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AGRESIVIDAD Y COMPETENCIA POR REFUGIOS EN EL MURCIÉLAGO *KERIVOULA HARDWICKII*

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado en Biología para optar al grado y título de Maestría Académica en Biología

CHRISTIAN ANGELO CASTILLO SALAZAR

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DEDICATORIA

Dedicado a mi familia que siempre ha sido mi sostén, a mi mamá y mi hermano que siempre han creído en mí y con mucho amor siempre han apoyado todas las locuras de este sueño de ser biólogo. Dedicado a mi abuela y mi abuelo que me enseñaron y me criaron con amor y vieron cómo empecé este proceso, pero no pudieron ver cómo termina; gracias por todas las enseñanzas y cariño. Ustedes tuvieron mucha influencia de seguir mis sueños de niño de ser biólogo; sin mi familia no hubiera podido tener las fuerzas de terminar este camino. También a mi padre que a pesar todo, también influyó en mi deseo de ser biólogo y cuando pudo me apoyó siempre en este camino.

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Posgrado en Biología de la Universidad de Costa Rica,
como requisito parcial para optar al grado y título de Maestría
Académica en Biología"

Dr. Gilbert Alvarado Barboza
Representante del Decano del Sistema de Estudios de Posgrado

Dra. Gloriana Chaverri Echandi
Directora de Tesis

Dr. Michael Schöner
Lector

Dr. Gerardo Ávalos Rodríguez
Lector

M.Sc. Paula Iturralde Polit
Representante del Director del Programa de Posgrado en Biología

Cristian C.

Christian Ángelo Castillo Salazar
Candidato

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RESUMEN

En la competencia por recursos, generalmente se ha pensado que los animales con mayor tamaño y masa corporal tienen más probabilidades de ganar batallas. Sin embargo, recientemente se ha descubierto que las diferencias de comportamiento individuales juegan un papel importante en la determinación del ganador de una pelea durante la competencia. *Kerivoula hardwickii* es un murciélago solitario que habita en el sudeste asiático. Esta especie utiliza estructuras de plantas como refugio, las cuales están disponibles por periodos cortos de tiempo. Sin embargo, se desconoce si los individuos compiten activamente por los refugios, qué tipos de interacciones pueden ocurrir durante esta competencia, y qué factores pueden ayudar a determinar quién gana y se queda con el refugio.

En el primer capítulo determino si la masa corporal, el sexo y las diferencias interindividuales en el nivel de agresividad influyen en la competencia por refugios en *K. hardwickii*. Realicé experimentos de competencia de los refugios, donde puse a prueba si un murciélago tenía la capacidad de desalojar a otro de un refugio ya ocupado, y cuantificamos si el éxito del desalojo estaba relacionado con la masa corporal y los niveles de agresividad individuales. Encontramos que *K. hardwickii* tiene la capacidad de desalojar a otro individuo de un refugio ya ocupado, y que se presentan interacciones físicas agresivas en los encuentros durante la competencia por el refugio. También encontramos que los niveles individuales de agresividad influyen en el éxito del desalojo en los machos, mientras que la masa corporal no tuvo influencia. Esto sugiere que el comportamiento juega un papel importante en la competencia por los recursos en los murciélagos y presenta evidencia de que cuán agresivas pueden ser estas interacciones, las cuales todavía no se han documentado para la mayoría de los murciélagos.

En el segundo capítulo describo las señales acústicas durante la competencia por los refugios en *K. hardwickii* y evaluamos si los parámetros acústicos tenían influencia sobre la capacidad de los individuos para defender un refugio. Analizamos las vocalizaciones emitidas durante los experimentos para determinar qué parámetros acústicos influían en la defensa exitosa del refugio. Encontramos que los llamados emitidos por los machos pueden influir en su capacidad para defender el refugio y que la entropía es el parámetro espectral que más explica una defensa exitosa.

Los altos niveles de entropía sugieren que los encuentros entre individuos de *K. hardwickii* escalan a altos niveles de agresividad. Sugerimos que las vocalizaciones de los murciélagos contienen información importante sobre las características individuales, tanto morfológicas como de comportamiento, que ayudan a los murciélagos a tomar decisiones durante la competencia por los recursos.

ABSTRACT

In competition for resources, animals with greater size and body mass have generally been considered to be more likely to win battles. However, it has recently been discovered that individual behavioral differences play an important role in determining the winner of a fight during competition. *Kerivoula hardwickii* is a solitary bat that lives in Southeast Asia. This species uses plant structures as roosts, which are available for short periods of time. However, it is unknown whether individuals actively compete for roosts, what types of interactions may occur during this competition, and what factors may help determine who wins and retains the roost.

In the first chapter we determined whether body mass and interindividual differences in the level of aggressiveness influence competition for roosts in *K. hardwickii*. We conducted roost competition experiments, where we tested whether one bat had the ability to evict another from an already occupied roost and quantified whether eviction success was related to individual body mass and aggressiveness levels. We found that *K. hardwickii* can evict another individual from an already occupied roost and that aggressive physical interactions occurred in encounters during contests for roost access. We also found that individual levels of aggressiveness influenced eviction success in males, while body mass had no influence. This suggests that behavior plays an important role in competition for resources in bats and presents evidence for how aggressive these interactions can be, which has yet to be documented for most bats.

In the second chapter we describe acoustic signals emitted during roost competition in *K. hardwickii* and assess whether acoustic parameters influence individuals' ability to defend a roost. We analyzed the vocalizations emitted during the competition experiments and analyzed them to determine which acoustic parameters influenced successful roost defense. We found that the calls emitted by males can influence their ability to defend the roost and that entropy is the spectral parameter that most explains a successful defense. The high levels of entropy detected suggest that encounters between *K. hardwickii* individuals escalate to high levels of aggressiveness. We suggest that bat vocalizations contain important information about individual characteristics, both morphological and behavioral, that help bats make decisions during competition for resources.

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LISTA DE ABREVIATURAS

Capítulo/ Chapter	Abreviatura/Abbreviation	Descripción/Description
I & II	km	kilometros/ kilometers
I & II	UK	Reino Unido/ United Kingdom
I & II	Fig	Figura /Figure
I	W	Prueba Wilcoxon / Wilcoxon test
I & II	P	Valor de P / P value
I	t	t de student / t student
I	df	Grados de libertad / Degrees of freedom
I	s.d.	Desviación estandar /Standard deviation
II	Hz	Herz
II	ms	milisegundos / milliseconds
II	PCA	Analisis de componentes principales / Principal Component Analysis
II	Pf	Frecuencia pico / Peak frequency
II	Minf	Frecuencia Minima / Minimun frequency
II	Maxf	Frecuencia maxima / Maximum frequency
II	Bandw	Ancho de banda /Bandwidth
II	AIC	Criterio de Información de Akaike / Akaike Information Criterion
II	SE	Error estandar/ Standard Error

INTRODUCCIÓN GENERAL

Las diferencias interindividuales de comportamiento o rasgos de personalidad pueden considerarse una fuerza motriz en la evolución de las poblaciones, ya que la variación individual en comportamiento tiene relación con la supervivencia y éxito reproductivo de los individuos (Sgoifo et al., 2005). Estos rasgos se definen como las diferencias en un determinado comportamiento a través de múltiples contextos (Carere et al., 2013). Se han reportado diferencias a nivel de personalidad en varios aspectos del comportamiento para varios taxones y se ha comprobado su influencia en la sobrevivencia y éxito reproductivo de grupos como reptiles (Cote et al., 2008), peces (Wilson et al., 2010), aves (van Oers et al., 2008), y mamíferos (Réale et al., 2009).

Cuando existen interacciones competitivas entre individuos, los animales con comportamientos más audaces son los que presentan los comportamientos más ofensivos, pues toman decisiones más rápidamente y corren mayores riesgos en busca de la victoria, y aumentan su audacia entre más victorias logren obtener, mientras que los individuos con comportamientos tímidos aumentan su timidez mientras más derrotas obtengan, aunque tengan una ventaja defensiva al correr menos riesgos (Briffa et al., 2015). Esta experiencia positiva de obtener victorias puede aumentar la audacia reforzando las decisiones estratégicas al iniciar y ganar peleas, mientras que la experiencia negativa de perder puede reducir el grado de audacia de un individuo debido a los efectos fisiológicos de la derrota que impactan en el resultado conductual posterior (Frost et al., 2007).

La agresión es un comportamiento común en los animales y ocurre comúnmente entre los congéneres en relación con la competencia por recursos como el alimento, el territorio, o las oportunidades de apareamiento (Briffa et al., 2015). Los niveles de agresividad varían a menudo dentro de las poblaciones y se incrementan con la densidad poblacional o cuando un recurso se vuelve más escaso (Carere et al., 2013). La agresividad no es directamente observable, pero se deduce de observaciones de conductas antagonísticas expresadas hacia uno o más congéneres (Øverli et al., 2004), y la expresión de estos comportamientos antagonísticos específicos estará determinada en parte por las características del individuo oponente hacia el cual se dirige la agresión (Carere et al., 2005). Entre algunos de los comportamientos agonísticos observados se encuentran las señales acústicas y visuales, así como acciones tanto ofensivas y defensivas, las cuales pueden influir en la posibilidad de victoria (Carere et al., 2013).

El murciélago lanudo, *Kerivoula hardwickii*, es una especie que se distribuye en el sudeste asiático que pertenece a la familia Vespertilionidae (Schöner et al. 2017). Esta especie utiliza como refugio una gran variedad de plantas carnívoras del género *Nepenthes*, así como hojas tubulares de las familias Zingiberaceae, Musaceae y Araceae (Grafe et al., 2011), pues estas plantas ofrecen hábitats tubulares que son utilizados como refugios efímeros. Las hojas tubulares en desarrollo que utiliza *K. hardwicki* permanecen como un refugio adecuado durante periodos cortos, ya que los refugios de hojas tubulares se abren rápidamente (Vonhof & Fenton, 2004), en algunas plantas similares usadas por otros murciélagos se ha estimado una duración aproximada de 24 horas (Findley &Wilson, 1974). Por lo tanto, estos refugios se convierten en recursos escasos y pueden generan una alta competencia intraespecífica de interferencia (Grover et al., 1997). Esta competencia está vinculada a la territorialidad interespecífica y las interacciones agresivas entre las especies, las cuales entran en contacto regularmente, y las especies más agresivas obtienen acceso prioritario a los recursos, mientras que las especies menos agresivas son desplazadas de los recursos (Grether et al., 2013). Sin embargo, no se conoce qué mecanismos o comportamientos individuales estén mediando la competencia entre *K. hardwickii,* y si existe una relación entre diferencias de comportamiento y competencia por recursos. El objetivo del primer capítulo fue evaluar si las diferencias interindividuales de agresividad y condición corporal determinan el acceso a refugios en *K. hardwickii*. El objetivo principal del capítulo dos fue describir las señales acústicas que emiten los individuos de *K. hardwickii* cuando compiten por un refugio y evaluar si los parámetros acústicos de las vocalizaciones influyen en la capacidad de los individuos para defender un refugio.

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CAPITULO 1

AGGRESSIVENESS PREDICTS ACCESS TO ROOSTS IN THE SOLITARY WOOLLY BAT KERIVOULA HARDWICKII

Aggressiveness predicts access to roosts in the solitary woolly bat *Kerivoula hardwickii*

Cristian Castillo-Salazar[1], Michael G. Schöner[2], Caroline R. Schöner[2], Gloriana Chaverri[3,4]

Affiliations: 1 Escuela de Biología, Universidad de Costa Rica, San Pedro, Costa Rica; 2 Zoological Institute and Museum, University of Greifswald, Loitzer Straße 26, 17489, Greifswald, Germany; 3 Sede del Sur, Universidad de Costa Rica, Golfito, Costa Rica; 4 Smithsonian Tropical Research Institute, Balboa, Ancón, Panamá

Abstract

When competing for resources, animals with the largest size and body mass are the most likely to win battles. Individual behavioral differences, however, have recently been discovered to play a significant role in determining the winner of a contest. *Kerivoula hardwickii* is a solitary bat that roosts in plant structures that are limited in number due to their ephemerality. It is unknown whether individuals actively compete for roosts in this species, what types of interactions may occur during this competition, and what factors may influence who wins and gets the roost. The goal of our study was to see if body mass and inter-individual differences in aggressiveness influence roost competition in *K. hardwickii*. We conducted roost competition experiments in a flight cage to see if one bat could evict another from an already occupied roost and to see if eviction success was related to body mass and individual aggressiveness levels. We discovered that *K. hardwickii* can evict another individual from an occupied roost and that individual levels of aggressiveness influence male eviction success while body mass has no effect. This suggests that behavior plays an important role in resource competition in bats, which until now, has not been well documented.

Introduction

Aggression is a common behavior in animals, and typically occurs among conspecifics in competition for resources such as food, territory, or mating opportunities (Briffa et al., 2015).

Levels of aggressiveness often vary within populations and increase with population density or when a resource becomes scarcer (Carere et al., 2013). Aggression is typically not directly observable but is deduced from observations of specific antagonistic and agonistic behaviors expressed towards one or more congeners (Øverli et al., 2004), and the expression of these specific antagonistic behaviors will be determined in part by the characteristics of the individual towards whom the aggression is directed (Carere et al., 2005). Some of the agonistic behaviors observed are associated with the emission of acoustic and visual signals, as well as offensive and defensive actions, which can influence the possibility of victory (Carere et al., 2013).

When there are competitive interactions between animals, the individuals with the boldest behaviors are the ones presenting the most aggressive behaviors and taking greater risks in the pursuit of victory (Briffa et al., 2015). These more aggressive individuals typically are the ones that gain priority access to resources, while the least aggressive individuals are displaced from them (Grether et al., 2013). An individual's aggressive behavior can be influenced by the animal's health status and energy reserves (Edeline et al., 2006). The method most used by most ecologists to evaluate these health parameters is through the body condition index (Green, 2001), which can be defined as the energy capital accumulated in the individual's body as a result of feeding, which is assumed to be an indicator of health and the functional performance of an animal (Peig & Green, 2009).

While our understanding of competition for resources has typically been focused on food, animals also need sites that provide some form of protection from inclement weather and predators, such as roosts, and would also need to compete for them, especially since some species are very selective and potential roost sites may be scarce. Roosts are, in fact, one of the most important resources for bats, as they spend at least half their time in these structures, and the roost plays a critical role in key bat activities like hibernation, reproduction, mating, and protection against predators. However, roosts have rarely been analyzed as a resource for which bats compete (Salinas-Ramos et al., 2020). Several species of bats have specialized and temporary roosts (Sagot & Chaverri, 2015), and so individuals must invest much of their time in the search for new roosts, which means a great energy expenditure and exposure to predation (Speakman et al., 1994). Therefore, it can be inferred that the more limited and temporary a roost is, the greater the competition between individuals will be for this resource.

The woolly bat, *Kerivoula hardwickii*, is a species found in Southeast Asia that belongs to the Vespertilionidae family (Francis 2019). This species uses the pitchers of carnivorous plants of the genus *Nepenthes* and leaves of the families Zingiberaceae, Musaceae and Araceae as roosts (Grafe et al., 2011; McArthur 2012). *K. hardwickii* is one of the few species of bats that are considered solitary because they do not form social groups and roost solitary in the pitchers and leaves, except when the mothers are with their pups. The developing tubular leaves used by *K. hardwickii* for roosting remain suitable for short periods of time, as tubular leaf-roosts generally open rapidly (Vonhof & Fenton, 2004). Therefore, these roosts are relatively scarce resources and thus it is expected that individuals should compete for access to them (Grover et al., 1997). However, it is not known what mechanisms or individual behaviors are mediating competition between *K. hardwickii*, and whether there is a relationship between differences in behavior and competition for resources. Therefore, the main goal of this study is to evaluate whether inter-individual differences in the level of aggression and body condition determine access to roosts in *K. hardwickii*. Our prediction is that individuals with a higher body condition index and higher aggressiveness will have greater access to roosts.

Methods

Study Site

The study was conducted in Gunung Mulu National Park, located in Sarawak, Malaysia, from December 2017 to March 2018. This park has an extension of 544 km^2 with altitudes that range from 50 to 2376 masl. It is characterized by having a wide variety of vegetation types with a large coverage of primary forest and abundance of karst areas (Proctor et al., 1983). Gunung Mulu was declared a World Heritage Site by UNESCO in 2000 due to its high biological and geomorphological diversity (UNESCO 2007). Research permits were granted by the Sarawak Forestry Department Kuching, Sarawak, Malaysia (research permit no. NPW.907.4.4(JLD.14)-189 and park permit no. WL91/2017).

Bat Sampling

Individuals were searched and captured in their roosts, the developing furled leaves of the plant families Zingiberaceae, Musaceae, and Araceae. Once a roost was located, we captured bats inside the furled leaf and placed them inside a cloth holding bag. For all the bats captured, we sexed and determined age as adult or juvenile based on the level of ossification of the union of the epiphysis in the phalanges. We classified the reproductive status of the adult individuals as active if the males had scrotal testis and if the females were pregnant or lactating. For individual recognition, all adult males and non-reproductive females were tagged with passive integrated transponders (ISO 11784/11785; Peddy-Mark, UK).

For all bats captured, we estimated the body condition index (BCI). The BCI is defined as the capital of energy accumulated in the individual's body, because of food. This index is an indicator of animal health, and it is considered closely related to fitness (Peig & Green, 2009). To determine the BCI of individuals, we used body mass as the value of the index; it has been shown that body mass is the most efficient way to estimate body condition in bats (McGuire et al., 2018). The mass of all the bats was measured using a balance.

Experiments

For the field experiments, a 3x3 m flight cage equipped with two video cameras (Sony HDR-CX560VE) was used. Juveniles, pregnant and lactating females were excluded from the experiments. All individuals selected for the experiments were returned to their original capture site in less than 24 hours. Water and insects, as a source of food, were provided *ad libitum* to individuals during and after the experiments.

Index of aggressiveness: To determine whether there are inter-individual differences in levels of aggressiveness in *K. hardwickii*, an index of aggressiveness per individual was calculated. For this, each individual was recorded for 1 min on video, where a soft object was placed near its mouth and the number of bites and their duration were quantified. Before the test, we waited 1 or 2 minutes for the bat to calm down if it was very excited. The amount of time the individual spent biting during 1 min was used to estimate the aggressiveness index, where 60 seconds corresponds to the maximum duration of bites and thus the highest level of aggressiveness, and 0 represents an individual that never bit the soft object and thus had the minimum level of aggressiveness. Only one video test was made per individual.

Competition for roosts: To determine if an individual can evict another from an already occupied roost, an experiment was carried out inside the flight cage in which a furled leaf of *Alpinia* sp. (Zingiberaceae) was placed. First, a bat was released inside the flight cage until it entered and settled within the roost; this bat was called the “owner”. Subsequently, a second bat was released (called the “intruder”) and all interactions between the two individuals were recorded. The cameras were placed as close as possible to the leaf to record all the interactions that occurred inside the roost between the owner and intruder. Another camera was placed over the opening of the leaf to determine if the individuals had physical contact during their interactions.

A successful eviction was defined as instances when the intruder managed to evict the owner completely from the roost and did not allow the owner to return to the roost. A successful defense of the roost was defined as when the owner bat was not evicted from the roost by the intruder within our experimental period of 30 min. Experiments were conducted only among same-sex individuals to avoid interactions between males and females that could not be directly associated with competition for roosts.

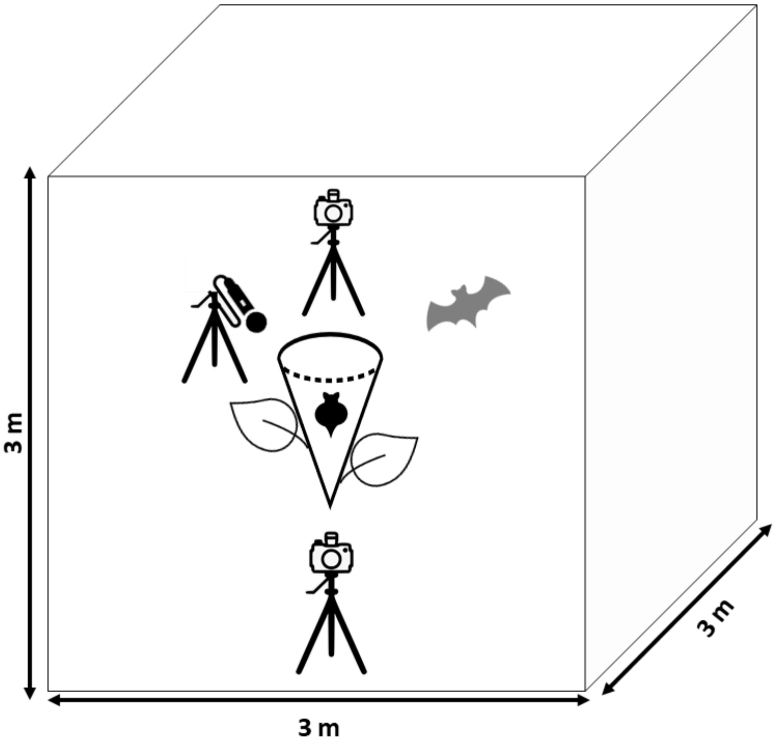

Figure 1. Schematic diagram of the eviction experiment. The owner bat is shown in black inside the furled leaf and the intruder bat is outside in grey.

Statistical analysis

A t-test was used to determine if there were differences in body condition between males and females. The Wilcoxon-Mann-Whitney test was used to determine if there were differences in aggressiveness between males and females. Logistic regressions were performed to determine the effect of the difference in aggressiveness, defined as the difference in aggressiveness between intruder and owner (where larger values indicate a more aggressive intruder), on eviction success. We also performed logistic regressions to determine if the difference in aggressiveness increases the probability of recording bites, which occurred when one of the individuals performed the action of biting the other during the experiment. Subsequently, the data sets were separated according to sex, and logistic regressions to determine if there is a differentiated effect according to sex. All statistical analyzes and graphs were performed with R software (R Core Team, 2021).

Results

We determined the body condition and aggressiveness index for a total of 55 bats, 31 females and 24 males. The body condition index data followed a normal distribution (W = 0.9808, P = 0.5241), while the aggressiveness index data did not (W = 0.9287, P = 0.0029). Significant differences in body condition were found between males and females (t = -4.6314, df = 52, $P < 0.05$), where females presented a higher average value of body condition (mean = 4.38, s.d. = 0.52) than males (mean = 3.78, s.d. = 0.43). No significant differences in aggressiveness were found between males and females (W = 292, $P > 0.05$). We also found a non-significant relationship between aggressiveness and body condition for males and females combined (R^2= 0.0087, $F_{1,53}$=0.47, P= 0.49). However, when analyzed separately, males exhibited less aggressiveness as their body condition increased (p = 0.04, Figure 2). Females did not show a significant correlation between aggressiveness and body condition (p = 0.54, Figure 2).

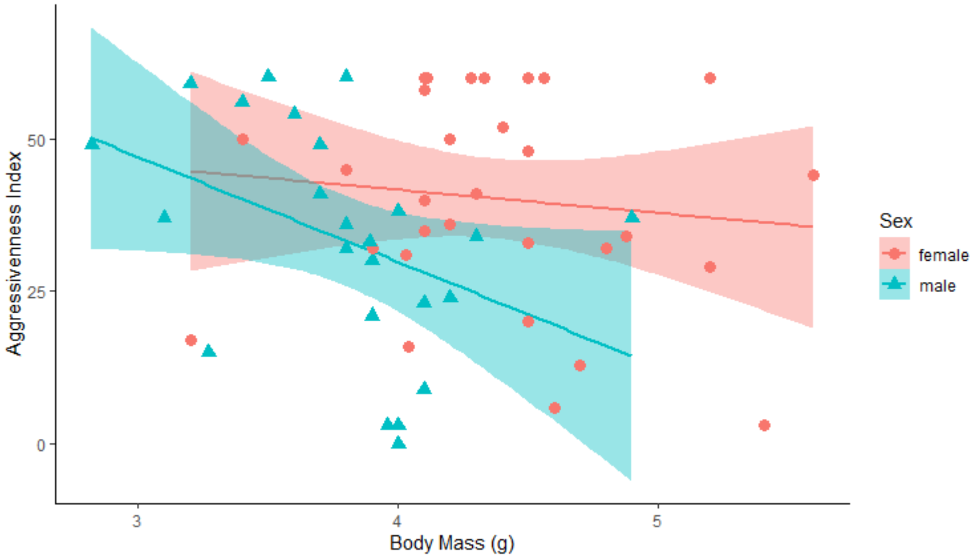

Figure 2. Relationship between the aggressiveness index and body condition (i.e., body mass).

When males compete for access to roosts, we found a general trend that eviction success increased when the intruder was increasingly more aggressive than the owner (p = 0.07, Figure 3, Table 1); this was not the case when females were competing. We found that eviction was not related to the difference in body condition between the intruder and owner (Table 1). We also found a trend between the number of bites and the level of aggressiveness in males; when male intruders were smaller, the probability of observing bites increased (p = 0.08, Figure 3, Table 1). We found no relationship between the difference in body condition between intruder and owner and the probability of eviction or the presence of bites (Table 1).

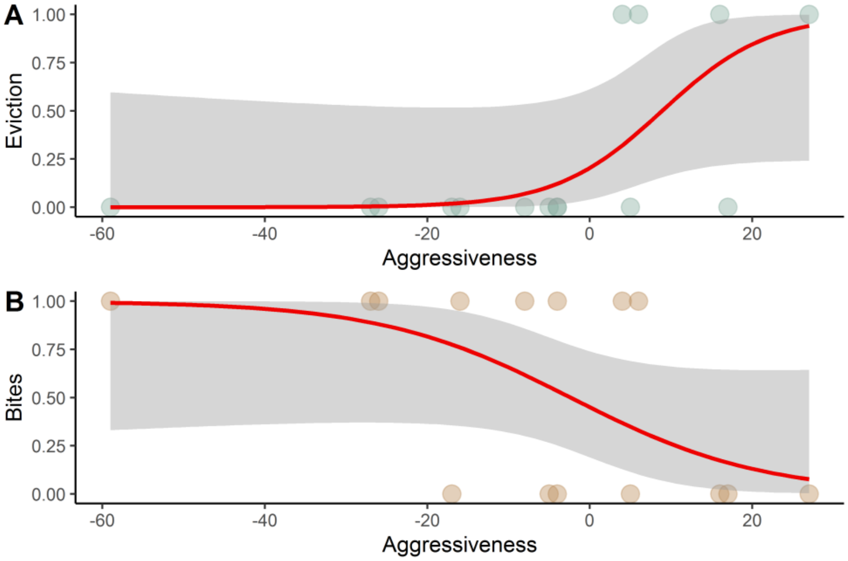

Figure 3. Logistic regressions showing the effect of the difference in aggressiveness between intruder and owner on eviction and number of bites for males only. Aggressiveness is calculated as the difference between the aggressiveness of the intruder minus the aggressiveness of the owner (larger values indicate a more aggressive intruder).

Table 1. Results of the logistic regressions (response ~ predictor) during the eviction experiments. Significant, or near-significant, effects are shown in bold.

Logistic model	Sex	St.error	Wald Chi^2	P-value
Eviction ~ Aggressiveness	Both	0.76	4.45	**0.0349***
	Male	0.08	1.81	**0.0702***
	Female	0.83	0.11	0.7424
Eviction ~ Body Cond	Both	0.69	2.36	0.1243
	Male	1.17	0.02	0.8761
	Female	0.95	3.56	0.518
Bites ~ Aggressiveness	Both	0.66	2.57	0.109
	Male	1.3	3.12	**0.0774***
	Female	0.83	1.05	0.3058

Bites ~ Body Cond	Both	0.64	0.38	0.5371
	Male	1.11	1.65	0.1988
	Female	0.81	0.34	0.5624

Discussion

In this work we found evidence of how intraspecific difference in a behavioral trait, such as aggressiveness, influences competition for resources. In the case of our experiments, we were able to show that access to a roost seems to be mainly determined by differences in the level of aggressiveness between competitors, and not so much by differences in body size. In addition, we were able to experimentally demonstrate how a bat can evict another individual from a previously occupied roost and even engage in physical confrontations, which may decide the winner.

During the competition for resources, individuals with the greatest body mass often have the highest probability of winning the match and therefore secure the resource in dispute (Areja-Gavina et al., 2021). For example, in the Asian elephant (*Elephas maximus*), individuals with a larger body size are more likely to win a match (Chelliah & Sukumar, 2013). This relationship has also been documented in various taxa such as fish (Rowland, 1989; Howard et al., 1998; Bolund, Schielzeth & Forstmeier, 2007), birds (Hagelin, 2002), and arthropods (Alcock, 1994; Nakata & Goshima, 2003; Serrano-Meneses et al., 2007). However, the victory of the encounter is not always determined by the heaviest or strongest individual, and it may be often decided by personality traits such as aggressiveness (Camerlink et al., 2015).

In our study, we did not find that body size has an effect on competitive encounters in *K. hardwickii*, but rather that access to the roost seems to be determined primarily by individual differences in aggressiveness. *K. hardwickii* then follows the trend of some studies (Cain & Ketterson, 2012; Rosvall, 2008) showing that differences in individual aggressiveness are the determining factor in deciding the winner in encounters when competing for a resource. For example, in spiny fish (*Gasterosteus* sp.), individual variation in behavior was shown to affect the outcome of interspecific competition for resources (Webster et al., 2009).

Few studies have looked at how differences in one or more behavioral traits may determine who wins access to resources (Harris & Siefferman, 2014). The majority of research on this topic has been conducted on fish (Colléter & Brown, 2011; David et al., 2011; Webster et al., 2009), so information on the topic is still limited in other groups of vertebrates. These studies are even more scarce in bats, a mammalian order in which it is frequently extremely difficult to observe behaviors associated with competition, such as access to roosts. The few competition studies on bats have concentrated on species that compete through interference and food exploitation (Arlettaz et al., 2000; Razgour et al., 2011; Roeleke et al., 2018). Because it uses an ephemeral roost, is a solitary species, and is amenable to experiments in relatively small spaces, *K. hardwickii* is an ideal model for understanding these processes.

Our study contributes to recent ideas of how behavior can be the determining factor for access to resources by competition, and how individuals of the same species can vary significantly in behavioral traits associated with competition (Cote et al., 2008; Réale et al., 2009; Wilson et al., 2010). Although not much is yet known about competitive interactions for access to roosts and their relationship to behavioral differences, we show that selecting a system that meets some basic requirements can allow us to generate valuable information on the relationship between behavior and the fight for essential resources in taxonomic groups that are often difficult to study. We suggest that future studies on the subject should try to select further species roosting in ephemeral roosts and tolerating temporary conditions of captivity. In our study we selected a solitary species that we assume may be aggressive towards conspecifics, but other studies could perform similar analyses in social species. Moreover, next studies could focus on interspecific competition for a resource. The results of this and future projects on the subject will allow us to broaden our knowledge of an area that is relatively little studied and that nevertheless is of great interest to understand the ecology of bats and other mammals and the behavior patterns associated with access to vital resources.

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CAPITULO 2

THE ROLE OF VOCALIZATIONS IN AGONISTIC INTERACTIONS DURING COMPETITION FOR ROOSTS IN A SOLITARY BAT

The role of vocalizations in agonistic interactions during competition for roosts in a solitary bat

Cristian Castillo-Salazar[1], Michael G. Schöner[2], Caroline R. Schöner[2], Gloriana Chaverri[3,4]

Affiliations: 1 Escuela de Biología, Universidad de Costa Rica, San Pedro, Costa Rica; 2 Zoological Institute and Museum, University of Greifswald, Loitzer Straße 26, 17489, Greifswald, Germany; 3 Sede del Sur, Universidad de Costa Rica, Golfito, Costa Rica; 4 Smithsonian Tropical Research Institute, Balboa, Ancón, Panamá

Abstract

Inter- and intraspecific competition for resources is common between individuals which share ecological niches. To avoid physical confrontations, individuals can use various types of signals to demonstrate their dominance including vocalizations. *Kerivoula hardwickii* is a solitary bat species that lives in highly ephemeral plant structures, which are therefore a limited resource. So far, it is unknown if individuals of *K. hardwickii* use vocalizations during competitive encounters for roosts, and if the intention of these vocalizations can be recognized by the competitors. We hypothesized that the calls emitted during roost competition contain information that influences the ability of an individual to defend its roost. We conducted roost competition experiments in a flight cage, where there was an individual roost owner and an intruder who would attempt to evict the owner from the roost. All the vocalizations emitted during these encounters were recorded and analyzed to determine which acoustic parameters had an influence on the successful defense of the roost. We found that the calls emitted by males can influence their ability to defend the roost and that entropy is the parameter that most strongly explains a successful defense. High entropy suggests that encounters between individuals of *K. hardwickii* escalate to high levels of aggressiveness and answers the question whether calls influence an individual's capacity to defend a roost. We suggest that bat vocalizations contain important information about individual characteristics, that help bats make decisions during resource competition.

Introduction

Competition for limited resources in the animal kingdom can escalate to physical contests that can lead to injury, increased energy expenditure, or even death (Briffa M & Elwood, 2004). That is why most animals prefer to resolve conflicts through signals instead of escalating to physical contests (Briffa, 2015). These signals demonstrate an animal's fighting ability and may allow it to gain or maintain its access to resources without battles (Bradbury & Vehrencamp, 2011). Some signals employed during contests can be color displays (Takeuchi, 2017), gestures (Call & Tomasello, 2020), facial expressions (Camerlink et al., 2018), body postures (Issa & Edwards, 2006), or movements (Reddon & Balshine, 2019). There are also chemical signals such as the release of pheromones (Blaul & Ruther, 2012), tactile signals such as small physical contacts, and acoustic signals (Reichert & Gerhardt, 2013) that may be used during confrontations.

Acoustic signals are widely used for various activities related to competition or defense of resources (Reichert & Quinn, 2017). It has been documented that some types of calls may contain critical information about an individual's competitive abilities, such as body size, aggressiveness, and even dominance rank in social species (Bradbury & Vehrencamp, 2011). These signals may also provide clues about a competitor's defense potential or fighting motivation (Vannoni & McElligott, 2008). All this information provided by the vocal signals facilitates the ability of competitors to decide which individuals to engage in a fight directly or against which individuals not to fight (Ratcliffe et al., 2007).

For example, male green frogs *Rana clamitans* (Anura, Ranidae) obtain information about an intruder's body size from the frequency of its calls, so they alter their behavior according to the perceived size of intruders based on differences in the frequency spectra of acoustic signals (Bee et al., 2000). In free-ranging male baboons (*Papio cynocephalus ursinus*), the acoustic parameters of the calls they emit during interactions contain information about the male's competitive ability. Dominant males emit higher fundamental frequencies and longer calls, while males that decrease in dominance emit shorter calls (Fischer et al., 2004). This use of acoustic signals to obtain information about individuals during competition has also been reported in other groups of mammals such as hyenas (Mathevon et al. 2010), deer (Reby & McComb 2003; Vannoni & McElligott, 2008), and rock hyraxes (Koren & Geffen, 2009). However, relatively few studies have been carried out on bats on this subject.

Bats are an ideal model for the study of acoustic communication in the context of social interactions given that sounds represent a vital signaling mode in this large mammalian taxon (Gillam & Fenton, 2016). Social calls in bats, for example, are known to contain information that is used in a wide variety of social contexts (Fernandez & Knörnschild, 2017; Jahelková et al., 2008). In bats that form groups, social calls may facilitate the formation and maintenance of the groups (Chaverri & Gillam, 2016), while in solitary bats it has been documented that these calls are used to defend space and resources (Sun et al., 2021). Despite the latter, there are only a handful of studies that have addressed the role of social calls in solitary species and the contexts in which they are used, and particularly in the context of roost defense.

The woolly bat, *Kerivoula hardwickii* is a bat of the family Vespertilionidae found in Southeast Asia (Francis 2019). *K. hardwickii* is one of the few species of bats that are considered solitary because individuals do not join conspecifics while roosting. This species uses as a roost the pitchers of carnivorous plants of the genus *Nepenthes* and leaves of the families Zingiberaceae, Musaceae and Araceae (Grafe et al., 2011; McArthur 2012). The plant structures used by *K. hardwickii* have a limited roosting space and only remain suitable for short periods of time (Schöner et al. 2017), so these types of roosts are relatively scarce resources. When resources are scarce it is expected that individuals should compete for their access and defend them (Grover et al., 1997). However, it is not known what acoustic signals can be emitted when several individuals attempt to secure access to a common roost. We hypothesized that the calls emitted during roost competition contain information that influences the ability of an individual to defend its roost and that some acoustic parameters could have an influence in defining who successfully accesses or defends the roost. Therefore, the main goal of this study is to describe the acoustic signals emitted by individuals of *K. hardwickii* when they compete for a roost and to evaluate if the acoustic parameters of the calls have an influence on the ability of individuals to defend a roost.

Methods

Study Site

The study was conducted in Gunung Mulu National Park, located in Sarawak, Malaysia, from December 2017 to March 2018. This park has an extension of 544 km^2 with altitudes that range from 50 to 2,376 masl. Gunung Mulu was declared a World Heritage Site by UNESCO in 2000 and it's characterized by having a wide variety of vegetation types and abundance of karst areas (Proctor et al., 1983). Research permits were granted by the Sarawak Forestry Department Kuching, Sarawak, Malaysia (research permit no. NPW.907.4.4(JLD.14)- 189 and park permit no. WL91/2017).

Bat Sampling

Individuals were searched and captured in their roosts, the developing furled leaves of the plant families Zingiberaceae, Araceae, and Musaceae. We captured bats and placed them inside a cloth holding bag. For all the bats captured, we sexed and classified the reproductive status of the adult individuals. We determined age as adult or juvenile based on the level of ossification of the union of the epiphysis in the phalanges. For individual recognition, all adult males and non-reproductive females were marked with passive integrated transponders (ISO 11784/11785; Peddy-Mark, UK).

Experiment

For the field experiments, a 3x3 m flight cage equipped with two video cameras (Sony HDR-CX560VE) was used. For sound recording, an Avisoft UltraSoundGate116Hn (Avisoft Bioacoustics, Berlin, Germany) was used with an ultrasonic condenser microphone (CM16/CMPA, Avisoft Bioacoustics). Pregnant and lactating females were excluded from the experiments. All individuals selected for the experiments were returned to their original capture site in less than 24 hours. Water and insects, as a source of food, were provided *ad libitum* to individuals during and after the experiments.

To record the calls emitted during the interactions in which bats competed for a single roost, we conducted experiments inside a 3x3 m flight cage equipped with two video cameras (Sony HDR-CX560VE). First, a bat was released inside the flight cage until it entered and settled within the roost, a furled leaf of *Alpinia* sp. (Zingiberaceaea). The bat inside the roost was called the "owner".

Later, a second bat was released and all interactions between the two individuals were recorded. The flying bat was called the “intruder”. A successful defense of the roost was defined as when the owner bat was not evicted from the roost by the intruder within our experimental period of 30 min. A successful eviction was defined as instances when the intruder managed to evict the owner completely from the roost and did not allow the owner to return to the roost. The cameras and the microphone were placed as close as possible to the leaf to record all the interactions and calls that occurred inside the roost between the bats. Experiments were conducted only among same-sex individuals to avoid interactions between females and males that could not be directly associated with competition for roosts.

Acoustic analysis

Acoustic analyzes were performed with the Avisoft-SASLab Pro version 5.1 software (Avisoft Bioacoustics, Berlin, Germany). We generated spectrograms with an FFT length of 512 (frequency resolution = 1465 Hz) and 93.75% overlap (temporal resolution = 0.0427 ms). For each of the recorded calls, the acoustic parameters were measured in the maximum amplitude of the element and the mean spectrum of the entire element. The acoustic parameters duration, number of elements, element rate, peak frequency, minimum frequency, maximum frequency, bandwidth, and entropy, were estimated. The number of calls were counted and classified into categories according to the position of their tonal component (figure 1). We defined 5 categories, or types of calls, according to the position of its tonal component, where T1 corresponds to a call without a tonal component, T2 is a call with the tonal component at the beginning, T3 has a tonal component in the middle of the call, T4 is a call with the tonal component at the end, and T5 is a call which only consists of the tonal component. To prevent tonal components from altering the measurements of the acoustic parameters, we manually excluded them from the measured calls and all calls were measured as T1 type (Figure 1).

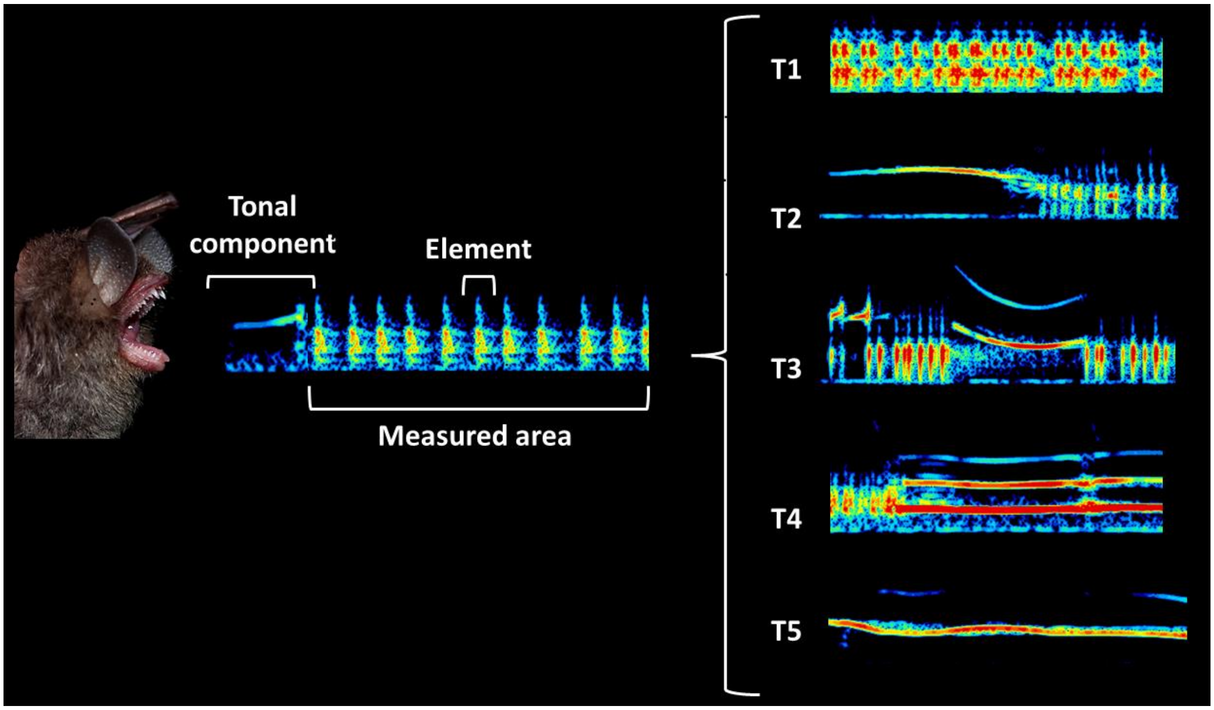

Figure 1. Classification of the calls according to the position of the tonal component emitted during the competition experiments for the roost in *K. hardwickii*.

Statistical analysis

To determine which factors can predict roost defense (i.e., when the owner bat was not evicted from the roost by the intruder within our experimental period), we run generalized mixed models with a binomial distribution. First, to reduce the dimensionality between acoustic parameters and remove correlations between independent variables, we conducted a principal component analysis (PCA). Then, the components that explained the greatest variability among the acoustic parameters, which had an eigenvalue > 1, were extracted and used as independent variables in the models. The model $Y(D) = m + S + P1 + P2 + P3 + P4 + I + O + error$ was used to determine which acoustic parameters of the calls could predict the successful defense of the refuge. Successful roost defense (D) was used as the dependent variable, Sex (S) and extracted components (P1, P2, P3, P4) as the independent variables, while Intruder ID (I) and Owner ID (O) were used as random variables.

The model $Y(D) = m + S + NC + NT + I + O + error$ was used to determine if the number of calls and the number of types of calls could predict the successful defense of the roost. The successful defense of the refuge (D) was used as the dependent variable, Sex (S), number of calls (NC) and number of types of calls (NT) as independent variables, while Intruder ID (I) and Owner ID (O) were used as the random variables. We performed the models for the complete data set first and then for the data separated by sex. The generalized mixed models were carried out with the lme4 package (Bates et al., 2015) and all statistical analysis were conducted in R v. 4.1.2 (R Core Development Team 2018).

Results

To calculate the acoustic parameters, 259 calls corresponding to 34 pairs of eviction experiments were analyzed, 31 experimental pairs were female and 24 pairs were male; 67 calls were emitted by female pairs, and 194 calls were emitted by male pairs. From the PCA analysis, 4 components were extracted with eigenvalues greater than 1. These 4 components explain 75.4% of the variance. The first component explains 29.2% of the variance and is related to the maximum frequency and bandwidth parameters. The second component explains 21.5% of the variance and is related to entropy. The third component explains 15.9% of the variance and is related to the peak frequency, while the fourth component explains 8.6% of the variance and is related to the duration of the call (Table 1).

Table 1. Call component loadings for the four components extracted of the PCA from the roost eviction experiments, with the percentages of variation explained by each component.

	Components			
Parameter	**PC1**	**PC2**	**PC3**	**PC4**
Duration	0.073327	0.050984	0.21927	**0.730061**
Element rate	0.062051	0.293912	-0.2927	0.17166
Pf_{max}	0.011846	0.289882	**0.505527**	-0.25248
$Minf_{max}$	0.181909	0.271237	0.396006	0.317312
$Maxf_{max}$	**-0.44993**	0.219253	0.13715	-0.04179
$Bandw_{max}$	**-0.48143**	0.058127	-0.0827	-0.19511
$Entropy_{max}$	-0.01189	**0.487469**	-0.34855	-0.11885
Pf_{mean}	0.087766	0.257223	**0.457404**	-0.34044
$Minf_{mean}$	0.203065	0.392958	-0.0615	-0.06676
$Maxf_{mean}$	**-0.48016**	0.068954	0.095332	0.187984
$Bandw_{mean}$	**-0.49263**	-0.00798	0.098966	0.191129
$Entropy_{mean}$	-0.03174	**0.488815**	-0.27359	0.164169

Variation$_{\%}$	29.287	21.573	15.914	8.638

Bold values indicate the highest loading associated with a particular component.

In the mixed model of both sexes (AIC = 47.589), which estimates the contribution of various acoustic parameters (based on the 4 principal components) to the defense of the roost, we found that sex, PC2 and PC3 are the variables that explain roost defense (Table 2). Therefore, the data were separated by sex to determine how acoustic parameters explained roost defense while removing the effect of sex. For females alone, we found that none of the acoustic parameters explained roost defense. For males, PC2 (i.e., entropy; Table 1) was significantly and positively associated with roost defense (Figure 2). The number and the types of calls did not seem to influence the defense of the roost (Table 2).

Table 2. Mixed generalized linear models between acoustic parameters and complexity of calls on successful roost defense.

Data	Model	Response	Predictor	Estimate	SE	Z	P
Acoustic Parameters	General	Defense	Intercept	-12.836	4.796	2.676	0.007
			Sex	34.872	6.635	5.256	**0.001**
			PC1	2.180	1.455	1.499	0.134
			PC2	5.943	1.905	3.120	**0.002**
			PC3	-3.719	1.885	-1.973	**0.048**
			PC4	0.948	2.151	0.441	0.660
	Females	Defense	Intercept	-12.140	4.891	-2.482	0.013
			PC1	0.889	4.169	0.213	0.831
			PC2	0.216	3.014	0.072	0.943
			PC3	-0.863	3.041	-0.284	0.777
			PC4	0.175	2.332	0.075	0.940
	Males	Defense	Intercept	22.879	5.150	4.443	0.000
			PC1	2.889	2.249	1.285	0.199
			PC2	6.560	2.498	2.626	**0.009**
			PC3	-3.857	2.233	-1.727	**0.084***
			PC4	0.268	3.430	0.078	0.938
Number and type of calls	General	Defense	Intercept	0.354	1.085	0.326	0.744
			Sex	0.615	0.893	0.689	0.491
			N Calls	0.151	0.089	1.687	0.092
			N types	-0.914	0.632	-1.446	0.148

Females	Defense	Intercept	0.112	1.513	0.074	0.941
		N Calls	0.232	0.226	1.027	0.304
		N types	-1.000	1.058	-0.946	0.344
Males	Defense	Intercept	13.662	10.249	1.333	0.183
		N Calls	0.567	0.378	1.499	0.134
		N types	-3.772	5.488	-0.687	0.492

* Indicates a slightly significant effect. Bold values indicate significant values of the models ($p < 0.05$).

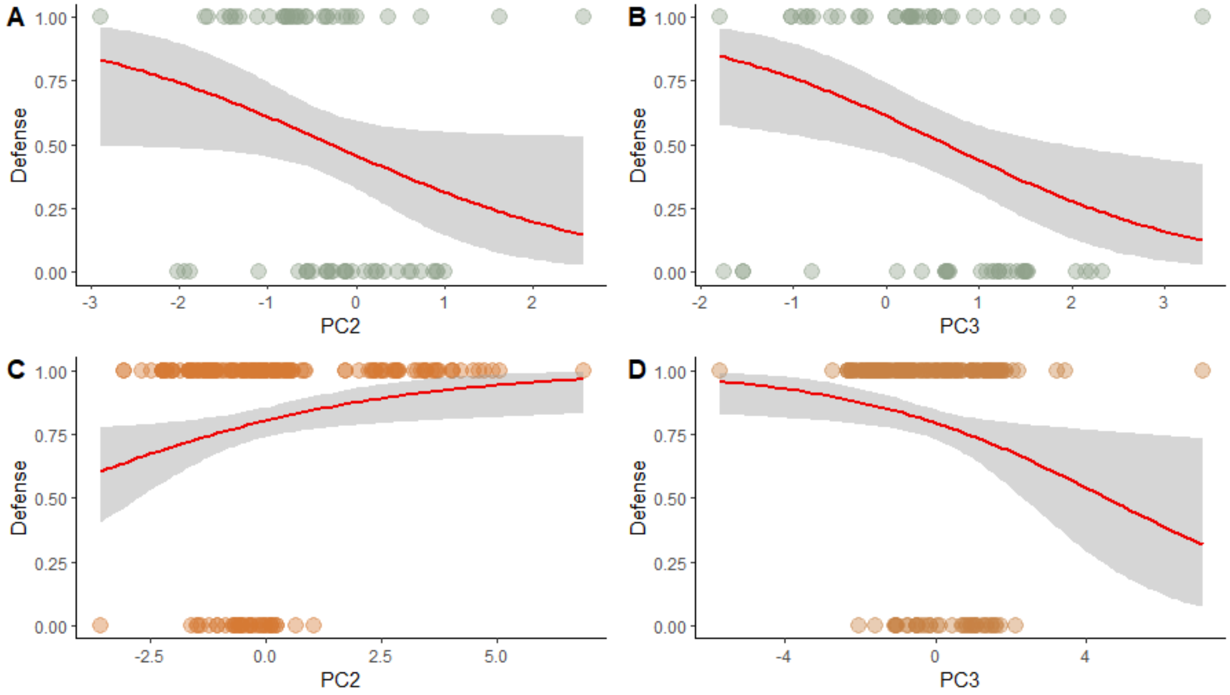

Figure 2. Mixed generalized linear models between acoustic parameters and successful roost defense (denoted by y-values = 1). Models A and B correspond to the effect of the variables PC2 and PC3 on the defense of the roost in experiments that only include females. Models C and D correspond to the effect of the variables PC2 and PC3 on the defense of roosts in male-only experiments.

Discussion

In this work we found that individuals of *K. hardwickii* can defend their roost and that the calls emitted by the males during their interactions may influence their ability to defend their roost sites. In addition, we found that entropy is the acoustic parameter that most strongly explains successful roost defense, supporting our hypothesis that calls emitted during roost competition contain information that may influence the result of competition for this resource in *K. hardwickii*.

The level of aggressiveness in the calls of several species has often been related to their frequency and complexity. For example, in the gray tree frog *Hyla versicolor*, males that won aggressive interactions tended to have lower-frequency aggressive calls than losers (Reichert & Gerhardt, 2013). In bats, it has been found that in the Great Himalayan leaf-nosed bat (*Hipposideros armiger*) the peak frequency of territorial calls indicates the dominance of the individual in the colony (Sun et. al, 2021). However, in *K. hardwickii* the parameters related to a call's frequency did not indicate roost defense; rather, it was the call's entropy that indicate roost defense. Basically, there was a greater probability of roost defense when the bat within the roost emitted calls with higher entropy.

Noisy, or high entropy, vocalizations have been documented to be related to the degree of arousal and alertness (Fitch et al. 2002). The latter states cause changes in physiology, such as increased respiration and heart rate, changes in the pressure of the glottis, and finally changes in the phonation of the call (Berry et al., 1996). The increase in pressure flow in the glottis generates calls with higher entropy and produce changes in the vocal structure of the individual (Goudbeek & Scherer, 2010; Lemasson et al., 2012). For example, it has been documented that in some primate species, such as Geoffroy's Spider Monkeys (*Ateles geoffroyi*) and rhesus macaques (*Macaca mulatta*), there is a transition from a low-entropy vocalization to a high-entropy vocalization when the aggression hanged from low severity to high severity (Fitch et al. 2002; Ordóñez-Gómez et al., 2015). It has also been documented that the Java sparrow (*Lonchura oryzivora*) produces similar calls in different situations of aggressiveness or affinity, but the entropy and sound pressure level were higher in aggressive calls than in affiliative calls (Furutani et al., 2018). This indicates that entropy increases considerably when the severity of the encounter is extremely high, and some research suggests that high-entropy vocalizations can produce negative responses in the mammalian auditory system and aim to discourage an opponent's aggressiveness (Gouzoules & Gouzoules, 2000). Documented calls with high entropy during encounters suggest that male *K. hardwickii* reach levels of high severity and aggressiveness during competitive interactions. As the entropy of the call emitted by the defending individual inside the roost increases, it is more likely that this individual will repel an eviction attempt.

Our study is, to the best of our knowledge, the first to describe aggressive competitive interactions in males of a strictly solitary bat. The few published studies on direct competition and aggressive interactions between bats have been carried out with species that live in colonies (Fernandez et al., 2014). In these groups, the bats have constant interactions with individuals of the colony, which generates dominance hierarchies in the group (Zhao et al., 2018). Calls emitted during aggressive interactions in these colonies have been shown to contain information about body size, dominance rank, and individual identity (Sun et al., 2021). In our case, *K. hardwickii* is a bat that roosts alone and does not form colonies. In addition, a previous study (C. Castillo-Salazar, unpublished data) showed that body mass did not influence aggressiveness or the eviction of individuals from roosts. Therefore, we suggest that the calls can have information about individual identity and levels of aggressiveness that help individuals to gauge each other's abilities during competition for roosting resources.

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