology* **151**:615-625 DOI 10.1007/s10336-010-0496-x.

Arizmendi MC. 2001. Multiple ecological interactions: Nectar robbers and hummingbirds in a highland forest in Mexico. *Canadian Journal of Zoology* **79**:997-1006 DOI 10.1139/z01-066.

Arizmendi MC, Berlanga H. 2014. *Colibríes de México y Norteamérica.* Tlalpan, DF, M: CONABIO.

Arizmendi MC, Ornelas JF. 1990. Hummingbirds and their floral resources in a tropical dry forest in Mexico. *Biotropica* **22**:172-180 DOI 10.2307/2388410.

Baker HG. 1975. Sugar Concentrations in Nectars from Hummingbird Flowers. *Biotropica* **7**:37-41 DOI 10.2307/2989798.

Biblioteca Digital de la Medicina Tradicional Mexicana. 2009. Chipiachi/*Cestrum thyrsoideum* Kunth. *Available at* *http://www.medicinatradicionalmexicana.unam.mx/monografia.php?l=3&t=&id=7225* (accessed on 02 August 2016).

Bribiesca R. 2012. Efecto de la abundancia floral y las interacciones intra y heteroespecíficas en el comportamiento territorial de *Hylocharis leucotis* Master's Universidad Nacional Autónoma de México.

Brown JH, Kodric-Brown A. 1979. Convergence, Competition, and Mimicry in a Temperate Community of Hummingbird-Pollinated Flowers. *Ecology* **60**:1022-1035 DOI 10.2307/1936870.

Calder WA. 2004. Rufous and Broad-Tailed Hummingbird. In: **Nabhan GP, Brusca RC, Van Devender TR, A. DM, eds.** *Conserving Migratory Pollinators and Nectar Corridors in Western North America.* Tucson, Arizona: The University of Arizona Press and The Arizona-Sonora Desert Museum, 204-224.

Cornejo-Tenorio G, Ibarra-Manríquez G. 2011. Diversidad y distribución del género Salvia (Lamiaceae) en Michoacán, México. *Revista Mexicana de Biodiversidad* **82** 1279-1296.

Cotton PA. 1998. Temporal partitioning of a floral resource by territorial hummingbirds. *Ibis* **140**:647-653 DOI 10.1111/j.1474-919X.1998.tb04710.x.

del Hoyo J, Elliot A, Sargatal J. 1999. *Barn-Owls to Hummingbirds*. Handbook of the Birds of the Worl. Barcelona: Lynx Edicions.

Des Ganges JL. 1979. Organization of a tropical nectar feeding bird guild in a variable environment. *The Living Bird* 17:199-236.

Díaz J. 2005. Tipos de vegetación y flora del ejido el Palmito, Concordia Sinaloa. Culiacan, Sinaloa, PRONATURA A.C. *Available at* *http://www.conabio.gob.mx/institucion/proyectos/resultados/VegetacionCQ014.pdf* (accessed on 02 August 2010).

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination Syndromes and Floral Specialization. *Annual Review of Ecology, Evolution, and Systematics* **35**:375-403 DOI 10.1146/annurev.ecolsys.34.011802.132347.

González-Gómez PL, Vásquez RA, Bozinovic F. 2011. Flexibility of Foraging Behavior in Hummingbirds: The Role of Energy Constraints and Cognitive Abilities. *The Auk* **128**:36-42 DOI 10.1525/auk.2011.10024.

Handelman C, Kohn JR. 2014. Hummingbird color preference within a natural hybrid population of Mimulus aurantiacus (Phrymaceae). *Plant Species Biology* **29**:65-72 DOI 10.1111/j.1442-1984.2012.00393.x.

Healy S, Calder WA. 2006. Rufous Hummingbird (*Selasphorus rufus*). *Available at* http://bna.birds.cornell.edu.bnaproxy.birds.cornell.edu/bna/species/053. (accessed on 02 February 2010).

Healy SD, Hurly TA. 1995. Spatial memory in rufous hummingbirds (*Selasphorus rufus*): A field test. *Animal Learning & Behavior* **23**:63-68 DOI 10.3758/bf03198016.

Healy SD, Hurly TA. 2001. Foraging and spatial learning in hummingbirds. In**: Chittka L, Thomson JD**, eds. *Cognitive Ecology of Pollination: Animal Behaviour and Floral Evolution.* Cambridge: Cambridge University Press.

Healy SD, Hurly TA. 2004. Spatial learning and memory in birds. *Brain Behavior Evolution* **63**:211-220 DOI 10.1159/000076782.

Heinemann D. 1992. Resource use, energetic profitability, and behavioral decisions in migrant rufous hummingbirds. *Oecologia* **90**:137-149 DOI 10.1007/BF00317819.

Henderson J, Hurly TA, Bateson M, Healy SD. 2006a. Timing in free-living rufous hummingbirds, *Selasphorus rufus*. *Current Biology* **16**:512-515 DOI 10.1016/j.cub.2006.01.054.

Henderson J, Hurly TA, Healy SD. 2006b. Spatial relational learning in rufous hummingbirds (*Selasphorus rufus*). *Animal Cognition* **9**:201-205 DOI 10.1007/s10071-006-0021-z.

Howell SNG, Webb S. 1995. *A guide to the birds of Mexico and northern Central America*. New York: Oxford University Press.

Hurly TA, Franz S, Healy SD. 2010. Do rufous hummingbirds (*Selasphorus rufus*) use visual beacons? *Animal Cognition* **13**:377-383 DOI 10.1007/s10071-009-0280-6.

Hurly TA, Healy SD. 2002. Cue learning by rufous hummingbirds (*Selasphorus rufus*). *Journal of Experimental Psychology: Animal Behavior Processes* **28**:209-223 DOI: 10.1037//0097-7403.28.2.209.

Jacobs J. 1974. Quantitative Measurement of Food Selection: A Modification of the Forage Ratio and Ivlev's Electivity Index. *Oecologia* **14**:413-417.

Janeček Š, Riegert J, Sedláček O, Bartoš M, Hořák D, Reif J, Padyšáková E, Fainová D, Antczak M, Pešata M, Mikeš V, Patáčová E, Altman J, Kantorová J, Hrázský Z, Brom J, Doležal J. 2012. Food selection by avian floral visitors: an important aspect of plant–flower visitor interactions in West Africa. *Biological Journal of the Linnean Society* **107**:355-367 DOI 10.1111/j.1095-8312.2012.01943.x.

Kearns CA, Inouye DW. 1993. *Techniques for pollination biologists*. Niwot, Colorado: University Press of Colorado.

Kessler D, Bhattacharya S, Diezel C, Rothe E, Gase K, Schottner M, Baldwin IT. 2012. Unpredictability of nectar nicotine promotes outcrossing by hummingbirds in *Nicotiana attenuata*. *The Plant Journal* **71**:529-538 DOI 10.1111/j.1365-313X.2012.05008.x.

Lara C, Gonzalez JM, Hudson R. 2009. Observational learning in the white-eared hummingbird (*Hylocharis leucotis*): experimental evidence**.** *Ethology* **115**:872-878 DOI 10.1111/j.1439-0310.2009.01668.x.

Lerch-Henning S, Nicolson SW. 2013. Bird pollinators differ in their tolerance of a nectar alkaloid. *Journal of Avian Biology* **44**:408-416 DOI 10.1111/j.1600-048X.2013.00079.x.

López-Calleja MV, Bozinovic F, Martinez del Rio C. 1997. Effects of sugar concentration on hummingbird feeding and energy use. *Comparative Biochemistry and Physiology Part A: Physiology* **118**:1291-1299 DOI http://dx.doi.org/10.1016/S0300-9629(97)00243-0.

López-Segoviano G. 2012. Comportamiento territorial y preferencias de forrajeo del colibrí migratorio *Selasphorus rufus* dentro de un sitio invernal Master's Universidad Nacional Autónoma de México.

Lunau K, Papiorek S, Eltz T, Sazima M. 2011. Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *Journal of Experimental Biology* **214**:1607-1612 DOI 10.1242/jeb.052688.

Maglianesi MA, Boehning-Gaese K, Schleuning M. 2015. Different foraging preferences of hummingbirds on artificial and natural flowers reveal mechanisms structuring plant-pollinator interactions. *Journal of Animal Ecology* **84**:655-664 DOI 10.1111/1365-2656.12319

Manly BFJ, McDonald LL, Thomas DL, McDonald TL, and Erickson WP. 2002. *Resource Selection by Animals: Statistical Design and Analysis for Field Studies.* Springer Netherlands.

Martínez del Rio C. 1990. Sugar Preferences in Hummingbirds: The Influence of Subtle Chemical Differences on Food Choice. *The Condor* **92**:1022-1030 DOI 10.2307/1368738.

McDade LA. 1983. Long-Tailed Hermit Hummingbird Visits to Inflorescence Color Morphs of Heliconia irrasa. *The Condor* **85**:360-364 DOI 10.2307/1367077.

Medina-Tapia N, Ayala-Berdon J, Morales-Perez L, Melo LM, Schondube JE. 2012. Do hummingbirds have a sweet-tooth? Gustatory sugar thresholds and sugar selection in the broad-billed hummingbird Cynanthus latirostris. *Comparative Biochemistry and Physiology Part A Molecular & Integrative Physiology* **161**:307- 314 DOI 10.1016/j.cbpa.2011.11.012.

Melendez-Ackerman E, Campbell DR, Waser NM. 1997. Hummingbird behavior and mechanisms of selection on flower colour in *Ipomopsis*. *Ecology* **78**:2532-2541 DOI 10.2307/2265912

Méndez Solís V. 2012. Intregraión floral fenotípica en plantas con sistemas de polinización especializados Master's. Universidad Nacional Autónoma de México.

Muchhala N, Johnsen S, Smith SD. 2014. Competition for hummingbird pollination shapes flower color variation in andean solanaceae. *Evolution* **68**:2275-2286 DOI 10.1111/evo.12441.

Newton I. 2007. *The Migration Ecology of Birds*. Oxford: Academic Press.

Ornelas JF, Lara C. 2015. Differential response to colour tasks on resident and migratory hummingbirds: a field test. *Ethology Ecology & Evolution 27*:357-378. DOI 10.1080/03949370.2014.948074.

Paige KN, Whitham TG. 1985. Individual and population shifts in flower color by scarlet gilia: a mechanism for pollinator tracking. *Science* **227**:315-317 DOI 10.1126/science.227.4684.315.

Phillips A. 1875. The migrations of Allen´s and other Hummingbird. *The Condor* **77**:196-205.

Roberts WM. 1996. Hummingbirds' nectar concentration preferences at low volume: the importance of time scale. *Animal Behaviour 52*:361-370 DOI http://dx.doi.org/10.1006/anbe.1996.0180

Rodríguez-Flores CI, Arizmendi MC. 2016. The dynamics of hummingbird dominance and foraging strategies during the winter season in a highland community in Western Mexico. *Journal of Zoology* **299**:262-274 DOI 10.1111/jzo.12360.

Sandlin EA. 2000a. Cue use affects resource subdivision among three coexisting hummingbird species. *Behavioral Ecology* **11**:550-559 DOI 10.1093/beheco/11.5.550.

Sandlin EA. 2000b. Foraging Information Affects the Nature of Competitive Interactions. *Oikos* **91**:18-28.

Scott WE, Ma RM, Schaffer PS, Fontaine TD. 1957. A survey of selected solanaceae for alkaloids. *Journal of the American Pharmaceutical Association* **46**:302-304 DOI 10.1002/jps.3030460511.

Schondube JE, Contreras-Martinez S, Ruan-Tejeda I, Calder WA, Santana C E. 2004. Migratory patterns of the rufous hummingbird in western Mexico. In: **Nabhan GP,** ed. *Conserving migratory pollinators and nectar corridors in western North America*. Tucson: University of Arizona Press, 80-95.

Schondube JE, del Rio CM. 2003. Concentration-dependent sugar preferences in nectar-feeding birds: mechanisms and consequences. *Functional Ecology* **17**:445-453.

Shrestha M, Dyer AG, Boyd-Gerny S, Wong BBM, Burd M. 2013. Shades of red: bird-pollinated flowers target the specific colour discrimination abilities of avian vision. *New Phytologist* **198**:301-310 DOI 10.1111/nph.12135.

Stiles FG. 1975. Ecology, Flowering Phenology, and Hummingbird Pollination of Some Costa Rican Heliconia Species. *Ecology* **56**:285-301 DOI 10.2307/1934961.

Stiles FG. 1976. Taste Preferences, Color Preferences, and Flower Choice in Hummingbirds. *The Condor* **78**:10-26 DOI 10.2307/1366912.

Stiles FG. 1981. Geographical Aspects of Bird-Flower Coevolution, with Particular Reference to Central America. *Annals of the Missouri Botanical Garden* **68**:323-351 DOI 10.2307/2398801.

Stiles FG, Wolf LL. 1970. Hummingbird Territoriality at a Tropical Flowering Tree. *The Auk* **87**:467-491DOI 10.2307/4083791.

Tadey M, Aizen MA. 2001. Why do flowers of a hummingbird-pollinated mistletoe face down? *Functional Ecology* **15**:782-790.

Temeles EJ, Koulouris CR, Sander SE, Kress WJ. 2009. Effect of flower shape and size on foraging performance and trade-offs in a tropical hummingbird. *Ecology* **90**:1147-1161.

Temeles EJ, Shaw KC, Kudla AU, Sander SE. 2006. Traplining by purple-throated carib hummingbirds: behavioral responses to competition and nectar availability. *Behavioral Ecology and Sociobiology* **61**:163-172 DOI 10.1007/s00265-006-0247-4.

Figure legends

Figure 1. Mean values (SE) for nectar A) volume, B) sugar concentration, and C) calories for *S. iodantha* and *C. thyrsoideum* flowers. The calorie content of *S. iodantha* and *C. thyrsoideum* flowers is similar.

Figure 2. Jacobs' selectivity indices (D) for the hummingbirds *A. beryllina* and *S. rufus* considering *S. iodantha* and *C. thyrsoideum* flowers in the field. The two species have different feeding preferences.

Figure 3. Mean values (SE) of the Jacobs' selectivity indices (D) for the hummingbirds *A. beryllina* and *S. rufus* considering a similar quantity of *S. iodantha* and *C. thyrsoideum* flowers under experimental conditions. There is a clear preference for the fuchsia flowers of *S. iodantha*.

Figure 4. Mean values (EE) of the Jacobs' selectivity indices (D) of the hummingbirds *A. beryllina* and *S. rufus* considering *S. iodantha* and *C. thyrsoideum* flowers when the latter is twice as abundant under experimental conditions. A preference is observed for the fuchsia flowers of *S. iodantha* by both species of hummingbirds.

Figure 1(on next page)

Figure 1.

Mean values (SE) for nectar A) volume, B) sugar concentration, and C) calories for *S. iodantha* and *C. thyrsoideum* flowers. The calorie content of *S. iodantha* and *C. thyrsoideum* flowers is similar.

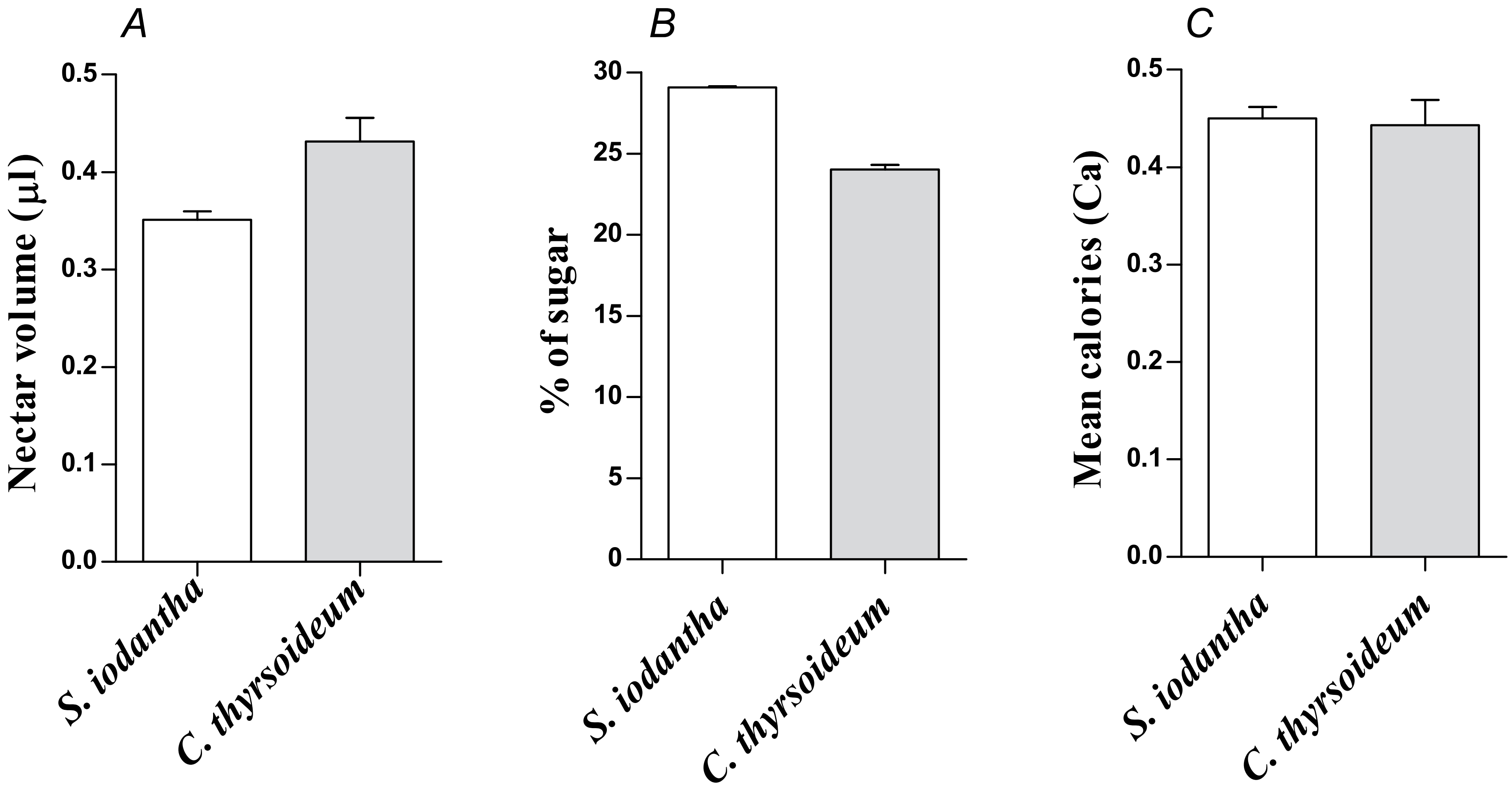
A
Nectar volume (µl)
0.5
0.4
0.3
0.2
0.1
0.0
S. iodantha
C. thyrsoideum
B
% of sugar
30
25
20
15
10
5
0
S. iodantha
C. thyrsoideum
C
Mean calories (Ca)
0.5
0.4
0.3
0.2
0.1
0.0
S. iodantha
C. thyrsoideum

Figure 2(on next page)

Figure 2.

Jacobs' selectivity indices (D) for the hummingbirds *A. beryllina* and *S. rufus* considering *S. iodantha* and *C. thyrsoideum* flowers in the field. The two species have different feeding preferences.

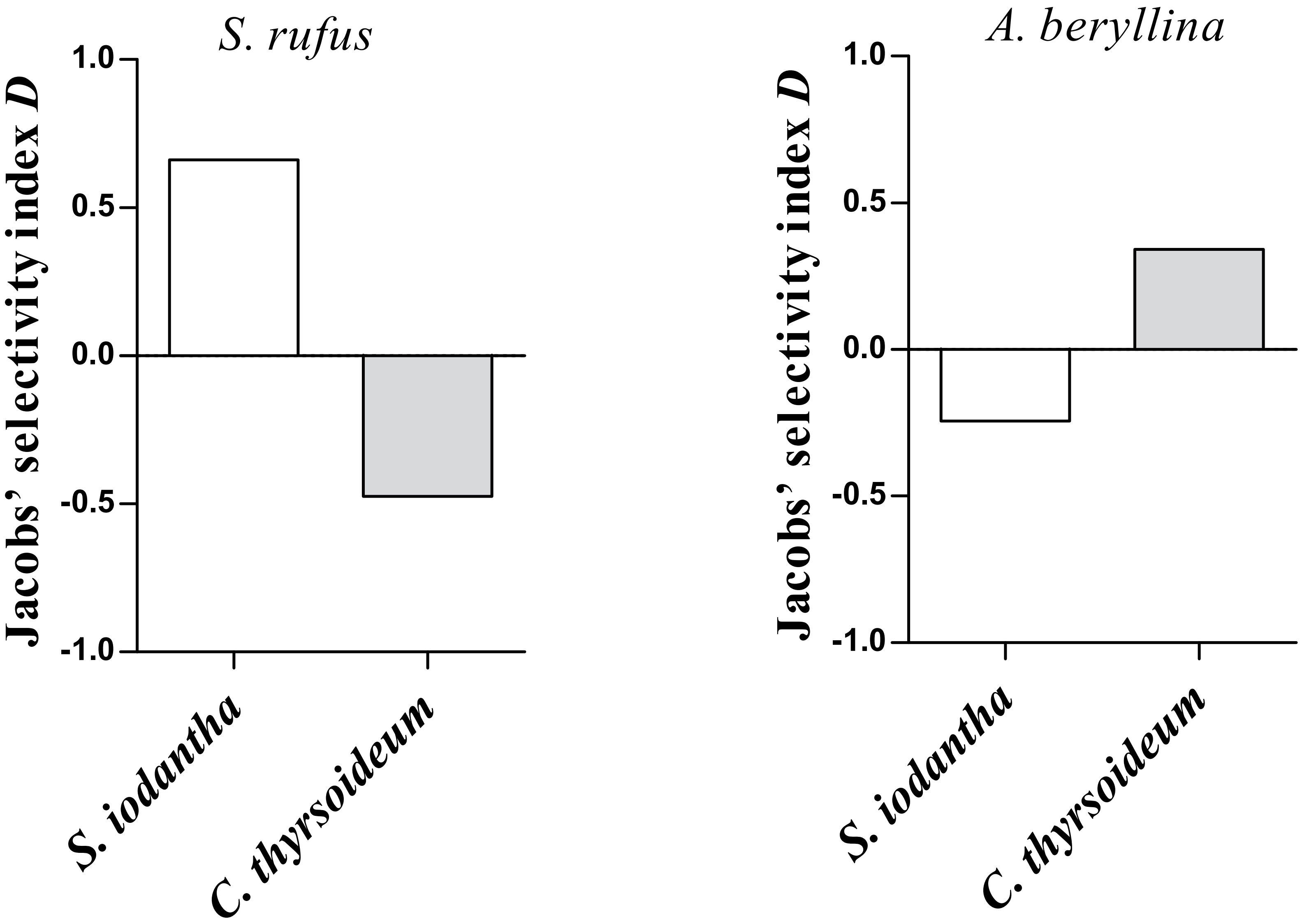

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Figure 3(on next page)

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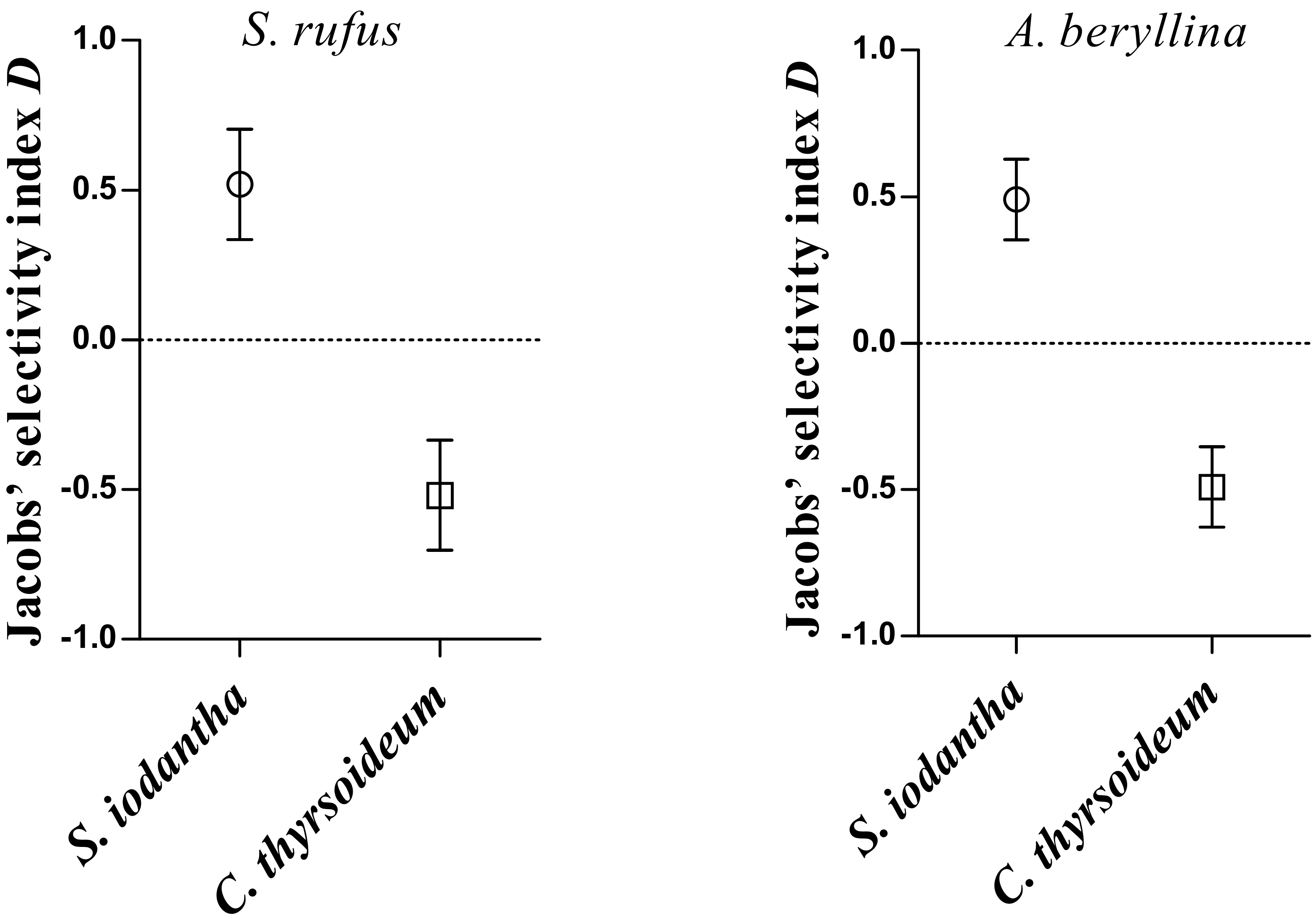

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Figure 4(on next page)

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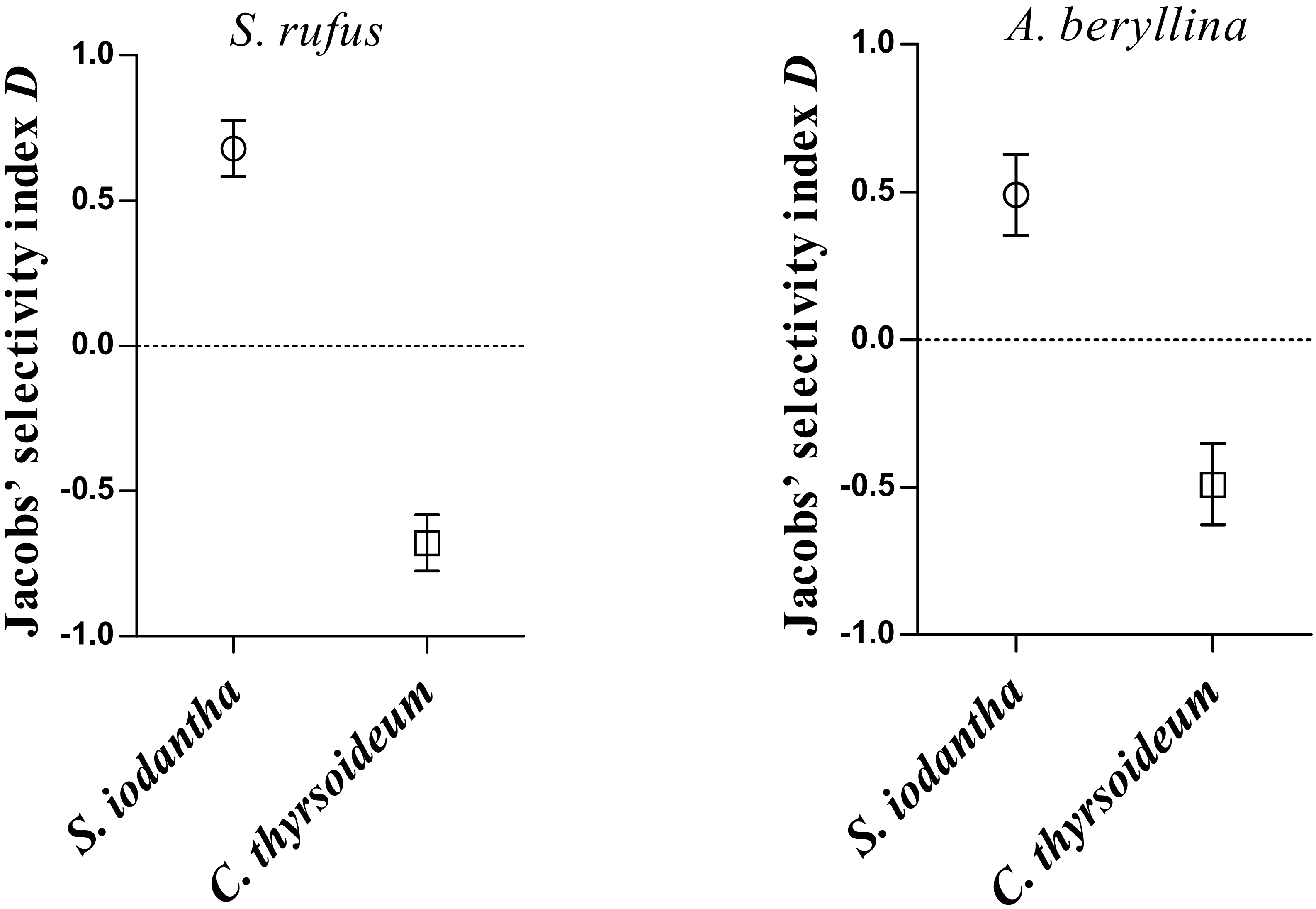

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