



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

Ecología

(PROYECTO)

**LOS RUGIDOS DEL MONO AULLADOR NEGRO (*ALOUATTA PIGRA*) SON SEÑALES
HONESTAS DEL TAMAÑO DEL CUERPO, PERO NO PRESENTAN CORRELACIÓN
NEGATIVA CON EL VOLUMEN TESTICULAR**

TESIS

(POR ARTÍCULO CIENTÍFICO)

**ACOUSTIC ALLOMETRY IN ROARS OF MALE BLACK HOWLER MONKEYS (*ALOUATTA
PIGRA*) BUT NO TRADE-OFF WITH TESTICULAR VOLUME**

QUE PARA OPTAR POR EL GRADO DE:

MAESTRO EN CIENCIAS BIOLÓGICAS

PRESENTA:

SAÚL DE LOS SANTOS MENDOZA

TUTOR PRINCIPAL DE TESIS: DR. ALEJANDRO ESTRADA MEDINA

INSTITUTO DE BIOLOGÍA, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

COMITÉ TUTOR: DR. ALEJANDRO RÍOS CHELÉN

CENTRO TLAXCALA DE BIOLOGÍA DE LA CONDUCTA, UNIVERSIDAD AUTÓNOMA DE TLAXCALA

DR. CARLOS RAFAEL CORDERO MACEDO

INSTITUTO DE ECOLOGÍA, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

TUTORA INVITADA: DRA. SARIE VAN BELLE

DEPARTMENT OF ANTHROPOLOGY, UNIVERSITY OF TEXAS

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Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **21 de agosto de 2023** se aprobó el siguiente jurado para el examen de grado de **MAESTRO EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de **ECOLOGÍA** del alumno **DE LOS SANTOS MENDOZA SAÚL** con número de cuenta **308173442** por la modalidad de graduación de **tesis por artículo científico** titulado: **“Acoustic Allometry in roars of male black howler monkeys (*Alouatta pigra*) but no trade-off with testicular volume”**, que es producto del proyecto realizado en la maestría que lleva por título: **“Los rugidos del mono aullador negro (*Alouatta pigra*) son señales honestas del tamaño del cuerpo, pero no presentan correlación negativa con el volumen testicular”**, ambos realizados bajo la dirección del **DR. JESUS ALEJANDRO ESTRADA MEDINA**, quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
“POR MI RAZA HABLARÁ EL ESPÍRITU”
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DR. ADOLFO GERARDO NAVARRO SIGÜENZA



c. c. p. Expediente del alumno
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Resumen en español

La teoría fuente-filtro sugiere que los rasgos de los animales, tales como el tamaño del cuerpo o edad, se encuentran codificados de manera confiable en sus vocalizaciones. Se piensa que estas señales vocales, con una probable utilidad previa a la cópula, son costosas. Debido a los límites energéticos en los animales, se espera que dichas señales se encuentren comprometidas con los rasgos de utilidad posterior a la cópula, como el volumen testicular. Aunque este compromiso evolutivo se ha puesto a prueba a través de estudios comparativos entre especies, pocos estudios han comprobado que se mantenga dentro de una única especie. Utilizando la técnica de fotogrametría de láser paralelo, realizamos un estudio de 9 meses en el Parque Nacional Palenque, México, para investigar si la frecuencia fundamental (F_0) o la dispersión de los formantes (ΔF) de los rugidos y ladridos de 14 machos de mono aullador negro (*Alouatta pigra*) codifican información del tamaño del cuerpo y si se encuentran comprometidos con su volumen testicular. Encontramos que solamente la ΔF de los rugidos está asociada con el tamaño del cuerpo, con machos más grandes produciendo menores ΔF , lo que sugiere un probable uso de los rugidos en la competencia entre machos o en la elección femenina. Por el contrario, ninguna asociación fue encontrada entre las características vocales y el volumen testicular luego de controlar por el efecto positivo del tamaño del cuerpo sobre el volumen testicular. Nuestros resultados muestran la presencia de alometría acústica en los rugidos de machos de mono aullador negro y sugieren la ausencia de un compromiso evolutivo dentro de una única especie, que previamente fue reportado a nivel de género.

Resumen en inglés

The source-filter theory suggests that animal traits, such as body size, are reliably encoded in vocalizations. These vocal signals, with a likely pre-copulatory function, are thought to be costly; given energetic constraints, they are expected to be in a trade-off with post-copulatory traits, such as testicular volume. Although this trade-off has been generally tested through comparative studies across species, it remains understudied if it holds within a single species. Using parallel-laser photogrammetry, we conducted a 9-month study at Palenque National Park, Mexico, to investigate whether fundamental frequency (F_0) or formant dispersion (ΔF) of roars and barks from 14 male black howler monkeys (*Alouatta pigra*) encode cues of body size, and if they are in a trade-off with testicular volume. We found that only roar ΔF is associated with body size, with larger males producing roars with lower ΔF , suggesting a likely use of roars in male-male competition or female mate choice. Similarly, only roar ΔF was associated with testicular volume, but contrary to our prediction, males producing roars with lower ΔF had larger testicles. This suggests they invest in both pre- and postcopulatory traits and might reflect males' inefficacy in undermining female mate choice.

Introducción general

La selección sexual es una de las principales fuerzas promotoras de la evolución de la comunicación acústica en los animales (Vehrencamp, 2000; Wilkins et al., 2013). Por ejemplo, las señales acústicas funcionan en la identificación de especies o del sexo, así como en la atracción de pareja. En algunas especies, las hembras muestran preferencia por ciertas características acústicas de las vocalizaciones de los machos, tales como la tasa de emisión de la vocalización o el tono (Simmons et al. 2010; 2011). Por otro lado, rivales conespecíficos pueden hacer uso de los despliegues acústicos para resolver conflictos antes de involucrarse en un enfrentamiento directo y así, evitar heridas potenciales (Brown et al., 2006). Por lo tanto, se asume que estas señales proveen información específica acerca del emisor, lo que puede incluir su edad, sexo, tamaño del cuerpo, habilidad para pelear o su estado de salud, características que el receptor puede interpretar como una pista y así actuar en consecuencia. Sin embargo, para que estas señales sean confiables, estas deben poseer alguna de las siguientes limitantes: 1) algún costo de desarrollo o producción (señal hándicap), 2) un gran costo de represalia cuando se señala un estado fuerte siendo en verdad débil (señal convencional), o 3) un límite físico o fisiológico que está funcionalmente enlazado con los atributos del emisor (señal índice) (Vehrencamp, 2000).

Las señales índice se han estudiado dentro del marco de la teoría “fuente-filtro” que establece una relación entre las características acústicas de las vocalizaciones y las estructuras anatómicas del emisor (Fitch 1997; Charlton et al., 2011). De acuerdo con esta teoría, las vocalizaciones son producidas a través de un proceso de dos pasos. Primero, un sonido “fuente” es producido en la glotis a partir de la apertura y cierre de las cuerdas vocales, cuya tasa determina la frecuencia fundamental (F_0) y el tono del sonido. Segundo, el sonido generado viaja a través de las cavidades supralaringeas, las cuales actúan como un “filtro”, amplificando o disminuyendo selectivamente los rangos de frecuencia conocidos como “formantes” (Fitch, 1997; Taylor & Reby, 2010). Por lo tanto, F_0 depende de la longitud y masa de las cuerdas vocales, las cuales se ven parcialmente afectadas por el tamaño del cuerpo, mientras que los formantes se encuentran limitados por la morfología del tracto vocal, que a su vez es determinada por los

huesos del cráneo, mismos que se encuentran estrechamente relacionados con el tamaño del cuerpo (Fitch 1997; 1999; Charrier et al., 2011). Consecuentemente, tanto la F0 como la dispersión de los formantes (la distancia media entre formantes; ΔF) podrían reflejar de manera confiable el tamaño del cuerpo. Esta correlación, conocida como “alometría acústica”, ha sido detectada en machos de múltiples especies, donde tanto la F0, ΔF o ambas características de sus vocalizaciones competitivas fueron correlacionadas negativamente con el tamaño del cuerpo. Esto es, a menor F0 o ΔF , mayor el tamaño del emisor (Bowling et al., 2017; Favaro et al., 2017; Fitch, 1997; Garcia et al., 2017).

Las vocalizaciones utilizadas en contextos competitivos confieren ventajas para ganar acceso a las hembras y, por lo tanto, funcionan previo a la cópula. En contraste, los rasgos con utilidad posterior a la cópula, como el volumen testicular, ayudan a asegurar la fertilización. Entre las especies, los machos usualmente están sesgados hacia uno de estos dos tipos de rasgos opuestos por dos razones principales. En primer lugar, el sistema de apareamiento principal de la especie puede favorecer la selección de rasgos con utilidad previa a la cópula cuando los machos tienen la capacidad de monopolizar el acceso a las hembras (poliginia), pero si por el contrario las hembras copulan con múltiples machos (poliginandria) los rasgos con utilidad posterior a la cópula pueden favorecerse (Lüpold et al. 2014; McCullough et al., 2018). En segundo lugar, el desarrollo y mantenimiento de los caracteres sexuales requiere una cantidad substancial de energía. Por esto, la inversión en rasgos con utilidad previa a la cópula puede prevenir el desarrollo de rasgos con utilidad posterior a la cópula y viceversa (Emlen et al., 2012; Simmons et al., 2017; Somjee et al., 2018). Este compromiso entre los caracteres con utilidad previa y posterior a la cópula ha sido confirmado en animales tan diversos como los acantocéfalos, anélidos, reptiles, aves, cetáceos, primates y pinnípedos (McCullough et al., 2018; Simmons & Fitzpatrick, 2012). Específicamente, un compromiso entre las señales vocales y las características de los testículos ha sido reportado para algunos mamíferos terrestres, aves e insectos (Charlton & Reby, 2016; Del Castillo & Gwynne, 2007; Simmons et al., 2011). Por ejemplo, los machos del ave avutarda hubara (*Chlamydotis undulata*) que se esfuerzan principalmente en la producción de extravagantes despliegues vocales

experimentan menor producción espermática comparados con machos de la misma especie que utilizan otras tácticas (Preston et al., 2011).

Los monos aulladores (*Alouatta spp.*) son considerados una de las especies de animales terrestres más ruidosas en el mundo. Poseen un aparato vocal especializado que incluye unas cuerdas vocales elongadas, sacos de aire, una laringe alargada, así como un hueso hioides alargado que funciona como una cámara resonante (Hilloowala, 1975; Schön, 1971). Aunque se han propuesto distintas hipótesis acerca de la función de sus vocalizaciones más fuertes (resumidas en Kitchen et al., 2015), estas probablemente juegan un papel en la competencia entre machos (Sekulic & Chivers, 1986). Esta hipótesis es apoyada por: (1) un tamaño del hueso hioides mayor en los machos que en las hembras (da Cunha et al., 2015); (2) la capacidad del hueso hioides para disminuir la ΔF (Dunn et al., 2015); (3) la capacidad de los machos de reconocer grupos vecinos a partir de sus vocalizaciones (Briseño-Jaramillo et al., 2014); (4) los machos adultos que poseen descendencia dentro de su grupo vocalizan más a menudo que machos sin descendencia (Van Belle et al., 2014a); y (5) el rol prominente de los episodios vocales durante los encuentros intergrupales, durando en promedio más tiempo que en cualquier otro contexto (Chiarello, 1995; Van Belle et al., 2013). Adicionalmente, un estudio de múltiples especies de monos aulladores reveló una relación positiva entre el tamaño del cuerpo y el volumen del hueso hioides, así como una correlación negativa entre el volumen del hueso hioides y la ΔF (Dunn et al., 2015). Sin embargo, hasta la fecha ningún estudio en especies de mono aullador ha evaluado de manera directa si la F_0 o la ΔF codifican de manera honesta el tamaño del cuerpo. Aunque no se ha demostrado si el tamaño del cuerpo confiere ventajas competitivas a los machos de mono aullador, su nivel de dimorfismo sexual en el tamaño del cuerpo sugiere considerables niveles de competencia entre machos y un rol en la fuerza individual (Kelaita et al., 2011).

Adicionalmente, el aparato vocal altamente especializado y el tiempo diario invertido en sus vocalizaciones en una especie con un estilo de vida por lo demás tranquilo sugiere la posibilidad de un compromiso evolutivo entre sus características vocales y testiculares en este género. En efecto, un estudio

de 6 de las 12 especies de mono aullador, reveló que los machos de especies que generalmente viven en grupos de un solo macho, y que por lo tanto enfrentan principalmente competencia intergrupala, poseen huesos hioides alargados, pero testículos pequeños, mientras que las especies que generalmente viven en grupos con múltiples machos, y por lo tanto que se enfrentan principalmente a competencia intra grupal, poseen huesos hioides pequeños, pero testículos grandes (Dunn et al., 2015). En otras palabras, en especies en las que los competidores principales viven en otros grupos la energía es invertida en el desarrollo de huesos hioides alargados que les permiten anunciar vocalmente su capacidad de defensa de recursos a rivales lejanos, mientras que en especies en las cuales los machos viven con sus competidores, la energía se invierte en una mayor producción espermática (Dunn et al., 2015). Estos resultados concuerdan con el sesgo del sistema de apareamiento hacia los rasgos con utilidad previa a la cópula y con la teoría del compromiso evolutivo. Sin embargo, aún se desconoce si este patrón se mantiene dentro de una única especie.

El mono aullador negro (*Alouatta pigra*) es una especie arborícola que vive en grupos cohesivos de uno a tres machos adultos, una a cuatro hembras adultas y múltiples crías; los grupos pueden contener hasta 16 individuos (Van Belle & Estrada, 2006). Su principal sistema de apareamiento es la poliginia, con un único macho conocido como el macho central, pero la poliginandria puede ocurrir también, lo que sugiere cierto nivel de competencia intragrupal entre los machos (Van Belle et al., 2014^a; Van Belle & Bicca-Marques, 2015). Los machos también enfrentan competencia extragrupal debido a que las hembras pueden copular con machos de grupos vecinos y que machos de otros grupos pueden inmigrar hacia grupos establecidos expulsando a los machos residentes (Van Belle et al., 2009a). Los rugidos y los ladridos constituyen la mayoría de su repertorio vocal y los producen de manera secuencial en episodios vocales que duran 15 minutos en promedio, pero que se pueden prolongar hasta por 59 minutos durante los encuentros intergrupales (Briseño-Jaramillo et al., 2017; Van Belle et al., 2013).

En este estudio, probamos las hipótesis de alometría acústica y del compromiso evolutivo entre las características vocales y el volumen testicular en el mono aullador negro en el Parque Nacional

Palenque, (Chiapas, México). Predecimos que, si los principales tipos vocales, rugidos y ladridos, son señales honestas del tamaño del cuerpo, es decir que existe alometría acústica, entonces la F_0 y/o la ΔF estarán negativamente correlacionadas con el tamaño del cuerpo. Nuestra segunda predicción fue que, si existe un compromiso evolutivo entre las características vocales y testiculares, entonces observaríamos una correlación positiva entre la F_0 y/o la ΔF y el volumen testicular. Esta última predicción es contra intuitiva (positiva en lugar de negativa) debido a la correlación negativa entre la ΔF y el volumen del hueso hioides (Dunn et al., 2015).

Acoustic allometry in roars of male black howler monkeys (*Alouatta pigra*) but no trade-off with testicular volume

Saúl De los Santos Mendoza¹ and Sarie Van Belle²

¹ Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico

² Department of Anthropology, University of Texas at Austin, Austin, TX, USA

Corresponding authors

Saúl de los Santos Mendoza: delossantoz@hotmail.com (+52 55 22 96 5380)

[Sarie Van Belle: sarievabelle@gmail.com](mailto:sarievabelle@gmail.com)

ORCID Saúl De los Santos Mendoza: 0009-0001-6902-3855

ORCID Sarie Van Belle: 0000-0003-3469-6619

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Conflict of interest declaration. We declare we have no competing interests.

Abstract. The source-filter theory suggests that animal traits, such as body size, are reliably encoded in vocalizations. These vocal signals, with a likely precopulatory function, are thought to be costly; given energetic constraints, they are expected to be in a trade-off with postcopulatory traits, such as testicular volume. Although this trade-off has been generally tested through comparative studies across species, it remains understudied if it holds within a single species. Using parallel-laser photogrammetry, we conducted a 9-month study at Palenque National Park, Mexico, to investigate whether fundamental frequency (F0) or formant dispersion (ΔF) of roars and barks from 14 male black howler monkeys (*Alouatta pigra*) encode cues of body size, and if they are in a trade-off with testicular volume. We found that only roar ΔF is associated with body size, with larger males producing roars with lower ΔF , suggesting a likely use of roars in male-male competition or female mate choice in black howlers. On the contrary, no association was found between vocal features and testicular volume, after accounting for the positive effect of body size on testicular volume. Our results show the presence of acoustic allometry within roars of male black howlers and suggest the absence of a trade-off within a single species, previously reported at the genus level.

Key words: bioacoustics, vocalization, honest acoustic signals, sexual selection, parallel-laser photogrammetry

Introduction

Sexual selection is a prominent driver of the evolution of acoustic communication in animals (Vehrencamp, 2000; Wilkins et al., 2013). For example, acoustic signals function in species and sex identification or in mate attraction, with females showing preferences for certain acoustic traits in males, such as calling rate or pitch (Simmons et al. 2010; 2011). Likewise, conspecific rivals might use acoustic displays to resolve conflicts prior to engaging in direct confrontation avoiding potential injuries (Brown et al., 2006). Hence, it is presumed that acoustic signals provide specific information about the caller, including its age, sex, body size, fighting ability or health status that the receiver can interpret as a cue to generate an adequate response. However, for these signals to be reliable, they should have either 1) some developmental or production cost (handicap signal), 2) a high retaliation cost of signalling a strong state when the individual is weak (conventional signal), or 3) a physical or physiological constraint that is functionally linked to the caller's attributes (index signal) (Vehrencamp, 2000).

Index signals have been studied under the framework of the “source-filter” theory that provides a link between acoustic features and anatomical structures of the caller (Fitch 1997; Charlton et al. 2011). According to this theory, vocalizations are generated through a 2-step process. First, a “source” base sound is produced in the glottis through the opening and closing of the vocal folds, with the rate determining the fundamental frequency (F_0) and pitch of the sound. Second, the generated sound travels through the supra-laryngeal cavities, which act as a “filter”, selectively amplifying or diminishing frequency ranges, known as “formants” (Fitch, 1997; Taylor & Reby, 2010). Therefore, F_0 depends on the length and mass of the vocal folds, which are partially affected by body size, while formants are limited by the vocal tract morphology bounded by the skull bones, which in turn are closely related to body size (Fitch 1997; 1999; Charrier et al. 2011). Consequently, F_0 or formant dispersion (i.e., the mean distance between formants; ΔF) may reliably reflect body size. Indeed, this correlation, known as “acoustic allometry”, has been observed in males of several species, where either F_0 , ΔF , or both of their

competitive vocalizations were negatively correlated with body size. That is, the larger the caller, the lower F0 or ΔF are (Bowling et al., 2017; Favaro et al., 2017; Fitch, 1997; Garcia et al., 2017).

Vocalizations used in competitive contexts confer advantages to gain access to females and thus function before copulation. In contrast, postcopulatory traits, such as enlarged testicles, help to secure fertilization. Among species, males are usually biased towards one of these opposing traits for two main reasons. First, a species modal mating system may either favour the selection of precopulatory traits when males can monopolize females (polygyny) but may otherwise favour postcopulatory traits if females mate with multiple partners (polygynandry) (Lüpold et al., 2014; McCullough et al., 2018). Second, the development and maintenance of sexual traits require a substantial amount of energy; given the limitation in energy, investment in precopulatory traits hinders the development of postcopulatory traits and vice versa (Emlen et al., 2012; Simmons et al., 2017; Somjee et al., 2018). This trade-off between pre- and postcopulatory traits has been confirmed in animals as diverse as acanthocephalans, annelids, reptiles, birds, cetaceans, primates, and pinnipeds (McCullough et al., 2018; Simmons & Fitzpatrick, 2012). Specifically, a trade-off between vocal signals and testicular features has been reported for some terrestrial mammals, birds, and insects (Charlton & Reby, 2016; Del Castillo & Gwynne, 2007; Simmons et al., 2011). For example, male houbara bustards (*Chlamydotis undulata*) that invest much in extravagant sexual vocal displays experience lower sperm production compared to conspecific males that use other tactics (Preston et al., 2011).

Howler monkeys (*Alouatta*) are considered one of the loudest terrestrial animals worldwide. They have a specialized vocal apparatus that includes elongated vocal folds, air sacs, an enlarged larynx, and an enlarged hyoid bone, which functions as a resonating chamber (Hilloowala, 1975; Schön, 1971). Though different hypotheses regarding the function of their loud vocalizations have been postulated (reviewed in Kitchen et al., 2015), they likely have a role in male-male competition (Sekulic & Chivers, 1986). This is supported by: 1) a larger hyoid bone in males than in females (da Cunha et al., 2015), 2) the capacity of the hyoid bone to lower ΔF (Dunn et al., 2015), 3) males' ability to recognize

neighbouring groups through their loud vocalizations (Briseño-Jaramillo et al., 2014), 4) the fact that adult males who have sired offspring in a group vocalize more than males without offspring (Van Belle et al. 2014a), and 5) the prominent role of loud calling bouts during intergroup encounters, lasting significantly longer than in any other context (Chiarello, 1995; Van Belle et al., 2013). Additionally, a multi-species analysis revealed a positive correlation between body size and hyoid volume and a negative correlation between hyoid volume and ΔF (Dunn et al., 2015). However, no study on any howler monkey species has assessed whether F0 or ΔF are honest signals of body size. Although it has not yet been assessed whether larger body size confer any competitive advantages to males in howler monkeys, their level of sexual dimorphism in body size is in line with considerable male-male competition and a presumed role of body size in individual strength (Kelaita et al. 2011).

Furthermore, the highly specialized vocal apparatus and the time invested in daily vocal displays within an otherwise tranquil lifestyle suggest the possibility of a trade-off between vocal and testicular features in this genus. Indeed, a study across six howler monkey species revealed that males of species generally living in unimale groups, and thus facing mainly intergroup male-male competition, possess large hyoid bones but small testicles, while males of species generally living in multimale groups, and thus facing higher intragroup male-male competition, have smaller hyoid bones but bigger testicles (Dunn et al., 2015). In other words, in species where males' main competitors live in other groups, energy is invested in developing an enlarged hyoid bone, presumably to be able to vocally announce their resource holding potential to out-of-sight competitors, while in species in which males co-reside with their main competitors, energy is rather invested in elevated sperm production (Dunn et al., 2015). These results fit both the mating system bias towards precopulatory traits and the trade-off theory. However, it remains to be tested if this pattern holds within a single species.

The black howler monkey (*Alouatta pigra*) is an arboreal species living in cohesive groups of one to three adult males, one to four adult females, and multiple offspring; groups can contain up to 16 individuals (Van Belle & Estrada, 2006). Their mating system is mainly polygynous, with a single male

recognized as the central male, but polygynandrous mating occurs as well, suggesting some level of intragroup male-male competition (Van Belle et al., 2014a; Van Belle & Bicca-Marques, 2015). Males also face intergroup male-male competition as females may copulate with neighbouring males and extragroup males can immigrate into established groups evicting resident males (Van Belle et al., 2009a). Roars and barks constitute most of their vocal repertoire; they are uttered in sequence during loud calling bouts that last, on average, 15 min, but that can be as long as 59 min during intergroup encounters (Briseño-Jaramillo et al., 2017; Van Belle et al., 2013).

In this study, we tested the acoustic allometry hypothesis and the trade-off between vocal and testes features in the black howler monkey during a 9-month non-invasive field study at Palenque National Park, Mexico. We predicted that, if the main vocal types, roars and barks, are honest signals of the caller's body size, then their F0 or ΔF will be negatively correlated with body size. Additionally, if a trade-off exists between vocal and testes features, then we predicted a positive correlation between F0 or ΔF of roars and barks and testicular volume. This latter predicted correlation is counterintuitive (positive instead of negative) due to the negative correlation between ΔF and hyoid bone size (Dunn et al., 2015).

Methods

Study site and Subjects

We conducted the study at Palenque National Park (PNP), Mexico (17° 28' N, 99° 03' W), from March through November 2022. PNP encompasses an area of 1771 ha, of which 1000 ha contain undisturbed or regenerating evergreen tropical rainforest. A long-term study on five groups of black howler monkeys has been established since 2006 (Van Belle & Di Fiore, 2022). Here, we recorded vocalizations and photographed 14 wild adult black howler males, six belonging to the five monitored groups and eight from eight unmonitored groups. We spent 1442 observation hours across 127 field days (05:00h to 17:00h) with these groups (mean = 111 ± SD 61.9 hours/group; mean = 10 ± SD 5.5

days/group; appendix Table S1). Monitored groups (Motiepa, Naha, Pakal, Picota, and Unites) were observed on more days than unmonitored groups to train and refine our techniques. Groups varied in size from two to 11 individuals, containing one to three adult males, one to three adult females, and none to six immatures (appendix Table S1). We recognized males by body size, permanent scars, genital pigmentation, and the presence of temporary botfly infections. We selected the known central male in monitored groups and the most likely central male in unmonitored groups, choosing the one adult male who called at higher rates and who was the only male to call alone (Van Belle et al., 2008). We did not include subadult males as they call less often and mostly together with other males (Van Belle et al., 2014a). We sampled two males in the Motiepa group as a new immigrant male (Elio) took over the central position in June 2022 while the former central male (Hugo) remained in the group.

Acoustic recordings

We used the all-occurrence sampling method to record all calling bouts by the focal male using a directional microphone (Sennheiser MKE600) and a digital audio recorder (Marantz PMD661-MKIII; sample rate 44.1 kHz, resolution 16-bit, WAV mono format). We simultaneously recorded spoken comments on caller identities with an additional voice recorder (iPhone 11). Calling bouts were considered separate when all group members remained silent for at least 10 minutes (Van Belle et al., 2013). All recordings were made at comparable distances (15 to 25 m). We recorded the context of the calling bouts as spontaneous when there was no noticeable external stimulus, as a response when calling was prompted by neighbouring groups calling out-of-sight, and as an intergroup encounter when they had visual contact with a neighbouring group (< 50 m; Van Belle et al., 2013). We recorded a total of 246 bouts (~46 hrs) from the 14 males (mean = $18.9 \pm \text{SD } 17.8$ bouts/male, median = 11 bouts/male, mean = $3.5 \pm \text{SD } 3.0$ hours/male; median = 3.0 hours/male; appendix Table S1).

We aligned the digital audio recordings and the voice recordings using Audacity 3.2.2. and subsequently selected isolated roars and barks of the focal males by inspecting all files visually and acoustically, selecting only high-quality recordings with minimum background noise. We inspected

spectrograms in PRAAT 6.2.1 (Boersma & Weenink, 2001), generated with a fast Fourier transformation, Gaussian window shape, 0.1 sec window length, 50 dB dynamic range, maximum formant of 4,000 Hz, resolutions of 1,500 time steps, and 250 frequency steps (following Bergman et al., 2016).

For acoustic analyses, we selected entire barks and the period at maximum amplitude of the longest syllable for roars (Bergman et al., 2016) (appendix Figure S1). We selected 10 roars and 10 barks per male, with a maximum of 5 calls of the same type from the same calling bout with a minimum of 20 s between selected calls. To calculate F0, we used the function “Get pitch” of PRAAT, which gives the mean pitch of the selected sound. For this, we set the voice threshold to 0.05 Hz, the window as Gaussian, and the pitch range to 15-150 Hz to control for the presence of chaos typical of these loud calls (Bergman et al., 2016). To calculate the formants for the selected calls we used the packages Seewave and phoneTools for R 4.2.3 (Sueur, 2018; Reby & McComb, 2003; R Core Team, 2022). Following the equation provided by Dunn et al., (2015), we calculated ΔF using the distances between the six lowest formants. Additionally, based on mean formant values across males, we estimate the vocal tract length with the package soundgen (Anikin, 2019; Reby & McComb, 2003).

Body and testes photographs

To non-invasively obtain estimates of body size and testicular volume, we used the parallel-laser photogrammetry technique. It consists of mounting two parallel lasers separated by a known distance onto a camera to project a scale within photographs from which sizes of photographed objects can be extrapolated (Barrickman et al., 2015; Rothman et al., 2008; Teichroeb et al., 2020). We adapted a digital camera (Panasonic Lumix DC-FZ80K with a 60x optical zoom) with a hand-crafted aluminium frame to which we attached two horizontally fixed parallel green lasers (GM-CW02) set 4 cm apart and powered by two AA batteries. The frame was joined to the camera with a bolt to the tripod socket. We ensured laser calibration at distances from 5 to 20 m.

Although this technique has been shown to estimate body part sizes accurately, estimating the size of small body parts might be less accurate due to parallax and variation across photographs in the relative position of the body parts within the 2D view of a photograph (Barrickman et al., 2015). In order to obtain as accurate of estimates as possible, we photographed males when we could position ourselves perpendicular to the subject at <15 m, and, whenever topography allowed, at the same height as the subject. We took multiple sessions of photographs every time the subject assumed an adequate position to either photograph its body or testicles. Different sessions captured different positions assumed by the subject at different times or similar/equal positions from different vantage points perpendicular to the subject. From each photograph session, we chose up to five high-quality photographs to estimate either body length or testes' length and width. We calculated the mean of these within each session, and then calculated the mean of these means across sessions of the same male. We took over 14,000 photographs from which we selected 150 for body size measurements (mean = $10.7 \pm \text{SD } 4.7$ photos/male) and 138 for testicular volume measurements (mean = $9.9 \pm \text{SD } 4.7$ photos/male; appendix Table S2 and Figure S2).

We estimated body size and testicular volume using the program ImageJ (Schneider et al., 2012). To set the scale, a line was drawn from the centre of each laser point and set to be 4 cm long. To measure body size, we drew a segmented line following the contour of the subject's back from the nape of the neck to the base of the tail. To measure testes' width and length, we first drew an oval delimiting each testicle, excluding scrotal folds. Then we measured the length and width as the largest vertical and horizontal line within the oval, taking into consideration the inclination of the oval. Finally, we obtained testicular volume with the formula: $W^2L\pi/6$, where W is the mean width and L is the mean length of the testicle per male and by summing the left and right testicle volumes (Dunn et al., 2015).

Statistical analysis

To test the acoustic allometry hypothesis we fitted a linear mixed-effect model for F0 and ΔF , each for roars and barks separately. Within each model, the response variable was either F0 or ΔF of the

10 calls (either roars or barks) measured per male. We entered body size as predictor variable, calling bout context as control variable, and male ID as random variable.

To test the trade-off theory, we fitted two linear mixed-effect models for F0 and ΔF , each for roars and barks separately. In both models, the response variable was either F0 or ΔF of the 10 calls (either roars or barks) measured per male. The first model was created only with testicular volume as predictor variable, while, in the second model, given that we hypothesized that both body size and testicular volume are related to these acoustic characteristics, we entered both as predictor variables (mean per male). In these models, calling bout context was used as a control variable and male identity as a random variable.

We looked for outliers using the interquartile range criterion in R. Testicular volume for the male Asim was identified as an outlier. He presented an abnormal protuberance on his right testicle, resulting in testicular volume more than two times larger than the average volume. We omitted him for the trade-off analysis (Table 1).

To create the models, we used the package “lme4” and the function “lmer” (Bates et al., 2015) in R. Proper model design was corroborated through the R package “performance” (Lüdtke et al., 2021). VIF values in all models was < 1.6 suggesting no collinearity between variables, a Pearson correlation test revealed a moderate correlation of 0.6 between body size and testicular volume. We obtained P-values through likelihood ratio tests by comparing the full model including the main predictor variable with a reduced model excluding that predictor variable. Model stability was corroborated by excluding individual males one at the time. No stability issues were found.

Ethical Note

This study involved non-invasively observing, recording, and photographing wild study subjects from a distance that did not disturb the behaviour of the howler monkeys in an obvious way. Laser points were only pointed towards subjects when they assumed adequate, stationary positions (when resting or feeding) at their rump or anogenital area, but never at their face. Howler monkeys have been regularly

monitored at this site for over 17 years. Research permissions to work in PNP were granted by the Mexican government (Dirección General de Vida Silvestre de la Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT SGPA/DGVS/07928/21, SPARN/DGVS/03723/2).

Results

Acoustic allometry

Across 14 males, mean body size was $49.4 \pm \text{SD } 4.3$ cm (range = 41.0 – 56.5 cm; Table 1), with mean intra- and inter-individual coefficients of variation (CV) of 4.4% and 8.7%, respectively. Roar F0 across males had a mean of $101.5 \text{ Hz} \pm \text{SD } 14.6$ (range = 33.2 – 141.0 Hz; N = 140 roars; Table 1), with intra- and inter-individual CV of 20.7 % and 14.4 %, respectively. Results from the linear mixed model for roar F0 and body size showed no association between them ($\chi^2 = 0.361$; P = 0.754).

The mean bark F0 was higher than in roars with $107.7 \pm \text{SD } 6.7$ Hz (range = 42.3 – 146.5 Hz; N = 140 barks; Table 1) and mean intra- and inter- individual CV of 17.2 % and 6.2 %, respectively. Also here, no significant association between bark F0 and body size was found ($\chi^2 = 0.956$; P = 0.328).

The mean roar ΔF across males was $377.3 \pm \text{SD } 18.0$ Hz (range = 334.3 – 457.9 Hz; N = 140 roars; Table 1), with mean intra- and inter-individual CV of 3.3 % and 4.7 %, respectively. In agreement with the source-filter theory and the allometry hypothesis, we found a negative and significant correlation between roar ΔF and body size (Table 2, Figure 1). An increase in one standard deviation in body size resulted in a decrease of 2.5 Hz in mean ΔF (Table 2; Figure 1). From the mean roar ΔF , we estimated a mean vocal tract length of $46.9 \pm \text{SD } 1.5$ cm (range = 43.3 – 49.5 cm; CV = 2.6 %).

The mean bark ΔF across males was $418.4 \pm \text{SD } 72.6$ Hz (range = 49.5 - 619.0 Hz; N = 140 barks; Table 1), higher than that for roars. Mean intra- and inter-individual CV for ΔF of barks were also higher with 13.3 % and 17.3 %, respectively. No evidence of an association was found for bark ΔF and body size ($\chi^2 = 0.084$; P = 0.771).

Precopulatory versus postcopulatory trade-off

Across 13 males, mean testicular volume was $25.9 \pm \text{SD } 6.6 \text{ cm}^3$ (range = 14.8 – 35.5 cm^3 ; CV = 25.3 %; Table 1). According to the linear mixed model of roar F0 and testicular volume as sole predictor variable, these traits showed no correlation ($\chi^2 = 0.003$; P = 0.955). The model of roar F0 and both testicular volume and body size as predictor variables was also not significant ($\chi^2 = 0.261$; P = 0.609). Similarly, bark F0 and testicular volume did not show a correlation either in the model with only testicular volume or in the model with testicular volume and body size as predictor variables ($\chi^2 = 0.745$ and 0.328 ; P = 0.388 and 0.566, respectively).

Results from the model of roar ΔF and testicular volume revealed a significant, but weak, negative correlation, resulting in males with larger testicular volumes showing smaller roar ΔF (Table 2; Figure 1). An increase in one standard deviation of testicular volume resulted in a decrease of 1.2 Hz in the mean ΔF of roars. However, the model of roar ΔF that included both testicular volume and body size as predictor variables showed no effect ($\chi^2 = 0.289$; P = 0.590). For bark ΔF , neither the model with only testicular volume, nor the model with both testicular volume and body size as predictors showed any correlation with testicular volume ($\chi^2 = 0.291$ and 4.525 ; P = 0.589 and 0.210, respectively).

Table 1 Summary of each male's mean \pm SD fundamental frequency (F0) and formant dispersion (ΔF) (N = 10 roars and 10 barks per male), total testicular volume per male (N = 3 – 17 photographs per male),

and body size (N = 4 – 18 photographs per male). Overall mean testicular volume excluded Asim as he was considered an outlier.

Male	Mean F0 (Hz)		Mean ΔF (Hz)		Body size (cm)	Total testicular volume (cm ³)
	Roars	Barks	Roar	Barks		
Ashur	103.5 ± 17.4	113.5 ± 20.1	382.9 ± 10.5	399.8 ± 18.3	44.0 ± 0.6	14.8 ± 0.8
Asim	101.9 ± 17.6	100.1 ± 15.6	374.4 ± 5.3	492.3 ± 68.8	48.1 ± 1.9	55.1 ± 12.9
Balam	79.1 ± 31.6	105.8 ± 26.8	373.7 ± 13.8	388.2 ± 18.5	51.0 ± 1.0	31.4 ± 4.8
Bolín	96.3 ± 20.9	105.5 ± 29.9	384.3 ± 11.3	411.3 ± 35.2	44.7 ± 0.8	19.7 ± 4.4
Elio	116.7 ± 10.3	92.9 ± 28.8	357.9 ± 5.7	443.6 ± 75.2	56.5 ± 3.2	27.7 ± 3.03
Goyo	111.9 ± 16.2	107.7 ± 20.8	365.0 ± 9.1	350.8 ± 146.4	49.6 ± 1.3	34.0 ± 9.3
Hugo	90.7 ± 28.3	106.7 ± 13.5	371.6 ± 12.6	467.9 ± 66.9	53.1 ± 2.8	35.5 ± 4.9
Lineo	101.7 ± 18.3	107.6 ± 10.4	369.8 ± 15.1	451.8 ± 38.7	53.5 ± 2.7	21.4 ± 2.4
Macho B	101.9 ± 16.7	111.4 ± 11.9	393.0 ± 16.6	373.7 ± 69.6	46.6 ± 1.5	21.0 ± 14.2
Nero	112.9 ± 15.4	103.2 ± 12.9	369.4 ± 12.4	377.4 ± 29.3	52.8 ± 4.3	34.0 ± 6.5
Orión	127.6 ± 14.5	115.1 ± 23.3	379.5 ± 11.4	403.6 ± 48.2	49.8 ± 4.0	27.6 ± 7.1
Tiglat	83.4 ± 24.3	117.8 ± 12.6	376.5 ± 29.5	401.8 ± 25.9	48.3 ± 1.4	26.7 ± 3.1
Voldi	79.4 ± 26.9	109.7 ± 12.6	375.0 ± 14.0	441.9 ± 89.5	53.1 ± 2.8	22.9 ± 4.6
Zod	113.7 ± 19.3	108.8 ± 17.8	409.2 ± 11.0	453.4 ± 34.3	41.0 ± 2.6	19.7 ± 2.3
Mean ± SD	101.5 ± 14.6	107.7 ± 6.7	377.3 ± 18.0	418.4 ± 72.6	49.4 ± 4.3	25.9 ± 6.5

Table 2 Likelihood ratio tests of the linear mixed models on the effect of body size and testicular volume on the formant dispersion (ΔF; Hz) of roars in adult males of black howler monkeys (*Alouatta pigra*) (N

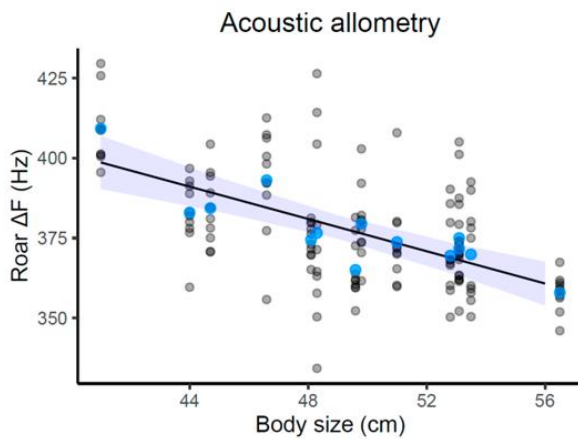
= 14 males in the model of body size; N = 13 males in the model of testicular volume, and testicular volume + body size). CI: lower (2.5%) and upper (97.5%) confidence intervals; DF: degrees of freedom.

Predictor	Estimate	SE	CI _{lower}	CI _{upper}	DF	χ^2	P
Roar ΔF ~ body size + context							
Intercept	501.63	22.14	457.83	545.43			
Body size	-2.52	0.44	-3.41	-1.64	1	18.67	0.00001
Context*							
response	0.33	3.53	-6.30	6.97	2	0.30	0.859
encounter	2.04	4.30	-6.47	10.55			
Roar ΔF ~ testicular volume + context							
Intercept	409.28	13.46	382.64	435.94			
Testicular volume	-1.20	0.48	-2.17	-0.23	1	5.76	0.016
Context*							
response	-1.00	3.95	-8.83	6.83	2	0.09	0.956
encounter	-0.95	4.69	-10.24	8.34			
Roar ΔF ~ testicular volume + body size + context							
Intercept	499.90	24.26	451.88	547.93			
Body size	-2.37	0.59	-3.56	-1.20	1	12.93	0.0003
Testicular volume	-0.19	0.40	-1.00	0.61	1	0.29	0.590
Context*							
response	0.66	3.55	-6.37	7.70	1	0.21	0.900
encounter	1.59	4.55	-7.42	10.61			

* Vocalization context was a control variable with the reference class being spontaneous calls.

Figure 1 Association between roar formant dispersion (ΔF) and body size. Diagonal line represents the fitted model after controlling for individual identity, while the shaded blue area represents confidence

intervals (95%). Blue circles represent the mean ΔF per male; opaque grey circles show the variation per male.



Discussion

We investigated whether fundamental frequency (F0) or formant dispersion (ΔF) within the maximum amplitude syllable of roars or within entire barks from wild *Alouatta pigra* males 1) encode acoustic cues of body size, and 2) if they, as a proxy for hyoid bone size, are in a trade-off with testicular volume. Through a source-filter theory approach, we found that, of the acoustic features and vocalization types considered in this study, only roar ΔF presents a negative correlation with body size. This result is in line with our prediction, making roar ΔF an honest acoustic signal of body size with a likely function in male-male competition or female mate choice. Results from the trade-off models showed that there is no trade-off among acoustic characteristics of their main vocal types and testicular volume, and variation in testicular volume is better explained by the variation in body size, with bigger males having larger testicles.

Acoustic allometry

The source-filter theory predicts a tight acoustic allometry between ΔF of calls and caller's body size due to the presence of multiple anatomical constraints in supra-laryngeal cavities (Fitch and Hauser, 1995; Rendall et al., 2005). Our study supports this prediction by finding a negative correlation between roar ΔF and body size, meaning that larger males produce roars with lower ΔF s in its longest syllable. In contrast, F0 of either roars or barks did not correlate with male body size and is thus unreliable at

predicting body size, most likely because the vocal folds that produce F0 are soft tissue not enclosed by bone structures (Charlton et al., 2010; Fitch, 1997; Gamba, 2014; Reber et al., 2017). Both, the allometric properties of ΔF and the lack of them in F0 are in line with results reported in many other species, including American alligators (*Alligator mississippiensis*; Reber et al., 2017), giant pandas (*Ailuropoda melanoleuca*; Charlton et al., 2010), baboons (*Papio hamadryas*; Rendall et al., 2005), humans (*Homo sapiens*; Pisanki et al., 2014), koalas (*Phascolarctos cinereus*; Charlton et al., 2011), and fallow deer (*Dama dama*; Vannoni & McElligott, 2008). Our results are the first to confirm the presence of acoustic allometry in any howler monkey species, which was previously inferred by the positive correlation between skull length and hyoid bone size across howler monkey species (Dunn et al., 2015).

The allometric properties of roars suggests that males are under specific social or sexual pressures to provide honest cues of body size. However, some species have evolved anatomical features that allow them to lower the frequency profiles of their vocalizations to exaggerate the acoustic impression of body size (Fitch, 1999; Harris et al., 2006). This is the case for howler monkeys, who have a specialized vocal apparatus that enables them to produce vocalizations with very low frequencies for their size (da Cunha et al., 2015; Dunn et al., 2015). Particularly, their enlarged hyoid bone has been shown to reduce formant dispersion (Dunn et al., 2015). Based on our mean roar ΔF across males, the length of male *A. pigra* vocal tract is predicted to be 46 cm. This is impossible with a mean body size of 49 cm, and thus underscores that the modifications of the vocal tract, particularly the hyoid bone, exaggerates the acoustic impression of body size. How then can roars be honest signals of body size? Possibly, the extent of deception is limited by the anatomical constraints on the hyoid bone size, which, even if capable of modifying formant profiles, is still varying in size according to the actual body size of the caller (Dunn et al., 2015). Moreover, if exaggeration is produced due to the presence of common permanent adaptations, i.e., the hyoid bone, this might lead receivers to adapt to such deception by shifting their perceptual scale to the exaggerated ranges (Pisanski & Reby, 2021; Smith & Price, 1973; Vehrencamp, 2000). As such, modifications of the vocal system may nonetheless transmit reliable information to

conspecifics, albeit convey an exaggerated body size to other species (Dunn et al., 2015; Fitzpatrick & Lüpold, 2015), which is also the case in koalas (Charlton et al., 2011) and black and white colobus (*Colobus guereza*; Harris et al., 2006).

The extent to which black howler monkeys attend to the acoustic information of body size is unknown. Considering that other primate species can recognize changes in ΔF (Fitch & Fritz, 2006; Gamba, 2014; Pisanski et al., 2016) and that roars prominently feature in howlers' loud vocalizations, it likely affects the behaviour of both males and females. Comparable to other species where females prefer acoustic signals conveying information about male body size (Charlton et al., 2007; Puechmaille et al., 2014), *A. pigra* females may assess males within or outside their group as potential mate. Similarly, dispersing females might use this information to decide which group to join (Van Belle & Di Fiore, 2022), a behaviour observed in mountain gorillas (*Gorilla beringei*) where females are presumed to actively choose to join groups with large alpha males based on honest acoustic cues of body size (Wright et al., 2021). Additionally, given the threat of infanticide in black howlers (Van Belle et al., 2010), females with vulnerable offspring may choose to participate in loud calling bouts during group defence based not only on the relative number of adult males between opposing groups, as has been reported before (Kitchen, 2006; Van Belle & Scarry, 2015), but also on the perceived relative body sizes of their resident versus rival males, derived from the formant dispersion cues within roars.

Male black howlers seem to be best adapted at resolving their contests through indirect aggressive displays such as branch breaking and loud calling bouts rather than direct combat, a strategy that can also be inferred by the longer calling bouts emitted during intergroup encounters than those in response to out-of-sight males (Van Belle et al., 2013; Van Belle et al., 2014b). Thus, *A. pigra* males could use the information on body size encoded within roars to assess the fighting ability or strength of other males, helping them to decide whether they should respond or initiate, escalate, or retreat from a contest (Sekulic, 1983; Van Belle et al., 2014b). Indeed, this could also explain the predominant presence of roars during calling bouts (Briseño-Jaramillo et al., 2017). Lastly, given the long-distance nature of their

vocalizations, acoustically encoded information on male size could contribute to group navigation decisions, helping them to decide whether to approach or avoid distant neighbouring groups or solitary males (Van Belle & Estrada, 2020). The importance of body size and roar ΔF in black howlers might be better understood by more studies that investigate the influence of those features within the context of male-male competition and female mate choice.

Although barks did not encode information on body size, they are the other main call type used during calling bouts, suggesting a complementary role that might be related to the transmission of different attributes or intentions through a distinct acoustic feature. A potential function of barks is vocal recognition of individual males or neighbouring groups, given their higher individual variation in 11 acoustic features – formant dispersion excluded – compared to roars (Briseño-Jaramillo et al., 2014). Similarly, our results showed higher coefficients of variation in ΔF for barks than for roars. Nonetheless, the tight correlation between roar ΔF and body size suggests that roars too may contribute to conveying information on identity as different studies have found that formant-related spectral characteristics are effective in assigning identity (Budka & Osiejuk, 2013; Charlton et al., 2011; Gamba et al., 2012). Indeed, in the black-and-gold howler monkey (*Alouatta caraya*), roar formant frequencies and call duration were found to reliably transmit identity among males (Holzmann & Córdoba, 2022). Additional studies are required to better understand the role of male roar ΔF and barks in individual vocal distinctiveness in black howler monkeys.

Finally, the absence of an association between F0 and body size does not necessarily imply an absence of utility within vocalizations. Some authors have suggested that F0 can affect perception of formant dispersion to listeners and thus enhancing its effectiveness (Charlton et al., 2010; Pisanski et al., 2016). Whether this is true in howlers requires additional investigations.

Pre-copulatory versus post-copulatory trade-off

We obtained a mean testicular volume of $25.9 \pm 6.6 \text{ cm}^3$. Compared to an invasive study that reported a mean of $11.3 \pm \text{SD } 3.8 \text{ cm}^3$ (Kelaita et al., 2011), our results seem to be an overestimation.

Nonetheless, our results of mean body size were highly comparable with those of that study ($49.4 \pm \text{SD } 4.3 \text{ cm}$ vs $48.5 \pm \text{SD } 3.2 \text{ cm}$, respectively; Kelaita et al., 2011). The difference in testicular volume between the two studies is likely explained by the different methods used and the fact that testicles are small body parts. In the invasive study, individuals were sedated and testicles' width and length were measured with a calliper, removing any scrotal folds (Kelaita et al., 2011). In our non-invasive study, estimates of testicles' width and length are likely less accurate given that 1) subjects were photographed from variable distances ($<15 \text{ m}$) and heights causing some degree of parallax, 2) the variation across photographs in the relative position and angle of the testicles in the 2D plane of photographs, which particularly affects the estimation of small body parts (Barrickman et al., 2015), and 3) the inability to physically remove scrotal folds, resulting in our estimates reflecting scrotal volume rather than testicular volume. To compensate for these shortcomings, we calculated a mean of testicle measurements estimated from multiple photographs portraying the testicles in adequate positions and angles, and fully dropped into the scrotal sacs. Thus, while we recognize that our results of testicular volume appear overestimated, and we recommend taking the subsequent interpretation of the results of the trade-off model with caution, we have no reason to suspect a bias in this overestimation towards particular males. Additionally, studies on other mammals have found that scrotal volume highly correlates with testicular volume and sperm producing capacity (Abdullahi et al. 2012; Rusk et al. 2002; Shende et al. 2019).

Since smaller values of ΔF reflect higher hyoid bone volumes (Dunn et al., 2015), a trade-off between ΔF and testicular volume should result in a positive correlation. Contrary to our prediction, we did not find an association between F_0 or ΔF of bark or roars and testicular volume. In the model with testicular volume as the only predictor variable, we found a negative, albeit weak, association with roar ΔF , which implies males investing in both enlarging the hyoid bone and enlarging testicles. However, when also considering body size as a predictor variable, the association between roar ΔF and testicular volume disappeared, suggesting that after accounting for the effect of body size on roar ΔF , no additional variation in roar ΔF could be explained by testicular volume. The significant effect found in the first

model likely results from the correlation between body size and testicular volume with bigger males tending to have larger testicular volumes. This has also been found in humans (Bahk et al. 2010; Innocent et al. 2016) and other mammals (Brito et al. 2012; Jacyno et al. 2015; Salhab et al. 2001). Therefore, our results rather suggest that in black howlers there is no trade-off between F0 or ΔF of barks or roars and testicular volume, reflecting an absence of a trade-off between hyoid bone size and testicular volume within this species.

Our results contrast with those reported by Dunn et al. (2015), where they found a negative association between hyoid bone size and testicular volume across five howler species, *A. pigra* included. However, this trade-off based on a small sample size is likely highly influenced by the species at both ends of the spectrum. On one side, there are *A. seniculus* and *A. guariba* who present an evident bias towards large hyoid bones and small testicles, with groups usually containing only one adult male who monopolizes access to females (Dunn et al., 2015; Van Belle & Bicca-Marques, 2015). On the other side, *A. caraya* and *A. palliata* show a clear bias towards large testicles and small hyoid bones, with groups usually containing multiple males, and a high rate of female promiscuity (Dunn et al., 2015; Kelaita et al., 2011; Van Belle & Bicca-Marques, 2015). Compared to these species, *A. pigra* presents intermediate values of testicular volume and hyoid bone size, groups most commonly have two adult males, and male monopolization of females is high, but incomplete (Van Belle et al., 2014a; Van Belle & Estrada, 2008). Given that hyoid bone size or testicular volume are fixed anatomical traits that are not readily changed by males in response to the varying social contexts they may face throughout their life, sizes of these body parts reflect the mean levels of intra- and intergroup male-male competition that species have experienced over evolutionary time (Dixson, 2018). This could explain why a trade-off between vocal and testicular traits was observed across howler species, who each have evolved different male mating strategies and associated anatomical traits driven by different relative levels of intra- and intergroup male-male competition, but not within a species, whose individuals cannot alter their anatomical traits but instead might display behavioural and physiological adjustments to variations in levels of intra- and

intergroup competition (Horwich et al. 2000; Rangel-Negrín et al. 2011; Van Belle et al. 2009b), particularly when experiencing intermediate levels of intra- and intergroup competition, as is likely the case for black howlers.

Indeed, trade-offs between pre- and postcopulatory traits appear more prevalent when there is a disproportional investment in a precopulatory trait, as when males can monopolize access to multiple females, making the potential gain so large that investment in postcopulatory traits has a markedly reduced payoff (Dines et al., 2015). Paternity analyses have corroborated that central male black howlers are unable to fully monopolize females despite the presence of mate-guarding behaviour and consortship (Van Belle et al., 2009b; Van Belle et al., 2014a). These analyses revealed that females most frequently mate with the central male of their group, but also mate with non-central males within their groups and males from neighbouring groups, resulting in occasional polygynandrous breeding (Van Belle et al., 2014a). The inability of central males to completely monopolize females exposes them to a certain degree of spermatic competition, preventing the sole investment in precopulatory traits, yet not high enough to trigger a disproportional investment in testicular volume, leading to a lack of a trade-off between vocal and testicular traits.

Furthermore, although *A. pigra* vocalizations likely play a role in direct male-male competition over access to females, they are hardly decisive; instead, they are mainly used as an indirect aggressive display that conveys information about individual and group-level strength, but do not dissuade females from copulating with non-central or neighbouring males (Van Belle et al., 2014b; Van Belle & Estrada, 2008). Probably, males also use vocalizations in other less direct competitive contexts, such as increasing their mating opportunities through the defence of a home range, its feeding resources, and their offspring, all of which are necessary for successful female reproduction (Kitchen, 2004; Van Belle et al., 2013; Van Belle & Estrada, 2020). Accordingly, the evolution of the specialized vocal apparatus of howler monkeys is most likely multifactorial including both social and environmental drives (Charlton & Reby, 2016; Kitchen et al., 2015).

Conclusions

We showed that roar formant dispersion is an honest signal of body size in males of the black howler monkey, while barks do not show any association with body size. Although our results do not assess a specific function (i.e., territorial/offspring/food/mate defence or intergroup spacing), they suggest that male roars are an adequate source of information to use in male-male competition or female mate choice due to their allometric properties. Meanwhile, our results from the trade-off model reveal both the benefits and limitations of non-invasive techniques and suggest that black howlers do not present a trade-off between acoustic features of their main call types and testicular volume. These findings underscore the importance of the relative pressure of the different forms of male-male competition in shaping sexual traits and reveal the absence of a trade-off within the black howler monkey, despite its presence at the genus level. Further research is necessary to determine 1) whether howlers can perceive formant related acoustic differences in roars, 2) how these vocalizations work in rival assessment, 3) the possibility of encoded individual signatures, and 4) if female mate choice is influenced by these calls.

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Appendices

Table S1.

Observation effort, number of sampled males, calling bouts, total recorded hours, and group composition per group.

Group	Observation time (hours)	Males sampled	N calling bouts	Recording time (hours)	Group composition*
Acueducto	90.1	1	11	3.2	2 AMs, 2 AFs, 3 IMM
Ajua	83.5	1	7	1.5	1 AM, 2 AFs, 2 IMM
Balam	48.3	1	5	0.4	1 AM, 2 AFs, 2 IMM
Bolas	46.5	1	9	2.1	2 AMs, 1 AF, 1 IMM
Calavera	86.1	1	5	0.9	1 AM, 1 AF, 2 IMM
Metzabok	84.9	1	13	3.0	2 AMs, 2 AFs, 1 IMM
Motiepa	179.7	2	32	6.2	3 AMs, 3 AFs, 4 IMM
Naha	212.9	1	24	4.0	1 AM, 3 AFs, 4 IMM
Pakal	188.9	1	65	9.0	1 AM, 3 AFs, 6 IMM
Picota	160.1	1	24	5.4	1 AM, 2 AFs, 4 IMM
Puras	95.8	1	7	1.2	2 AMs, 1 AF, 1 IMM
Ribereños	12.1	1	5	1.1	1 AM, 2 AFs, 2 IMM
Unites	152.9	1	39	7.5	1 AM, 1 AF
Total	1442.3	14	246	46.0	

* AM: adult male, AF: adult female, IMM: immature.

Table S2 Male IDs, their groups, number of photograph sessions, and total number of photographs used for the measurements of body size and testicular volume.

Male	Group	Body size		Testicles	
		Sessions	Photographs	Sessions	Photographs
Ashur	Puras	2	4	1	3
Asim	Picota	4	16	3	9
Balam	Balam	2	4	3	5
Bolín	Bolas	4	8	3	6
Elio	Motiepa	3	15	3	8
Goyo	Unites	7	18	4	17
Hugo	Motiepa	3	5	2	9
Lineo	Naha	5	15	4	17
Macho B	Metzabok	4	12	5	15
Nero	Pakal	4	15	2	6

Orión	Ajua	5	11	5	11
Tiglat	Acueducto	4	7	4	13
Voldi	Calavera	3	11	3	5
Zod	Ribereños	3	9	4	14
Total		53	150	46	138

Figure S1. An entire roar of *Alouatta pigra* taken from a longer calling bout. A) Oscillogram (amplitude vs. time) with spectrogram (frequency vs. time) generated in PRAAT. The section shaded in red represents the maximum amplitude (MA) of the longest syllable, while the yellow line shows the intensity. IP: Inhalation phase. B) The six formants within the maximum amplitude section shown in (A), each in a different color, calculated in R.

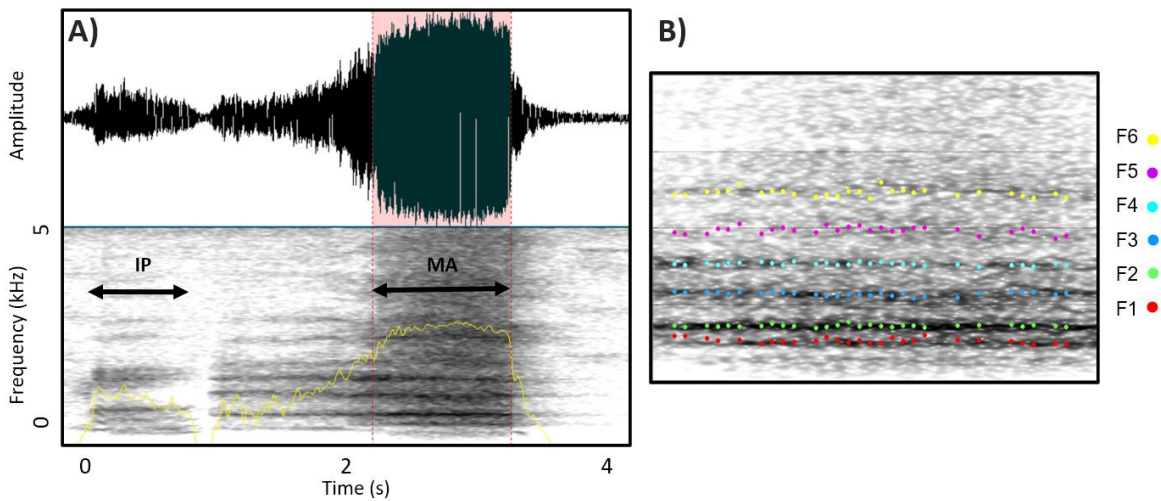


Figure S2. Parallel laser photogrammetry technique to measure non-invasively body size (A) and testicular volume (B) from 14 adult *Alouatta pigra* males. Green laser points positioned parallel and 4 cm apart are projected into both pictures. To measure body size, we drew a segmented line (yellow dotted line) following the contour of the male's back from the nape of his neck to the base of his tail. To measure testicular volume, we first drew an oval vector (yellow) delimiting each testicle. We then drew relatively vertical and horizontal lines, considering the inclination of the oval, to measure length and width of each testicle (yellow crossed lines).



Discusión general y conclusiones

En este trabajo evaluamos si la frecuencia fundamental (F0) y/o la dispersión de los formantes (ΔF) en la sílaba de mayor amplitud de los rugidos o ladridos de machos salvajes de *Alouatta pigra* 1) contienen información acústica relacionada al tamaño del cuerpo, y 2) si dichas características, que funcionan como un indicador del tamaño del hueso hioides, se encuentran comprometidas con el volumen testicular. A través de un enfoque basado en la teoría fuente-filtro encontramos que, de las características acústicas y tipos vocales analizados en este estudio, únicamente la ΔF de los rugidos presenta una correlación negativa con el tamaño del cuerpo. Este resultado apoya nuestra predicción inicial, demostrando que la ΔF de los rugidos es una señal honesta del tamaño del cuerpo con una probable función en la competencia entre machos o la elección femenina. Los resultados del modelo de compromiso evolutivo mostraron que no existe un compromiso entre las características acústicas de sus principales vocalizaciones y el volumen testicular, y que la variación en volumen testicular se explica mejor a través de la variación en tamaño del cuerpo, con machos más grandes presentando mayores volúmenes.

Alometría acústica

La teoría fuente-filtro predice una estrecha alometría acústica entre la ΔF de las vocalizaciones y el tamaño del cuerpo debido a la presencia de múltiples limitantes anatómicas en las cavidades supra laríngeas (Fitch & Hauser, 1995; Rendall et al., 2005). Nuestro estudio corroboró esta predicción al encontrar una fuerte correlación negativa entre la ΔF de los rugidos y el tamaño del cuerpo ($\chi^2 = 13.84$; $P = 0.0001$) es decir, que machos más grandes producen menores dispersiones de los formantes en la

sílaba de mayor duración de sus rugidos. En contraste, la F0 tanto de los rugidos como de los ladridos no presentó asociación con el tamaño del cuerpo y por lo tanto no transmite información de dicha característica. Esta falta de asociación probablemente se debe a que las cuerdas vocales que se encargan de generar la F0 son un tejido suave que no está limitado por estructuras óseas (Charlton et al., 2010; Gamba, 2014; Reber et al., 2017). Tanto las propiedades alométricas de la ΔF como la ausencia de las mismas en la F0 son resultados que otros estudios también han obtenido, por ejemplo, en los lagartos americanos (*Alligator mississippiensis*; Reber et al., 2017), pandas gigantes (*Ailuropoda melanoleuca*; Charlton et al., 2010), babuinos (*Papio hamadryas*; Rendall et al., 2005), humanos (*Homo sapiens*; Pisanki et al., 2014), koalas (*Phascolarctos cinereus*; Charlton et al., 2011), y en los gamos (*Dama dama*; Vanoni & McElligott, 2008). Nuestros resultados son también los primeros en confirmar la presencia de alometría acústica en los monos aulladores, lo que previamente era inferido debido a la correlación positiva entre el tamaño del cráneo y el volumen del hueso hioides entre distintas especies del género (Dunn et al., 2015).

Las propiedades alométricas de los rugidos sugieren que los machos de esta especie se encuentran bajo presiones sociales o sexuales específicas para proporcionar información honesta del tamaño del cuerpo. Sin embargo, en algunas especies han sido seleccionadas características anatómicas que permiten a los emisores disminuir ciertas frecuencias de sus vocalizaciones como una manera para exagerar su impresión acústica del tamaño del cuerpo (Fitch 1999; Harris et al., 2006). Este es también el caso de los monos aulladores quienes poseen un aparato vocal altamente especializado que les permite producir vocalizaciones con muy bajas frecuencias para su tamaño (da Cunha et al., 2015; Dunn et al., 2015). En específico, se ha demostrado que su hueso hioides puede reducir el espaciado entre formantes (Dunn et al., 2015). En este estudio, basándonos en el promedio de la ΔF de sus rugidos, calculamos que el promedio del largo del tracto vocal de un macho de *A. pigra* es de 46 cm. Dado que el promedio del largo del cuerpo es de 49 cm, la estimación del largo del tracto vocal muestra claramente el efecto del aparato vocal modificado, particularmente del hueso hioides, en la exageración de la impresión acústica del

tamaño del cuerpo. Entonces, ¿por qué sus rugidos son señales honestas del tamaño del cuerpo? Es posible que el grado de exageración se encuentre limitado por un propio límite anatómico en el tamaño del hueso hioides, el cual, incluso si es capaz de modificar los formantes, sigue variando en su tamaño de acuerdo con el tamaño del emisor (Dunn et al., 2015). Asimismo, si la exageración es un producto de una adaptación permanente común a todos los monos como lo es el hueso hioides, entonces probablemente los monos aulladores se han adaptado a ese nivel de exageración, cambiando su escala perceptual del tamaño a ese rango exagerado (Pisanki & Reby, 2021; Smith & Price, 1973; Vehrencamp, 2000). De este modo, las modificaciones del aparato vocal pueden transmitir información honesta del tamaño del cuerpo a sus conespecíficos, aunque información exagerada se transmitirá a otras especies (Dunn et al., 2015; Fitzpatrick & Lüpold, 2015), como es el caso de los koalas (Charlton et al., 2011) y el colobo blanco y negro (*Colobus guereza*) (Harris et al., 2006).

Se desconoce hasta qué punto los monos aulladores negros usan la información del tamaño del cuerpo codificada en sus rugidos. Sin embargo, considerando que otras especies de primates pueden reconocer cambios en la ΔF (Fitch & Fritz, 2006; Gamba, 2014; Pisanki et al., 2016) y que los rugidos se encuentran de manera predominante en las vocalizaciones de los monos aulladores, probablemente las variaciones en la ΔF influyen el comportamiento tanto de machos como hembras. Comparable a otras especies en las que las hembras muestran preferencia por señales acústicas que proveen información del tamaño del cuerpo (Charlton et al., 2007; Puechmaille et al., 2014), las hembras de *A. pigra* podrían utilizar dicha información para elegir pareja dentro o fuera de su grupo. De manera similar, las hembras que se dispersan y se encuentran buscando grupo podrían utilizar esa información para decidir el grupo al que se unen (Van Belle & Di Fiore, 2022). Este comportamiento se ha observado en los gorilas de montaña (*Gorilla beringei*), en donde se presume que las hembras eligen de manera activa a qué grupo unirse basándose en la percepción acústica del tamaño de los machos alfa (Wright et al., 2021). Adicionalmente, debido al riesgo de infanticidio en los monos aulladores negros (Van Belle et al., 2010), las hembras con descendientes vulnerables pueden elegir participar en los episodios vocales grupales de

defensa del grupo basándose no solamente en la cantidad de machos rivales, como ha sido mostrado previamente (Kitchen, 2006; Van Belle & Scarry, 2015), sino también en la percepción relativa del tamaño del cuerpo de los machos residentes y rivales, derivada de la dispersión de los formantes dentro de los rugidos.

Los machos de mono aullador negro parecen estar adaptados a resolver conflictos de manera indirecta a través de despliegues agresivos tales como la ruptura de ramas o episodios vocales, en lugar del conflicto directo, una estrategia que puede inferirse por los episodios vocales de mayor duración durante los encuentros intergrupales que en cualquier otro contexto (Van Belle et al., 2013; Van Belle et al., 2014b). Así, los machos de *A. pigra* podrían usar la información del tamaño del cuerpo contenida en sus rugidos para calcular la habilidad para pelear o fuerza de otros machos, ayudándoles a decidir si deben iniciar, responder, escalar o retroceder de un encuentro (Sekulic, 1983; Van Belle et al., 2014b). Esto mismo podría explicar la presencia predominante de los rugidos durante los episodios vocales (Briseño-Jaramillo et al., 2017). Finalmente, debido a la naturaleza de larga distancia de sus vocalizaciones, la información del tamaño del cuerpo podría contribuir a la toma de decisiones para la navegación grupal, ayudándoles a decidir si deben acercarse o evitar algún grupo vecino o macho solitario (Van Belle & Estrada, 2020). La importancia específica del tamaño del cuerpo y la ΔF de los rugidos en los monos aulladores negros podrá ser mejor comprendida a través de estudios que investiguen la influencia de dichas características en el contexto de la competencia entre machos o elección femenina.

A pesar de que los ladridos no contienen información del tamaño del cuerpo, siguen siendo el segundo tipo vocal más común luego de los rugidos, lo que sugiere un rol complementario que podría estar relacionado a la transmisión de distintos atributos o intenciones mediante una característica acústica distinta. Otra función potencial de los ladridos puede ser el reconocimiento vocal o transmisión de la identidad de los machos debido a su mayor variación entre individuos en 11 características acústicas – sin incluir la dispersión de los formantes – en comparación a los rugidos (Briseño-Jaramillo et al., 2014). En este sentido, nuestros resultados mostraron mayores coeficientes de variación en la ΔF de los ladridos

que en los rugidos. No obstante, debido a la estrecha asociación entre la ΔF de los rugidos y el tamaño del cuerpo sugerimos que los rugidos también pueden contribuir a transmitir información acerca de la identidad de los individuos ya que distintos estudios han encontrado que los formantes son efectivos en la asignación de identidad (Budka & Osiejuk, 2013; Charlton et al., 2011; Gamba et al., 2012). En efecto, en el mono aullador negro y dorado (*Alouatta caraya*), se encontró que los formantes contenidos en sus rugidos y la duración de estos transmitían de manera confiable la identidad del emisor entre distintos machos (Holzmann & Córdoba, 2022). Se requieren estudios adicionales para entender mejor el rol de la ΔF en los rugidos de los machos en la transmisión de identidad dentro de los monos aulladores.

Finalmente, la ausencia de asociación entre la F_0 y el tamaño del cuerpo no necesariamente implica una ausencia de utilidad dentro de las vocalizaciones. Algunos autores han sugerido que la F_0 puede afectar la percepción de la dispersión de los formantes y, así, amplificar su efectividad (Charlton et al., 2010; Pisanski et al., 2016). Si esto es cierto en los monos aulladores requiere investigaciones adicionales.

Compromiso evolutivo entre los caracteres con utilidad previa y posterior a la cópula

El promedio del volumen testicular que obtuvimos es $25.9 \pm 6.6 \text{ cm}^3$. Comparado al promedio obtenido en un estudio invasivo de $11.3 \pm \text{SD } 3.8 \text{ cm}^3$ (Kelaita et al., 2011), nuestros resultados parecen ser una sobre estimación. Sin embargo, nuestros resultados del promedio del tamaño del cuerpo son altamente comparables con aquellos reportados en el mismo estudio invasivo ($49.4 \pm \text{SD } 4.3 \text{ cm}$ vs $48.5 \pm \text{SD } 3.2 \text{ cm}$, respectivamente; Kelaita et al., 2011). La diferencia en el volumen testicular de ambos estudios probablemente se explique por las diferentes metodologías utilizadas y el hecho de que los testículos son una parte pequeña del cuerpo. En el estudio invasivo, los machos fueron sedados y el ancho y largo de sus testículos se obtuvo a través de la medición con un calibrador, removiendo los pliegues escrotales (Kelaita et al., 2011). En nuestro estudio no invasivo, las estimaciones del ancho y largo de los testículos son probablemente menos precisas dado que 1) los machos fueron fotografiados a distancias y alturas variables ($<15 \text{ m}$), causando un grado de paralaje, 2) la variación entre las fotografías en la

posición relativa y ángulo de los testículos en un plano 2D, lo que afecta especialmente la estimación de partes pequeñas del cuerpo (Barrickman et al., 2015), y 3) la incapacidad de remover físicamente los pliegues escrotales y, en consecuencia, nuestras estimaciones del volumen testicular pueden reflejar en su lugar el volumen escrotal. Para compensar estos problemas, calculamos una media para las medidas de los testículos a partir de múltiples fotografías que les capturaban en posiciones y ángulos adecuados, siempre con los testículos por completo dentro del escroto. Así, si bien reconocemos que nuestros resultados del volumen testicular parecen sobre estimados y recomendamos tomar la subsecuente interpretación de los resultados del modelo del compromiso evolutivo con precaución, no tenemos razones para sospechar un sesgo en la sobre estimación hacia algún macho en particular. Asimismo, estudios en otros mamíferos han encontrado que el volumen escrotal se correlaciona altamente con el testicular y la capacidad de producción espermática (Rusk et al. 2002; Abdullahi et al. 2012; Shende et al. 2019).

Considerando que valores más pequeños de ΔF reflejan volúmenes mayores del hueso hioides (Dunn et al., 2015), el compromiso evolutivo entre ΔF y el volumen testicular debería resultar en una correlación positiva. Contrario a nuestra predicción, no encontramos una asociación entre la F_0 o ΔF de ladridos o rugidos y el volumen testicular. En el modelo con solo el volumen testicular como variable predictora, encontramos una correlación negativa, aunque débil, con la ΔF de los rugidos, lo que implica que los machos invierten tanto en el agrandamiento del hueso hioides como de sus testículos. Sin embargo, cuando también consideramos al tamaño del cuerpo como variable predictora, la asociación entre la ΔF de los rugidos y el volumen testicular desaparece, lo que sugiere que después de tomar en cuenta el efecto del tamaño del cuerpo sobre la ΔF de los rugidos, no existe variación adicional de la ΔF de los rugidos que pueda ser explicada por el volumen testicular. El efecto significativo que encontramos en el primer modelo probablemente resulta de la correlación positiva entre el tamaño del cuerpo y el volumen testicular, con machos más grandes presentando mayores volúmenes testiculares. Esto es algo que se ha encontrado también en humanos (Bahk et al. 2010; Innocent et al. 2016) y otros mamíferos

(Salhab et al. 2001; Brito et al. 2012; Jacyno et al. 2015). Por lo tanto, nuestros resultados sugieren que en los monos aulladores negros no existe un compromiso entre la F_0 o ΔF de sus ladridos o rugidos y el volumen testicular, lo que refleja una ausencia de compromiso entre el hueso hioides y el volumen testicular en esta especie.

Nuestros resultados contrastan con los reportados por Dunn et al., (2015), donde encontraron una asociación negativa entre el tamaño del hueso hioides y el volumen testicular entre cinco especies de mono aullador, *A. pigra* incluida. Sin embargo, ese trade-off es probablemente altamente influenciado por las especies a ambos extremos del espectro. En un lado, se encuentran *A. seniculus* y *A. guariba*, quienes presentan un sesgo evidente hacia huesos hioides agrandados y testículos pequeños, sus grupos usualmente contienen solo un macho adulto quien monopoliza el acceso a las hembras (Dunn et al., 2015; Van Belle & Bicca-Marques, 2015). Por el otro lado, *A. caraya* y *A. palliata* muestran un claro sesgo hacia testículos grandes y huesos hioides pequeños, con grupo usualmente de múltiples machos, y una alta tasa de promiscuidad femenina (Dunn et al., 2015; Kelaita et al., 2011; Van Belle & Bicca-Marques, 2015). Comparado con estas especies, *A. pigra* presenta valores intermedios de volumen testicular y tamaño del hueso hioides, sus grupos usualmente contienen un par de machos, y la monopolización femenina es alta, pero incompleta (Van Belle & Estrada, 2008; Van Belle et al., 2014a). Dado que el tamaño del hueso hioides o el volumen testicular son rasgos anatómicamente fijos que no cambian en los machos de acuerdo con los varios contextos sociales que enfrentan en su vida, los tamaños de estas partes del cuerpo reflejan la tasa promedio de competición intra o intergrupala que la especie ha experimentado en escala evolutiva (Dixson, 2018). Esto podría explicar porque el compromiso entre los rasgos vocales y testiculares fue encontrado entre las especies de mono aullador, ya que cada una ha evolucionado diferentes estrategias reproductivas para los machos en conjunto con sus rasgos anatómicos asociados, ambas promovidas por los diferentes niveles de competencia entre machos intra- e intergrupala, lo que no sucede dentro de una especie, donde los individuos no pueden alterar sus rasgos anatómicos, en su lugar, muestran ajustes de comportamiento y fisiología de acuerdo a las variaciones en los niveles de

competencia intra- e intergrupala (Horwich et al. 2000; Rangel-Negrín et al. 2011; Van Belle et al. 2009b), particularmente cuando se experimentan niveles intermedios de competencia intra- e intergrupala, como es probablemente el caso de los monos aulladores negros.

En efecto, los compromisos entre los rasgos con utilidad previa y posterior a la cópula parecen ser más prevalentes cuando existe una inversión desproporcional en un rasgo con utilidad previa a la cópula, como cuando los machos puede monopolizar el acceso a múltiples hembras, volviendo la ganancia potencial tan grande que la inversión en rasgos con utilidad posterior a la cópula representa una marcada mala inversión (Dines et al., 2015). Los análisis de paternidad han corroborado que los machos centrales de mono aullador negro no son capaces de monopolizar a las hembras a pesar de la presencia de comportamiento de guardia y consorcios (Van Belle et al., 2009b; Van Belle et al., 2014a). Estos análisis revelaron que las hembras copulan más frecuentemente con el macho central de su grupo, pero también con machos no centrales dentro de su grupo y con machos de grupos vecinos, lo que resulta en un sistema de apareamiento ocasional de poliginándria (Van Belle et al., 2014^a). La incapacidad de los machos centrales para monopolizar a las hembras les ha expuesto a un grado de competición espermática, previniendo la inversión única en rasgos con utilidad previa a la cópula, aunque no lo suficiente para disparar la inversión desproporcional en volumen testicular, lo que puede resultar en la ausencia del compromiso evolutivo entre los rasgos vocales y testiculares.

Asimismo, aunque las vocalizaciones de *A. pigra* probablemente juegan un rol en la competencia directa entre machos por acceder a las hembras, estas son poco decisivas; en su lugar, se utilizan principalmente como un despliegue agresivo indirecto que transmite información acerca de la fuerza individual y grupal, pero no disuade a las hembras de copular con machos no centrales o de grupos vecinos (Van Belle et al., 2014b; Van Belle & Estrada, 2008). Probablemente, los machos también utilizan sus vocalizaciones en otros contextos competitivos de manera menos directa, por ejemplo, al incrementar sus oportunidades de apareamiento a través de la defensa de un territorio, sus recursos comestibles, y su descendencia, todo ello necesario para una reproducción femenina exitosa (Van Belle

et al., 2013; Van Belle & Estrada, 2020; Kitchen, 2004). En consecuencia, la evolución del aparato vocal especializado de los monos aulladores es sobre todo multifactorial, incluyendo factores sociales y del medio ambiente (Charlton & Reby, 2016; Kitchen et al., 2015).

Conclusiones

Hemos mostrado que la dispersión de los formantes en los rugidos son una señal honesta del tamaño del cuerpo en machos de mono aullador, mientras que sus ladridos no muestran ninguna asociación con el tamaño del cuerpo. Aunque nuestros resultados no proporcionan evidencia de la función específica de los rugidos (defensa de territorio, descendientes, alimentos, pareja o espaciado intergrupar), sugieren que los rugidos de los machos son un recurso particularmente útil para utilizarlo en la competencia entre machos o la elección femenina debido a sus propiedades alométricas. Mientras tanto, nuestros resultados del modelo de compromiso evolutivo revelan tanto los beneficios como las limitaciones de las técnicas no invasivas, y sugieren que los monos aulladores negros no presentan un compromiso entre las características acústicas de sus principales vocalizaciones y el volumen testicular. Estos hallazgos remarcan la importancia de la presión relativa de las diferentes formas de competencia entre machos en la formación de los rasgos sexuales y revelan la ausencia de un compromiso evolutivo dentro de una especie, a pesar de su presencia a nivel de género. Se necesita investigación adicional para determinar 1) si los monos aulladores pueden percibir cambios en el espaciado entre formantes de los rugidos, 2) cómo pueden funcionar sus rugidos en la evaluación de rivales, 3) la posibilidad de que los formantes codifiquen la identidad del emisor, y 4) si la elección femenina es influenciada por la información de los rugidos.

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