The ecological, evolutionary, and behavioural context of ultrasonic vocalizations in flying squirrels

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Abstract

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Acoustic communication is vital for mediating interactions between individuals and their environment. From echolocating bats to isolated rodent pups, ultrasonic vocalizations are a useful mechanism for producing localized and cryptic acoustic signals. Flying squirrels have been demonstrated to produce almost exclusively ultrasonic calls which is rare in both squirrels and mammals, though the significance of this acoustic range is unknown. To investigate the context of ultrasonic calls in these gliding mammals, I created phylogenetic comparative analyses to compare ecological and morphological traits against call frequencies. I found that nocturnality, a trait entangled with gliding in squirrels, was related to higher-frequency calls. Furthermore, by comparing all gliding mammals and their non-gliding counterparts, I found that gliding mammals produce significantly higher frequencies than these relatives. This form of cryptic communication is likely used to avoid predation, which was further supported by behavioural experiments wherein flying squirrels used significantly higher frequencies when predation risk increased. While high frequency communication was used by most gliders, I found that ultraviolet-induced photoluminescence, another potential form of crypsis, was strongly associated with nocturnality, with half of the tested gliding mammals showing evidence of this unique colouration. While ultrasonic vocalizations are widespread in echolocating bats and echonavigating mammals, I did not find evidence to support echonavigation in flying squirrels. Instead, I found that ultrasonic vocalizations are used in a variety of social contexts and during solitary foraging and exploration. These combined results demonstrate the unique ecological and evolutionary pressures acting on small-bodied, nocturnal gliding taxa and the resulting cryptic behaviours and communication.

Keywords: communication, flying squirrels, gliding mammals, Sciuridae, ultrasound, ultraviolet-induced photoluminescence

Preface

This thesis is written in manuscript format, and I am the first author on each manuscript. I am the sole author of Chapters One and Five (General Introduction and Discussion), while Chapters Two to Four have additional co-authors, and this is reflected in the pronouns used in each chapter. Chapters Two to Four contain significant contributions from Jeff Bowman, while Chapter Three contains additional contributions from Irena Schneiderová and Bryan Hughes. At the time of writing, Chapters Two and Three have been published in peer-reviewed journals (i.e., Frontiers in Ecology and Evolution and PeerJ, respectively). Chapters Four has yet to be submitted for publication but is formatted for the Journal of Mammalogy.

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Chapter 1 - General Introduction

[Animals] do speak, but only to those who know how to listen. (Orhan Pamuk, 1998)

Communication is essential for interactions between organisms. Species can use visual, tactile, electrical, chemical, and acoustic cues to transfer critical information from one individual to another. The transferred information can range in purpose, from sexual, social, territorial, familial, predatory, and so on. Without communication, individuals wouldn't be able to facilitate group interactions, warn conspecifics of predators, defend territories without fighting, or properly interpret sexual cues.

Acoustic communication is incredibly useful for transmitting information over large distances. Unlike visual communication, acoustic communication does not require seeing the producer or receiver. Unlike chemical cues, acoustic cues almost instantaneously transmit across a multidimensional space and can be controlled temporally. Acoustic communication is often used by every age-class within a vocal species, starting with mammalian pups producing calls to attract their mothers (Blake, 1992; Blumberg et al., 1992; Zaytseva et al., 2019) and ending with fear screams used in a last attempt to escape predation (Högstedt, 1983). In some species, acoustic communication has been observed at the embryonic level, with parental calls sharing environmental information (i.e. temperature; (Mariette & Buchanan, 2016)) and facilitating auditory development (Gottlieb, 1968) and vocal complexity (Kleindorfer et al., 2018). Additionally, there are rare occurrences of prenatal individuals using acoustic communication to facilitate hatching synchrony and parental care in some reptiles (i.e. crocodiles; (Vergne et al., 2009)) and birds (Berlin & Clark, 1998; Rumpf & Tzschentke, 2010).

The way that different species communicate acoustically is dictated by their physical structures. Many insects rely on external structures to produce sounds which they may vibrate, click (i.e. tymbals), percuss, or move against each other (i.e. stridulation) to produce sounds (Neil & Holderied, 2021). In contrast, most vertebrates produce sounds via internal structures contained in the respiratory, phonary, and filter systems (Lattenkamp, 2020). In vocal vertebrates, sound originates from the respiratory system when air is expelled via the lungs. The air passes through the phonary system where the air is transformed into an auditory sound by opening and contracting muscles either in the larynx, as in mammals, or syrinx, as in birds. The combination of the respiratory and phonary systems creates the sound ‘source’, resulting in the fundamental frequency (Taylor & Reby, 2010). The fundamental frequency is then modified in the filter systems (i.e. the trachea, mouth, and nasal cavities) to suppress or emphasize the harmonics of the fundamental frequency, resulting in formants. The exact structures associated with the source-filter theory may vary among species, but this is the general vocal production mechanism of all vocal vertebrates (Taylor & Reby, 2010).

All acoustic systems, regardless of taxa, result in sound waves which vibrate molecules in the surrounding environment to transmit the acoustic signal. The distance between peaks in the sound wave, caused by the rate of vibration of acoustic structures (e.g. rate of vocal folds opening and closing in mammals, rate of ear drum vibration), define the call frequency which is measured in hertz (Hz). The faster the acoustic structures are vibrated, the closer the distance will be between the peaks, resulting in a higher-frequency vocalization. The physical size of the acoustic structures dictates the maximum frequency that can be produced as more energy is required to move larger acoustic structures at faster rates (Martin et al., 2017). This results in a strong relationship between vocal frequency and body size in mammals, with elephants, the largest land mammals, capable of vocalizing lower than humans can hear (McComb et al., 2003) and small mammals, such as shrews, producing vocalizations above the hearing range of humans (Siemers et al., 2009; Tomasi, 1979). Interestingly, this relationship is less pronounced in aquatic environments because signals can travel more easily in water, allowing much larger animals to produce higher frequencies in the water than similarly sized terrestrial mammals (Martin et al., 2017). Ultimately, the anatomy of both the sender and the receiver of acoustic information influence the evolution of signals that are ultimately employed in communication.

In acoustics, the human hearing range defines the categorization of all frequencies. Humans have the capacity to hear between 20Hz and 20kHz, though this range deteriorates over a human's life, with most older adults unable to hear above 10kHz (Masterton et al., 1969). Sounds below the human hearing range are termed infrasonic (<20Hz) and sounds above that range are termed ultrasonic (>20kHz). As already mentioned, elephants can use infrasonic frequencies due to their large body size. These infrasonic sound waves have greater distance between their peaks and can more easily move through solid structures than higher frequencies – these acoustic characteristics allow elephants to communicate over several kilometers (McComb et al., 2003). However, the sound wave will deteriorate as it moves through multiple structures, meaning that the information carried becomes less specific, especially in spatially complex environments (Hedwig et al., 2018). Over short distances, elephants can distinguish between female social groups and levels of sexual maturation in males (McComb et al., 2003; Stoeger & Baotic, 2016). Over longer distances, even in ideal atmospheric and environmental conditions, information deteriorates to simply call presence (Garstang et al., 2005; McComb et al., 2003).

In contrast, ultrasound is restricted to very short distances because the narrow sound waves are easily attenuated by the environment around them (Smith, 1979). This attenuation means that ultrasonic calls are limited by not only the caller's size (Martin et al., 2017), but also the environment and distance over which they are communicating. Echolocation, the rapid production of mostly ultrasonic calls to locate prey and environmental features (Jones, 2005), takes advantage of the directional nature of ultrasonic calls. Echolocation is used by a wide variety of bats, including one branch of Yinpterochiroptera (*Rousettus* (Springer et al., 2001; Yovel et al., 2011)), but Yangochiroptera display the most precise narrowband forms of echolocation of all mammals (Fenton, 1984). Cetaceans are also able to echolocate to hunt and navigate, though cetaceans rely on a dense lipid structure (i.e. melon) to amplify calls passing through the nasal passage (Tyack & Miller, 2002) and like *Rousettus*, cetaceans also utilize broadband clicks.

For many years, echolocation was the main type of ultrasonic communication that was studied, with the assumption that most mammals are unable to echolocate. However, behavioural studies in recent decades have demonstrated that a variety of other mammals can use simplistic echolocation to orient and travel through their environment. This click-based echolocation is used by animals that have reduced visual input, such as the previously mentioned *Rousettus* that navigate dark cave systems (Yovel et al., 2011). Unlike the click-based echolocation of cetaceans, fruit bats do not employ a terminal buzz (as also seen in Yangochiroptera) when nearing an obstacle. The lack of this buzz places *Rousettus* in the category of 'echonavigators' – a term borrowed here from Medway (1967) who described similar click-based echolocation in cave-nesting swiftlets (*Aerodramus* and *Collocalia*). Other echonavigators include a variety of shrews (Gould et al., 1964; Siemers et al., 2009; Tomasi, 1979), arboreal blind mice (*Typhlomys*; (Panyutina et al., 2017)), and even humans with visual impairments (Thaler & Goodale, 2016). All these species have been demonstrated to produce simple calls in sequence while navigating mazes and complex spaces, allowing them to move through these systems without visual input. The discovery of echonavigation in multiple, unrelated species suggests that while complex echolocation is limited to select groups, echonavigation may be widely used by mammals in select physical or environmental conditions, especially when visual input is severely reduced.

Ultrasonic calls are not limited to echolocation or navigation and have been demonstrated as useful for social communication in many mammals. Firstly, as previously mentioned, many rodent pups rely on ultrasound to communicate with their mothers. Ultrasonic calls are readily induced by changes in their environment (Blake, 1992; Blumberg et al., 1992) and isolation (Arriaga & Jarvis, 2013; Branchi et al., 2001), meaning that these pups are more likely to vocalize when alone and vulnerable. However, the use of ultrasound acts as an acoustic camouflage as the calls are quickly attenuated in complex environments (e.g., in areas covered in grass or leaves) and not readily heard by many predators, including most birds (Branchi et al., 2001; Knudsen, 1981). Other mammals, as demonstrated in the Richardson's ground squirrels (*Spermophilus richardsonii*), derive similar benefits of ultrasound when alarm calling. These ground squirrels use ultrasonic alarm calls when predators are farther away, taking advantage of the attenuation and localization of ultrasonic calls to warn conspecifics when predators are less likely to eavesdrop (Wilson & Hare, 2006). Ultrasound has also been demonstrated in a variety of other social interactions, specifically in myomorphic rodents, including courtship (Egnor & Seagraves, 2016; Miller & Engstrom, 2007), mating (Pasch et al., 2017), and aggressive encounters (Corrigan & Flannelly, 1979). While ultrasonic calls have been identified in many other mammals, such as tarsiers (*Tarsier* spp. (Gursky, 2015; Ramsier et al., 2012)), slow lorises (*Nycticebus javanicus* (Geerah et al., 2019)), hazel dormice (*Muscardinus avellanarius* (Ancillotto et al., 2014)), colugos (*Galeopterus variegatus* (Miard et al., 2019)), and flying squirrels (*Glaucomys* spp. (Diggins et al., 2020; Eisinger et al., 2016; Gilley et al., 2019; Murrant et al., 2013)), the behaviours related to ultrasound production are undocumented for most species.

In 2013, the first records of ultrasonic calls in North American flying squirrels (*Glaucomys*) were published by two research groups (Gilley, 2013; Murrant et al., 2013). Prior to these studies, flying squirrels were assumed to not produce many, if any, vocalizations. However, findings reported in several publications reveal that flying squirrels produce over 20 unique, mostly ultrasonic, vocalizations which vary between northern (*Glaucomys sabrinus*) and southern (*G.volans*) flying squirrels (Diggins et al., 2020; Eisinger et al., 2016; Gilley et al., 2019; Murrant et al., 2013). Despite the numerous publications addressing the vocal production of these squirrels, no researcher has identified why flying squirrels are signalling in the ultrasonic range or the behaviours associated with ultrasonic calls. One researcher attempted to record echolocation in these squirrels prior to any ultrasonic calls being reported and was unsuccessful in their experiments (Chattin, 1969).

Ultrasound production is not the only unique phenotype expressed by flying squirrels. Firstly, flying squirrels are the only group of squirrels that are strictly nocturnal (Arbogast, 2007) with about 50 species of flying squirrels around the globe which all share this trait (Jackson & Schouten, 2012). Additionally, as their name implies, they are the only group of squirrels that have a patagium which allows them to glide between trees and conserve energy (Scheibe et al., 2006). Gliding is shared by only a few extant mammalian clades, all of which are also strictly nocturnal (Jackson & Schouten, 2012). Nocturnality and gliding may be related to the use of ultrasonic calls, due to some nocturnal predators (i.e., owls have not been shown to hear ultrasonic calls (Brittan-Powell et al., 2005; Knudsen, 1981)) and the visual limitations of gliding in dark conditions, which may mirror evolutionary pressures of other visually-limited echonavigators.

In my thesis, I address the ecological and evolutionary pressures that could explain the use of ultrasonic communication in flying squirrels and other lineages that occupy a similar niche. In my first chapter, I explore the history of vocal production studies in Sciuridae and the life history traits that relate to the type of frequencies employed by all squirrels. In my second chapter, I dive into the relationship between gliding and ultrasonic calls by comparing all gliding mammals to their non-gliding counterparts. Interestingly, flying squirrels aren’t the only mammalian gliders to produce ultrasonic calls and this may be a form of acoustic camouflage necessary for social, nocturnal gliders. In my third chapter, I use behavioural studies to explore the contexts in which ultrasonic calls are used in northern and southern flying squirrels, investigating both social contexts and the potential for echonavigation. Finally, in my discussion chapter, I compare vocalizations of several non-gliding mammals to explore the prevalence of ultrasonic calls and summarize the ecological function of ultrasound production in flying squirrels. Within this discussion, I summarize the findings from my primary data chapters and provide avenues for future research.

References

Ancillotto L, Sozio G, Mortelliti A, Russo D. 2014. Ultrasonic communication in Gliridae (Rodentia): the hazel dormouse (*Muscardinus avellanarius*) as a case study. *Bioacoustics* 23(2):129-141. DOI: 10.1080/09524622.2013.838146.

Arbogast BS. 2007. A brief history of the new world flying squirrels: phylogeny, biogeography, and conservation genetics. *Journal of Mammalogy* 88(4):840-849. DOI: 10.1644/06-mamm-s-322r1.1.

Arriaga G, Jarvis ED. 2013. Mouse vocal communication system: are ultrasounds learned or innate? *Brain & Language* 124(1):96-116. DOI: 10.1016/j.bandl.2012.10.002.

Berlin KE, Clark AB. 1998. Embryonic calls as care-soliciting signals in budgerigars, *Melopsittacus undulatus*. *Ethology* 104(6):531-544. DOI: 10.1111/j.1439-0310.1998.tb00088.x

Blake BH. 1992. Ultrasonic vocalization and body temperature maintenance in infant voles of three species (Rodentia: Arvicolidae). *Developmental Psychobiology* 25(8):581-596. DOI: 10.1002/dev.420250805.

Blumberg MS, Efimova IV, Alberts JR. 1992. Ultrasonic vocalizations by rat pups: the primary importance of ambient tempertaure and the thermal significance of contact comfort. *Developmental Psychobiology* 25(4):229-250. DOI: 10.1002/dev.420250402.

Branchi I, Santucci D, Alleva E. 2001. Ultrasonic vocalisation emitted by infant rodents: a tool for assessment of neurobehavioural development. *Behavioural Brain Research* 125(1-2): 49-56. DOI: 10.1016/s0166-4328(01)00277-7.

Brittan-Powell EF, Lohr B, Hahn DC, Dooling RJ. 2005. Auditory brainstem responses in the eastern screech owl: an estimate of auditory thresholds. *The Journal of the Acoustical Society of America* 118(1):314-321. DOI: 10.1121/1.1928767.

Chattin D. 1969. Acoustical echolocation in flying squirrels. B. A. Thesis, Reed College.

Corrigan JG, Flannelly KJ. 1979. Ultrasonic vocalizations of defeated male rats. *Journal of Comparative and Physiological Psychology* 93(1):105-115. DOI: 10.1037/h0077565.

Diggins CA, Gilley LM, Turner GG, Ford WM. 2020. Ultrasonic acoustic surveys of state endangered northern flying squirrels in the Pocono Mountains, Pennsylvania. *Journal of Fish and Wildlife Management* 11(2):644-653. DOI: 10.3996/JFWM-20-020.

Egnor SER, Seagraves KM. 2016. The contribution of ultrasonic vocalizations to mouse courtship. *Current Opinion in Neurobiology* 38(1):1-5. DOI: 10.1016/j.conb.2015.12.009.

Eisinger JW, Scheibe JS, Flaherty EA. 2016. Novel *Glaucomys volans* vocalizations in Indiana and evidence of geographic variation in high frequency communication. *Journal of Mammalogy* 97(4):1219-1227. DOI: 10.1093/jmammal/gyw076.

Fenton MB. 1984. Echolocation: implications for ecology and evolution of bats. *The Quarterly Review of Biology* 59(1):33-53.

Garstang M, Fitzjarrald DR, Fristrup K, Brain C. 2005. The daily cycle of low-frequency elephant calls and near-surface atmospheric conditions. *Earth Interactions* 9(14):1-21. DOI: 10.1175/EI147.1.

Geerah DR, O'Hagan RP, Wirdateti W, Nekaris KAI. 2019. The use of ultrasonic communication to maintain social cohesion in the Javan slow loris (*Nycticebus javanicus*). *Folia Primatologica* 90(5):392-403. DOI: 10.1159/000495077.

Gilley LM. 2013. Discovery and characterization of high-frequency calls in North American flying squirrels (*Glaucomys sabrinus* and *G. volans*): implications for ecology, behavior, and conservation. D. Phil. Thesis, Auburn University.

Gilley LM, Diggins CA, Pearson SM, Best TL. 2019. Vocal repertoire of captive northern and southern flying squirrels (*Glaucomys sabrinus* and *G. volans*). *Journal of Mammalogy* 100(2):518-530. DOI: 10.1093/jmammal/gyz064.

Gottlieb G. 1968. Prenatal behavior of birds. *The Quarterly Review of Biology* 43(2):148-174. DOI: 10.1086/405726.

Gould E, Negus NC, Novick A. 1964. Evidence for echolocation in shrews. *Journal of Experimental Zoology* 156(1):19-37. DOI: 10.1002/jez.1401560103.

Gursky S. 2015. Ultrasonic vocalizations by the spectral tarsier, *Tarsius spectrum*. *Folia Primatologica* 86(3):153-163. DOI: 10.1159/000371885.

Hedwig D, DeBellis M, Wrege PH. 2018. Not so far: attenuation of low-frequency vocalizations in a rainforest environment suggests limited acoustic mediation of social interaction in African forest elephants. *Behavioural Ecology and Sociobiology* 72(33). DOI: 10.1007/s00265-018-2451-4.

Högstedt G. 1983. Adaptation unto death: function of fear screams. *The American Naturalist* 121(4):562-570.

Jackson SM, Schouten P. 2012. *Gliding Mammals of the World*. Melbourne: CSIRO.

Jones G. 2005. Echolocation. *Current Biology* 15(13):R484-R488. DOI: 10.1016/j.cub.2005.06.051.

Kleindorfer S, Evans C, Hauber ME, Colombelli-Négrel D. 2018. Could prenatal sound discrimination predict vocal complexity later in life? *BMC Biology* 3(11). DOI: 10.1186/s40850-018-0038-1.

Knudsen EI. 1981. The hearing of the barn owl. *Scientific American* 245(6):112-125. DOI: 10.1038/scientificamerican1281-112.

Lattenkamp EZ. 2020. Vocal Learning in the Pale Spear-Nosed Bat, *Phyllostomus discolor*. D. Phil. Thesis, Radboud University.

Mariette MM, Buchanan KL. 2016. Prenatal acoustic communication programs offspring for high posthatching temperatures in a songbird. *Science*, 353(6301):812-814. DOI: 10.1126/science.aaf7049.

Martin K, Tucker MA, Rogers TL. 2017. Does size matter? Examining the drivers of mammalian vocalizations. *Evolution* 71(2):249-260. DOI: 10.1111/evo.13128.

Masterton B, Heffner H, Ravizza R. 1969. The evolution of human hearing. *The Journal of the Acoustical Society of America* 45(4):966-985. DOI: 10.1121/1.1911574.

McComb K, Reby D, Baker L, Moss C, Sayialel S. 2003. Long-distance communication of acoustic cues to social identity in African elephants. *Animal Behaviour* 65(2):317-329. DOI: 10.1006/anbe.2003.2047.

Medway L. 1967. The function of echonavigation among swiftlets. *Animal Behaviour* 15(4):416-420. DOI: 10.1016/0003-3472(67)90038-3.

Miard P, Lim LS, Abdullah NI, Elias NA, Ruppert N. 2019. Ultrasound use by Sunda colugos offers new insights into the communication of these cryptic mammals. *Bioacoustics* 28(5):397-403. DOI: 10.1080/09524622.2018.1463294.

Miller JR, Engstrom MD. 2007. Vocal stereotypy and singing behavior in Baiomyine mice. *Journal of Mammalogy* 88(6):1447-1465. DOI: 10.1644/06-MAMM-A-386R.1.

Murrant MN, Bowman J, Garroway CJ, Prinzen B, Mayberry H, Faure PA. 2013. Ultrasonic vocalizations emitted by flying squirrels. *PLoS ONE* 8(8), 1-6. DOI: 10.1371/journal.pone.0073045.

Neil TR, Holderied MW. 2021. Sound production and hearing in insects. In: Jurenka R, ed. *Advances in Insect Physiology, Volume 61*. Academic Press, 101-139. DOI: 10.1016/bs.aiip.2021.10.001.

Panyutina AA, Kuznetsov AN, Volodin IA, Abramov AV, Soldatova I B. 2017. A blind climber: the first evidence of ultrasonic echolocation in arboreal mammals. *Integrative Zoology* 12(2):172-184. DOI: 10.1111/1749-4877.12249.

Pasch B, Tokuda IT, Riede T. 2017. Grasshopper mice employ distinct vocal production mechanisms in different social contexts. *The Royal Society Proceedings B* 284(1859):20171158. DOI: 10.1098/rspb.2017.1158.

Ramsier MA, Cunningham AJ, Moritz GL, Finneran JJ, Williams CV, Ong PS, Gursky-Doyen SL, Dominy NJ. 2012. Primate communication in the pure ultrasound. *Biology Letters* 8(4):508-511. DOI: 10.1098/rsbl.2011.1149.

Rumpf M, Tzschentke B. 2010. Perinatal acoustic communication in birds: why do birds vocalize in the egg? *The Open Ornithology Journal* 3:141-149. DOI: 10.2174/1874453201003010141.

Scheibe JS, Smith WP, Bassham J, Magness D. 2006. Locomotor performance and cost of transport in the northern flying squirrel *Glaucomys sabrinus*. *Acta Theriologica* 51(2):169-178. DOI: 10.1007/BF03192668.

Siemers BM, Schauermann G, Turni H, von Merten S. 2009. Why do shrews twitter? Communication or simple echo-based orientation. *Biology Letters* 5(5):593-596. DOI: 10.1098/rsbl.2009.0378.

Smith JC. 1979. Factors affecting the transmission of rodent ultrasounds in natural environments. *Integrative and Comparative Biology* 19(2):432-442. DOI: 10.1093/icb/19.2.432.

Springer MS, Teeling EC, Madsen O, Stanhope MJ, de Jong WW. 2001. Integrated fossil and molecular data recontruct bat echolocation. *Proceedings of the National Academy of Sciences* 98(11):6241-6246. DOI: 10.1073/pnas.11155199.

Stoeger AS, Baotic A. 2016. Information content and acoustic structure of male African elephant social rumbles. *Scientific Reports* 6:27585. DOI: 10.1038/srep27585.

Taylor AM, Reby D. 2010. The contribution of source-filter to mammal vocal communication research. *Journal of Zoology* 280(3):221-318. DOI: 10.1111/j.1469-7998.2009.00661.x.

Thaler L, Goodale MA. 2016. Echolocation in humans: an overview. *WIREs Cognitive Science* 7(6):382-393. DOI: 10.1002/wcs.1408.

Tomasi TE. 1979. Echolocation by the short-tailed shrew *Blarina brevicauda*. *Journal of Mammalogy* 60(4):751-759. DOI: 10.1121/10.0020071.

Tyack PL, Miller EH. 2002. Vocal anatomy, acoustic communication and echolocation. In: Hoelzel AR, ed. *Marine Mammal Biology: An Evolutionary Approach*. Oxford: Blackwell Publishing, 142-184.

Vergne AL, Pritz MB, Mathevon N. 2009. Acoustic communication in crocodilians: from behaviour to brain. *Biological Reviews* 84(3):391-411. DOI: 10.1111/j.1469-185X.2009.00079.x.

Wilson DR, Hare JF. 2006. The adaptive utility of Richardson's ground squirrel (*Spermophilus richardsonii*) short-range ultrasonic alarm signals. *Canadian Journal of Zoology* 84(9):1322-1330. DOI: 10.1139/Z06-120.

Yovel Y, Geva-Sagiv M, Ulanovsky N. 2011. Click-based echolocation in bats: Not so primitive after all. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 197(5):515-530. DOI: 10.1007/s00359-011-0639-4.

Zaytseva AS, Volodin IA, Ilchenko OG, Volodina EV. 2019. Ultrasonic vocalization of pup and adult fat-tailed gerbils (*Pachyuromys duprasi*). *PLoS ONE* 14(7):e0219749. DOI: 10.1371/journal.pone.0219749.

Chapter 2 - Think before they squeak: Vocalizations of the squirrel family

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Abstract

Squirrels (Sciuridae) are a diverse group in behaviour, morphology, and ecology. This variation is typified by the wide range of vocalizations spanning ground squirrels (Marmotini and Xerini), tree squirrels (Callosciurinae and Sciurini), and flying squirrels (Pteromyini). Squirrels produce calls that range in frequency, modulation, and function, with a complex set of social calls occurring across the family. We review the history of recording methods used in the development of squirrel vocalization repertoires, with emphasis on how the ecology and methodology impact the frequency values reported. The fundamental (F_0 – the mean frequency of the fundamental harmonic), dominant (F_{Dom} – the frequency of maximum energy or amplitude), minimum (F_{Min} – the minimum frequency of the fundamental harmonic), maximum (F_{Max} – the maximum frequency of the dominant harmonic), and highest harmonic (F_{Harm} – the mean frequency of the highest visible harmonic) frequencies were considered against popular hypotheses that have attempted to explain the evolution of vocal frequency characteristics in terrestrial mammals. These hypotheses include body size, predator avoidance, habitat type, and diel activity pattern. Phylogenetic generalized least squares modelling revealed that body mass and the frequency limits of the methods were strong determinants of perceived high-frequency communication. Consistent with popular hypotheses, social squirrels exhibited a broader range of F_0, F_{Dom}, and F_{Max} than solitary squirrels while habitat openness promoted higher F_{Dom} and F_{Harm}. Additionally, nocturnality was significantly associated with higher F_0, F_{Dom}, and F_{Max}, suggesting that flying squirrels, the only nocturnal squirrels, commonly use high-frequency acoustic signals, a finding that merits further investigation. In conclusion, our review provides unique insight into the role of behavioural ecology on vocal repertoires and the importance of accurate equipment selection for sampling across a diverse taxon.

Contribution to Field

We carry out the first review of determinants of vocal frequency characteristics in the squirrel family (Sciuridae), controlling for phylogeny, and testing for effects of technological limit such as recording equipment. We found that vocal frequency characteristics are affected by diel activity (i.e., nocturnality), body size, habitat openness, and sociality, though the strength and relevance of the factors vary between the characteristics. The technological method limits (i.e., technology) also had a significant effect on the detection of higher frequencies. Our study suggests the need for better global coverage of vocalizations in the squirrel family and of the need to test hypotheses about the evolution of high-frequency communication in the nocturnal flying squirrel tribe.

Keywords: Sciuridae, squirrels, vocalizations, alarm call, ultrasonic vocalizations, frequency characteristics

Introduction

Mammalian bioacoustics is an expansive field in which biology, physics, and psychology are used to study the taxonomic diversity of signalers and interpret the complexity of vocal communication. Over the last century or so, the bioacoustic community has seen the development, implementation, and specialization of their research technology. Ever-improving recording devices have allowed advancements from phonetic descriptions of non-human animal calls to the first spectrographic visualizations to the development of software allowing quantitative analysis of calls in minute detail (Terhune, 2011). While phonetic descriptions of the calls served an important purpose at one point in time, the recording of calls provided the first opportunity for a detailed comparison of the differences between species and the evolution of vocal production in mammals through spectrographic analyses. The first acoustic spectrograph was developed in 1946 to study human speech, though the authors suggested that this new device could be used to study environmental sounds, musical instruments and orchestral composition, and potentially animal sounds under the right conditions (Koenig, Dunn & Lacy, 1946).

Spectrographs were, and continue to be, important because they allowed researchers to study the shape and structure of the sounds being produced, but they also allowed the visualization of calls that cannot be heard by the human ear. Humans are limited to a hearing frequency range of about 20 Hz to a maximum of 20 kHz, though 15-18 kHz is the cut-off for the average human (Masterton, Heffner & Ravizza, 1969). Given that it is difficult for even the best human ears to hear sounds near the upper limit (Masterton et al., 1969), some have argued that 15 kHz should be the cut-off for differentiating between the audible range (what can be heard by humans) and the ultrasonic range (calls that exceed the upper limit of human hearing).

However, most publications describe 20 kHz, the absolute maximum of human hearing, as the frequency at which calls can be described as ultrasonic.

Ultrasonic vocalizations (USVs), calls that completely lie in the ultrasonic range (>20 kHz herein) were first studied in cetaceans and bats that had been observed navigating and hunting without visual cues. USVs were first recorded with the aid of frequency-altering heterodynes in bats (Pierce & Griffin, 1938) and were first visualized in porpoises (Wood Jr., 1952). Bats and cetaceans largely remained the focus of ultrasonic studies for decades, due in particular to interest in echolocation. The intensive echolocation focus has likely overshadowed ultrasound-production in many other wild species such as shrews (*Blarina* and *Sorex*: Gould, Negus & Novick, 1964; *Sorex*: Tomasi, 1979), singing mice (*Baiomys* and *Scotinomys*: Miller & Engstrom, 2007; *Scotinomys*: Fernández-Vargas, Tang-Martinez & Phelps, 2011), and even several species of squirrel (*Urocitellus richardsonii*: Wilson & Hare, 2004; *Glaucomys*: Murrant et al., 2013). Additionally, the widespread availability of lab mice and rats has led to many studies investigating the function and biomechanical processes involved with USV production. The study of USV production in these rodents has been quite active since the early 1970s (Corrigan & Flannelly, 1979; Geyer, 1979; Geyer & Barfield, 1978; Geyer, Barfield & McIntosh, 1978; Hofer & Shair, 1978, 1980; Oswalt & Meier, 1975; Smith, 1979), leading to a wealth of publications, but also, along with bats, a taxonomic bias in publications about ultrasound use. While still crucial in understanding the physiological and cognitive processes associated with USVs, captive rats and mice have been isolated from natural processes for generations and are not known to produce well-studied wild calls such as predator alarm calls (Blanchard et al., 1991; Hahn & Lavooy, 2005; Kalcounis-Rueppell, Petric & Marler, 2018; Sirotin, Elias Costa & Laplagne, 2014).

Ultrasound can be a useful communication tool because the short sound waves are highly directional and are not readily heard by most predatory birds, reptiles, and amphibians (Knudsen, 1981; Yamazaki et al., 2004). It has been proposed that the main drivers of ultrasound use for all terrestrial vertebrates are body size, predator avoidance, and increasing the signal-to-noise ratio (Arch & Narins, 2008). Several studies support the hypothesis that body size has a strong relationship to the frequency of vocalizing animals (avian mass: Ryan & Brenowitz, 1985; mammalian mass: Charlton & Reby, 2016, Martin, Tucker &Rogers, 2016; mass of mole rats: [*Cryptomys, Heterocephalus, Sorex*], Credner, Burda & Ludescher, 1997; length of ground squirrels: *Spermophilus,* Nikol'skii, 2017). The mass-call frequency relationship is influenced directly by the physical mechanisms of vocal production (i.e., lung capacity, larynx size) and through indirect effects like species ecology (e.g., larger animals using different environments than smaller animals; Ryan & Brenowitz, 1985). In contrast, predator-avoidance is more difficult to quantify and this hypothesis seems to be more supported by anecdotal evidence of vocal shifts toward ultrasonic frequencies when animals are in the presence of predatory birds (Digweed & Rendall, 2009; Kotler, 1984; Tamura & Yong, 1993). Finally, the signal-to-noise ratio hypothesis has been supported by only a few examples, such as the Kihansi spray toad (*Nectophyrynoides asperginis*) which has shifted all vocal production to USVs to reduce interference from nearby roaring, low-frequency waterfalls (Arch, Richards-Zawaki & Feng, 2011). Similarly, the acoustic adaptation hypothesis, a version of the signal-to-noise idea that focuses on sound propagation relative to environmental characteristics (e.g., open versus closed) instead of ambient sound, postulates that higher frequencies will be employed in open habitats where the shorter sound waves are less likely to be attenuated by the environment before reaching the receiver (Ey &

Fischer, 2009). In contrast to the acoustic adaptation hypothesis, it has also been shown that some mammals inhabiting forested habitats have improved hearing sensitivities to continue to use high-frequency calls in closed habitats (Charlton, Owen & Swaisgood, 2019).

Our review will focus on literature covering the recording and reporting of the highly vocal family, Sciuridae. With spectrographic reports of squirrel vocalizations beginning in the 1960s, squirrel genera have been well sampled, and many vocal libraries have been developed and updated with improving technologies. We apply popular and novel hypotheses related to vocal characteristics (Table 2.1) to observe which behavioural and ecological traits drive frequency use in squirrels. We also challenge the currently established repertoires in light of new reports of USVs in flying squirrels and call for future research to employ equipment that is intended for non-human animals, and not subject to the limitations of equipment designed for use with humans.

Squirrel Vocalizations – A Short History

The first published spectrographic analyses of squirrels appeared in 1966; the authors used these visualizations to describe a variety of calls of the Uinta ground squirrel (*Urocitellus armatus*; Balph & Balph, 1966) and the yellow-bellied (*Marmota flaviventris*) and hoary marmots (*M. caligata*; Waring, 1966). While the authors published separately, they used the same equipment to record the squirrels: a unidirectional microphone with a relatively narrow frequency bandwidth (Electrovoice-644 Sound Spot, frequency response of ~0.05-12kHz; Balph & Balph, 1966; Waring, 1966) attached to a relatively large portable tape recorder. Waring (1966) used a W-610-EV battery-operated tape-recorder (weight ~7.7 kg; Amplifier

Corporation of America, 1959) whereas Balph and Balph (1966) used an 11.8 kg Wollensak T 1700 tape recorder (Revere-Wollsenak Division, 2010). The fundamental frequency (F_0 – the vibration frequency of the vocal folds which is determined by the shape of the larynx as well as airflow; Charlton & Reby, 2016; Fitch, Neubauer & Herzel, 2002; Riede, Borgard & Pasch, 2017) and the dominant frequency (F_{Dom} – the frequency with the maximum amplitude or energy; (Fig. 2.1)) of the calls produced by the ground squirrels and marmots were captured within this narrow frequency range as demonstrated on the spectrographs. However, Waring noted at least one call produced by the yellow-bellied marmot had strong harmonics (resonant frequencies produced at intervals above F_0) exceeding 40 kHz, well beyond the dynamic range of the microphone and only detectable with a heterodyne. The dynamic range of the recording microphone is limited by the physical shape of the transducer; this element vibrates when sound is produced, translating the physical sound wave into an electronic signal (Obrist et al., 2010). Similarly, heterodynes (handheld ultrasonic detectors that transpose USVs down to the human hearing range so that researchers can listen to USVs in real-time) can detect a broad range of frequencies with a specialized transducer. However, the transposer (which translates the vibration into an audible sound) is restricted to a narrow bandwidth and the researcher must tune the transducer to listen to different frequencies (Ahlén, 2002).

As Waring (1966) demonstrated, harmonics can lead to vocalizations that partially exceed human hearing abilities (i.e., ultrasonic harmonics that exceed 20 kHz). F_0 is produced as air exits the lungs (called the glottal flow) and without any interruptions, this tone would only consist of F_0 (Titze, 2009). However, if the glottal flow collides with the vocal tract (vocal folds, upper larynx, tongue, mouth, etc.), infinite harmonics will be produced, decreasing in strength as they increase in frequency (Monsen & Engebretson, 1977; Titze, 2009). Harmonics can be strengthened or dampened, depending on the shape of the vocal tract (like the addition of harmonic resonance chambers; Riede et al., 2017) and the presence of strong high-frequency harmonics can be indicative of active manipulation of the vocal tract (Fenton et al., 2011; Titze, 2009). Harmonics can allow animals to express a larger bandwidth (as in bats; Fenton et al., 2011; Kingston & Rossiter, 2004) or to communicate at a higher frequency (as in anurans and birds; Arch et al., 2011; Narins et al., 2004), though the function of harmonics in most squirrels remains unclear. The yellow-bellied marmot is not the only squirrel for which ultrasonic harmonics have been described. Other genera include *Callospermophilus* (Eiler & Banack, 2004), *Glaucomys* (Gilley, 2013; Gilley et al., 2019; Murrant et al., 2013), *Sciurus* (Lishak, 1982; Zelley, 1971), *Spermophilus* (Koshev & Pandourski, 2008; Matrosova et al., 2011, 2012, 2016; Schneiderová, 2008, 2012; Schneiderová & Policht, 2012; Schneiderová, Štefanská & Kratochvíl, 2019), *Tamias* (Blake, 1992), and *Urocitellus* (Manno et al., 2007; Melchior, 1971). The function of harmonics has been discussed in the context of a few species of ground squirrel (*Spermophilus*). California ground squirrels (*Spermophilus beecheyi*) will dampen the strength of higher harmonics to increase the clarity of F_0 and improve the ability for receivers to locate the sender (Leger, Owings & Gelfand, 1980). As well, California ground squirrels habituating to noisy environments have developed stronger harmonics than their counterparts inhabiting quieter environments, improving the signal-to-noise ratio of their calls (Rabin et al., 2003). In contrast, some ground squirrels with larger geographic ranges or with multiple subspecies may alter the strength or number of harmonics to accommodate different habitat or social constraints, resulting in distinct dialects (Eiler & Banack, 2004). So, while all species would have infinite ultrasonic harmonics if recorded under ideal conditions (i.e., facing the recorder directly, maintaining close proximity to reduce attenuation of higher frequencies, remove all background noises, microphone with infinite sensitivity), we consider it of some interest that strong ultrasonic harmonics have only been detected in a subset of free-roaming squirrels.

Since the first spectrographic publications, the calls of over 70 extant squirrel species have been reported. There has been a strong emphasis on alarm calls (reported in 74/89 publications), likely because these are often the highest-amplitude calls, they are produced in response to human observers, and they are most obvious to humans given our own sensory bias (Masterton et al., 1969). Therefore, alarm calls are likely the easiest to record. Alarm calls function to notify conspecifics of the presence of a predator and usually prompt a behavioural response from the receivers. Blumstein (1999) spent over a decade looking at the role of alarm calls in marmots (*Marmota* spp.), noting that several species can communicate the threat level of a predator by modifying the speed or repetitive pattern of the alarm calls (Blumstein, 1999; Blumstein & Armitage, 1997a). Additionally, many authors have reported that squirrels will produce different alarm calls (distinct in structure and frequency) to identify unique predator types and to elicit a specific behavioural response in the receivers (Rendall, Owren & Ryan, 2009). For example, Richardson's ground squirrels (*Urocitellus richardsonii*) produce a short chirp (lasting only 75 ms and rapidly decreasing in frequency from 8 kHz to 3.5 kHz) to warn of aerial predators and evoke an immediate retreat response in conspecifics. In contract, a terrestrial predator is identified by a long whistle (lasting around 400 ms with a constant frequency of 10 kHz) which provokes conspecifics to become for alert stand erect (Davis, 1984). Additionally, these squirrels can add a chuck syllable (only 22.5 ms long and decreasing in frequency from about 6 kHz to 2 kHz) to the end of a whistle when predators are nearby, eliciting increased vigilance from receivers after their initial behavioural response (Sloan, Wilson & Hare, 2005) and like marmots, Richardson's ground squirrels will increase the repetition of their calls as the threat level increases (Warkentin, Keeley & Hare, 2001). When predator-specific alarm calls are used in a species, the caller most often only distinguishes between terrestrial and aerial predators (*Callosciurus* (Tamura, 1995; Tamura & Yong, 1993), *Cynomys* (Ackers & Slobodchikoff, 1999; Loughry et al., 2019), *Marmota* (Blumstein, 1999; Blumstein & Arnold, 1995; Davis, 1991; Taulman, 1977), *Otospermophilus* (Owings & Leger, 1980; Owings & Virginia, 1978), and *Urocitellus* (Balph & Balph, 1966; Melchior, 1971)), though some Asian tree squirrels have a third call which elicits snake mobbing behaviour in conspecifics (Tamura & Yong, 1993).

While alarm calls have been the focus of most studies, squirrels have many distinct call types (Table 2.2) varying in function from mating (male courtship, pre- and postcopulatory calls, and female mating calls), territoriality, facilitating aggressive (threat, combat) or amicable (maternal, group-cohesion) interactions, and communicating internal states (distress). Several species of flying squirrel have also been observed producing calls during their glides and while exploring their environment (*Glaucomys sabrinus* and *G. volans*: Murrant et al., 2013; *Petaurista leucogenys*: Ando & Kuramochi, 2008), suggesting this nocturnal tribe (Pteromyini; Brandt, 1855) may be using echo-based orientation (Siemers et al., 2009). While this hypothesis has been explored once (Chattin, 1969), the recent discovery of ultrasonic vocalizations in North American flying squirrels (Eisinger, Scheibe & Flaherty, 2016; Gilley, 2013; Gilley et al., 2019; Murrant et al., 2013) and the vast improvement in ultrasonic recording since the 1960s warrants a re-examination of the hypothesis.

It was not until 2004 that a USV (i.e., a call where F_0 entirely exceeds 20 kHz) was first reported in a squirrel species: the Richardson's ground squirrel (*Urocitellus richardsonii*; Wilson & Hare, 2004). Richardson's ground squirrels are known to both produce predator-specific calls that differentiate between terrestrial and aerial predators (Davis, 1984) and vary the repetition and call structure to communicate perceived risk (Sloan et al., 2005; Warkentin et al., 2001). USVs were found to be another way for this species to convey the perceived predation risk by varying their calls between weaker, ultrasonic vocalizations and louder, audible vocalizations as the threat imposed by the presumptive predator increases (Wilson & Hare, 2006). When a predator is farther away, it is less likely to detect highly directional USVs and therefore prey individuals can call altruistically while remaining inconspicuous. As the predator becomes more of a threat, the colony benefits from louder, more omnidirectional alarm calls to ensure that the maximum number of receivers are notified. In addition to this, it was found that there is a significant increase in the frequency of ultrasonic calls when the emerging juveniles reach their highest density, implying that ultrasonic communication may be most beneficial for social cohesion (Wilson & Hare, 2006). It is key to note that the authors did not use a secondary microphone in the audible range (< 20 kHz) to confirm that the calls were not biphonic (two unrelated fundamental frequencies are produced at the same time; Matrosova et al., 2012) with one fundamental in the audible range and a second in the ultrasonic range. However, the authors published a follow-up report in which they attempted to quantify biophonic versus ultrasonic calls in the same population by noting ultrasonic call behaviours (thoracic cavity expanding and mouth held in communicative position, sometimes a very faint whistle can be heard by the observer) while recording with an audible microphone and they were able to differentiate between ultrasonic and biphonic calls (termed 'mixed calls' by the authors; Wilson & Hare, 2006).

Wilson and Hare's (2004) publication challenges the squirrel vocal literature because most of the published repertoires have used microphones that are not sensitive to the ultrasonic range (85% of studies reviewed in our report did not use equipment that encompassed ultrasonic frequencies). By constraining the microphones to ranges that maximize human voice recording, the published repertoires have been unintentionally biased by methodology. While the detection of ultrasonic calls can be extremely rare even when the correct equipment is being used (as in some *Spermophilus;* Matrosova et al., 2012)*,* since 2004, three new Sciuridae species have been observed to produce USVs. USVs have now been reported in northern (*Glaucomys sabrinus*: Murrant et al., 2013) and southern flying squirrels (*G. volans*: Eisinger, Scheibe & Flaherty, 2016; Murrant et al., 2013) and noted, though rarely, in the speckled ground squirrel (*Spermophilus suslicus*: Matrosova et al., 2012). Calls where F_0 partially extend into the ultrasonic range have been also been noted in both the southern (*G. volans*: Eisinger et al., 2016; Gilley, 2013; Gilley et al., 2019; Murrant et al., 2013) and northern flying squirrels (*G. sabrinus*: Gilley, 2013; Gilley et al., 2019) as well as the Carolina flying squirrel (*G. sabrinus coloratus*: Gilley, 2013).

Despite the underrepresentation of USVs in published squirrel vocal repertoires owing to technological limitations and difficulties associated with recording free-ranging individuals, squirrels are a useful taxonomic group to preliminarily evaluate factors controlling the frequency limits of mammalian vocal signals. Squirrels have been well sampled across the taxa and represent a diverse range of social structure, habitat use, and behaviour. We used phylogenetic methods to investigate the following five popular and novel hypotheses relating to different frequency characteristics of the vocalizing members of Sciuridae (Table 2.1). (1) The role of body size, which has been shown to drive frequencies higher as body size decreases, will be explored (Arch & Narins, 2008; Martin et al., 2016). However, it has already been noted in Sciuridae that body size differences attributed to age class (pup versus adult) do not result in within-species differences in vocal characteristics (Matrosova et al., 2007; Swan & Hare, 2008), therefore this relationship is only expected to be significant when making cross-species comparisons. (2) To investigate the potential role of predator avoidance (prey species will favour acoustic ranges that exceed the detection of common predators; Arch & Narins, 2008), we used sociality as a proxy because species that exhibit social complexity are more likely to exhibit increased vocal complexity (Blumstein, 2007; Blumstein & Armitage, 1997b). Primates that exhibit more complex social systems are sensitive to higher frequencies (Ramsier et al., 2012) and mammals with complex social systems are more likely to produce higher frequency vocalizations associated with submissiveness and appeasement (Charlton & Reby, 2016). Additionally, Wilson and Hare (2006) suggest that squirrel social systems in which kin are more clustered, either spatially or temporally from juvenile emergence, are more likely to favour USV production which is less likely to be detected at a distance by an eavesdropping predator. Therefore, we predicted that more socially complex squirrels will vocalize at higher frequencies. (3) We explored the acoustic-adaptation hypothesis (terrestrial animals will modify call structure depending on their environment; Blumstein, 2007; Ey & Fischer, 2009) by comparing the frequency limits of squirrels in open versus closed habitats, with open habitats expected to promote the use of higher frequencies. (4) The role of diel activity patterns is interesting as the only nocturnal squirrels are the flying squirrels which might be using vocalizations for echolocation as echo-based orientation (or 'echonavigation') is associated with reduced visual cues in other rodents (Ancillotto et al., 2014; Panyutina et al., 2017). Therefore, we predicted that nocturnal species would have higher frequencies than diurnal species. (5) Finally, the relevance of the equipment used will be analyzed by incorporating the method detection limits (defined as the limits of either the microphone or spectrographic analysis, hereinafter 'method limits') of the publication methods into the models defined below. We predicted that method limits, particularly the upper-frequency limit, will constrain the perceived vocal ranges of recorded species.

Reviewing Frequency Characteristics

We developed a database beginning with a list of publications that described the vocalizations of squirrels up to 2019 (summarized in Table A1.1). The minimum requirement for each publication was the description of at least one call with either a spectrographic analysis or numerical data, though the majority of publications described multiple calls per species or described multiple species per publication (493 calls from 72 species represented in 89 publications; summarized in Table A1.1). The databases used to search for these publications were Google Scholar, JSTOR, Web of Science, and Wiley Online Library. We used the keywords acoustics, acoustic repertoire, calls, frequency, Hz, vocalizations, and ultrasound paired with Sciuridae, squirrel, or an exhaustive list of currently valid and invalid genera (the most updated nomenclature was taken from the Integrated Taxonomic Information System http://www.itis.gov/). For each call described in the selected publication, the following characteristics were taken: the fundamental frequency (F_0: the mean frequency of the primary vibrational frequency of the vocal membrane; kHz), dominant frequency (F_{Dom}: the frequency with the greatest energy, power or amplitude; kHz), minimum frequency (F_{Min}: the minimum frequency of the fundamental frequency; kHz), maximum frequencies (F_{Max}: the maximum frequency of the fundamental frequency (or of harmonic on which F_{Dom} is measured); kHz), and the highest visible harmonic (F_{Harm}: mean frequency of the highest complete harmonic visible on the spectrograph; kHz) (Fig. 2.1).

Once our review of vocalization publications was complete, we searched for the body mass (g), diel activity pattern (diurnal or nocturnal), social complexity, and habitat openness of the dominant habitat (open or closed) of each species from the relevant vocalization papers. If not provided, other resources including Mammalian Species accounts, PanTHERIA (Jones et al., 2009), and the Animal Diversity Web (Myers et al., 2020) were reviewed. Both male and female body masses were initially recorded, but male body size could not be found for *Spermophilus taurensis*. Male and female body mass were strongly correlated ($r = 0.98$, $p < 0.001$), therefore female body mass was chosen to represent squirrel body size. Because we could only assign an adult female body mass to all species, calls that are exclusively produced by males or pups were removed from the dataset before analysis. We pooled all other calls (calls produced by both sexes or females only as well as calls produced by juveniles and adults) as there is little evidence to suggest that juveniles and adults produce acoustically distinct calls across the family (Matrosova et al., 2007, 2011; Schneiderová, 2012; Volodina, Matrosova & Volodin, 2010; but see: Nikol'skii, 2007). While the initial database included a five-tiered social classification ranging from solitary to colonial (based on the social grades of ground squirrels described by Matějů et al., (2016)), social classes were reduced to social or solitary living to reduce model parameters. Species that exhibit dynamic social structures, such as flying squirrels that engage in social nesting to a greater extent during one portion of the year (Garroway, Bowman & Wilson, 2013), were treated as socially living. Two subspecies (*Marmota baibacina centralis* and *Tamias dorsalis dorsalis*) could not be used in the subsequent analyses because ecological data and body mass-specific to each subspecies could not be found; similarly, the species *Spermophilus pallidicauda* could not be included as body mass for either sex could not be found.

Phylogeny

VertLife, an online resource that allows the user to extract pruned trees from vertebrate supertrees, was used to produce 100 pruned trees from the Mammalian supertree (Fig. 2.2; Upham, Esselstyn & Jetz, 2019). Three subspecies had to be incorporated under their parent species, so branch tips were broken in two and subspecies were treated as equivalent to parent species, with branch lengths identical between the parent and subspecies (the addition of a subspecies did not create any polytomies in the tree). Three species are represented by subspecies only: *Sciurus aberti kaibensis, Sciurus niger rufiventer,* and *Callosciurus erythraeus thaiwanensis*.

Statistical Analysis

Phylogenetic generalized least square (PGLS) modelling was used to account for the variation in acoustic repertoire that may be explained by phylogenetic relatedness. PGLS models produce a lambda parameter, λ, that represents the degree to which the variance of traits is explained by the phylogenetic relationships in the model. The λ parameter varies between 0 and 1, with 0 representing no phylogenetic trace and 1 representing absolute Brownian motion (Freckleton, Harvey & Pagel, 2002; Martin et al., 2016).

PGLS modelling restricts each species to a single observation (i.e., no subsampling of species permitted). Therefore, the numerous data entries per species had to be reduced. For the fundamental, dominant, maximum, and highest harmonic frequencies, the absolute maximum value for each characteristic reported among all publications was chosen. Likewise, for minimum frequency, the absolute minimum reported frequency was chosen. We use maximum and minimum values rather than the median for a more rigorous test of our hypothesis about method limits.

Body mass and all frequency characteristics were log-transformed to achieve normal distributions. Additive models were built for each frequency type (β_0 + body mass (β_{Mass}) + diel activity pattern (β_{Diel}) + sociality ($\beta_{Sociality}$) + habitat openness (β_{Open}) + method limits (β_{Lim})) using the caper package in R (ver 3.6.2). We reported the test statistics of the regression to evaluate significance and effect size (*F*-statistic, *p*-value, and adjusted R^2).

Evaluating Correlates of Frequency Characteristics

The effect of body mass and method limits were significant for all five call descriptors (F_0, F_{Dom}, F_{Min}, F_{Max}, and F_{Harm}) whereas habitat openness only had a significant effect for F_{Dom} and F_{Harm} (Table 2.3). The PGLS models fit the data well for most frequency characteristics, with effect sizes (adjusted R^2) of 0.64 ($p < 0.001$), 0.57, ($p < 0.001$), 0.61 ($p < 0.001$), and 0.41 ($p < 0.001$) for F_0, F_{Dom}, F_{Max}, and F_{Harm} accordingly. The minimum frequency PGLS model did not fit the data well, yielding an effect size of only 0.12 ($p < 0.05$), however it was the only model that yielded a significant phylogenetic signal (Pagel's λ with 95% confidence intervals) of 0.65 [0, 0.88]. In contrast, the phylogenetic signal was estimated to be 0 for F_0 (0 [0, 0.96]), F_{Dom} (0 [0, 0.32]), F_{Max} (0 [0, 0.95]), and F_{Harm} (0 [0, 0.43]) which suggests that these traits are highly variable across these taxa. A summary of model parameters for secondary candidate models (ΔAICc < 2; Mazerolle 2004) selected through stepwise regression can be found in the appendix (Table A1.2). Notably, harmonic frequency values could only be found for 43 of the 73 species, with species reported in only one publication being significantly less likely to have reported harmonics (Chi-square = 17.1, $p < 0.001$) than those appearing in multiple publications.

Body Mass

Body mass (Fig. 2.3) had a significant effect on F_0 (slope = -0.22 (±SE = 0.05; Table 2.4), $F_{1,58} = 23.14$, $p < 0.001$), F_{Dom} (slope = -0.20 (±0.05), $F_{1,60} = 12.37$, $p < 0.001$), F_{Min} (slope = -0.19 (±0.09), $F_{1,58} = 6.24$, $p < 0.05$), F_{Max} (slope = -0.25 (±0.05), $F_{1,59} = 37.61$, $p < 0.001$), and F_{Harm} (slope = -0.29 (±0.08), $F_{1,37} = 9.18$, $p < 0.01$). These results are consistent with our prediction that increased body mass results in the production of lower frequencies as already shown in mammals (Charlton & Reby, 2016) and birds (Ryan & Brenowitz, 1985). The avian mass-frequency relationship focused on the 'emphasized' frequency, similar to F_{Dom} reported here (Ryan & Brenowitz, 1985). Our results are somewhat consistent with Martin et al., (2016) who also found that F_{Min} was constrained by mammalian body mass, but the mass-F_{Min} relationship reported was twice as strong as reported here (slope = 0.41). This is likely because their dataset ranged from microbats (weighing less than 0.1 kg) to whales (larger than 100 000 kg), representing a much larger range of body sizes and therefore showing a stronger effect of body size on frequency types. In comparison, this dataset covered only a range of about 36 g (*Tamias alpinus*) to 8.0 kg (*Marmota sibirica*). In contrast to our results, Martin et al., (2016) found that F_{max} was not constrained by body size, though this was largely attributed to the interaction between environment and body size. They suggest that because sound waves travel differently in the water, aquatic species are freed from the constraints of body size on the maximum frequency compared to their terrestrial relatives. Like Martin et al., (2016), Ryan and Brenowitz

(1985) also found that there was an interaction between environment and body size in avian song frequency, but they compared habitat openness as we did here. Interestingly, when we also added the interaction between habitat openness and body mass did not change the model outcome (AICc values or variable effects) for most frequency characteristics, but it did yield a significantly better model for F_{Harm} (ΔAICc = -9.78). The effect size of the model increased (adjusted $R^2 = 0.55$, $p < 0.001$), the effect of habitat openness increased (slope = 3.70 (±0.91), $F_{1,36} = 6.80$, $p < 0.05$) and the interaction between habitat openness and body mass was significant (slope = -0.55 (±0.16), $F_{1,36} = 12.51$, $p < 0.01$). However, the slope estimate of body mass was reduced (slope = -0.06 (±0.12), $F_{1,36} = 12.04$, $p < 0.01$) and the effect of sociality became non-significant (slope = -0.10 (±0.19), $F_{1,36} = 1.67$, $p = 0.21$). By observing an interaction plot, we determined that as body size increased in the closed habitat, there was little change in F_{Harm}, whereas there was a large decrease in F_{Harm} with increasing body size in the open habitat.

Sociality

Sociality (social (0) or solitary (1)) had a significant effect on F_0 (slope = -0.007 (±0.13), $F_{1,58} = 9.54$, $p < 0.01$), F_{Dom} (slope = -0.04 (±0.14), $F_{1,60} = 8.60$, $p < 0.01$), and F_{Max} (slope = -0.07 (±0.13), $F_{1,59} = 8.57$, $p < 0.01$). Notably, sociality had a significant effect on these models while yielding a non-significant slope; investigating the models further, there was no evidence of collinearity between sociality and other variables. However, there was a greater variation in frequencies used by social squirrels compared to solitary squirrels as minimum frequencies were similar and maximum frequencies were higher in social squirrels. These results are consistent with Blumstein and Armitage (1997) who found that social complexity was related to increased use of alarm calls in the repertoire in marmots. In our results, we found that alarm calls are widespread across the taxa, appearing in 64 of the 73 vocalizing Sciuridae species. While social squirrels might not be more likely to have alarm calls in their vocal repertoire than solitary squirrels (Chi-Square = 3.51, $p = 0.06$), they likely maintain shorter distances between the sender and receiver and increase their production of alarm calls (ex. *Urocitellus beldingi*; Sherman, 1985). This reduced distance could facilitate the incorporation of higher frequencies into the repertoire as these frequencies are highly directional but are more likely to be attenuated over longer distances (Smith, 1979). As well, the increased proximity not only results in more altruistic alarm calling (Sherman, 1985) but also the production of more vocalizations to facilitate group cohesion and maintain social hierarchies, particularly between emerging juveniles and established adults. For example, calls in adult female yellow-bellied marmots (*Marmota flaviventris*) are directly correlated with the emergence of juveniles (Blumstein et al., 1997). Primates, another group of highly communicative and socially variable mammals, also trend toward increased vocal complexity and overall improved hearing sensitivities in more social species and larger groups (Ramsier et al., 2012). To compensate for increased exposure via sound, social animals benefit from communicating in ranges less readily heard by predatory species (Arch & Narins, 2008). In contrast, solitary squirrels would benefit from louder calls if they are warning surrounding conspecifics of potential danger, especially if the species maintains relatively large home ranges. However, the maintenance of these territories would likely lead to vocal production that maximizes the effectiveness of territorial displays (which we only found reports of in solitary and socially alternating species), meaning that lower frequencies would be more beneficial to maximize amplitude over longer distances and to increase omnidirectionality. In addition to social structure, mating systems may be complicating the results observed.

We grouped monogamous and polygynous species. However, Charlton (2016) suggests that F_0 is a sexually selected trait and is higher in polygynous species and lower in monogamous species. While we excluded calls produced exclusively by males, we kept calls produced by both sexes, therefore not eliminating calls produced by both sexes that are also under sexual selection. We suggest further research into the role of sociality and mating systems in vocal complexity across Sciuridae.

Habitat

The habitat type (open (1) or closed (0)) only had a significant effect on F_{Dom} (slope = 0.31 (±0.14), $F_{1,60} = 4.22$, $p < 0.05$) and F_{Harm} (slope = 0.59 (±0.25), $F_{1,37} = 5.65$, $p < 0.05$), with open habitats facilitating higher dominant and higher maximum harmonic frequencies than closed habitats. We expected that the F_{Max} would be most affected by habitat type because higher frequencies are attenuated more rapidly in closed environments (Smith, 1979) resulting in a shift of vocal range (minimum to maximum frequency). Instead, we found that squirrels in open habitats spend their maximum acoustic energy (F_{Dom}) on higher frequencies than squirrels in closed habitats, with little effect on range, as F_{Min} and F_{Max} were not significantly influenced by habitat openness (Table 2.3 and 2.4). Charlton et al., (2019) have shown that forest-dwelling animals may not be hindered by their ability to produce or detect high frequencies as many have been shown to co-evolve improved acoustic structures to not lose their access to these frequencies in a closed environment. They suggest that forest-dwelling animals may boost the amplitude and increase the frequency band of their calls to help counteract the increased attenuation of their environment and improve the ability of receivers to locate the sender (Charlton et al., 2019). This hypothesis would explain why squirrels in closed habitats continue to use high frequencies, despite the limitations. Based on this hypothesis, we would also predict

F_{Harm} would not be affected by habitat openness as increased harmonic strength would improve the bandwidth of calls in closed environments and high harmonics would be unhindered in open habitats, making them favourable in both conditions. We found instead that higher harmonics are detected in open habitats, supporting the more traditional acoustic adaptation hypothesis (Blumstein, 2007; Ey & Fischer, 2009). However, we think it is important to note that the detection of the highest harmonic is largely dependent on the recording techniques of the researcher and how the calls have been displayed on spectrographs. It may likely be that high harmonics are easier to record in open environments, whether because the acoustics are better for recording (reduced attenuation of harmonics before reaching the microphone as in bats; Fenton et al., 2011) or the individual squirrel ecology lends to better study subjects (i.e. congregated ground squirrels allow microphones to be placed close to predictable burrow holes compared to free-ranging tree squirrels which have many temporary nests and retreats across a forest canopy). While not explored in this study, environments with greater humidity transmit higher frequencies better than arid environments where calls are attenuated faster (Nikol'skii, 1984). This was partially shown in ground squirrels (*Spermophilus*; Nikol'skii, 2017) when the strength of the relationship between the F_{Dom} and body mass was significantly improved when habitat aridity was incorporated as a factor into the models. Overall, our findings suggest that higher dominant frequencies are more easily incorporated and recorded in squirrels in open habitats, but high frequencies are not necessarily unused or completely inhibited by closed habitats.

Diel Activity Pattern

As predicted, nocturnality resulted in significantly higher values for F_0 (slope = 0.79 (±0.22), $F_{1,58} = 51.22$, $p < 0.001$), F_{Dom} (slope = 0.96 (±0.24), $F_{1,60} = 40.84$, $p <$

0.001), and F_{Max} (slope = 0.61 (±0.23), $F_{1,59} = 33.10$, $p < 0.001$). Though the frequency traits between diel activity patterns was not significant for F_{Min} (slope = 0.43 (±0.56), $F_{1,58} = 1.63$, $p = 0.21$) and F_{Harm} (slope = 2.96 (±), $F_{1,37} = 2.96$, $p = 0.09$), all frequency characteristics trend toward nocturnal species using higher frequencies than diurnal species (Fig. 2.4). Unfortunately, the lack of behavioural observations associated with the published flying squirrel repertoires, the only nocturnal squirrels, means that interpretation of these results is mostly hypothetical (as nocturnal patterning is only represented by 5 different (sub)species). Both species of Asian giant flying squirrel (*Petaurista lena* and *P. leucogenys*) stand out as the only squirrels with movement-associated vocalizations that were recorded while observing free-ranging individuals (Ando & Kuramochi, 2008; Shen, 2013). Additionally, one call associated with the exploration of a novel environment was reported in the southern flying squirrel (*Glaucomys volans*; Murrant et al., 2013). While there has yet to be any direct evidence of echo-based navigation (Siemers et al., 2009) or echolocation in flying squirrels (Chattin, 1969), we propose that the strong effect of nocturnality on multiple frequency characteristics, correlated to calls in higher frequencies, as well as the recent discovery of USVs in North American flying squirrels (Gilley, 2013, Murrant et al., 2013), warrants a re-examination of the function of USVs in these squirrels with specific echo-based navigation experiments included in the research.

Method Limits

As we predicted, the limits of the methods (microphone or analysis ranges) did significantly influence all the frequency characteristics as shown in the PGLS models. As equipment limits increased, so did the F_0 (slope = 0.45 (±0.08), $F_{1,58} = 37.91$, $p <$ 0.001), F_{Dom} (slope = 0.39 (±0.08), $F_{1,60} = 23.78$, $p < 0.001$), F_{Max} (slope = 0.40 (±0.08), $F_{1,59} = 25.16$, $p < 0.001$), and F_{Harm} (slope = 0.51 (±0.15), $F_{1,37} = 11.01$, $p <$

0.01; similarly, lower method limits were related to a lower F_{Min}, though this relationship was not as strong (slope = 0.12 (±0.05), $F_{1,37} = 4.63$, $p < 0.05$) Additionally, the AICc of all secondary PGLS models were improved by about 10% when method limits were included (note that all secondary candidate models include β_{Lim}; Table A1.2). We conclude that the type of equipment being used is crucial for detecting all frequency characteristics and that using equipment with large bandwidths (the entire range of frequencies that could be detected) is important for all squirrel acoustics research as we continue to discover novel USV production in Sciuridae.

Summary

We predicted that body mass, sociality, habitat openness, diel activity pattern, and method limits would influence the fundamental, dominant, minimum, maximum, and highest harmonic frequencies (kHz; Table 2.1). We found at least some support for all of these hypotheses. We found that across the squirrel family, there was a significant effect of body mass with all frequency characteristics, a finding that is mostly consistent with other literature (Arch & Narins, 2008; Charlton & Reby, 2016; Martin et al., 2016). Social squirrels used higher fundamental, dominant, and maximum frequencies which aligns with the predator-avoidance hypothesis (Arch & Narins, 2008). Social squirrels also used a larger range of frequencies which can be partially attributed to increased communication amongst kin (Blumstein & Armitage, 1997b) or sexual selection acting on vocal traits (Charlton & Reby, 2016). As predicted by the acoustic adaptation hypothesis (Blumstein, 2007; Ey & Fischer, 2009), we found that species inhabiting open habitats used higher dominant and harmonic frequencies than those in closed habitats where high frequencies are more rapidly attenuated by the environment (Smith, 1979); however, the maximum frequency was not affected by habitat type which suggests that squirrels in closed habitats may have co-evolved structures to maintain access to USVs despite the environmental complications (Charlton et al., 2019). Nocturnality was associated with higher frequencies for the fundamental, dominant, and maximum frequencies, suggesting that nocturnal, gliding squirrels are under unique selection pressures for using USVs. An explanation for this general pattern of higher frequencies in nocturnal squirrels remains elusive. Finally, the significant effect of method limits on all frequency characteristics and the presence of method limits in all candidate models (Table A1.2) suggest that method limits are important for framing the ranges detected during recording sessions.

Think Before They Squeak – Conclusion

Squirrels are vocally complex taxa, displaying a wide range of call frequencies and functions. While there has been a representative sampling across the genera, there are large gaps in the geographic sampling of squirrels (Fig. 2.5). The USA alone accounts for over 50% of the published vocal repertoires. In contrast, only a single study has occurred in Africa and no repertoires have been published from Central or South America. In the face of global extinction crises and habitat loss, it is more important than ever to archive the traits of rare and cryptic species so that we can maximize the utility of all available monitoring and conservation tools. Bioacoustic monitoring is an effective tool for cryptic species (Gilbert, McGregor & Tyler, 1994; Tripp & Otter, 2006; Walker, 1964) and has been shown to improve the monitoring of flying squirrels which are notoriously difficult to detect (Diggins et al., 2016). Considering these analyses, we encourage researchers to record squirrel vocalizations in under-sampled locations. We further suggest the use of recording equipment that can resolve frequencies that extend into the ultrasonic range so that repertoires accurately reflect the ranges used by the species being studied. Additionally, we caution that researchers studying the vocalizations of previously reported squirrels should consider the accuracy of previous literature, especially when there is possible evidence of partial USVs or ultrasonic harmonics present. Squirrels that are socially complex or inhabiting open areas are of interest for studies focused on high-frequency vocalizations; we also strongly encourage the examination of the role of USVs in nocturnal, flying squirrels. We also caution that while USV research requires specialized technology, this work also requires ideal recording conditions and patience as USVs can be rare and difficult to detect in some Sciuridae species (Matrosova et al., 2012). In conclusion, while rodent bioacoustic studies are typified by controlled environments and lab animals, studying the vocalizations of free-ranging rodents can reveal new information about the natural world, providing informative findings and new insights that apply to real-time conservation efforts.

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Data Availability

The raw data, phylogenies, and R script that support the findings of this study are openly available on Dryad at https://doi.org/10.5061/dryad.vt4b8gtpm.

References

Ackers SH, Slobodchikoff CN. 1999. Communication of stimulus size and shape in alarm calls of Gunnison's prairie dogs, *Cynomys gunnisoni*. *Ethology* 105(2):149-162. DOI: 10.1046/j.1439-0310.1999.00381.x.

Ahlén I. 2002. Heterodyne and time-expansion methods for identification of bats in the field and through sound analysis. *Bat Echolocation Research: Tools, Techniques and Analysis* 72-78.

Amplifier Corporation of America. 1959. *The Audio Tape Recorder Directory.* New York: Audio Devices, Inc.

Ancillotto L, Sozio G, Mortelliti A, Russo D. 2014. Ultrasonic communication in Gliridae (Rodentia): the hazel dormouse (*Muscardinus avellanarius*) as a case study. *Bioacoustics* 23(2):129-141. DOI: 10.1080/09524622.2013.838146.

Ando M, Kuramochi Y. 2008. Vocal communication of the Japanese giant flying squirrel *Petaurista leucogenys*. *The Journal of Agricultural Science* 53(2):176-183.

Arch VS, Narins PM. 2008. 'Silent' signals: selective forces acting on ultrasonic communication systems in terrestrial vertebrates. *Animal Behaviour* 76(4):1423-1428. DOI: 10.1016/j.anbehav.2008.05.012.

Arch VS, Richards-Zawaki CL, Feng AS. 2011. Acoustic communication in the Kihansi spray toad (*Nectophrynoides asperginis*): insights from a captive population. *Journal of Herpetology* 45(1):45-49. DOI: 10.1670/10-084.1.

Balph DM, Balph DF. 1966. Social communication of Uinta ground squirrels. *Journal of Mammalogy* 47(3):440-450. DOI: 10.2307/1377685.

Blake BH. 1992. Estrous calls in captive Asian chipmunks, *Tamias sibricus*. *Journal of Mammalogy* 73(3):597-603. DOI: 10.2307/1382031.

Blanchard RJ, Blanchard DC, Agullana R, Weiss SM. 1991. Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow system. *Physiology and Behavior* 50(5):967-972. DOI: 10.1016/0031-9384(91)90423-l.

Blumstein DT. 1999. Alarm calling in three species of marmots. *Behaviour* 136(6):731-757.

Blumstein DT. 1999. The evolution of functionally referential alarm communication. *Evolution of Communication* 3(2):135-147. DOI: 10.1075/eoc.3.2.03blu.

Blumstein DT. 2007. The evolution of alarm communication in rodents: structure, function, and the puzzle of apparently altruistic calling. In: Wolff JO, Sherman PW, eds. *Rodent Societies: An Ecological and Evolutionary Perspective*. Chicago: University of Chicago Press, 317-327.

Blumstein DT, Armitage KB. 1997a. Alarm calling in yellow-bellied marmots: I. The meaning of situationally variable alarm calls. *Animal Behaviour* 53(1):143-171. DOI: 10.1006/anbe.1996.0285.

Blumstein DT, Armitage KB. 1997b. Does sociality drive the evolution of communicative complexity? A comparative test with ground-dwelling sciurid alarm calls. *The American Naturalist* 150(2):179-200. DOI: 10.1086/286062.

Blumstein DT, Arnold W. 1995. Situational specificity in alpine-marmot alarm communication. *Ethology* 100(1):1-13. DOI: 10.1111/j.1439-0310.1995.tb00310.x.

Blumstein DT, Steinmetz J, Armitage KB, Daniel JC. 1997. Alarm calling in yellow-bellied marmots: II. The importance of direct fitness. *Animal Behaviour* 53(1):173-184. DOI: 10.1006/anbe.1996.0286.

Charlton BD, Owen MA, Swaisgood RR. 2019. Coevolution of vocal signal characteristics and hearing sensitivity in forest mammals. *Nature Communications* 10(1):2778. DOI: 10.1038/s41467-019-10768-y.

Charlton BD, Reby D. 2016. The evolution of acoustic size exaggeration in terrestrial mammals. *Nature Communications* 7:12739. DOI: 10.1038/ncomms12739.

Chattin D. 1969. Acoustical echolocation in flying squirrels. B. A. Thesis, Reed College.

Corrigan JG, Flannelly KJ. 1979. Ultrasonic vocalizations of defeated male rats. *Journal of Comparative and Physiological Psychology* 93(1):105-15. DOI: 10.1037/h0077565.

Credner S, Burda H, Ludescher F. 1997. Acoustic communication underground: vocalization characteristics in subterranean social mole-rats (*Cryptomys* sp., Bathyergidae). *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 180:245-55. DOI: 10.1007/s003590050045.

Davis LS. 1984. Alarm calling in Richardson’s ground squirrels (*Spermophilus richardsonii*). *Zeitschrift Für Tierpsychologie* 66(2):152-64. DOI: 10.1111/j.1439-0310.1984.tb01362.x.

Davis RO. 1991. Semantical communication in anti-predator alarm calls. In Hall Jr. CA, Doyle-Jones V, Widawski B, eds. *The Natural History of Eastern California and High Altitude Research.* White Mountain Research Station: University of California, 275-312.

Digweed SM, Rendall D. 2009. Predator-associated vocalizations in North American red squirrels (*Tamiasciurus hudsonicus*): To whom are alarm calls addressed and how do they function? *Ethology* 115(12):1190-1199. DOI: 10.1111/j.1439-0310.2009.01709.x.

Eiler KC, Banack SA. 2004. Variability in the alarm call of golden-mantled ground squirrels (*Spermophilus lateralis* and *S. saturatus*). *Journal of Mammalogy* 85(1):43-50. DOI: 10.1644/1545-1542(2004)085<0043:VITACO>2.0.CO;2.

Eisinger JW, Scheibe JS, Flaherty EA. 2016. Novel *Glaucomys volans* vocalizations in Indiana and evidence of geographic variation in high frequency communication. *Journal of Mammalogy* 97(4):1219–1227. DOI: 10.1093/jmammal/gyw076.

Ey E, Fischer J. 2009. The "acoustic adaptation hypothesis" - a review of the evidence from birds, anurans and mammals. *Bioacoustics* 19(1-2):21-48. DOI: 10.1080/09524622.2009.9753613.

Fenton MB, Skowronski MD, McGuire LP, Faure PA. 2011. Variation in the use of harmonics in the calls of laryngeally echolocating bats. *Acta Chiropterologica*

13(1):169-178. DOI: 10.3161/150811011X578714.

Fernández-Vargas M, Tang-Martinez Z, Phelps SM. 2011. Singing, allogrooming, and allomarking behaviour during inter- and intra-sexual encounters in the neotropical short-tailed singing mouse (*Scotinomys teguina*). *Behaviour* 148(8):945-965. DOI: 10.2307/23034277.

Fitch W, Tecumseh JN, Herzel H. 2002. Calls out of chaos: The adaptive significance of nonlinear phenomena in mammalian vocal production. *Animal Behaviour* 63(3):407-418. DOI: 10.1006/anbe.2001.1912.

Freckleton RP, Harvey PH, Pagel M. 2002. Phylogenetic analysis and comparative data: A test and review of evidence. *American Naturalist* 160(6):712-726. DOI: 10.1086/343873.

Garroway CJ, Bowman J, Wilson PJ. 2013. Complex social structure of southern flying squirrels is related to spatial proximity but not kinship. *Behavioral Ecology and Sociobiology* 67(1):113-122. DOI: 10.1007/s00265-012-1431-3.

Geyer LA. 1979. Olfactory and thermal influences on ultrasonic vocalization during development in rodents. *Integrative and Comparative Biology* 19(2):419-431. DOI: 10.1093/icb/19.2.419.

Geyer LA, Barfield RJ. 1978. Influence of gonadal hormones and sexual behavior on ultrasonic vocalization in rats: I. Treatment of females. *Journal of Comparative and Physiological Psychology* 92(3):438-446. DOI: 10.1037/h0077480.

Geyer LA, Barfield RJ, McIntosh TK. 1978. Influence of gonadal hormones and sexual behavior on ultrasonic vocalization in rats: II. Treatment of males. *Journal of Comparative and Physiological Psychology* 92(3):447-456. DOI: 10.1037/h0077487.

Gilbert G, McGregor PK, Tyler G. 1994. Vocal individuality as a census tool: practical considerations illustrated by a study of two rare species (Individualidad vocal como herramienta en los censos: Consideraciones prácticas ilustradas por un estudio de dos especies raras. *Journal of Field Ornithology* 65(3):335-348.

Gilley LM. 2013. Discovery and characterization of high-frequency calls in North American flying squirrels (*Glaucomys sabrinus* and *G. volans*): implications for ecology, behavior, and conservation. D. Phil. Thesis, Auburn University.

Gilley LM, Diggins CA, Pearson SM, Best TL. 2019. Vocal repertoire of captive northern and southern flying squirrels (*Glaucomys sabrinus* and *G. volans*). *Journal of Mammalogy* 100(2):518-530. DOI: 10.1093/jmammal/gyz064.

Gould E, Negus NC, Novick A. 1964. Evidence for echolocation in shrews. *Journal of Experimental Zoology* 156(1):19-37. DOI: 10.1002/jez.1401560103.

Hahn ME, Lavooy MJ. 2005. A review of the methods of studies on infant ultrasound production and maternal retrieval in small rodents. *Behavior Genetics* 35(1):31–52. DOI: 10.1007/s10519-004-0854-7.

Hofer MA, Shair H. 1978. Ultrasonic vocalization during social interaction and isolation in 2-week-old rats. *Developmental Psychobiology* 11(5):495–504. DOI: 10.1002/dev.420110513.

Hofer MA, Shair H. 1980. Sensory processes in the control of isolation-induced ultrasonic vocalization by 2-week-old rats. *Journal of Comparative and*

Physiological Psychology 94(2):271-279. DOI: 10.1037/h0077665.

Jones KE, Bielby J, Cardillo M, Fritz SA, O'Dell J, Orme CDL, Safi K, Sechrest W, Boakes EH, Carbone C, Connolly C, Cutts MJ, Foster JK, Grenyer R, Habib M, Plaster CA, Price SA, Rigby EA, Rist J, Teacher A, Bininda-Emonds ORP, Gittleman JL, Mace GM, Purvis A. 2009. PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. *Ecology* 90(9):2648-2648. DOI: 10.1890/08-1494.1.

Kalcounis-Rueppell MC, Petric R, Marler CA. 2018. The bold, silent type: predictors of ultrasonic vocalizations in the genus *Peromyscus*. *Frontiers in Ecology and Evolution* 6(NOV):1-10. DOI: 10.3389/fevo.2018.00198.

Kingston T, Rossiter SJ. 2004. Harmonic-hopping in Wallacea's bats. *Nature* 429(6992):654-657. DOI: 10.1038/nature02487.

Knudsen EI. 1981. The hearing of the barn owl. *Scientific American* 245(6):112–125. DOI: 10.1038/scientificamerican1281-112.

Koenig W, Dunn HK, Lacy LY. 1946. The sound spectrograph. *Journal of the Acoustical Society of America* 18(1):19-49. DOI: 10.1121/1.1916342.

Koshev Y, Pandourski I. 2008. Structure and variability of alarm calls of European ground squirrel *Spermophilus citellus* L. 1766 (Mammalia: Rodentia) from western Bulgaria. *Acta Zoologica Bulgarica* 60(1):99-105.

Kotler BP. 1984. Risk of predation and the structure of desert rodent communities. *Ecology* 65(3):689-701. DOI: 10.2307/1938041.

Leger DW, Owings DH, Gelfand DL. 1980. Single-note vocalizations of California ground squirrels: graded signals and situation-specificity of predator and socially evoked calls. *Zeitschrift Für Tierpsychologie* 52(3):227-246. DOI: 10.1111/j.1439-0310.1980.tb00714.x.

Lishak RS. 1982. Gray squirrel mating calls : a spectrographic and ontogenic analysis. *Journal of Mammalogy* 63(4):661-663. DOI: 10.2307/1380274.

Loughry WJ, Oeser M, Anderson CD, Hoogland JL. 2019. The importance of individual variation in the alarm calls of Gunnison's prairie dogs. *Animal Behaviour* 150:59-68. DOI: 10.1016/j.anbehav.2019.01.019.

Manno TG, Nesterova AP, Debarbieri LM, Kennedy SE, Wright KS, Dobson FS. 2007. Why do male Columbian ground squirrels give a mating call ? *Animal Behaviour* 74(5):1319-1327. DOI: 10.1016/j.anbehav.2007.02.033.

Martin K, Tucker MA, Rogers TL. 2016. Does size matter? Examining the drivers of mammalian vocalizations. *Evolution* 71(2):249-260. DOI: 10.1111/evo.13128.

Masterton B, Heffner H, Ravizza R. 1969. The evolution of human hearing. *The Journal of the Acoustical Society of America* 45(4):966-985. DOI: 10.1121/1.1911574.

Matějů J, Kratochvíl L, Pavelková Z, Řičánková VP, Vohralík V, Němec P. 2016. Absolute, not relative brain size correlates with sociality in ground squirrels. *Proceedings of the Royal Society B: Biological Sciences* 283(1827). DOI: 10.1098/rspb.2015.2725.

Matrosova VA, Blumstein DT, Volodin IA, Volodina EV. 2011. The potential to encode sex, age, and individual identity in the alarm calls of three species of

Marmotinae. *Naturwissenschaften* 98(3):181-192. DOI: 10.1007/s00114-010-0757-9.

Matrosova VA, Rusin MY, Volodina EV, Proyavka SV, Savinetskaya LE, Shekarova ON, Rashevska HV, Volodin IA. 2016. Genetic and alarm call diversity across scattered populations of speckled ground squirrels (*Spermophilus suslicus*). *Mammalian Biology* 81(3):255-265. DOI: 10.1016/j.mambio.2016.01.001.

Matrosova VA, Schneiderová I, Volodin IA, Volodina EV. 2012. Species-specific and shared features in vocal repertoires of three Eurasian ground squirrels (genus *Spermophilus*). *Acta Theriologica* 57(1):65-78. DOI: 10.1007/s13364-011-0046-9.

Matrosova VA, Volodin IA, Volodina EV, Babitsky AF. 2007. Pups crying bass: vocal adaptation for avoidance of age-dependent predation risk in ground squirrels? *Behavioral Ecology and Sociobiology* 62(2):181-191. DOI: 10.1007/s00265-007-0452-9.

Mazerolle MJ. 2004. APPENDIX 1: Making sense out of Akaike's information criterion (AIC): its use and interpretation in model selection and inference from ecological data. Université Laval.

Melchior HR. 1971. Characteristics of arctic ground squirrel alarm calls. *Oecologia* 7(2):184-190. DOI: 10.1007/BF00346360.

Miller JR, Engstrom MD. 2007. Vocal stereotypy and singing behavior in Baiomyine mice. *Journal of Mammalogy* 88(6):1447-1465. DOI: 10.1644/06-MAMM-A-386R.1.

Monsen RB, Engebretson AM. 1977. Study of variations in the male and female glottal wave. *Journal of the Acoustical Society of America* 62(4):981-993. DOI: 10.1121/1.381593.

Murrant MN, Bowman J, Garroway CJ, Prinzen B, Mayberry H, Faure PA. 2013. Ultrasonic vocalizations emitted by flying squirrels. *PLoS ONE* 8(8):e73045. DOI: 10.1371/journal.pone.0073045.

Myers P, Espinosa R, Parr CS, Jones T, Hammond GS, Dewey TA. 2020. The Animal Diversity Web (Online). *Avaible at https://animaldiversity.org*.

Narins PM, Feng AS, Lin W, Schnitzler H-U, Denzinger A, Suthers RA, Xu C. 2004. Old world frog and bird vocalizations contain prominent ultrasonic harmonics. *The Journal of the Acoustical Society of America* 115(2):910-913. DOI: 10.1121/1.1636851.

Nikol'skii AA. 1984. *Звуковые Сигналы Млекопитающих в Эволюционном Процессе / Sound Signals of Mammals in Evolutionary Process*. Moscow: Nauka.

Nikol'skii AA. 2007. A comparative analysis of the alarm call frequency in different age rodent groups (in Russian). *Zoologicheskii Zhurnal* 86(4):499-504.

Nikol'skii AA. 2017. Two models of the sound-signal frequency dependence on the animal body size as exemplified by the ground squirrels of Eurasia (Mammalia, Rodentia). *Doklady Biological Sciences* 477(1):227-231. DOI: 10.1134/S0012496617060059.

Obrist MK, Pavan G, Sueur J, Riede K, Llusia D, Márquez R. 2010. Chapter 5.

Bioacoustics approaches in biodiversity inventories. In: Eymann J, Degreef J, Häuser C, Monje JC, Samyn Y, VandenSpiegel D, eds. *Volume 8 - Manual on Field Recording Techniques and Protocols for All Taxa Biodiversity Inventories*. Belgium: The Journal Dedicated to Capacity Building in Taxonomy and Collection Management, 68-99

Oswalt GL, Meier GW. 1975. Olfactory, thermal, and tactual influences on infantile ultrasonic vocalization in rats. *Developmental Psychobiology* 8(2):129-135. DOI: 10.1002/dev.420080205.

Owings DH, Virginia RA. 1978. Alarm calls of California ground squirrels (*Spermophilus beecheyi*). *Zeitschrift Fur Tierpsychologie* 46:58-70. DOI: 10.1111/j.1439-0310.1978.tb01438.x.

Owings DH, Leger DW. 1980. Chatter vocalizations of California ground squirrels: predator- and social-role specificity. *Zeitschrift Für Tierpsychologie* 54(2):163-184. DOI: 10.1111/j.1439-0310.1980.tb01070.x.

Panyutina AA, Kuznetsov AN, Volodin IA, Abramov AV, Soldatova IB. 2017. A blind climber: the first evidence of ultrasonic echolocation in arboreal mammals. *Integrative Zoology* 12(2):172-184. DOI: 10.1111/1749-4877.12249.

Pierce GW, Griffin DR. 1938. Experimental determination of supersonic notes emitted by bats. *Journal of Mammalogy* 19(4):454-455. DOI: 10.2307/1374231.

Rabin LA, Mccowan B, Hooper SL, Owings DH. 2003. Anthropogenic noise and its effect on animal communication: an interface between comparative psychology and conservation biology. *International Journal of Comparative Psychology* 16(2):172-192. DOI: 10.46867/C4F59P.

Ramsier MA, Cunningham AJ, Finneran JJ, Dominy NJ. 2012. Social drive and the evolution of primate hearing. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367(1597):1860-1868. DOI: 10.1098/rstb.2011.0219.

Rendall D, Owren MJ, Ryan MJ. 2009. What do animal signals mean? *Animal Behaviour* 78(2):233-240. DOI: 10.1016/j.anbehav.2009.06.007.

Revere-Wollsenak Division. 2010. Service Manual: Wollensak T-1500, T- 1500U, T-1515-4, T-1520, T-1700 Recorders. Chicago: Minnesota Mining & Manufacturing Co.

Riede T, Borgard HL, Pasch B. 2017. Laryngeal airway reconstruction indicates that rodent ultrasonic vocalizations are produced by an edge-tone mechanism. *Royal Society Open Science* 4(11):170976. DOI: 10.1098/rsos.170976.

Ryan MJ, Brenowitz EA. 1985. The role of body size, phylogeny, and ambient noise in the evolution of bird song. *The American Naturalist* 126(1):87-100. DOI: 10.1086/284398.

Schneiderová I. 2008. Acoustics communication and alarm calls in two species of ground squirrels *Spermophilus citellus* and *Spermophilus taurensis* (Sciuridae; Rodentia). D. Phil. Thesis, Charles University.

Schneiderová I. 2012. Frequency-modulated second elements of two-element alarm calls do not enhance discrimination of callers in three Eurasian ground squirrels. *Current Zoology* 58(5):749-757. DOI: 10.1093/czoolo/58.5.749.

Schneiderová I, Policht R. 2012. Acoustic analysis of the alarm call of the Anatolian ground squirrel *Spermophilus xanthoprymnus*: a description and comparison with alarm calls of the Taurus *S. taurensis* and European *S. citellus* ground squirrels. *Naturwissenschaften* 99(1):55-64. DOI: 10.1007/s00114-011-0870-4.

Schneiderová I, Štefanská L, Kratochvíl L. 2019. Geographic variability in the alarm calls of the European ground squirrel. *Current Zoology* 66(4):407-415. DOI: 10.1093/cz/zoz055.

Sherman PW. 1985. Alarm calls of Belding's ground squirrels to aerial predators : nepotism or self-preservation? *Behavioral Ecology and Sociobiology* 17(4):313-323. DOI: 10.1007/BF00293209.

Siemers M, Schauermann G, Turni H, von Merten S. 2009. Why do shrews twitter? Communication or simple echo-based orientation. *Biology Letters* 5(5):593-596. DOI: 10.1098/rsbl.2009.0378.

Sirotin YB, Costa ME, Laplagne DA. 2014. Rodent ultrasonic vocalizations are bound to active sniffing behavior. *Frontiers in Behavioral Neuroscience* 8. DOI: 10.3389/fnbeh.2014.00399.

Sloan JL, Wilson DR, Hare JF. 2005. Functional morphology of Richardson's ground squirrel, *Spermophilus richardsonii*, alarm calls: the meaning of chirps, whistles, and chucks. *Animal Behaviour* 70(4):937-944. DOI: 10.1016/j.anbehav.2005.01.013.

Smith WJ. 1979. The study of ultrasonic communication. *American Zoologist* 19(2):531-538. DOI: 10.1093/icb/19.2.531.

Swan DC, Hare JF. 2008. Signaler and receiver ages do not affect responses to Richardson's ground squirrel alarm calls. *Journal of Mammalogy* 89(4):889-894. DOI: 10.1644/07-MAMM-A-228.1.

Tamura N. 1995. Postcopulatory mate guarding by vocalization in the Formosan squirrel. *Behavioral Ecology and Sociobiology* 36(6):377-386. DOI: 10.1007/BF00177333.

Tamura N,Yong H-S. 1993. Vocalizations in response to predators in three species of Malaysian Callosciurus (Sciuridae). *Journal of Mammalogy* 74(3):703-714. DOI: 10.2307/1382292.

Taulman JF. 1977. Vocalizations of the hoary marmot, *Marmota caligata*. *Journal of Mammalogy* 58(4):681-683. DOI: 10.2307/1380026.

Terhune JJ. 2011. From switches to menus and students to HQPs-The evolving world of marine mammal bioacoustics. *Aquatic Mammals* 37(1):95-100. DOI: 10.1578/AM.37.1.2011.94.

Titze IR. 2009. How are harmonics produced at the voice source? *Journal of Singing* 65(5):575-576.

Tomasi TE. 1979. Echolocation by the short-tailed shrew *Blarina brevicauda*. *Journal of Mammalogy* 60(4):751-759. DOI: 10.2307/1380190.

Tripp TM, Otter KA. 2006. Vocal individuality as a potential long-term monitoring tool for western screech-owls, *Megascops kennicottii*. *Canadian Journal of Zoology* 84(5):744-753. DOI: 10.1139/z06-055.

Upham NS, Esselstyn JA, Jetz W. 2019. Inferring the mammal tree: species-level sets of phylogenies for questions in ecology, evolution, and conservation. *PLoS*

Biology 17(12):e3000494. DOI: 10.1371/journal.pbio.3000494.

Volodina EV, Matrosova VA, Volodin IA. 2010. An unusual effect of maturation on the alarm call fundamental frequency in two species of ground squirrels. *Bioacoustics* 20(1):87-98. DOI: 10.1080/09524622.2011.9753634.

Walker TJ. 1964. Cryptic species among sound-producing ensiferan Orthoptera (Gryllidae and Tettigoniidae). *The Quarterly Review of Biology* 39(4):345-355. DOI: 10.1086/404325.

Waring GH. 1966. Sounds and communications of the yellow-bellied marmot (*Marmota flaviventris*). *Animal Behaviour* 14(1):177-183. DOI: 10.1016/S0003-3472(66)80028-3.

Warkentin KJ, Keeley ATH, Hare JF. 2001. Repetitive calls of juvenile Richardson's ground squirrels (*Spermophilus richardsonii*) communicate response urgency. *Canadian Journal of Zoology* 79(4):569-573. DOI: 10.1139/cjz-79-4-569.

Wilson DR, Hare JF. 2004. Ground squirrel uses ultrasonic alarms. *Nature* 430(6999):523. DOI: 10.1038/430523a.

Wilson DR, Hare JF. 2006. The adaptive utility of Richardson's ground squirrel (*Spermophilus richardsonii*) short-range ultrasonic alarm signals. *Canadian Journal of Zoology* 84(9):1322-1330. DOI: 10.1139/z06-120.

Wood Jr. FG. 1952. Porpoise Sounds. A phonograph record of underwater sounds made by *Tursiops truncatus* and *Stenella plagiodon.* Florida: Marineland Research Lab.

Yamazaki, Y, Yamada H, Murofushi M, Momose H, Okanoya K. 2004. Estimation of hearing range in raptors using unconditioned responses. *Ornithological Science* 3(1):85-92. DOI: 10.2326/osj.3.85.

Zelley RA. 1971. The sounds of the fox squirrel, *Sciurus niger rufiventer*. *Journal of Mammalogy* 52(3):597-604. DOI: 10.2307/1378595.

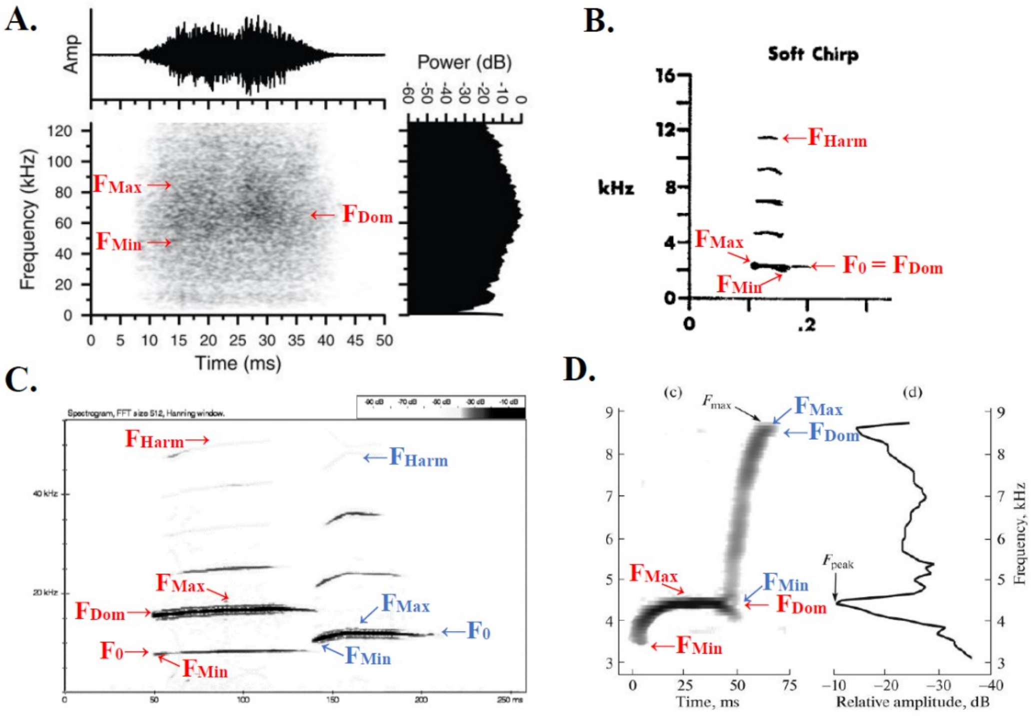


Figure 2.1 Examples highlighting the variation of how Sciuridae calls are presented as well as how frequency characteristics were measured on different spectrographs and call types. Frequency characteristics measured for all call types: **F_0** (fundamental frequency: the mean frequency of the primary vibrational frequency of the vocal membrane; when the dominant frequency occurs on the fundamental harmonic, $F_0 = (F_{Max}+F_{Min})/2$), **$F_{Dom}$** (dominant frequency: the frequency with the greatest energy, power or amplitude), **F_{Min}** (minimum frequency: the minimum frequency of the fundamental frequency), **F_{Max}** (maximum frequency: the maximum frequency of the fundamental frequency (or of harmonic on which **F_{Dom}** is measured)) and **F_{Harm}** (frequency of highest visible harmonic: mean frequency of the highest complete harmonic visible on the spectrograph). (A) Broadband noise produced by a female southern flying squirrel (*Glaucomys volans;* Murrant et al., 2013). (B) Alarm call ("shrill chirp") recorded in a colony of Columbian ground squirrels (*Urocitellus columbianus*; Betts 1976). (C) Alarm call (two call elements identified by the initial researchers and therefore recorded separately, red = element 1, blue = element 2) recorded in a population of European ground squirrels in the Ponor Mountains (*Spermophilus citellus*; Koshev and Pandourski 2008). (D) Alarm call (also with two distinct elements identified by red and blue) emitted by a female little ground squirrel (*Spermophilus pygmaeus*; Nikol'skii 2019).

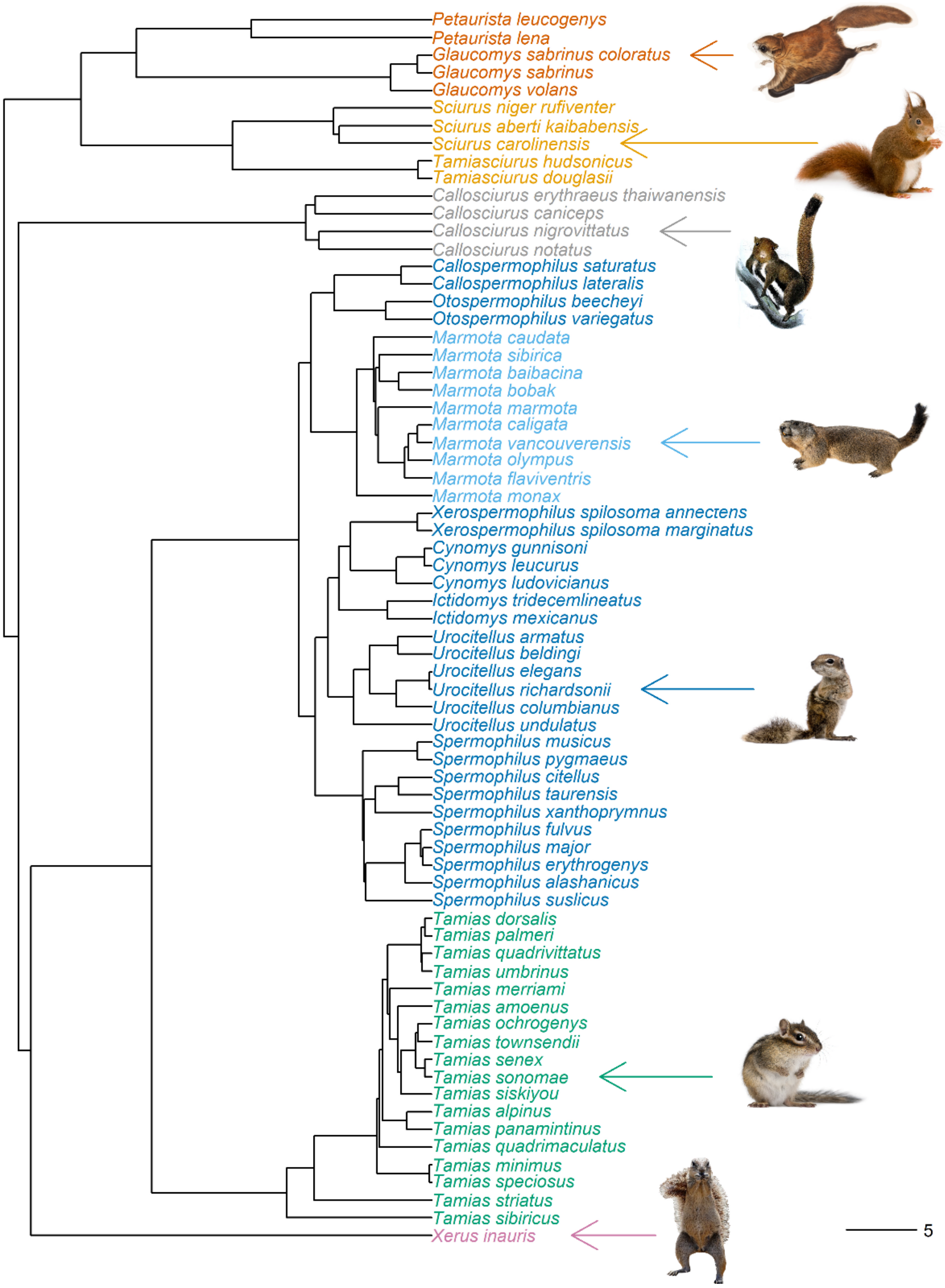


Figure 2.2 Phylogeny of Sciuridae reduced to all species used in analysis (n = 70). Species present represent the subfamily Callosciurinae (grey: southern Asian tree squirrels), Sciurinae (orange: Pteromyini, flying squirrels; yellow: Sciurini, Holarctic tree squirrels) and Xerinae (pink: tribe Xerini, African ground squirrels; Marmotini, Holarctic ground squirrels). Marmotini is subdivided into the genera *Tamias* (green: chipmunks), *Marmota* (light blue: marmots) and the remaining Holarctic ground squirrels (dark blue). Edge lengths to scale; scale bar represents 5 million years from present. Tree downloaded from VertLife.org.

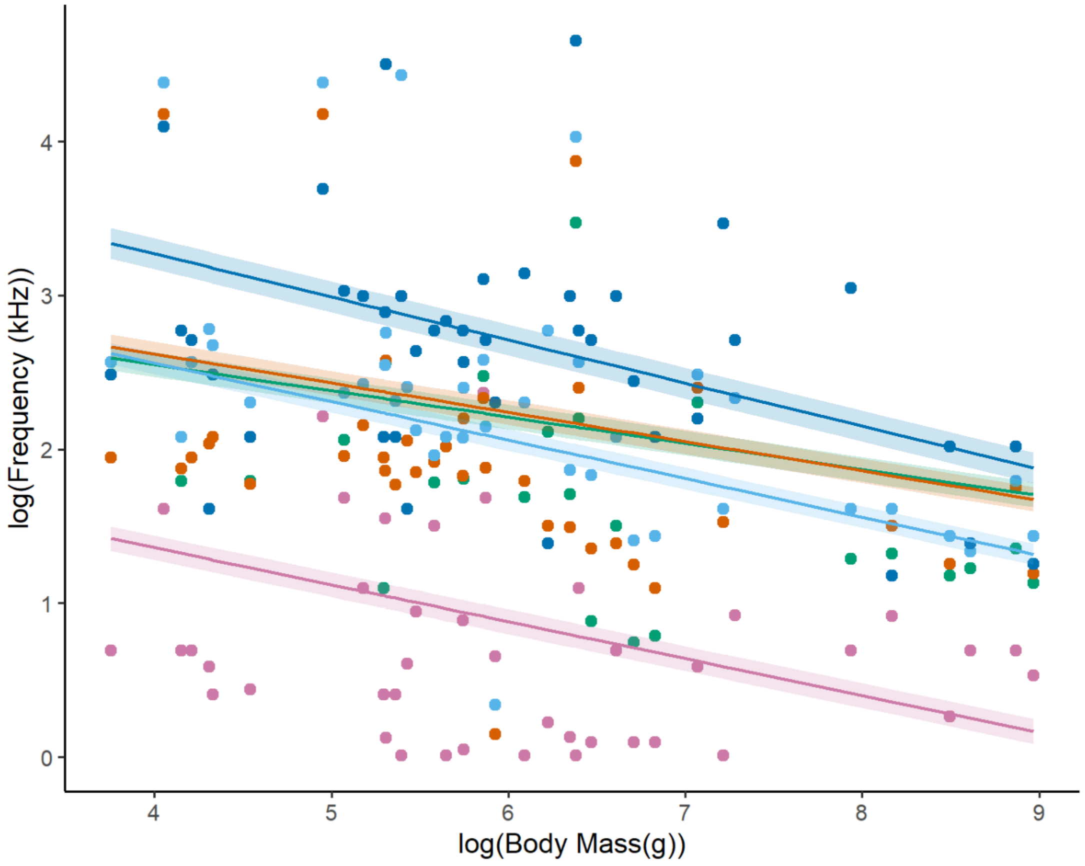


Figure 2.3 Frequency measurements for squirrel (n = 42) vocalizations as function of body mass. Frequencies measured as absolute minimum (pink), fundamental (green), dominant (orange), maximum (light blue), and highest harmonic (dark blue) (all variables log-transformed for normality; SE of slopes represented as shaded ribbons). Phylogenetic generalized least square (PGLS) models were used to describe the relationships between the variables and are represented by the following linear trend line: minimum (pink, $\log(Y) = -0.24*\log(X) + 2.3$), fundamental (green, $\log(Y) = -0.17*\log(X) + 3.2$), dominant (orange, $\log(Y) = -0.19*\log(X) + 3.4$), maximum (light blue, $\log(Y) = -0.18*\log(X) + 3.6$), and highest harmonic (dark blue, $\log(Y) = -0.28*\log(X) + 4.4$).

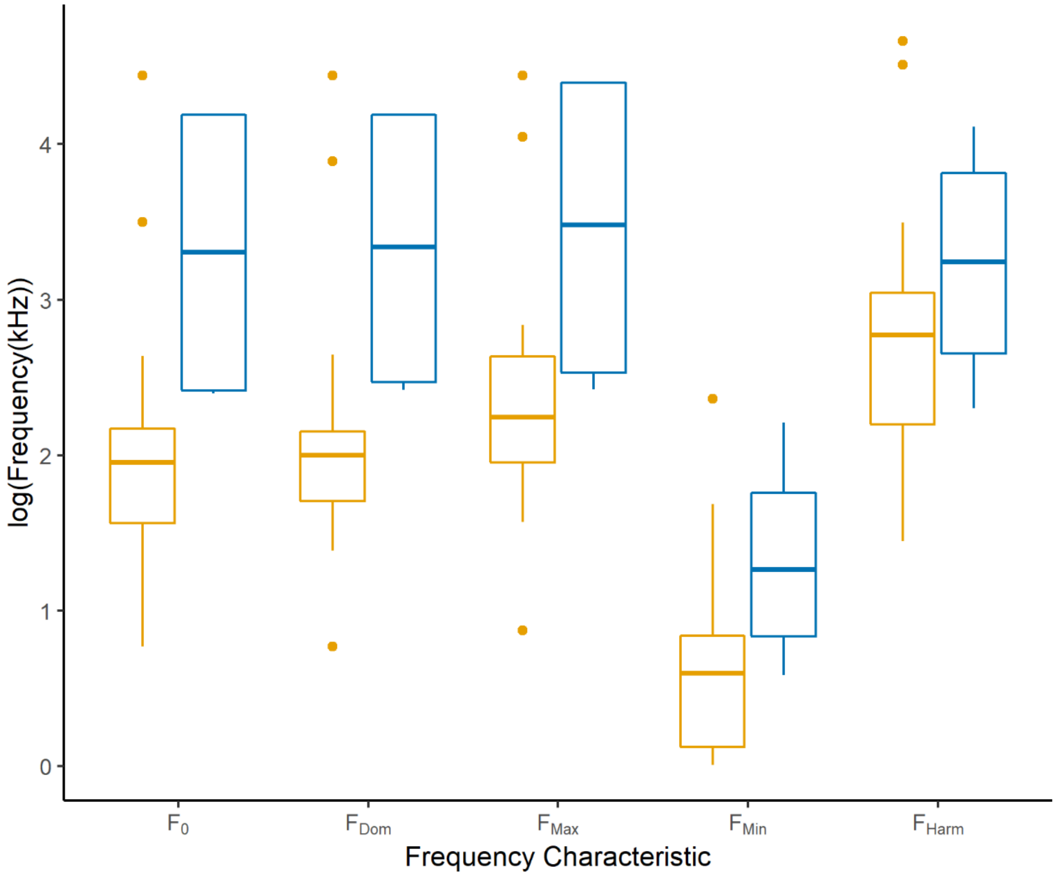


Figure 2.4 The frequency characteristics of calls produced by diurnal (orange) versus nocturnal (blue) squirrels (species of the family Sciuridae; n = 43). Frequency characteristics presented are the fundamental (F_0), dominant (F_{Dom}), maximum (F_{Max}), minimum (F_{Min}), and maximum harmonic (F_{Harm}) frequencies and all are log-transformed for normality.

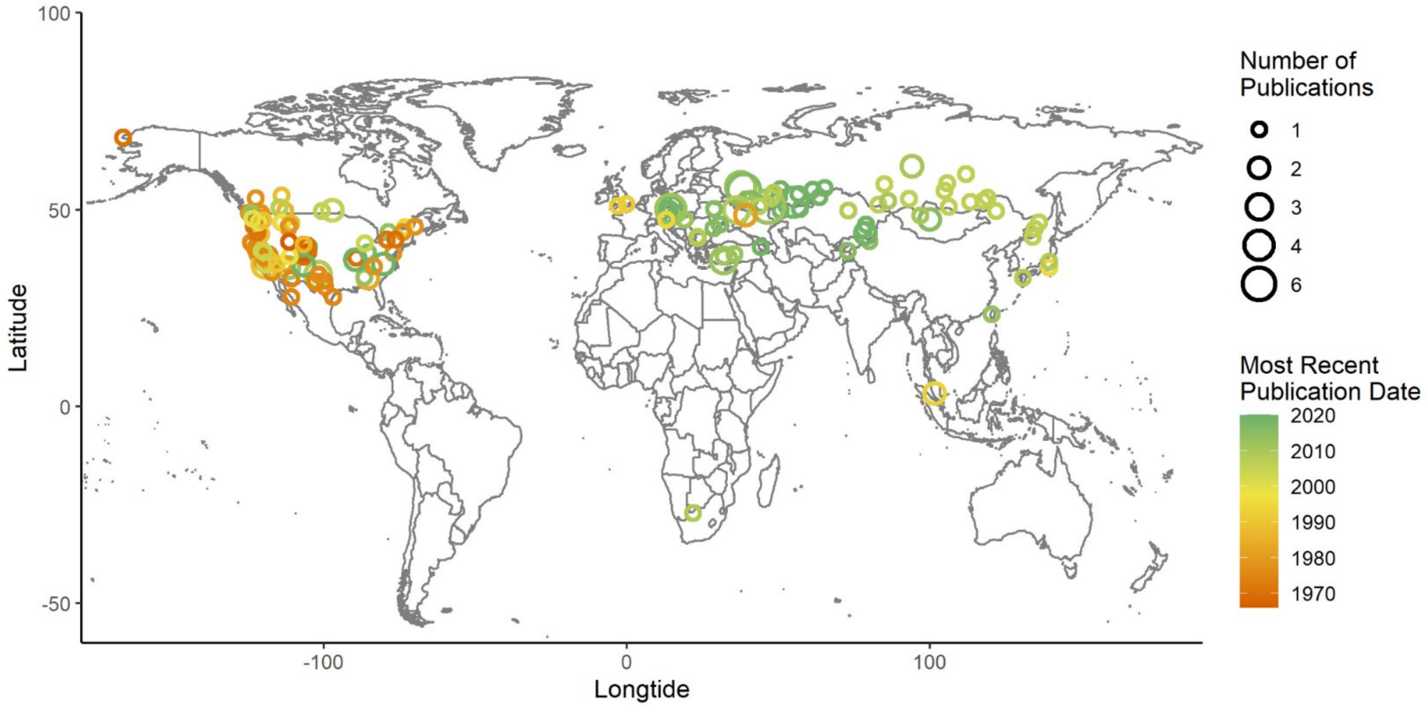


Figure 2.5 The recording locations of squirrel vocal repertoires published between 1966 and 2020. The year of publication is represented along a colour gradient and the number of studies recorded at each location is represented along a size gradient. The epicenter of each circle represents the location; circles are translucent to minimize overlap. The USA alone has hosted 52% of all published studies; Russia, Canada, and the Czech Republic are also notable contributors representing 18%, 11%, and 9% each. North America, Europe, Asia, and Africa represent 61%, 30%, 13%, and 1% respectively, with no record of South America, India, or the majority of Africa hosting a repertoire study.

Table 2.1 Summary of parameters used in the phylogenetic least square (PGLS) analyses and how they relate to popular or novel vocal range hypotheses. The predicted direction of the relationship to frequency is indicated as positive (+) or negative (-).

Parameter	Hypothesis	Citation
Body Mass (-)	Body size controls the size of vocal producing structures; body size estimated through body mass.	(Arch and Narins 2008; Charlton and Reby 2016; Martin et al., 2016)
Sociality: Social (+)	Social species communicate more often and rely on conspecifics for predator avoidance; higher frequencies are less likely to detected by predatory birds, reptiles, and amphibians.	(Arch and Narins 2008; Ramsier et al., 2012)
Habitat: Openness (+)	Acoustic adaptation hypothesis: species will alter call structure to maximize transmission in different environments; closed habitats attenuate high-frequency sound waves rapidly while open habitats do not inhibit transmission.	(Ey and Fischer 2009)
Diel Activity Pattern: Nocturnality (+)	Gliding and nocturnality are intertwined for Sciuridae; nocturnality facilitates the use of echolocation as visual cues are greatly reduced.	Novel
Method Limits (+)	The frequency limits of the microphone and spectrograph restrict the detection range of the equipment.	Novel

Table 2.2 Summary of call functions used by all reported vocalizing squirrel genera of Sciuridae (n = 73).

Genus	**Alarm**	**Territorial**	**Distress**	**Threat**	**Friendly**	**Mating**	**Movement**
Callosciurus	X*					X	
Callospermophilus	X						
Cynomys	X*		X	X	X	X	
Glaucomys							X
Ictidomys	X		X	X	X		
Marmota	X*		X	X	X	X	
Otospermophilus	X*		X	X			
Petaurista	X	X		X		X	X
Sciurus	X	X	X	X		X	
Spermophilus	X		X	X			
Tamias	X	X	X	X		X	
Tamiasciurus	X	X	X		X		
Urocitellus	X*		X	X	X	X	
Xerospermophilus	X		X				
Xerus	X						

*Indicated predator-specific vocalizations

Table 2.3 Phylogenetic generalized least square (PGLS) results for the effects of all factors on the frequency characteristics (fundamental, dominant, minimum, maximum, and highest harmonic frequency; kHz) of Sciuridae.

Variable	Fundamental	Dominant	Minimum	Maximum	Highest Harmonic
Body Mass	**$F_{1,58}$ = 23.14*** **	**$F_{1,60}$ = 12.37*** **	**$F_{1,58}$ = 6.24* **	**$F_{1,59}$ = 37.61*** **	**$F_{1,37}$ = 9.18** **
Sociality	**$F_{1,58}$ = 9.54** **	**$F_{1,60}$ = 8.60** **	$F_{1,58}$ = 1.14, p = 0.29	**$F_{1,59}$ = 8.57** **	**$F_{1,37}$ = 5.31* **
Habitat	$F_{1,58}$ = 1.87, p = 0.18	**$F_{1,60}$ = 4.22* **	$F_{1,58}$ = 0.32, p = 0.57	$F_{1,59}$ = 0.22, p = 0.64	**$F_{1,37}$ = 5.65* **
Diel Activity Pattern	**$F_{1,58}$ = 51.22*** **	**$F_{1,60}$ = 40.84*** **	$F_{1,58}$ = 1.63, p = 0.21	**$F_{1,59}$ = 33.10*** **	$F_{1,37}$ = 2.96, p = 0.09
Method Limits	**$F_{1,58}$ = 37.91*** **	**$F_{1,60}$ = 23.78*** **	**$F_{1,58}$ = 4.63* **	**$F_{1,59}$ = 25.16*** **	**$F_{1,37}$ = 11.01** **

Significant effects are given in bold.

*$p < 0.05$ **$p < 0.01$ ***$p < 0.0001$

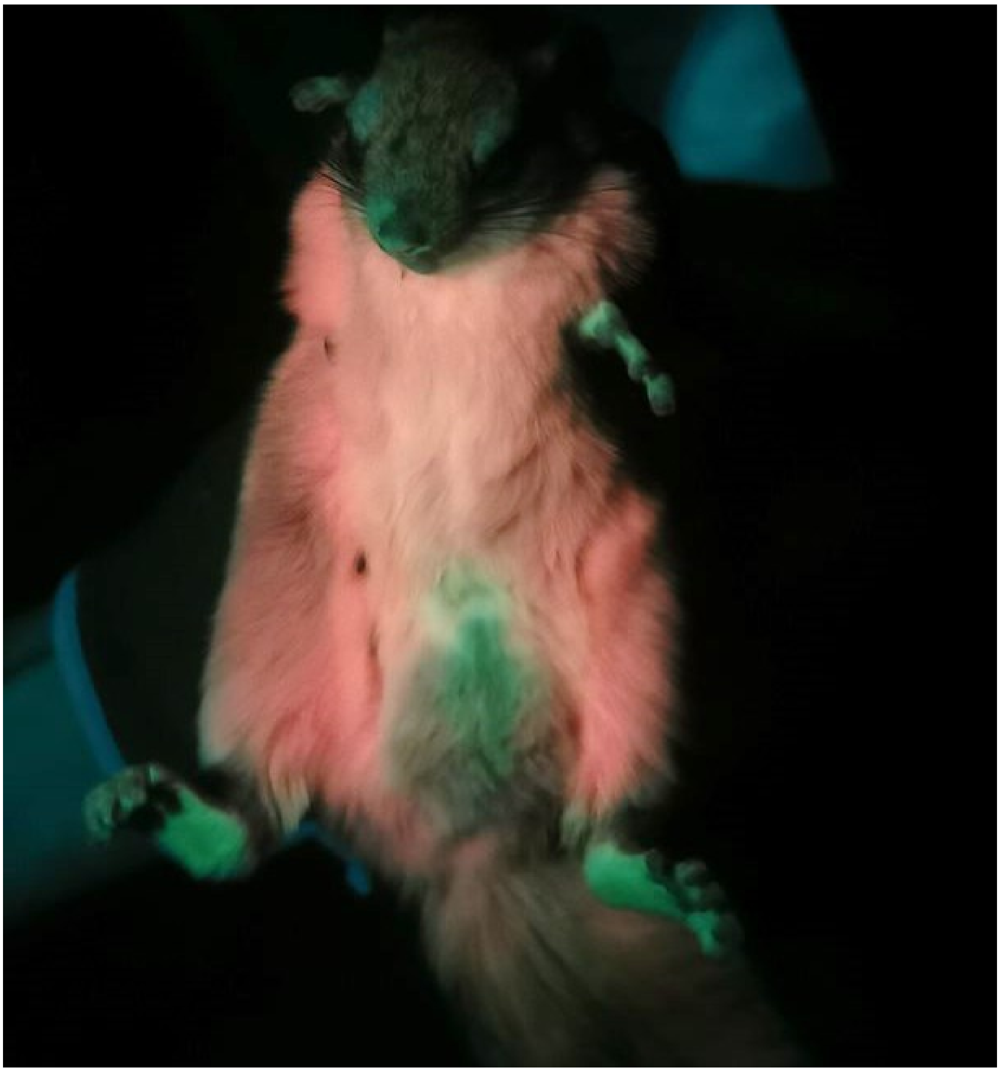
Table 2.4 Summary of slope (±SE) and phylogenetic signal (λ [95% CI]) estimates for all variables taken from phylogenetic generalized least square (PGLS) models of the frequency characteristics (fundamental, dominant, minimum, maximum, and highest harmonic frequency; kHz) of Sciuridae.

Variable	Fundamental	Dominant	Minimum	Maximum	Highest Harmonic
Intercept	-1.33 (±0.87), $p = 0.13$	-0.88 (±0.91), $p = 0.34$	**1.46 (±0.65)***	-0.19 (±0.90), $p = 0.84$	-1.50 (±1.87), $p = 0.43$
Body Mass	**-0.22 (±0.05)*****	**-0.20 (±0.05)*****	-0.19 (±0.09), $p = 0.05$	**-0.25 (±0.05)*****	**-0.29 (±0.08)****
Sociality	-0.01 (±0.13), $p = 0.96$	-0.04 (±0.14), $p = 0.75$	0.19 (±0.17), $p = 0.26$	-0.07 (±0.13), $p = 0.61$	-0.06 (±0.22), $p = 0.78$
Habitat	0.18 (±0.13), $p = 0.17$	**0.31 (±0.14)***	-0.16 (±0.29), $p = 0.59$	0.07 (±0.13), $p = 0.61$	**0.59 (±0.25)***
Diel Activity Pattern	**0.79 (±0.22)*****	**0.96 (±0.24)*****	0.43 (±0.56), $p = 0.45$	**0.61 (±0.23)***	0.31 (±0.38), $p = 0.43$
Method Limits	**0.45 (±0.08)*****	**0.39 (±0.08)*****	**0.12 (±0.05)***	**0.40 (±0.08)*****	**0.51 (±0.15)****
λ	0 [0, 0.96]	0 [0, 0.32]	0.65 [0, 0.88]	0 [0, 0.95]	0 [0, 0.43]

Significant estimates are given in bold.

*$p < 0.05$ **$p < 0.01$ ***$p < 0.0001$

Chapter 3 - Ultrasound and ultraviolet: Crypsis in gliding mammals



A version of this chapter has been published by PeerJ.

Newar SL, Schneiderová I, Hughes B, Bowman J. 2024. Ultrasound and ultraviolet: crypsis in gliding mammals. *PeerJ* 12:e17048. DOI: 10.7717/peerj.17048.

Abstract

Gliding is only present in six extant groups of mammals – interestingly, despite divergent evolutionary histories, all mammalian gliders are strictly nocturnal. Gliding mammals also seem to have relatively high rates of ultrasound use and ultraviolet-induced photoluminescence (UVP) in contrast with their close relatives. Therefore, we hypothesized that, despite diverging lineages, gliding mammals use similar modes of cryptic communication compared to their non-gliding counterparts. We developed two datasets containing the vocal range (minimum-maximum of the dominant harmonic; kHz) and UVP of 73 and 82 species, respectively; we report five novel vocal repertoires and 57 novel observations of the presence or absence of UVP. We complemented these datasets with information about body size, diel activity patterns, habitat openness, and sociality to explore possible covariates related to vocal production and UVP. We found that the maximum of the dominant harmonic was significantly higher in gliding mammals when vocalizing than their non-gliding relatives. Additionally, we found that nocturnality was the only significant predictor of UVP, consistent with the previous hypothesis that luminophores primarily drive UVP in mammal fur. In contrast, however, we did not find UVP ubiquitous in nocturnal mammals, suggesting that some unknown process may contribute to variation in this trait.

Keywords: mammalian phylogeny, nocturnal, photoluminescence, vocal range, vocal repertoire

Introduction

Gliding mammals are physically unique: their most notable shared trait, a thin membrane (the patagium), stretches between limbs, digits, necks, and tails, allowing them to glide between trees and other elevated features in their arboreal habitats (Jackson & Schouten, 2012). Gliding has independently evolved at least nine times in mammals and is represented by six extant taxa (Thorington & Heaney, 1981; Dudley et al., 2007; Jackson & Schouten, 2012; Jackson & Thorington, 2012): colugos (Cynocephalidae; Dermoptera), flying squirrels (Pteromyini; Rodentia), scaly-tailed flying squirrels (Anomaluridae; Rodentia), lesser gliding possums (*Petaurus*; Diprotodontia), greater gliders (*Petauroides*; Diprotodontia) and the feather-tailed glider (*Acrobates*; Diprotodontia). Despite what some of their common names imply (e.g., flying squirrels), gliding mammals are incapable of true flight, as exhibited by birds or bats. Instead, these mammals extend their patagium as they jump to convert gravitational velocity to forward momentum, allowing traversal of complex environments farther and faster than would occur through other means (e.g., walking and climbing; Dudley et al., 2007; Byrnes & Spence, 2011). This locomotive advantage has been hypothesized to aid with predator avoidance (Emmons & Gentry, 1983), traversing vertical habitat structures (Emmons & Gentry, 1983; Dial, 2003), and improved foraging efficiency (Paskins et al., 2007; Scheibe et al., 2007).

While gliding mammals all share this unique locomotive trait, it is not the only trait they have evolved to share. All gliding mammals are strictly nocturnal (Thorington & Heaney, 1981; Fokidis & Risch, 2008; Jackson & Schouten, 2012); this is particularly notable in the flying squirrels, the only extant lineage of nocturnal squirrels (Chapter 2; Newar & Bowman, 2020). Interestingly, unlike other gliders, which share some close nocturnal relatives, phylogenetic reconstructions suggest that traits associated with diurnality are the ancestral state of all squirrels (Menéndez et al., 2021), meaning that the emergence of nocturnality and gliding are entangled in flying squirrels. Even the oldest flying squirrel fossil, which was estimated to have originated 11.6Mya, already had well-developed wrist spurs (a key adaptation that both keeps the patagium tucked when climbing and holds the patagium open to increase surface area when gliding) and large orbital processes like current nocturnal species (Casanovas-Vilar et al., 2018). For the only other group of volant mammals, bats, it is also unclear if nocturnality evolved first, as previously assumed, or if nocturnality emerged in tandem with flight (Anderson & Ruxton, 2020). So, while the order in which these traits evolved in gliding mammals may be unclear, nocturnality appears to be an important covariate of volancy in mammals.

In bats, echolocation has evolved to aid in navigating complex environments and capturing moving prey in mid-flight. Echolocation is the (often) rapid production of calls that echo off solid objects, allowing the caller to interact with their environment in poor light conditions (Panyutina et al., 2017). The frequency of calls used to echolocate can vary from auditory (within the human auditory range from approximately 20Hz to 20kHz; Masterton et al., 1969) to ultrasonic (>20kHz). Most echolocating mammals commonly use ultrasonic vocalizations (USVs), including cetaceans, most bats, and small terrestrial mammals with poor eyesight, because the shorter wavelengths of USVs allow for greater detection accuracy (Panyutina et al., 2017). The evolutionary relationship between echolocation and volancy in bats is complex, with at least six competing hypotheses relating to the evolutionary past of these traits (Anderson & Ruxton, 2020). The most widely accepted of these hypotheses is that bats began as gliders, with echolocation likely developing in tandem as gliding evolved to the more complex behaviour of flying (Anderson &

Ruxton, 2020). Interestingly, several gliding mammals have also been shown to produce USVs, including colugos (Miard et al., 2019), feather-tailed gliders (Martin, 2019), and flying squirrels (Gilley, 2013; Murrant et al., 2013; Gilley et al., 2019; Diggins et al., 2020). While the function of these calls is still unclear, due largely to relatively few in-situ studies of mammalian gliders, there has been some evidence that gliders produce high-frequency (>10kHz) calls in tandem with gliding behaviours (Miard et al., 2019). Additionally, flying squirrels produce significantly higher calls than non-gliding squirrels, suggesting that gliding may be linked to USV production (Chapter 2; Newar & Bowman, 2020). However, nocturnality and gliding are entangled in Sciuridae, with all nocturnal squirrels belonging to the flying squirrel tribe, Pteromyini, meaning that it is unclear whether nocturnality or gliding is more closely associated with high-frequency calls.

Aside from nocturnality and gliding, higher frequencies are also commonly associated with various other traits: smaller body sizes (Martin, Tucker & Rogers, 2017; Chapter 2, Newar & Bowman, 2020) and open and structurally uncomplicated habitats (Boncoraglio & Saino, 2007; Ey & Fischer, 2009; Fischer, Wadewitz & Hammerschmidt, 2017). Additionally, some primates have demonstrated increased sensitivity to higher frequencies as social complexity increases (Ramsier et al., 2012) and a wide range of small mammals can exploit high frequencies for social contexts (Arch & Narins, 2008), such as alarm calling that’s undetectable by focal predators (Wilson & Hare, 2006). These higher frequency calls are not restricted to the ultrasonic range, with birds, anurans, and mammals all displaying frequency shifts within the sonic ranges. While gliding mammals are relatively small-bodied and exhibit social behaviours, they exclusively inhabit forested habitats and even the largest gliders still need to navigate closed canopies. However, forested habitats should greatly restrain USVs as these environments easily attenuate high-frequency sounds. Yet, bird songs are acoustically complex despite forested habitats (Boncoraglio & Saino, 2007), and squirrels in open habitats only have a slightly higher peak frequency than those in closed habitats, with no effect on the maximum frequency of the dominant harmonic (Chapter 2; Newar & Bowman, 2020). Additionally, bats foraging along edge habitats and within narrow spaces use higher frequencies than bats foraging in open habitats to optimize echolocating behaviour (Schnitzler, Moss & Denzinger, 2003).

Flying squirrels have been shown to exhibit another obscure trait: ultraviolet-induced photoluminescence (UVP) in fur (Kohler et al., 2019; Reinhold et al., 2023; Touissant et al., 2023). Recent interest in this topic has led to the discovery of UVP in several species, including other gliding mammals (Reinhold, 2021) and relatives, such as springhares (Olson et al., 2021; shared suborder with scaly-tailed flying squirrels) and dormice (Nummert, Ritson & Nemvalts, 2023; shared suborder with flying squirrels). UVP occurs when ultraviolet (UV) light from the environment is absorbed and then re-emitted as visible light by excited particles, which, in the case of mammals, can be expressed in the fur (Kohler et al., 2019; Reinhold, 2021), quills (Hamchand et al., 2021), scales (Jeng, 2019), and teeth and bone (Levin & Flyger, 1973). It has been proposed that porphyrins and tryptophan metabolites (henceforth luminophores), both of which are known to photoluminesce under UV light and are ubiquitous across mammals, are the likely cause of UVP in the fur of some mammals (Nicholls and Rienits, 1971; Olson et al., 2021; Hughes et al., 2022; Toussaint et al., 2023). There is some evidence to suggest that most photoluminescent fur is the result of photodegradable porphyrins (Toussaint et al., 2023). However, tryptophan metabolites that are often associated with vivid fluorescent pigments in possums and some diurnal animals are not as readily photodegradable as porphyrins (Pine et al., 1985; Schäfer et al., 1997; Toussaint et al., 2023). Thus, we might expect a greater prevalence of UVP in nocturnal species compared to diurnal species that experience increased photodegradation of porphyrins and lack sufficient concentrations of tryptophan metabolites within their fur. However, melanin can mask the photoluminescent properties of luminophores found in mammal fur (Huang et al., 2006); therefore, mammals with darker fur should exhibit weaker or no UVP compared to mammals with lighter fur (Rebell, 1966). Notably, while the fundamental processes associated with UVP are understood in some species, there has yet to be a comprehensive review of which mammals exhibit (and perhaps more importantly, do not exhibit) UVP.

While empirical evidence demonstrating the behavioural relevance of UVP in mammals has yet to be presented in the literature, this trait has received substantial media attention, with several hypotheses aiming to describe a behavioural function. Marshall and Johnsen (2017) suggested the following criteria to conceptualize the communication potential of photoluminescent colouration in any taxa: visible location of colours, wavelengths of excitement and emission, viewer sensitivity, behavioural changes regarding photolumination, and natural light availability. While some mammals may exhibit UVP internally (fox squirrels exhibiting UVP in their bones (Levin and Flyger, 1973)), UVP in fur is easily visible to potential viewers. There is a broad excitation range for visible-spectrum photoluminescence emission, with excitation spectra from ~320 – 650 nm (Huang et al., 2010; Hamchand et al., 2021). While many nocturnal mammals are sensitive to the ultraviolet portion of this range via short-wave cone sensitivity ~ 360 nm (Gerkema et al., 2013), photoluminescence emission can occur at almost any colour in the visible spectrum. Therefore, UV sensitivity is not necessarily required for UVP to be biologically relevant and instead, UVP is restricted by the availability of the environmental UV-light to excite the photoluminescent structures. UV light drastically decreases during the night which suggests that nocturnal mammals have a lower potential for UVP to be relevant compared to diurnal mammals. However, UV reflectance of moonlight has been shown to change the relevance of UVP in some nocturnal non-mammalian species (Kloock, 2005; Marshall and Johnsen, 2017). Nocturnal species also lack the UV-filtering lens present in diurnal mammals, potentially allowing for a larger colour range of UVP to be used when there is enough UV light to cause UVP (Yolton et al., 1974).

Given the strong relationship between high-frequency sound production and gliding in squirrels and the recent discovery of UVP in flying squirrels, we wanted to further investigate these traits across all gliding mammals. The link between nocturnality and gliding in mammals allowed these species to exploit a particular niche; the communication methods used by nocturnal gliders might be constrained by the features associated with this niche. For example, gliding mammals are exposed to fewer predators than their diurnal relatives, but their predators are specialized for nocturnal prey detection (Jackson, 2012). Owls are common predators of North American flying squirrels (*Glaucomys*) and employ large, low-light sensitive eyes to aid in prey detection (Dice, 1945). At the same time, owl ears are adapted for detecting low-frequencies (Knudsen, 1981), which would be advantageous for detecting movement-related sounds. Therefore, it would be beneficial for flying squirrels, which are socially complex species, to communicate with conspecifics at a frequency higher than what an owl is specialized to receive. Additionally, given the communication potential of UVP, we might expect UVP to be used either as a visual cue to conspecifics or as a conspicuous visual camouflage (e.g., Batesian mimicry; Kohler et al., 2019), as owls are another known group to exhibit UVP (Weidensaul et al., 2011).

Crypsis is the ability of an animal to avoid detection by other animals, including visual (Vignieri, Larson & Hoekstra, 2010; Ruxton et al., 2018) and auditory (Ruxton, 2009; Legett, Hemingway & Bernal, 2020) concealment, such as using camouflaged signals or signals outside of the perceptual range of a predator (Marples, Kelly & Thomas, 2005). We consider the use of high-frequency communication and UVP as potentially cryptic traits because of evidence or hypotheses that the traits might be camouflaged or outside of the perceptual range of predators. Given the potential vulnerability of gliding mammals to predators and the apparent selective pressure toward nocturnality of the gliding trait, we were interested in the potential that crypsis was widespread among this group of species. Given their unique ecological niches and evolutionary pressures, we hypothesized that gliding mammals are more likely to exhibit these potentially cryptic traits than their close phylogenetic relatives. We selected a range of squirrels (to contrast with flying squirrels), rodents (to contrast with the scaly-tailed flying squirrels), primates (to contrast with colugos), and marsupials (to contrast with marsupial gliders) with similar body sizes to compare UVP and vocal ranges across gliding and non-gliding mammals. We predicted that physical (body size), behavioural (sociality, nocturnality), and environmental (habitat openness) traits would impact vocal range across all species, but higher frequencies would be most associated with gliding mammals. In contrast, given the current limited understanding of UVP, we did not expect UVP to be strongly associated with physical or environmental variables. UVP may also play a role in the communication of social species inhabiting visibly difficult or low-light environments. However, given the photodegradability of some luminophores that accumulate in fur and the communication potential of UVP in some nocturnal species, we also predicted that the pink-orange-red UVP would be strongly associated with nocturnality and sociality. Additionally, while we expected to find UVP in all gliding mammals, we predicted that UVP would be found in most nocturnal mammals tested. Our overall aim was to investigate the relationship between acoustic (vocal range) and visual (UVP) traits in gliding mammals in contrast with related species.

Methods

Vocalizations

We developed a database beginning with a list of publications describing gliding mammal vocalizations (summarized in Table A2.1). The minimum requirement for each publication was describing at least one call with either a spectrographic analysis or numerical data. However, most publications described multiple call types per species or multiple species per publication (7 gliding mammals represented across 9 publications, summarized in Table A2.1). The databases used to search for these publications were Google Scholar, JSTOR, Web of Science, and Wiley Online Library. We used the keywords acoustics, acoustic repertoire, calls, frequency, Hz, vocalizations, and ultrasound paired with an exhaustive list of currently valid and invalid genera (the most updated nomenclature was taken from the Integrated Taxonomic Information System http://www.itis.gov/). Across all published calls, we took the absolute minimum and maximum frequencies (kHz) of the dominant harmonic for the final analyses (this often corresponded to the fundamental harmonic, if multiple harmonics were present; following Chapter 2; Newar &

Bowman, 2020). For noisy calls, such as broadband calls, where the harmonics are not well defined, we estimated the minimum and maximum of the loudest parts of the call. We did not include calls produced by neonates or juveniles as there is evidence of some frequencies and calls being different in younger individuals (Nikol'skii, 2007; Schneiderová et al., 2015).

To compare gliding mammals to closely related species, we systematically searched for vocalization data using the same methodology described above (Fig. 3.1). Flying squirrels are unique amongst the gliders as there are many extant species that occupy the same family (Sciuridae); therefore, we kept all relatives from the same subfamily (Sciurinae) and a random subset of squirrels from the other subfamilies (26 squirrels across 62 publications). Other gliders have few extant relatives and we strategically chose taxa that shared similar evolutionary histories and traits. For the scaly-tailed gliders, we selected springhares (*Pedetes capensisi*), the only other extant taxa of the Anomaluromorpha suborder, and a variety of small-bodied rodents (12 species across 16 publications) exhibiting a range of vocal frequencies (maximum dominant frequency range: 9.86 (*Sicista subtilis*; Volodin et al., 2019a) - 109.8kHz (*Mus musculus*; Hoffmann, Musolf & Penn, 2012)). For colugos, the only extant members of the order Dermoptera, we selected tree shrews (*Tupaia belangeri*) which form a sister clade with Dermoptera (Nie et al., 2008) and similarly sized taxa from the order Primates (19 primates across 27 publications), which are the next closest sister taxa (Beard, 1993). For marsupial gliders, we expanded our search to include similarly sized taxa of the order Diprotodontia as there were few records of marsupial vocalizations (5 marsupials across 6 publications). The vocalization data for two marsupial gliders (*Petaurus breviceps*, and *P. norfolcensis*) and two glider relatives (*Pedetes capensis* and *Pseudocheirus peregrinus*; Fig. 3.1) were not available in the literature, and we worked with co-authors and collaborators to develop novel call descriptions for our study (methods in Article S1). We also provide vocalization data from free-ranging *Petaurus australis* (methods in Articles S1) to opportunistically contrast our recordings with previously reported calls in the literature (Kavanagh & Rohan-Jones, 1982; Whisson et al., 2021). In the literature, four species were represented by a single subspecies only: *Otolemur garnettii lasiotis*, *Petaurista alborufus lena*, *Sciurus aberti kaibabensis*, and *Sciurus niger rufiventer*.

Ultraviolet-Induced Photoluminescence

To expand on our vocalization dataset, we assessed the UVP of pelage for 83 species. Previous literature accounted for 19 species in our dataset; we sampled an additional 64 species from the mammal collections at the Canadian Museum of Nature and the Royal Ontario Museum (one mounted specimen (*Sicista subtilis*), otherwise all dry-preserved pelts; specimen and museum information available at https://doi.org/10.5061/dryad.3n5tb2rp4). We sampled species from the vocalization dataset preferentially. However, we opportunistically added ten species (bold type in Table A2.1) to increase the sample size of luminescing species. We followed the same vocalization protocol detailed above for both opportunistic and previously published UVP species; we found vocalization data for eight additional species (four opportunistic and four from previous UVP literature).

We used a Vansky UV flashlight (395nm wavelength) to illuminate museum specimens (held 75 cm above the individual) and a Huawei P30 Pro phone (held directly beside the light) to capture any luminescence. A yellow gel filter was held in front of the camera lens to reduce the input of purple-blue light (Kohler et al., 2019; Nummert, Ritson & Nemvalts, 2023). To minimize the additional yellow hue created by the filter, we manually colour-corrected the photos in Photoshop (details in A2).

We took pictures of each specimen's ventral and dorsal sides under white-light conditions, UV-light only, UV-light + filter, and UV-light + filter + correction (example provided in Fig. 3.2; complete photoset available at https://doi.org/10.5061/dryad.3n5tb2rp4). We additionally photographed a few live *Glaucomys* individuals trapped in the Kawartha Highlands, Ontario, following the same protocol (animals studied under Trent University animal care protocol 27909).

In our investigation, some species expressed visible photoluminescence in white pelage or in some cases, the white ends of guard hairs. While "white" UVP has been noted in some species, this "white" colouration has been reported as a bluish-white (as seen in the striped possum (*Dactylopsila trivirgata*) and some marsupial gliders; Reinhold, 2021). The underlying cause of UVP expressed as distinct colours have been linked to porphyrins (red or pink) or tryptophan metabolites (cyan, blue, lavender; Reinhold et al., 2023). However, the expression of exclusively "white" colouration is not commonly reported, nor has a clear explanation been proposed for producing UVP without a dominant colour. Furthermore, white human hair may emit a bluish hue similar to the pelage of minks, rabbits and goats and sheep, which have been described as being photoluminescent due to the presence of tryptophan metabolites (Millington, 2020). Given that we could not photograph museum specimens in complete darkness, the available visible light may have excited white hairs that would otherwise not express UVP. Therefore, to remove the potential bias of visible light, we removed individuals that only expressed "white" photoluminescence (but model outputs for all species, including those with "white" UVP, are included in Table A2.2). While UVP varied dramatically in colour (e.g., pink, blue/green), placement, and patterning across museum specimens and published literature, we reduced variability to absence/presence to increase the sample size in each category.

Dataset Assembly

Once we had assembled our database of vocalizing mammals with UVP records, we searched for the body mass (g), diel activity pattern (diurnal or nocturnal), social complexity, and habitat openness of the dominant habitat (open or closed) of each species. We preferentially took these data from the relevant vocalization or UVP papers, though this information was rarely provided; therefore, other resources, including articles and online databases such as Mammalian Species accounts and the Animal Diversity Web (Myers et al., 2023), were reviewed to complete our dataset. If a range was provided for the body mass, we took the mean of the given values; we took a mean of male and female body masses as we were not capturing the effect of sex on vocalization frequencies or UVP. Social variability was reduced to social or solitary living to reduce model parameters; species that exhibit dynamic social structures, where adult individuals will seasonally or cyclically shift between solitary and social living (e.g., flying squirrels engaging in social nesting during the winter only; Garroway et al., 2013), were treated as socially living.

Phylogeny

While multiple subspecies were present in the vocalization dataset, we calculated the vocalization maxima at the species level for the final dataset and analyses (Fig. 3.1; subspecies-specific information noted in Table A2.1). Only one subspecies was excluded from analyses (*Peromyscus maniculatus bairdii*) due to a binary variable inconsistency with the parent species – this subspecies only occurs in open habitats (Wecker, 1963), while the parent species is most commonly found in closed habitats. In addition, Masters et al., (2017) recently proposed that the

Paragalago genus is a distinct clade from the *Galagoides* genus to which the *Paragalago* species had been previously assigned; we reassigned these species accordingly.

For the final species dataset (n = 93), we pruned 1000 node-dated completed trees from the mammalian supertree on VertLife, an online database used to produce pruned random distribution trees of vertebrate species (Upham, Esselstyn & Jetz, 2019). The nexus outputs were compiled into a consensus tree using the *phytools* (Revell, 2012) package in R (R Core Team, 2022) (Fig. 3.1). *Petaurus notatus* is a recently described species (previously incorporated within *P. breviceps*), and therefore, it was the only species not available on Vertlife; we incorporated this species into the final consensus tree by splitting the *P. breviceps* lineage at a divergence time of 1Ma (Cremona et al., 2021).

Analyses

We built phylogenetic generalized least square (PGLS) models to account for variation in the vocal range that could be explained by phylogenetic relatedness. PGLS models estimate phylogenetic relatedness as lambda (λ), which varies between 0 (no phylogenetic trace) and 1 (absolute Brownian motion) (Freckleton et al., 2002; Martin, Tucker & Rogers, 2017). Full models were built for each frequency limit (β_0 + body mass (β_{Mass}) + diel activity pattern (β_{Diel}) + sociality ($\beta_{Sociality}$) + habitat openness (β_{Open}) + UVP (β_{UVP})) using the *caper* (Orme et al., 2018) package in R (R Core Team, 2022). We reported the regression coefficient estimates ($\bar{x} \pm SE$) to evaluate significance and effect size (F-statistic, P-value, and adjusted R^2).

We also built a phylogenetic generalized linear mixed (PGLM) model for binary data using the *ape* (Paradis & Schliep, 2019) package in R to assess the presence of UVP. This binary PGLM model accounted for variation in UVP while dealing with the bimodal distribution that violates other tests (Ives & Garland, 2010). The same independent variables were used ($\beta_0 + \beta_{\mathrm{Mass}} + \beta_{\mathrm{Diel}} + \beta_{\mathrm{Sociality}} + \beta_{\mathrm{Open}}$); β_{Mass} was standardized to have a mean of 0 and variance of 1, while the categorical variables were reconstructed into dummy variables (2 categories = 0, 1) for the PGLM model. We standardized the variables to improve the interpretation of regression coefficients as they more accurately represent the effect size of the independent variables (Ives & Garland, 2010). The PGLM model represents the phylogenetic signal (s^2) as the scalar magnitude of the phylogenetic variance across all species comparisons (estimated from the phylogenetic variance-covariance matrix; Ives and Garland 2010).

Results

Phylogeny

Our final phylogeny (Fig. 3.1) contained 92 species from three mammalian lineages: primates and relatives (Dermoptera, Scandentia, and Primates), rodents (Rodentia), and marsupials (Diprotodontia). Stochastic character mapping with marginal frequencies of UVP and maximum likelihood of the maximum frequency (kHz) projected onto our phylogeny (estimated from 1000 simulations each) supported the hypothesis that high-frequency communication is species-specific (with high-frequencies only showing up on branch tips; Fig. 3.1). This was further supported by the weak phylogenetic signal (λ [95% CI]) detected for the minimum frequency (0 [0, 0.40]) and variable phylogenetic signal for the maximum frequency (0.77 [0, 0.95]). Interestingly, we found a significantly stronger phylogenetic signal for UVP ($s^2 = 0.39$, $p < 0.001$), suggesting that UVP is not species-specific and is, instead, a broader taxonomic trait. This is demonstrated in the phylogeny, where primates rarely exhibit UVP, most rodents (except for diurnal squirrels and some *Peromyscus*) exhibit UVP, and UVP is variable among marsupials. Despite this variation among the orders, UVP appears in the marginal frequencies of all nodes for the first ~100My, further supporting the finding that UVP is likely ancestral to some extent in most mammals.

Vocalizations

Our final vocalization dataset consisted of 73 species, of which nine were gliding mammals. In this chapter, we contributed call descriptions for five species (*Pedetes capensis*, *Petaurus australis, Petaurus breviceps, Petaurus norfolcensis*, and *Pseudocheirus peregrinus*), four of which have not been published previously (*Petaurus australis* has been previously reported; Table A2.1). We found that our collaborator recorded calls consistent with previous literature for *Petaurus* australis, which provides confidence for our novel descriptions reported here. All five species were recorded with microphones sensitive to the human auditory range (20Hz-20kHz); however, we also opportunistically recorded sugar gliders (*Petaurus breviceps*) with ultrasonic detectors. We found that sugar gliders produced at least one truly ultrasonic call type along with two calls that extended into the ultrasonic range (bark, broadband burst) and three additional high-frequency calls (>10kHz) that could be detected on the ultrasonic microphones (high frequency, sniffing, whistle; Fig. A2.1 & Table A2.3). The ultrasonic microphones used can distort calls in the sonic range, therefore, the values presented in Table A2.3 should be further investigated with sonic microphones. Additionally, given that we were unable to remove pups from the recording space, we did not include the call type ‘ultrasonic’ in the final analysis as they were rare and had very low amplitude; additionally, many mammalian pups produce ultrasonic isolation calls that are lost (or decrease in frequency) later in life. Therefore, we conservatively removed the ultrasonic call type from our dataset. In addition to the high-frequency calls, sugar gliders also produced a low-frequency vocalization (yap) similar to the other marsupials recorded (Fig. A2.2 & Table A2.4). Of the four additional species recorded, non-gliding springhares (*Pedetes capensis*) had the most consistent repertoire, with only one confirmed call type (growl) recorded over 100 times (Fig. A2.2 & Table A2.4). Yellow-bellied gliders (*Petaurus australis*) produced the longest vocalizations, with almost all calls lasting longer than one second (Fig. A2.2 & Table A2.4).

Across all species in the dataset, we found that body size had a negative effect on the minimum (Estimate: $\bar{x}$ $(\pm SE)$ = -0.48 ± 0.15; Effect Size: $F_{df} = 22.07_{1,56}$, $p <$ 0.001) and maximum (-0.39 (±0.09); $F_{df} = 19.48_{1,56}$, $p <$ 0.001) frequencies. Additionally, gliding was positively related to the maximum frequency (1.14 (±0.50); $F_{df} = 6.81_{1,56}$, $p =$ 0.01). We also found that nocturnal mammals produced significantly higher minimum frequencies than their diurnal counterparts (1.32 (±0.62); $F_{df} = 5.10_{1,56}$, $p =$ 0.03), as did solitary mammals (1.01 (±0.48); $F_{df} = 4.20_{1,56}$, $p =$ 0.05). Additionally, we did not detect a relationship between UVP and either frequency limit.

Ultraviolet-Induced Photoluminescence

Our final UVP dataset consisted of 82 species, of which 27 were photoluminescent under UV conditions (16 from literature, 11 novel reports). We found an additional 7 species which expressed strictly white UVP which were treated as non-photoluminescent in the analysis (Fig. 3.1). Nocturnality was the only trait with a significant relationship to UVP, with nocturnal species significantly more likely to exhibit UVP (Estimate: 8.09 (±3.79), $p =$ 0.03). However, despite nocturnality being a significant covariate, we found that a similar number of nocturnal species did not exhibit UVP, with 23 species not exhibiting UVP compared to the 26 that did (blue-green-yellow-pink). Out of the nocturnal mammals that expressed UVP, 20 displayed pink (occasionally with orange or red) photoluminescence while 10 expressed blue or green, with 5 of these species expressing both blue and pink (Fig. 3.1). We did not find any instances of UVP in the diurnal mammals used in this study (Fig. 3.1). We also found a weak effect for gliding, sociality, size, and habitat openness on the absence or presence of UVP (Table 3.1). Finally, unlike the vocalization data, we found a significant phylogenetic signal for UVP ($s^2 = 0.39, p < 0.001$).

Discussion

In support of our hypothesis, we found that gliding mammals exhibited significantly higher vocal ranges than their non-gliding counterparts. We were also able to demonstrate for the first time high-frequency calls in sugar gliders, which further supports our finding that high-frequency communication is common across gliding mammals. Despite discovering UVP in several new species, we found did not find a strong relationship between UVP and vocal limits or gliding, despite some flying squirrels and glider relatives exhibiting both traits. However, we found a significant relationship between nocturnality and non-white UVP, further providing evidence for the hypothesis that nocturnal species will exhibit UVP because of the accumulation of porphyrin luminophores (i.e. pink-orange-red photoluminescence) without photodegradation observed in diurnal species (Toussaint et al., 2023).

Vocalizations

As predicted, we found that the capacity to emit high-frequency vocalizations is a common trait across gliding mammals. We recorded high-frequency vocalizations in sugar gliders (Fig. A2.1), which is the first record of calls reaching ultrasonic frequencies in marsupial gliders. Notably, despite being unable to test for USVs in other marsupial gliders (i.e., yellow-bellied or squirrel gliders), we still found that gliding was one of only two key traits significantly associated with higher maximum frequency use. Body size is a common predictor for vocal limits and has been explored across various taxa (Ryan & Brenowitz, 1985; Evans et al., 2006; Pfefferle & Fischer, 2006; Cui, 2012; Charlton & Reby, 2016; García-Navas & Blumstein, 2016; Martin, Tucker & Rogers, 2017). Vocal limits are highly controlled by vocal-producing structures that increase with body size and produce larger sound waves, perceived as lower frequencies (Martin, Tucker & Rogers, 2017). Therefore, we expected body size to be a significant predictor in our dataset. However, an important limitation of our study is that we did not separate ultrasonic and audible calls in our analysis. Ultrasonic calls are often produced via an aerodynamic whistle mechanism in the ventral pouch on the larynx (Riede, Borgard & Pasch, 2017; Abhirami et al., 2023), which may circumvent the negative body size relationship seen across most mammals. However, further investigation into the role and variation of the ventral pouch across many ultrasound-producing mammals would provide further insight into whether purely ultrasonic calls are also limited by body size.

The positive relationship between gliding and vocal limits was previously explored in the squirrel family (Sciuridae; Chapter 2, Newar and Bowman, 2020), however, within Sciuridae, nocturnality and gliding are entangled traits, with gliding squirrels also being the only extant nocturnal species. Therefore, our current investigation, which incorporates gliding mammals and their nocturnal and diurnal relatives, allowed us to disentangle the nocturnal and gliding traits associated with all gliding mammals (Jackson, 2012). When we modelled this larger dataset, we found nocturnality was positively related to increased minimum vocal frequencies while gliding was positively related to increased maximum frequencies (Table 3.1).

The function of higher vocal limits in gliding mammals is likely complex, though these functions remain unclear, with few behavioural accounts linked to vocal recordings. USVs have been predominantly reported in echolocating mammals like bats and cetaceans (Ahlén, 2004; Yovel et al., 2011; Parker et al., 2013; Thomas & Hahn, 2015; Carter & Adams, 2016), with some hypothesizing that bats began as nocturnal gliders. Our investigation into cryptic communication in non-flying mammals supports this hypothesis, with gliding mammals producing USVs without a clear echolocation function. The first record of USVs in gliding mammals was in the North American flying squirrels (*Glaucomys*; Murrant et al., 2013), with subsequent reporting in the feather-tailed pygmy glider (Martin, 2019) and colugo (Miard et al., 2019) and novel USVs reported here in the sugar glider (Fig. A2.1 & Table A2.3). While the vocal repertoire of many previously and newly reported gliding mammals do not contain USVs (Ando & Kuramochi, 2008; Shen, 2013; Poje, 2016), these calls require specialized recording equipment, and the frequency limits of recording equipment are highly associated with the maximum frequency limits detected (Chapter 2; Newar & Bowman, 2020). Given the recent reporting of USVs in multiple gliding mammals and the strong association between higher vocal limits and gliding reported here, we encourage researchers with access to these low-frequency gliders (including scaly-tailed flying squirrels, most marsupial gliders, and giant flying squirrels) to record individuals with ultrasonic equipment to determine if USVs are also present in these species. The presence (or absence) of USVs in other gliding mammals and any novel behavioural contexts for these calls should clarify the role of high frequencies in gliding mammals.

Given the currently available data, gliding mammals use significantly higher maximum frequencies than their non-gliding relatives. It may be intuitive to assume that these higher frequencies play an essential role in gliding, particularly given the predominant role of USVs in bats. Bats use USVs for echolocation, wherein USVs are rapidly produced to detect objects and often prey while both the individual and the object are moving (Jones & Siemers, 2011). This trait is highly specialized to both the vocal-producing structures and auditory receptors that must vibrate fast enough to produce and detect USVs (Anderson & Ruxton, 2020). The rate of call production is not nearly rapid enough in gliding mammals to mimic echolocation in bats (which varies between 2-20 pulses/s; Jones and Siemers, 2011). However, the frequency and production rate are like that of echonavigating shrews (Gould, Negus & Novick, 1964; Tomasi, 1979; Siemers et al., 2009) and blind mice (Panyutina et al., 2017; Volodin et al., 2019b), who use USVs to navigate complex spaces (Siemers et al., 2009; Panyutina et al., 2017). Both taxa have reduced vision perhaps as a result of their dark environments, which may explain why acoustic signals have been selected as a navigation tool; like echolocation, echonavigating calls are produced to help orient an individual to their environment and do not seem to require the same physical specializations to be produced. Given the nocturnal behaviour of all gliding mammals, which navigate complex, arboreal environments in reduced light conditions, similar selection pressures may have allowed for echonavigation to develop in this system. However, experiments like those shown in other echonavigating mammals (Gustafson & Schnitzler, 1979; Siemers et al., 2009) would be required to explore this hypothesis further. I explore this issue further in Chapter 4.

Aside from echonavigation, many other mammals produce USVs for non-navigating purposes. Even within our dataset, 18 species produced calls at least partially in the ultrasonic range. The function of USVs in other species has been explored across several hypotheses, many of which were incorporated into our analyses. Interestingly, we found that habitat openness, which has been shown to be associated with higher frequency production, did not have a significant relationship with higher frequencies in our dataset. Previous studies exploring the role of habitat openness and attenuation of sound waves across the landscape have heavily biased their examples to open habitat species (Koeppl, Hoffmann & Nadler, 1978; Blumstein, 2007; García-Navas & Blumstein, 2016). Indeed, many species produce high frequencies and a variety of USVs in closed habitats despite these calls being easily absorbed by the spatially complex habitat in which they are produced. Furthermore, a truly subterranean rodent, the mole vole (*Ellobius talpinus*), has been shown to produce ultrasonic vocalizations (Volodin et al., 2022) despite the assumed acoustic restrictions of living underground, where acoustic signals are quickly absorbed by the dense surrounding environment. Therefore, while other researchers have predicted that open habitats may be more conducive to the evolution of USV production (Boncoraglio & Saino, 2007; Ey & Fischer, 2009; Fischer, Wadewitz & Hammerschmidt, 2017), it may be that open habitats are better for recording USVs (as previously proposed in Chapter 2; Newar and Bowman; 2020). Sociality has also been previously investigated as a driver of vocal behaviours in mammals (Hauser 1993; Blumstein & Armitage 1997; Arch & Narins, 2008; Ramsier et al., 2012; Faure et al., 2017). In our dataset, we found that solitary mammals produced significantly higher minimum frequencies than their social counterparts; this is an interesting finding as literature points to social mammals using higher frequencies, with high-frequency hearing in primates increasing with vocal complexity (Ramsier et al., 2012) and ultrasound being used in a variety of social contexts in small mammals (Arch &

Narins, 2008). However, Hauser (1993) demonstrated that frequencies can vary with different social encounters, particularly that frequencies decrease with aggressiveness and increase with fearfulness. We also found that maximum frequencies were lower in solitary species, thought this relationship was bordering non-significant (Table 3.1). Therefore, our findings suggest that the solitary mammals in our dataset have less frequency variability than their social counterparts due to decreased social complexity.

Ultraviolet-Induced Photoluminescence

We did not find that ultraviolet-induced photoluminescence (UVP) was associated with vocal limits, nor did we find that UVP was associated with gliding (Table 3.1). While UVP has been recently described in North American flying squirrels (*Glaucomys sabrinus* and *G. volans*), the Australian Krefft's glider (*Petaurus notatus*), the red-cheeked flying squirrel (*Hylopetes spadiceus*) and the smoky flying squirrel (*Pteromyscus pulverulentus*) we were unable to confirm UVP in any other gliding mammals (Fig. 3.1). However, we noticed substantial variation in the presence of UVP in North American flying squirrel museum specimens when confirming UVP presence in mammals known to express detectable levels of photoluminescence. We tested four dry-preserved museum specimens from *Glaucomys sabrinus* and *G. volans* each and observed very weak pink and blue UVP in one individual from each species as well as considerable variation in the dorsal UVP across all eight individuals (the dorsal UVP is weaker in both species). Comparing UVP in dry-preserved *Glaucomys* specimens to live individuals, there is a striking difference in the strength and variation of colours observed under UV light, with live individuals producing very strong UVP colouration (Fig. 3.2). Therefore, not observing UVP in our study may be an artifact of poor preservation or specimen age rather than a lack of UVP. This key finding is important for researchers considering the use of museum specimens for UVP studies. Museum specimens are already more likely to produce false-positives due to the use of chemicals during preservation and mounting processes, as seen in birds (Pohland & Mullen, 2006). This is the first study to directly compare live and dry-preserved individuals using the exact same methods and demonstrate that false-negatives are just as likely, if not more likely, to occur when using museum specimens. Similarly, due to the photosensitive nature of porphyrins, reddish photoluminescence is generally not expected to be retained in museum specimens (Hill 2010). However, pink UVP was the most common colour detected in our museum specimens, with 12 exhibiting pink compared to only 2 cases of blue and blue/green. Additionally, the pink UVP in the dry-preserved flying squirrels was much more pronounced than the blue, especially when contrasted against the live individuals (Fig. 3.2). We encourage researchers with access to other mammalian gliders (particularly giant flying squirrels, colugos, and marsupial gliders) to assess UVP with live specimens to either confirm a lack of UVP or to challenge our findings (Reinhold et al., 2023). We also note that the pelt preservation processes are unknown in the species used and there is a possibility that we have detected false positives due to chemicals and not natural photoluminescence, particularly in the mounted *Sicista subtilis.* Therefore, we further encourage other researchers to confirm investigate UVP in live-specimens whenever possible; developing a more comprehensive record of live-specimen UVP is crucial to understanding the ecological importance.

Despite our limitations, we found a significant relationship between nocturnality and the presence of UVP in our dataset. Several researchers have proposed that porphyrin induced UVP in fur should be highly associated with nocturnality (Kohler et al., 2019; Olson et al., 2021; Toussaint et al., 2023). Specifically, porphyrins and tryptophan metabolites, which readily accumulate in mammalian fur through various physiological pathways, have been identified as the main compounds associated with UVP in mammal pelage (Toussaint et al., 2023; Reinhold et al., 2023). Some of these luminophores are easily degraded by UV rays emitted by the sun (e.g., porphyrins), while others are not as photodegradable (e.g., tryptophans) and have been shown to cause UVP in some diurnal mammals. Additionally, heavy melanin loads in the fur can mask UVP in any mammal, regardless of temporality. Therefore, we had expected that there may be a greater prevalence of UVP in nocturnal species compared to diurnal species when there are low melanin loads in the fur. Consistent with this hypothesis, nocturnal species in our analysis with dark fur (e.g., *Aotus trivigatus*, *Otolemus garnettii*) did not exhibit UVP. However, we found considerable variation in UVP across nocturnal species concerning both occurrence (26 present, 23 absent including 4 white-only species) and colouration (from our methods: nine predominantly pink, one predominantly blue/green, three with mixed blue and pink colouration). This variation in UVP is greater than what we would expect if UVP is ubiquitous among nocturnal mammals and suggests that the mechanism behind pelage UVP is likely more complicated than luminophore degradation.

Interestingly, UVP has been proposed as a method for social communication (Kohler et al., 2019). However, we found no evidence for UVP being associated with social species compared to solitary species. Looking at our data, half of the UVP species were solitary, including *Didelphis virginiana*, *Marmosa* spp., and *Peromyscus eremicus*. The role of UVP as a social trait is challenging to reconcile with our finding that several UVP species exhibit alternate social systems (Garroway, Bowman &

Wilson, 2013), where they cyclically spend significant portions of their lives as solitary individuals. It remains possible that UVP can be a form of crypsis by contributing to visual camouflage (Ruxton et al., 2018; Kohler et al., 2019), particularly in North American flying squirrels which display similar colouration to some native owl species (Weidensaul et al., 2011). Consistent with our hypothesis regarding predation avoidance in gliders, we found that 6 of the tested gliders exhibit pink UVP on their ventral pelage. Including the white-only UVP shown in *Petauroides* volans, seven of the 14 gliders exhibited UVP; notably, the gliders not exhibiting UVP in this study are all dry-preserved specimens while those gliders exhibiting UVP has been mostly confirmed in live or recently deceased individuals (with the exception of *Hylopetes spadiceus* and *Ptermyscus pulverulentus*). This further supports our finding that false negatives may be prolific in museum specimens and live animals should be used whenever possible.

Conclusions

We found that gliding mammals emitted significantly higher vocal frequencies than their non-gliding relatives. Additionally, we found strong support for the role of body mass in reducing vocal frequencies across all taxa and no evidence for sociality, habitat openness, or UVP as key correlates of vocal limits. We propose that habitat openness and sociality may not be as crucial for predicting frequency limits as previously proposed. We contributed novel vocal repertoires for five species, and novel UVP reports for 57 species, of which 11 displayed non-white UVP. Finally, we found that nocturnality was the only significant predictor of UVP, with half of the nocturnal mammals tested exhibiting UVP of various colours (blue-green-yellow-pink). While UVP was not significantly more associated with gliding mammals compared to non-gliders, we also found that half of the gliders tested exhibited coloured UVP. We conclude that gliding mammals have shifted to higher vocal frequencies to conceal themselves from potential eavesdroppers and while some gliders may be exploiting UVP to camouflage themselves in their environment, UVP is not as ubiquitous in gliders as high-frequency communication. While gliding mammals can be cryptic, their cryptic traits are likely just as influenced by their varied phylogenetic histories as they are by their convergent evolution.

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Data Availability

The raw data, complete UVP dataset, museum specimen information, raw phylogenies, and R script that support the findings of this study are openly available on Dryad at https://doi.org/10.5061/dryad.3n5tb2rp4.

References

Ahlén I. 2004. Heterodyne and time-expansion methods for identification of bats in the field and through sound analysis. In: Brigham RM, Kalko EKV, Jones G, Parsons S, Limpens HJGA, eds. *Bat Echolocation Research: Tools, Techniques and Analysis*. Austin: Bat Conservation International, 72–78.

Anderson SC, Ruxton GD. 2020. The evolution of flight in bats: a novel hypothesis. *Mammal Review* 50(4):426–439. DOI: 10.1111/mam.12211.

Ando M, Kuramochi Y. 2008. Vocal communication of the japanese giant flying squirrel *Petaurista leucogenys*. *The Journal of Agricultural Science* 53(2):176–183.

Arch VS, Narins PM. 2008. "Silent" signals: selective forces acting on ultrasonic communication systems in terrestrial vertebrates. Animal Behaviour 76(4):1423–1428. DOI: 10.1016/j.anbehav.2008.05.012.

Beard KC. 1993. Phylogenetic systematics of the Primatomorpha, with special reference to Dermoptera. In: Szalay FS, Novecaek MJ, McKenna MC eds. *Mammal Phylogeny: Placentals.* Verlag: Springer, 129–150. DOI: 10.1007/978-1-4613-9246-0_10.

Blumstein DT. 2007. The evolution of alarm communication in rodents: Structure, function, and the puzzle of apparently altruistic calling. In: Wolff JO, Sherman PW eds. *Rodent Societies: An Ecological and Evolutionary Perspective*. Chicago: University of Chicago Press, 317–327. DOI: 10.7208/chicago/9780226905389.001.0001.

Blumstein DT, Armitage KB. 1997. Does sociality drive the evolution of communicative complexity? A Comparative Test with Ground-Dwelling Sciurid Alarm Calls. *The American Naturalist* 150(2):179–200. DOI: 10.1086/286062.

Boncoraglio G, Saino N. 2007. Habitat structure and the evolution of bird song: A meta-analysis of the evidence for the acoustic adaptation hypothesis. *Functional Ecology* 21(1):134–142. DOI: 10.1111/j.1365-2435.2006.01207.x.

Byrnes G, Spence AJ. 2011. Ecological and biomechanical insights into the evolution of gliding in mammals. *Integrative and Comparative Biology* 51(6):991–1002. DOI: 10.1093/icb/icr069.

Carter RT, Adams RA. 2016. Integrating ontogeny of echolocation and locomotion gives unique insights into the origin of bats. *Journal of Mammalian Evolution* 23(4):413–421. DOI: 10.1007/s10914-016-9324-2.

Casanovas-Vilar I, Garcia-Porta J, Fortuny J, Scar Sanisidro O′, Rô Me Prieto J, Querejeta M, Llá Cer S, Robles JM, Bernardini F, Alba DM. 2018. Oldest skeleton of a fossil flying squirrel casts new light on the phylogeny of the group. *eLife* 7:e39270. DOI: 10.7554/eLife.39270.001.

Charlton BD, Reby D. 2016. The evolution of acoustic size exaggeration in terrestrial mammals. *Nature Communications* 7(1):12739. DOI: 10.1038/ncomms12739.

Chávez AE, Bozinovic F, Peichl L, Palacios AG. 2003. Retinal spectral sensitivity, fur coloration, and urine reflectance in the genus *Octodon* (Rodentia): Implications for visual ecology. *Investigative Ophthalmology and Visual Science* 44(5):2290–2296. DOI: 10.1167/iovs.02-0670.

Cremona T, Baker AM, Cooper SJB, Montague-Drake R, Stobo-Wilson AM, Carthew SM. 2021. Integrative taxonomic investigation of *Petaurus breviceps* (Marsupialia: Petauridae) reveals three distinct species. *Zoological Journal of the Linnean Society* 191(2):503–527. DOI: 10.1093/zoolinnean/zlaa060.

Cui J. 2012. Effects of body size and environmental factors on the acoustic structure and temporal rhythm of calls in *Rhacophorus dennysi*. *Asian Herpetological Research* 3(3):205–212. DOI: 10.3724/SP.J.1245.2012.00205.

Dial R. 2003. Energetic savings and the body size distributions of gliding mammals. *Evolutionary Ecology Research* 5(8):1151–1162.

Dice LR. 1945. Minimum intensities of illumination under which owls can find dead prey by sight. *The American Naturalist* 79(784):385–416. DOI: 10.1086/281276.

Diggins CA, Gilley LM, Turner GG, Ford WM. 2020. Ultrasonic acoustic surveys of state endangered northern flying squirrels in the Pocono Mountains, Pennsylvania. *Journal of Fish and Wildlife Management* 11(2):644–653. DOI: 10.3996/JFWM-20-020.

Dudley R, Byrnes G, Yanoviak SP, Borrell B, Brown RM, McGuire JA. 2007. Gliding and the functional origins of flight: Biomechanical novelty or necessity? *Annual Review of Ecology, Evolution, and Systematics* 38(1):179–201. DOI: 10.1146/annurev.ecolsys.37.091305.110014.

Emmons LH, Gentry AH. 1983. Tropical forest structure and the distribution of gliding and prehensile-tailed vertebrates. *The American Naturalist* 121(4):513–524. DOI: 10.1086/284079.

Evans S, Neave N, Wakelin D. 2006. Relationships between vocal characteristics and body size and shape in human males: An evolutionary explanation for a deep male voice. *Biological Psychology* 72(2):160–163. DOI: 10.1016/j.biopsycho.2005.09.003.

Ey E, Fischer J. 2009. The "acoustic adaptation hypothesis"— A review of the evidence from birds, anurans and mammals. *Bioacoustics* 19(1):21–48. DOI: 10.1080/09524622.2009.9753613.

Faure A, Pittaras E, Nosjean A, Chabout J, Cressant A, Granon S. 2017. Social behaviors and acoustic vocalizations in different strains of mice. *Behavioural Brain Research* 320:383–390. DOI: 10.1016/j.bbr.2016.11.003.

Fischer J, Wadewitz P, Hammerschmidt K. 2017. Structural variability and communicative complexity in acoustic communication. *Animal Behaviour* 134(6):229–237. DOI: 10.1016/j.anbehav.2016.06.012.

Fokidis HB, Risch TS. 2008. The burden of motherhood: gliding locomotion in mammals influences maternal reproductive investment. *Journal of Mammalogy* 89(3):617–625. DOI: 10.1644/07-mamm-a-116r1.1.

Freckleton RP, Harvey PH, Pagel M. 2002. Phylogenetic analysis and comparative data: A test and review of evidence. *The American Naturalist* 160(6):712–726. DOI: 10.1086/343873.

García-Navas V, Blumstein DT. 2016. The effect of body size and habitat on the evolution of alarm vocalizations in rodents. *Biological Journal of the Linnean Society* 118(4):745–751. DOI: 10.1111/bij.12789.

Garroway CJ, Bowman J, Wilson PJ. 2013. Complex social structure of southern flying squirrels is related to spatial proximity but not kinship. *Behavioral Ecology and Sociobiology* 67:113–122. DOI: 10.1007/s00265-012-1431-3.

Gerkema MP, Davies WI, Foster RG, Menaker M, Hut RA. 2013. The nocturnal bottleneck and the evolution of activity patterns in mammals. *Proceedings of the Royal Society B: Biological Sciences* 280(1765):20130508. DOI: 10.1098/rspb.2013.0508.

Gilley LM. 2013. Discovery and characterization of high-frequency calls in North American flying squirrels (*Glaucomys sabrinus* and *G. volans*): Implications for ecology, behavior, and conservation. D. Phil. Thesis, Auburn University.

Gilley LM, Diggins CA, Pearson SM, Best TL. 2019. Vocal repertoire of captive northern and southern flying squirrels (*Glaucomys sabrinus* and *G. volans*). *Journal of Mammalogy* 100(2):518–530. DOI: 10.1093/jmammal/gyz064.

Gould E, Negus NC, Novick A. 1964. Evidence for echolocation in shrews. *Journal of Experimental Zoology* 156(1):19–37. DOI: 10.1002/jez.1401560103.

Gustafson Y, Schnitzler H-U. 1979. Echolocation and obstacle avoidance in the hipposiderid bat *Asellia tridens*. *Journal of Comparative Physiology* 131(2):161–167. DOI: 10.1007/BF00619076.

Hamchand R, Lafountain AM, Büchel R, Maas KR, Hird SM, Warren M, Frank HA, Brückner C. 2021. Red fluorescence of European hedgehog (*Erinaceus europaeus*) spines results from free-base porphyrins of potential microbial origin. *Journal of Chemical Ecology* 47(6):588–596. DOI: 10.1007/s10886-021-01279-6.

Hauser MD. 1993. The evolution of nonhuman primate vocalizations: Effects of phylogeny, body weight, and social context. *The American Naturalist* 142(3):528–542. DOI: 10.1086/285553.

Hoffmann F, Musolf K, Penn DJ. 2012. Ultrasonic courtship vocalizations in wild house mice: spectrographic analyses. *Journal of Ethology* 30(1):173–180. DOI: 10.1007/s10164-011-0312-y.

Huang W, Liu Q, Zhu E-Y, Shindi AAF, Li Y-Q. 2010. Rapid simultaneous determination of protoporphyrin IX, uroporphyrin III and coproporphyrin III in human whole blood by non-linear variable-angle synchronous fluorescence technique coupled with partial least squares. *Talanta* 82(4):1516-1520. DOI: 10.1016/j.talanta.2010.07.034.

Huang Z, Zeng H, Hamzavi I, Alajlan A, Tan E, McLean DI, Lui H. 2006. Cutaneous melanin exhibiting fluorescence emission under near-infrared light excitation. *Journal of Biomedical Optics* 11(3):034010. DOI: 10.1117/1.2204007.

Hughes B, Bowman J, Stock NL, Burness G. 2022. Using mass spectrometry to investigate fluorescent compounds in squirrel fur. *PLoS ONE* 17(2) :e0257156. DOI: 10.1371/journal.pone.0257156.

Ives AR, Garland T. 2010. Phylogenetic logistic regression for binary dependent variables. *Systematic Biology* 59(1):9–26. DOI: 10.1093/sysbio/syp074.

Jackson SM, Schouten P. 2012. *Gliding Mammals of the World*. Melbourne: CSIRO.

Jackson SM, Thorington RW. 2012. *Gliding Mammals: Taxonomy of Living and Extinct Species*. Washington: Smithsonian Contributions to Zoology.

Jeng M-L. 2019. Biofluorescence in terrestrial animals, with emphasis on fireflies: A review and field observation. In: Suzuki H, ed. *Bioluminescence - Analytical Applications and Basic Biology*. London: IntechOpen, 1-25. DOI: 10.5772/intechopen.86029.

Johnsen S, Kelber A, Warrant EJ, Sweeney AM, Widder EA, Lee RL, Hernández-Andrés J. 2006. Crepuscular and nocturnal illumination and its effects on color perception by the nocturnal hawkmoth *Deilephila elpenor*. *Journal of Experimental Biology* 209(5): 789–800. DOI: 10.1242/jeb.02053.

Jones G, Siemers BM. 2011. The communicative potential of bat echolocation pulses. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 197(5):447–457. DOI: 10.1007/s00359-010-0565-x.

Kavanagh RP, Rohan-Jones WG. 1982. Calling behaviour of the yellow-bellied glider, *Petaurus australis* Shaw (Marsupialia: Petauridae). *Australian Mammalogy* 5(2):95–111. DOI: 10.1071/AM82011.

Kloock CT. 2005. Aerial insects avoid fluorescing scorpions. *Euscorpius* 21: 1–7. DOI: 10.18590/euscorpius.2005.vol2005.iss21.1.

Knudsen EI. 1981. The hearing of the barn owl. *Scientific American* 245(6):112–125. DOI: 10.1038/scientificamerican1281-112.

Koeppl JW, Hoffmann RS, Nadler CF. 1978. Pattern analysis of acoustical behavior in four species of ground squirrels. *Journal of Mammalogy* 59(4):677–696. DOI: 10.2307/1380133.

Kohler AM, Olson ER, Martin JG, Anich PS. 2019. Ultraviolet fluorescence discovered in new world flying squirrels (*Glaucomys*). *Journal of Mammalogy* 100(1):21–30. DOI: 10.1093/jmammal/gyy177.

Legett HD, Hemingway CT, Bernal XE. 2020. Prey exploits the auditory illusions of eavesdropping predators. *The American Naturalist* 195(5):927–933. DOI: 10.1086/707719.

Levin EY, Flyger V. 1973. Erythropoietic porphyria of the fox squirrel *Sciurus niger*. *The Journal of Clinical Investigation* 52(1):96–105. DOI: 10.1172/JCI107178.

Marples NM, Kelly DJ, Thomas RJ. 2005. Perspective: The evolution of warning coloration is not paradoxical. *Evolution* 59(5):933–940. DOI: 10.1111/j.0014-3820.2005.tb01032.x.

Marshall J, Johnsen S. 2017. Fluorescence as a means of colour signal enhancement. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372(1724): 20160335. DOI: 10.1098/rstb.2016.0335.

Martin K. 2019. The vocal repertoire of feathertail gliders (*Acrobates pygmaeus*) and how macrocomparisons can shape future research on acoustic communication in mammals. D. Phil. Thesis, University of New South Wales.

Martin K, Tucker MA, Rogers TL. 2017. Does size matter? Examining the drivers of mammalian vocalizations. *Evolution* 71(2):249–260. DOI: 10.1111/evo.13128.

Masters JC, Génin F, Couette S, Groves CP, Nash SD, Delpero M, Pozzi L. 2017. A new genus for the eastern dwarf galagos (Primates: Galagidae). *Zoological Journal of the Linnean Society* 181(1): 229–241. DOI: 10.1093/zoolinnean/zlw028.

Masterton B, Heffner H, Ravizza R. 1969. The evolution of human hearing. *The Journal of the Acoustical Society of America* 45(4):966–985. DOI: 10.1121/1.1911574.

McGregor DC, Padovan A, Georges A, Krockenberger A, Yoon H-J, Youngentob KN. 2020. Genetic evidence supports three previously described species of greater glider, *Petauroides volans*, *P. minor*, and *P. armillatus*. *Scientific Reports* 10(1):19284. DOI: 10.1038/s41598-020-76364-z.

Menéndez I, Gómez Cano AR, Cantalapiedra JL, Peláez-Campomanes P, Álvarez-Sierra MA, Hernández Fernández M. 2021. A multi-layered approach to the diversification of squirrels. *Mammal Review* 51(1):66–81. DOI: 10.1111/mam.12215.

Miard P, Lim LS, Abdullah NI, Elias NA, Ruppert N. 2019. Ultrasound use by Sunda colugos offers new insights into the communication of these cryptic mammals. *Bioacoustics* 28(4):397–403. DOI: 10.1080/09524622.2018.1463294.

Millington KR. 2020. Anomalous fluorescence of white hair compared to other unpigmented keratin fibers. *International Journal of Cosmetic Science* 42(3):289–301. DOI: 10.1111/ics.12614.

Murrant MN, Bowman J, Garroway CJ, Prinzen B, Mayberry H, Faure PA. 2013. Ultrasonic vocalizations emitted by flying squirrels. *PLoS ONE* 8(8):1–6. DOI: 10.1371/journal.pone.0073045.

Myers P, Espinosa R, Parr C, Jones T, Hammond G, Dewey T. 2023. The Animal Diversity Web (online). *Available at* https://animaldiversity.org (accessed 18 September 2023).

Newar SL, Bowman J. 2020. Think before they squeak: Vocalizations of the squirrel family. *Frontiers in Ecology and Evolution* 8:1–15. DOI: 10.3389/fevo.2020.00193.

Nicholls EM, Rienits KG. 1971. Tryptophan derivatives and pigment in the hair of some Australian marsupials. *International Journal of Biochemistry* 2(11):593–603. DOI: 10.1016/0020-711X(71)90031-0.

Nie, W, Fu B, O'Brien PCM, Wang J, Su W, Tanomtong A, Volobouev V, Ferguson-Smith MA, Yang F. 2008. Flying lemurs - the 'flying tree shrews'? Molecular cytogenetic evidence for a Scandentia-Deromptera sister clade. *BMC Biology* 6:18. DOI: 10.1186/1741-7007-6-18.

Nikol'skii AA. 2007. A comparative analysis of the alarm call frequency in different age rodent groups (in Russian). *Zoologicheskii Zhurnal* 86:499–504.

Nummert G, Ritson K, Nemvalts K. 2023. Photoluminescence in the garden dormouse (*Eliomys quercinus*). *Zoology* 157:126075. DOI: 10.1016/j.zool.2023.126075.

Olson ER, Carlson MR, Ramanujam VMS, Sears L, Anthony SE, Anich PS, Ramon L, Hulstrand A, Jurewicz M, Gunnelson AS, Kohler AM, Martin JG. 2021. Vivid biofluorescence discovered in the nocturnal Springhare (Pedetidae). *Scientific Reports* 11:4125. DOI: 10.1038/s41598-021-83588-0.

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pease W. 2018. caper: Comparative analyses of phylogenetics and evolution in R. *Available at https://cran.r-project.org/web/packages/caper/index.html* (accessed 01 September 2023)

Panyutina AA, Kuznetsov AN, Volodin IA, Abramov A V., Soldatova IB. 2017. A blind climber: The first evidence of ultrasonic echolocation in arboreal mammals. *Integrative Zoology* 12(2):172–184. DOI: 10.1111/1749-4877.12249.

Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35(3):526–528. DOI: 10.1093/bioinformatics/bty633.

Parker J, Tsagkogeorga G, Cotton JA, Liu Y, Provero P, Stupka E, Rossiter SJ. 2013. Genome-wide signatures of convergent evolution in echolocating mammals. *Nature* 502(7470):228–231. DOI: 10.1038/nature12511.

Paskins KE, Bowyer A, Megill WM, Scheibe JS. 2007. Take-off and landing forces and the evolution of controlled gliding in northern flying squirrels *Glaucomys sabrinus*. *Journal of Experimental Biology* 210(8):1413–1423. DOI: 10.1242/jeb.02747.

Pfefferle D, Fischer J. 2006. Sounds and size: identification of acoustic variables that reflect body size in hamadryas baboons, *Papio hamadryas*. *Animal Behaviour* 72(1):43–51. DOI: 10.1016/j.anbehav.2005.08.021.

Pine RH, Rice JE, Bucher JE, Tank DH Jr., Greenhall AM. 1985. Labile pigments and fluorescent peage in didelphid marsupials. *Mammalia* 49(2):249–256. DOI: 10.1515/mamm.1985.49.2.249.

Pohland G, Mullen P. 2006. Preservation agents influence UV-coloration o plumage in museum bird skins. *Journal of Ornithology* 147:464-467. DOI: 10.1007/s10336-005-0038-0.

R Core Team. 2022. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. *Available at http://www.R-project.org/*.

Ramsier MA, Cunningham AJ, Finneran JJ, Dominy NJ. 2012. Social drive and the evolution of primate hearing. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367(1597):1860–1868. DOI: 10.1098/rstb.2011.0219.

Rebell G. 1966. Kynurenine in Rat Hair. *Nature* 209:913–914. DOI: 10.1038/209913a0.

Reinhold L. 2021. Mammals with fluorescent fur: Observations from the Wet Tropics. *North Queensland Naturalist* 51:1–8. DOI: 10.53060/prsq23.5.

Reinhold LM, Rymer TL, Helgen KM, Wilson DT. 2023. Photoluminescence in mammal fur: 111 years of research. *Journal of Mammalogy* 104(4):892-906. DOI: 10.1093/jmammal/gyad027.

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3(2):217–223. DOI: 10.1111/j.2041-210X.2011.00169.x.

Ruxton GD. 2009. Non-visual crypsis: A review of the empirical evidence for camouflage to senses other than vision. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364(1516):549–557. DOI: 10.1098/rstb.2008.0228.

Ruxton GD, Allen WL, Sherratt TN, Speed MP. 2018. *Avoiding Attack: The Evolutionary Ecology of Crypsis, Aposematism, and Mimicry*. Oxford University Press. DOI: 10.1093/oso/9780199688678.001.0001.

Ryan MJ, Brenowitz EA. 1985. The role of body size, phylogeny, and ambient noise in the evolution of bird song. *The American Naturalist* 126(1):87–100. DOI: 10.1086/284398.

Schäfer K, Goddinger D, Höcker H. 2008. Photodegradation of tryptophan in wool. *Journal of the Society of Dyers and Colourists* 113(12):350–355. DOI: 10.1111/j.1478-4408.1997.tb01862.x.

Scheibe JS, Paskins KE, Ferdous S, Birdsill D. 2007. Kinematics and functional morphology of leaping, landing, and branch use in *Glaucomys sabrinus*. *Journal of Mammalogy* 88(4):850–861. DOI: 10.1644/06-MAMM-S-331R1.1.

Schneiderová I, Schnitzerová P, Uhlíková J, Brandl P, Zouhar J, Matějů J. 2015. Differences in alarm calls of juvenile and adult European ground squirrels (*Spermophilus citellus*): findings on permanently marked animals from a semi-natural enclosure. *Zoo Biology* 34(6):503–512. DOI: 10.1002/zoo.21233.

Schnitzler H-U, Moss CF, Denzinger A. From spatial orientation to food acquisition in echolocating bats. *Trends in Ecology & Evolution* 18(8): 386–394. DOI: 10.1016/S0169-5347(03)00185-X.

Shen P-S. 2013. Acoustic behavior of white-faced flying squirrel (*Petaurista lena*) in Guanghua Village, Alishan. M.Sc. Thesis. National Sun Yat-sen University.

Siemers M, Schauermann G, Turni H, von Merten S. 2009. Why do shrews twitter? Communication or simple echo-based orientation. *Biology Letters* 5(5):593–596. DOI: 10.1098/rsbl.2009.0378.

Thomas GWC, Hahn MW. 2015. Determining the null model for detecting adaptive convergence from genomic data: A case study using echolocating mammals. *Molecular Biology and Evolution* 32(5):1232–1236. DOI: 10.1093/molbev/msv013.

Thorington RW, Heaney LR. 1981. Body proportions and gliding adaptations of flying squirrels (Petauristinae). *Journal of Mammalogy* 62:101–114. DOI: 10.2307/1380481.

Tomasi TE. 1979. Echolocation by the short-tailed shrew *Blarina brevicauda*. *Journal of Mammalogy* 60:751–759. DOI: 10.2307/1380190.

Toussaint SLD, Ponstein J, Thoury M, Métivier R, Kalthoff DC, Habermeyer B, Guilard R, Bock S, Mortensen P, Sandberg S, Gueriau P, Amson E. 2023. Fur glowing under ultraviolet: in situ analysis of porphyrin accumulation in the skin appendages of mammals. *Integrative Zoology* 18(1):15–26. DOI: 10.1111/1749-4877.12655.

Upham N, Esselstyn J, Jetz W. 2019. Inferring the mammal tree: species-level sets of phylogenies for questions in ecology, evolution, and conservation. *PLoS Biology* 17(12):e3000494. DOI: 10.1371/journal.pbio.3000494.

Vignieri SN, Larson JG, Hoekstra HE. 2010. The selective advantage of crypsis in mice. *Evolution* 64(7):2153–2158. DOI: 10.1111/j.1558-5646.2010.00976.x.

Volodin IA, Dymskaya MM, Smorkatcheva AV, Volodina EV. 2022. Ultrasound from underground: cryptic communication in subterranean wild-living and captive northern mole voles (*Ellobius talpinus*). *Bioacoustics* 31:414–434. DOI: 10.1080/09524622.2021.1960191.

Volodin IA, Klenova AV, Ilchenko OG, Volodina EV. 2019. High frequency audible calls in northern birch mice *Sicista betulina* in response to handling: effects of individuality, sex and body mass on the acoustics. *BMC Research Notes* 12(1):677. DOI: 10.1186/s13104-019-4719-9.

Volodin IA, Panyutina AA, Abramov A V., Ilchenko OG, Volodina E V. 2019. Ultrasonic bouts of a blind climbing rodent (*Typhlomys chapensis*): acoustic analysis. *Bioacoustics* 28(6):575–591. DOI: 10.1080/09524622.2018.1509374.

Wecker SC. 1963. The role of early experience in habitat selection by the prairie deer mouse, *Peromyscus maniculatus bairdi*. *Ecological Monographs* 33(4):307–325. DOI: 10.2307/1950749.

Weidensaul CS, Colvin BA, Brinker DF, Huy JS. 2011. Use of ultraviolet light as an aid in age classification of owls. *The Wilson Journal of Ornithology* 123(2):373-377. DOI: 10.1676/09-125.1.

Whisson DA, McKinnon F, Lefoe M, Rendall AR. 2021. Passive acoustic monitoring for detecting the yellow-bellied glider, a highly vocal arboreal marsupial. *PLOS ONE* 16(5):e0252092. DOI: 10.1371/journal.pone.0252092.

Wilson DR, Hare JF. 2006. The adaptive utility of Richardson’s ground squirrel (*Spermophilus richardsonii*) short-range ultrasonic alarm signals. *Canadian Journal of Zoology* 84(9):1322-1330. DOI: 10.1139/Z06-120.

Yolton RL, Yolton DP, Renz J, Jacobs GH. 1974. Preretinal absorbance in sciurid eyes. *Journal of Mammalogy* 55(1):14–20. DOI: 10.2307/1379253.

Yovel Y, Franz MO, Stilz P, Schnitzler HU. 2011. Complex echo classification by echolocating bats: A review. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 197(5):475–490. DOI: 10.1007/s00359-010-0584-7.

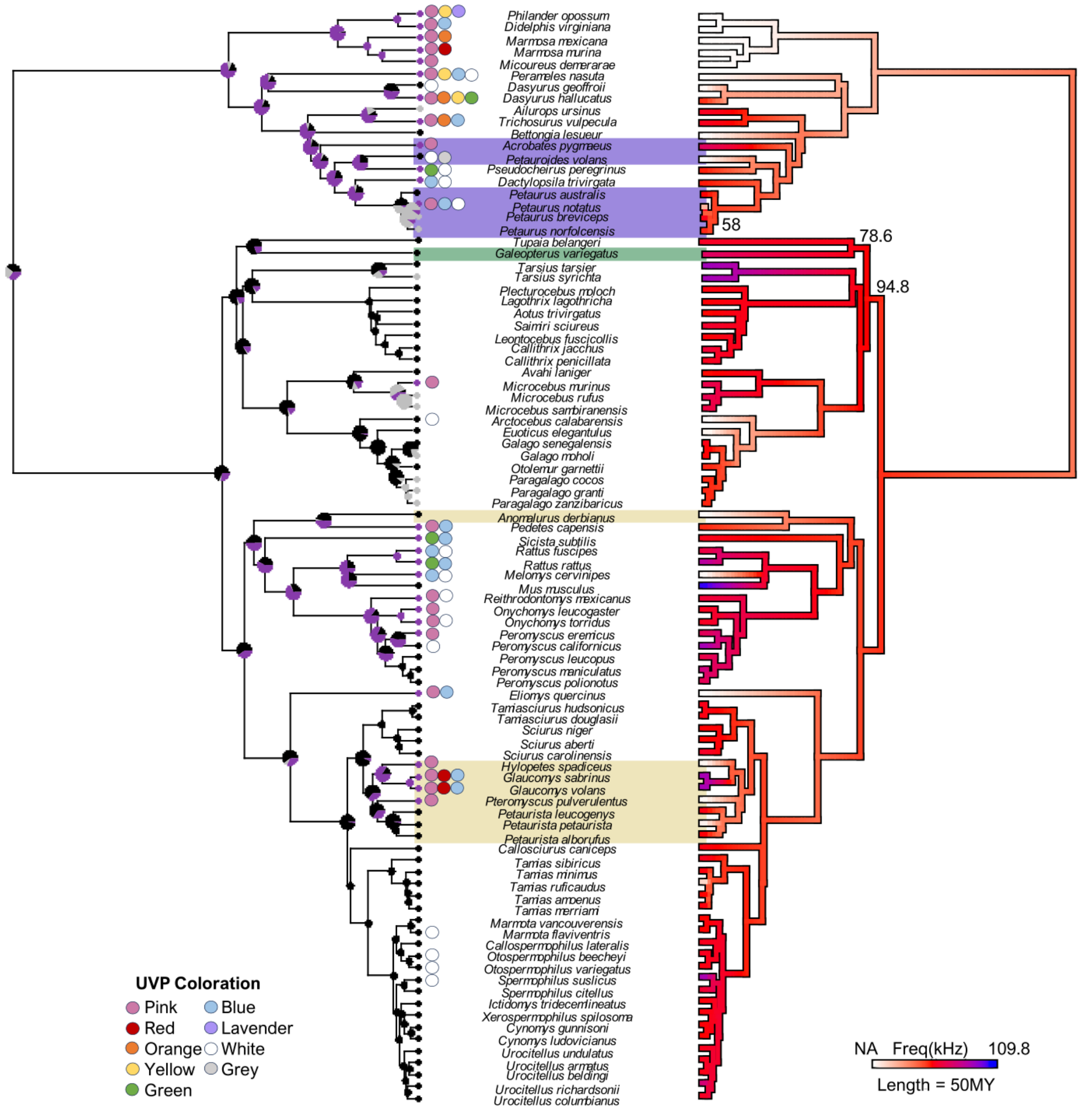

Fig. 3.1 The final consensus tree representing traits associated with ultraviolet-induced photoluminescence (UVP) and vocal range limits (kHz) in gliding mammals and their relatives (n = 92). Phylogeny estimated from mean edge lengths across 1000 trees; bootstrap values <100 represented on the right phylogeny. The left stochastic character map represents UVP presence (purple = yes, black = no, grey = untested) with marginal frequencies at the nodes; circles along the left tips represent dominant UVP colouration. The right maximum likelihood map represents the character history of the maximum frequency (kHz; [0.12-84]) with white indicating species without vocal data (NA = 19). Gliding mammals are highlighted (marsupials = purple, colugos = green, rodents = yellow; n = 15; *Petauroides* included); scale bar represents 75My.

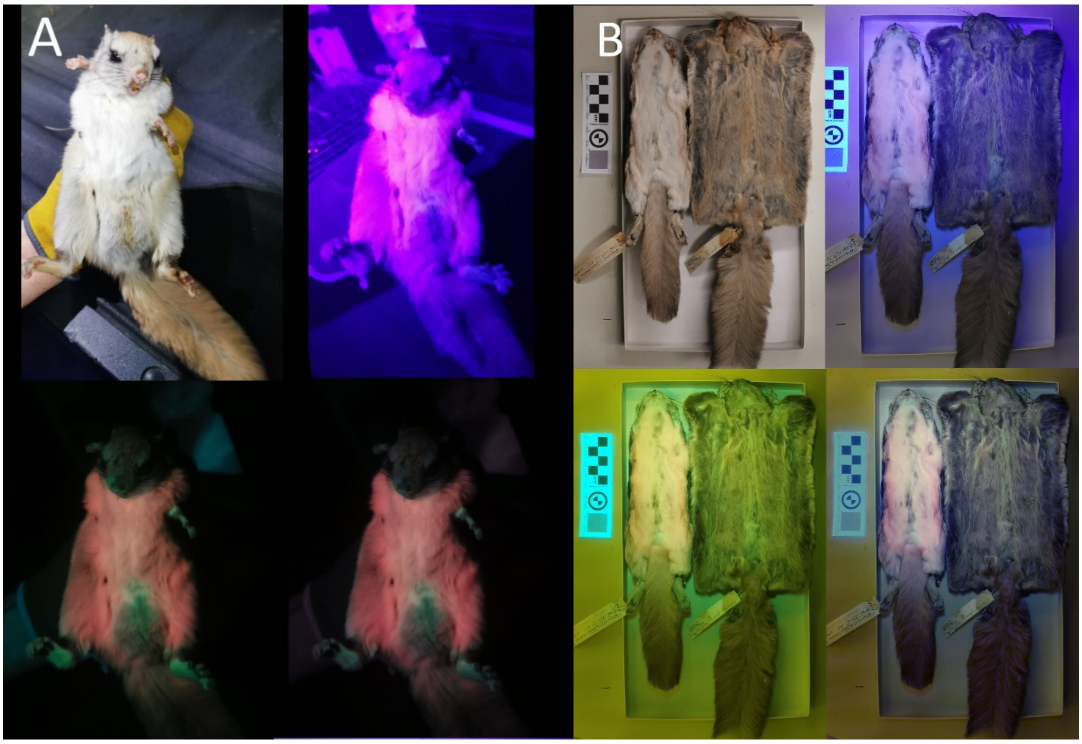

Fig. 3.2 Variation in ultraviolet-induced photoluminescence demonstrating the variability within museum specimens and between live and preserved individuals. (A) Live adult male *Glaucomys sabrinus* trapped in the Kawartha Highlands in 2023. (B) Dry-preserved *Glaucomys sabrinus* from the Canadian Museum of Nature. Top left: white light; top right: ultraviolet light; bottom left: ultraviolet light and yellow gel filter; bottom right: ultraviolet light, yellow gel filter, and colour edit (A3).

Table 3.1 Model results for the frequency range (minimum and maximum frequencies; kHz; n = 73) and ultraviolet-induced photoluminescence (UVP; n = 82) of gliding Mammalia and their relatives. Phylogenetic least square (PGLS) models were conducted with the frequency ranges while a binary phylogenetic generalized linear mixed (PGLM) model was used for the UVP dataset. Effect size ($F_{df,n}$; PGLS only) and slopes ($\bar{x}$ ± SE) are estimated for each variable: gliding (Y/N), diel activity pattern (Diel A.P.; nocturnal/ diurnal), habitat openness (Habitat; Closed/Open), sociality (solitary/social), and UVP (Y/N). The phylogenetic signal (PGLS: λ [95% CI], PGLM: *s2*; estimated from 1000 trees) and estimations of model fit (R^2, PGLS: *F*) are provided. Significant estimates are given in bold.

	Minimum frequency		**Maximum frequency**		**UVP**
	$\bar{x}$ (±*SE*)	**F_{df}**	**$\bar{x}$ (±*SE*)**	**F_{df}**	**($\bar{x}$ ± *SE*)**
Intercept	1.60 (±1.01), $p = 0.12$	-	**4.56 (±0.99)** **$p < 0.001$**	-	**-7.77 (±3.93),** **$p = 0.05$**
log(Body mass (g))	**-0.48 (±0.15)** **$p = 0.002$**	**$22.07_{1,56}$** **$p < 0.001$**	**-0.39 (±0.09)** **$p < 0.001$**	**$19.48_{1,56}$** **$p < 0.001$**	-0.66 (±0.53) $p = 0.21$
Gliding: *Y*	0.37 (±0.77) $p = 0.64$	$3.75_{1,56}$ $p = 0.06$	**1.14 (±0.50)** **$p = 0.03$**	**$6.81_{1,56}$** **$p = 0.01$**	-1.13 (±0.96) $p = 0.24$
Diel A.P.: *nocturnal*	**1.32 (±0.62)** **$p = 0.04$**	**$5.10_{1,56}$** **$p = 0.03$**	-0.13 (±0.48), $p = 0.79$	$0.23_{1,56}$ $p = 0.63$	**8.09 (±3.79)** **$p = 0.03$**
Habitat: *open*	-0.23 (±0.48) $p = 0.63$	$0.25_{1,56}$ $p = 0.62$	-0.29 (±0.29), $p = 0.33$	$1.25_{1,56}$ $p = 0.27$	0.39 (±0.99) $p = 0.70$
Sociality: *solitary*	**1.01 (±0.48)** **$p = 0.04$**	**$4.20_{1,56}$** **$p = 0.05$**	-0.46 (±0.24), $p = 0.06$	$3.87_{1,56}$ $p = 0.05$	0.72 (±0.75) $p = 0.34$
UVP: *Y*	-0.37 (±0.64) $p = 0.56$	$0.34_{1,56}$ $p = 0.56$	-0.14 (±0.35), $p = 0.69$	$0.16_{1,56}$ $p = 0.69$	
λ	0.00 [0.00, 0.37]		0.82 [0, 0.96]		**s^2** **0.39, $p < 0.001$**
R^2	0.32		0.29		R^2 0.64
F, P	**$5.95_{6,56}$, <0.001**		**$5.30_{6,56}$, <0.001**		

Chapter 4 - Chirps in the Dark: Effects of behavioural context on ultrasonic vocalizations emitted by flying squirrels (*Glaucomys* spp.)

Abstract

Flying squirrels (*Glaucomys sabrinus and G. volans*) produce ultrasonic vocalizations (USVs), though, unlike bats or mice, USVs have not been behaviourally contextualized in these gliding mammals. We hypothesized that the behavioural contexts of echonavigation, social communication, and predator avoidance would affect call production by flying squirrels. In the field, we recorded flying squirrels during a T-maze and natural gliding experiment; we also brought squirrels temporarily into captivity to record them in a novel arena. Flying squirrels did not vocalize in the T-maze or during glides which suggests USVs are not related to echonavigation; however, we found evidence for social communication between individuals in various contexts. We also found that lunar illumination negatively affected the number, mean peak frequency (kHz), and duration (s) of chirps, consistent with reducing their vulnerability to predators during periods of higher illumination. We conclude that flying squirrels acoustically camouflage their vocalizations to facilitate communication with familiar conspecifics.

Key Words: cryptic communication, echonavigation, gliding, high-frequency, moonlight, T-maze

Introduction

From echolocating bats to alarm-calling marmots, mammals demonstrate an impressive range of high-frequency vocalizations (HFVs; >10kHz). Ultrasonic vocalizations (USVs; >20kHz) are of particular interest to humans as our auditory range is restricted to frequencies between 20Hz-20kHz, with considerable sensitivity lost after 10kHz (hence HFVs; Masterton, Heffner & Ravizza, 1969). With ultrasonic technology becoming more accessible, there has been an increase in the reporting of novel mammalian USVs, though contextualizing these calls has been primarily focused on echolocating and lab mammals, with comparably few studies in other mammals.

Echolocating mammals produce highly directional, high-energy USVs to detect moving prey and navigate complex environments (Jones, 2005). Researchers have found several unrelated taxa however, that use a basic form of echolocation, 'echonavigation,' to avoid obstacles. Species that echonavigate include blind mice (*Typhlomys*: Panyutina et al., 2017), several shrew species (*Blarina*: Tomasi, 1979; *Sorex*: Gould et al., 1964; *Sorex* and *Crocidura*: Siemers et al., 2009), and even macro bats which are genetically distant from their smaller, echolocating relatives (*Eidolon*: Schoeman & Goodman, 2012; *Rousettus*: Yovel et al., 2011). It has been proposed by the researchers studying these mammals that their echonavigation has developed due to permanent or temporary reduction in visual information, facilitating the evolution of compensatory behaviours. Significantly, while most echolocating bats and cetaceans use USVs for echolocation signals (Jones, 2005; Ketten, 1992), echonavigating calls range from the relatively long (8-16ms), low-frequency (4-8kHz) twitters produced by shrews (Siemers et al., 2009) to the short (50-100μs), ultrasonic (30kHz) clicks produced by Egyptian fruit bats (Yovel et al., 2011). These differences in call characteristics are mainly due to the function of the call, with echolocating mammals using short wavelengths to reflect off small, precise surfaces (e.g., prey items) and echonavigating mammals using longer wavelengths to detect larger surfaces (e.g., environment).

Aside from echolocation or navigation, various mammals use USVs to communicate socially. Again, this has been well-documented in echolocating bats, who use social calls (Boughman & Wilkinson, 1998) and eavesdrop on echolocating calls to gather social information (Fenton et al., 2004). There is also a wealth of literature on calls in mice (Arriaga & Jarvis, 2013; Castellucci et al., 2018; Holy & Guo, 2005) and rats (Blumberg et al., 2000; Corrigan & Flannelly, 1979; Oswalt & Meier, 1975) who use USVs to attract mates, express aggression or submission, and facilitate parental care. USVs are used as alarm calls by some ground squirrels (Wilson & Hare, 2004) as these calls are only detectable at short distances and therefore less likely to attract distant predators (Wilson & Hare, 2006); regardless of distance, some predatory taxa cannot hear ultrasonic calls at all (Yamazaki et al., 2004; Young et al., 2013).

Flying squirrels are a monophyletic group within the squirrel family, characterized by their nocturnal behaviour and the presence of a patagium (i.e., a thin membrane stretched between limbs) that allows them to glide through the air among trees and other elevated structures in their habitat. Both northern (*Glaucomys sabrinus*) and southern flying squirrels (*G. volans*) produce USVs (Gilley, 2013; Gilley et al., 2019; Murrant et al., 2013), though the behavioural contexts associated with these repertoires have not been studied. Flying squirrels (hereafter *Glaucomys*) are strictly nocturnal (Jackson & Schouten, 2012) and use the patagium to glide between trees, conserving energy and time (Byrnes & Spence, 2011). Flying squirrels are a particularly interesting group to better understand USV function as they are potentially good candidates for echonavigation. The nocturnal habits of flying squirrels limit visual information as ambient light is significantly reduced compared to daytime; these visual cues are likely further limited during different stages of the lunar cycle as reflected in the cyclical activity patterns observed in *G. sabrinus* (Radvanyi, 1959). Flying squirrels have also been shown to produce several types of USVs in isolation (Murrant et al., 2013).

Flying squirrels are socially dynamic, forming larger nesting groups to improve thermoregulation during winter (Garroway et al., 2013; O'Brien et al., 2021). While they often forage alone, flying squirrels can also forage with nestmates (Murrant et al., 2014) and are anecdotally known to gather in groups to forage at sites with abundant food (e.g., at bird feeders). Foraging squirrels reconstitute into familiar nesting groups after a foraging bout (Murrant et al., 2014). These social aspects of foraging and nesting make flying squirrels good candidates for social communication as they rely on their conspecifics for survival (e.g., thermoregulation). They may be more inclined to produce alarms (as seen in many other squirrels; (García-Navas & Blumstein, 2016; Wilson & Hare, 2004)), and acoustic signals may be required in the reassembly of nest groups after foraging bouts (Murrant et al., 2014).

The predator-prey dynamics of flying squirrels and their primary predators, owls, may also play a role in flying squirrel USV production. While measured in few species, the auditory thresholds of owls are <12kHz (Brittan-Powell et al., 2005; Knudsen, 1981; Krumm et al., 2017), suggesting that owls cannot acoustically detect flying squirrels using most HFVs. Additionally, flying squirrels and owls rely on visual cues from their environment, which increase in availability as moonlight increases. In different owl species, increased moonlight has been demonstrated to increase hunting effectiveness (*Asio flammeus*; Clarke, 1983) and increased calling behaviour (*Bubo bubo*; Penteriani et al., 2010). In contrast, many rodents are known to change their behaviour in response to moonlight to reduce predation risk (Kronfeld-Schor et al., 2013). Given that owls are important predators of flying squirrels, the squirrels would be under selective pressure to reduce their exposure during periods associated with increased risk. Therefore, USVs could be beneficial to communicate with conspecifics while minimizing predation risk, especially during periods of greater lunar illumination.

We investigated variation in call characteristics of flying squirrels (*Glaucomys*) to contextualize the usage of USVs. We developed three, non-exclusive, hypotheses: (1) flying squirrels use USVs to echonavigate, (2) flying squirrels use USVs to communicate with conspecifics, and (3) flying squirrels use USVs to avoid predators. We did not expect these hypotheses to be exclusive, as squirrels could be varying frequencies in social and solitary contexts to avoid predators, or squirrels could use USVs to both echonavigate and communicate. If flying squirrels use high frequencies to echonavigate, we expected flying squirrels to vocalize in novel environments and before gliding. Additionally, if flying squirrels echonavigate, we expected high-frequency vocalizations to aid in the navigation of basic tasks, as demonstrated for shrews (Gould et al., 1964; Tomasi, 1979). If flying squirrels use high frequencies to communicate, we expected squirrels to produce few calls in isolation, with squirrels producing more and higher frequencies in social contexts. If flying squirrels use high frequencies to both echonavigate and communicate, we expected high-frequency vocalizations to be common in both social and solitary environments, with a variation in the type of calls produced in either context. Finally, if flying squirrels use high frequencies to avoid predators, we expected higher frequencies to be used with increased lunar illumination corresponding to increased vulnerability to predators, regardless of social context.

Methods

Trapping

We trapped flying squirrels (*Glaucomys sabrinus* and *G. volans*) either 40km north of Peterborough, Ontario, Canada, at two long-term research stations (Kawartha Highlands (44°41’20.3” N, 78°20’02.2” W) and James McLean Oliver Ecological Centre (44°34’26.9” N, 78°29’23.0” W)), or in and around Algonquin Provincial Park, Ontario (45°34’59.6” N, 78°28’04.1” W). We used Tomahawk 102 live traps (Tomahawk Live Trap Co., Hazelhurst, Wisconsin, USA) attached to shelf brackets mounted at a height of approximately 2m on trees in primarily mature mixed wood forests. We baited the traps using sunflower hearts, peanut butter, bacon grease, or nuts. If the weather was poor, we added plastic sheeting on top of the traps to keep captured squirrels dry. We set traps before sunset and either checked them every hour and closed them shortly after midnight or left them open all night and simultaneously checked and closed them shortly after sunrise. If we left traps open overnight, we added cotton (for warmth) and a slice of apple (for hydration).

Upon the initial capture of a target individual (*Glaucomys*), we recorded morphological measurements (skull length and width, foot length, tail length) and collected a small tissue sample from the tip of the ear for genetic analysis. We noted mass, as well as sex and reproductive status, upon the initial and subsequent recaptures. Depending on trapping location individuals were tagged with either a unique passive integrated transponder (PIT) tag (model TX1411SST; Biomark Inc., Boise, Idaho, USA) or an ear tag with a unique alphanumeric combination (1005-1

Monel ear tags; National Band and Tag Co., Newport, Kentucky, USA) so that we could identify recaptured individuals. Before behavioural trials (detailed below), we outfitted some individuals with radio collars (1.8 g model BD-2C; Holohil Systems Ltd., Carp, Ontario, Canada) for another project at the Kawartha Highlands field site. We conducted all behavioural trials after the squirrel had been measured and identified; squirrels were temporarily held and transported alone in their initial traps.

In-Situ Recordings

We evaluated the role of USVs during navigation by introducing flying squirrels ($n = 69$) to a T-maze we assembled in the field upon capture of a squirrel. The T-maze (Figure A3.1) was made of 7.6cm black ABS piping (IPEX HomeRite Products, Oakville, Ontario, Canada), which was large enough for squirrels to turn around in while exploring the maze freely; each arm was 1.2m long, which made the arena approximately 0.27 m^2. Individuals would enter at the bottom of the maze and travel to the T-junction (we used an ABS Tee to connect all three arms), where they would decide between either arm of the maze; we outfitted the ends of both arms with a 90° ABS elbow which obscured the exits from the individual while at the T-junction. The end of one arm was randomly assigned a cover, while the other was left open for squirrels to leave and return to the surrounding habitat. The cover was made of a transparent acrylic plate with holes drilled in either a low or high density to allow for airflow and natural olfactory input while still creating a mostly solid surface; we randomly selected hole density for each trial. We visually and audibly recorded individuals with a trail camera (Ultrafire XP9; Reconyx Inc., Holmen, Wisconsin, USA) placed at the base of the maze and an ultrasonic microphone (SMM-U1 attached to Song Meter SM4BAT FS recorders; Wildlife Acoustics Inc., Maynard, Massachusets, USA) placed through a small hole we cut into the top of the T-junction.

We used a curved ABS tee at the base of the maze, which allowed us to attach the trap and the camera to the outside of the maze without obscuring the camera's view; the curve in this junction encouraged individuals to turn toward the main maze instead of toward the camera. Once in the maze, we allowed squirrels to explore and leave freely; after 5 minutes, we stopped the recordings and carefully disassembled the maze so the individual could return to the surrounding habitat if they had not yet exited. We later reviewed the trail camera videos to determine which arm the individual chose to explore first: if the individual chose the arm with no cover, we assigned this as a 'correct' choice. We assigned every other option as an 'incorrect' choice, even if they eventually escaped the maze. We conducted all T-maze experiments in the summer of 2018.

To evaluate the role of USVs during gliding, we opportunistically released flying squirrels (n = 17) after capture on a branch (~5m long) on a hill in the middle of the Kawartha Highlands field site (we only used squirrels from this location for these recordings). Individuals were placed at the base of the branch by a handler. We allowed the squirrel to explore the branch freely; ideally, as occurred in most trials, the squirrel would glide from the distal end of the branch into the forest canopy. We recorded throughout subsequent gliding trials with ultrasonic microphones (SMM-U1 attached to Song Meter SM4BAT FS recorders) located underneath and in front of the supported branch. During each trial, individuals were also recorded with an Echo Meter Touch (Wildlife Acoustics Inc.; attached to a Huawei P30 Pro) held by an observer with the microphone pointed directly at the squirrel throughout the trial. We also visually recorded the squirrels on three nights with a trail camera (Ultrafire XP9). We conducted all gliding recording sessions over seven nights in the fall of 2020.

Captive Recordings

During the summer 2018 and fall of 2019, we brought individuals from the Kawartha Highlands and the Oliver Property into temporary captivity at Trent University. After capture, we immediately transported the squirrels to an outdoor aviary at the university (the same one used by Olson et al., (2018) and Stead et al., (2024)), where we subsequently transferred squirrels to individual holding cages (39.3 cm x 24.1 cm x 38.7cm). Squirrels were provided water and food (seeds, nuts, commercial squirrel chow) *ad libitum*. We lined cages with pine wood chips and shredded paper; we provided cotton for nest building, a short tube (3-inch ABS piping with a cap on one end) to nest in, and a wheel for enrichment. Squirrels were held in captivity for a maximum of two weeks before being released back at the location where we initially trapped them.

To test for vocal behaviour in individual and social conditions, we introduced squirrels to a 3D recording arena for 24-hour recording sessions. The arena was outfitted from a steel mesh storage cabinet (85cm x 152cm x 165cm) with a middle shelf that partially divided the arena horizontally. Two wooden nest boxes were placed on the shelf and a variety of large wooden branches and vegetation added for transport between the floor and the shelf. We added water and food to the enclosure at the beginning of the recording sessions. At the beginning of the recording session, squirrels would be transported from the holding cage to the arena via their nesting tube (squirrels would always be sleeping in the tube during this transport period); the tube would be placed on the floor of the arena and left undisturbed for squirrels to emerge on their own time.

Squirrels were first introduced individually into the arena for 24 hours to explore the novel environment. After introducing two individuals to the novel environment separately, we reintroduced both individuals in tandem (pairings were opposite or same sex and either both *G.v.* or *G.v./G.s.* depending on the squirrels currently in captivity; we were unable to record *G.s./G.s.* pairings due to low catch numbers during our recording periods). For the individual and paired trials, we recorded the squirrels with an ultrasonic microphone (SMM-U1) mounted on the arena and two trail cameras (Ultrafire XP9) mounted outside the upper and lower levels. During the fall of 2019, we placed a third camera inside the arena on the shelf facing the clear acrylic side of one nest box (these sides usually allow researchers to view inside the box without disturbing the inhabitants). In the summer of 2018, we set the camera recording time to 10 minutes when activated; however, the large video files resulted in the SD cards filling before many of the trials had ended. Therefore, in the fall of 2019, recording times were changed to 10 seconds, resulting in shorter activity videos scattered throughout the trials.

Acoustic Analyses

We first analyzed all sound files with Avisoft-SASLab Pro (Avisoft Bioacoustics e.K., Glienicke/Nordbahn, Germany); an observer (S.N.) listened to each file at a sample rate of 48kHz and cut-off frequencies of 5 and 125kHz to identify potential sound events. We used Avisoft to measure the duration of the entire call and the fundamental, peak, minimum, and maximum frequencies (kHz) taken at the start, centre, and end and calculated for the mean of the entire duration for each call.

After identifying all the potential vocalizations, we classified them based on classes previously established in the literature (Gilley, 2013; Gilley et al., 2019; Murrant et al., 2013). For the captive recordings, the observer re-listened to the potential calls at a rate of 22.05kHz to confirm that the sound was biophonic. Given that captive squirrels created many non-vocalizing sounds by physically interacting with the metal arena (i.e. nails scraping on solid metal surfaces produced many high-frequency sounds with narrow bandwidths), we wanted to be conservative with the final dataset. Therefore, we added a confidence scale for each previously identified sound, with 0 being sounds that the observer could not confidently assign as biophonic (i.e. produced during lots of movement and noise), one being calls that were possibly biophonic (produced near other noises, recorded immediately at the beginning or end of the file so surrounding noises could not be identified, etc.), and two being calls that were biophonic (calls produced in total isolation of other sounds, calls that were repeated at predictable intervals, or calls in which the frequency was heavily modulated). We filtered the captivity call dataset to only include level two confidence calls for the remaining analyses.

Statistical Analyses

We performed all statistical analyses in *R* (R Core Team, 2023). For the T-maze, we used a binomial generalized linear model to compare the likelihood of making a correct choice relative to the amount of external light (day or night), the side with the cover (left or right), the number of holes drilled in the cover for airflow (low or high density), the trial number for the individual, and the total time to exit the maze (s). We also used *rptR* (Stoffel et al., 2017) to estimate the individual repeatability of making a successful exit or making a correct choice over repeated trials to estimate habituation to the experiment. Finally, we used Fisher's exact test (R Core Team, 2023) to compare the total number of each species or sex used in the maze and sequence design.

For the gliding recordings, we used a zero-inflated binomial regression to look at the relationship between the number of calls produced during the recording session and species, sex, and trial sequence for the individual squirrel; a zero-inflated model was chosen as many trials did not result in any recordings. We also incorporated proportional lunar illumination using *lunar* (Lazaridis, 2022) and temperature (°C) recorded at the Trent University Climate Station, approximately 40km from the Kawartha Highlands field site. Temperature is only taken every hour so the reading closest to the gliding trial was used for analysis; lunar illumination is averaged over the entire night. We included temperature as this can impact the characteristics of acoustic signals directly (i.e. sound propagation) and indirectly (i.e. behavioural and physiological changes). While we did not expect this to have a strong effect on flying squirrels, very few studies have investigated the impact of ambient temperature on vocal characteristics, with most studies focused on anurans (Gambale & Bastos, 2014; Guerra Batista et al., 2012; Lingnau & Bastos, 2007), with a few additional studies on the effects of temperature inversions on elephant calling behaviour (Garstang et al., 2005; Larom et al., 1997).

We also used a linear mixed effects linear model to examine the relationship between the calls' mean peak frequency (kHz) and the abovementioned variables. However, we removed temperature as it was highly correlated with lunar illumination in this dataset ($R = 0.71$). We also included time before glide which was extracted from the recordings. While we initially identified six call types (Table A3.1), warble and broadband calls (n = 1 each) were re-categorized as chirps based on similar frequency and temporal characteristics; we removed screams as they were rapidly produced by one individual (female *G. volans*) while being handled and were not directly related to gliding. This resulted in three final call classes which we term 'superclasses': chirps, high-frequency calls, and trills (described in Table A3.1).

For the recordings of captive squirrels, we extracted individual calls and uploaded them to R using *tuneR* (Ligges et al., 2023); we filtered calls with a duration <0.01s and classes with fewer than five observations. We then used *gibbonR* (Clink, 2023) to calculate Mel-frequency cepstral coefficients (MFCCs) for the individual calls. We used *e1071* (Meyer et al., 2023) and *randomForest* (Liaw & Wiener, 2002) accordingly to run support-vector machine (SVM) and random forest (RF) models to assess the differentiation of call classes. Our initial classifications (n = 10; Table A3.2 & A3.3) were differentiated at an average error rate of 54.5% (SVM = 55%, RF = 54%); we subsequently grouped similar classes to improve the assignment accuracy. Our reduced classifications (superclasses = 3) were considerably more distinct, with an average error rate of only 23.2% (SVM = 28.6%, RF = 17.8%; Fig. 4.1), and we used them in the remaining analyses.

Using the same methodology as the gliding experiments, we added lunar illumination and temperature for each call. We created a poisson regression to compare the total number of calls recorded during each trial relative to the trial type (individual or paired) and the average lunar illumination and temperature recorded over the entire trial. We then used linear mixed effects models (*lme4* (Bates et al., 2015)) to look at the mean peak frequency (kHz) and duration (s) for each class relative to the variables listed above (i.e., lunar illumination, temperature, trial type) while including the trial ID as a random effect; these were modeled independently as barks were only recorded during paired sessions. Species, sex, and behaviour could not be included in the primary captivity dataset as we could not identify the individual that produced the call for most vocalizations. However, we filtered our dataset twice: 1) to only include calls that we could synchronize with an activity on the trail cameras (n = 37) to look at the relationship between behavioural categories and call types (which were compared with a chi-square analysis) and 2) to only include calls produced during individual trials to compare the effects of sex and species on the frequency and duration of the classes.

Ethics Statement

All methods were approved by the Trent University Animal Care Committee under trapping protocols #25668 and #27909, and captivity protocol #25283. We focused on animal welfare throughout the various procedures, building a protocol that would allow us to temporarily bring free-ranging squirrels into captivity and freely release them at the end of the recording sessions. All procedures were in accordance with the Canadian Council on Animal Care and follow the ASM guidelines (Sikes & Gannon, 2011).

Results

In-Situ Recordings

We conducted 100 T-maze trials (n) with 59 individuals (N); however, we removed 11 trials because the individual escaped (n = 8), made it back into the trap (n = 2), or because in one instance we terminated the trial early when a second flying squirrel glided onto and beside the T-maze during the trial. The final dataset was comprised of 89 trials from 56 individuals; we captured fewer individual *Glaucomys sabrinus* (n = 12, N = 6, F: M = 4:2) than *G. volans* (n = 77, N = 50, F:M = 23:27). However, the sex ratio (*OR* = 2.31, *p* = 0.41) and sequence distribution (*p* = 0.54) were not significantly different between the species.

We found no evidence of vocalizations on either the ultrasonic recorder or the acoustic trail camera during the T-maze trials. We found the likelihood of making a correct choice decreased during night trials compared to day trials ($\bar{x}$ (±*SE*) = -

1.26(±0.55), $p = 0.02$) and as time to exit (s) increased (-0.07(±0.02), $p < 0.001$). The sequence, airflow, and cover side were not significantly related to the likelihood of making a correct choice, nor were species or sex (Table 4.1). We also found that exit success (R = 8.31e-16, $p = 0.46$) and choice ($R = 0.003$, $p = 0.32$) were not repeatable.

We recorded 17 individuals (N) for the *in situ* gliding recordings over seven nights, resulting in 37 unique gliding trials (n). Of the 37 trials, only 26 ended with a successful glide and all other trials were not included in the final dataset (one individual was therefore excluded from the final analysis as they never successfully glided). Trials were terminated by squirrels either not leaving the branch over the five-minute trial (n = 2) or leaving the branch without gliding (n = 8). As in the T-maze, the final gliding dataset had fewer *Glaucomys sabrinus* (n = 9, N = 5, F: M = 2:3) than *G. volans* (n = 17, N = 11, F:M = 7:4), but the sex ratio ($OR = 0.21$, $p = 0.28$) and sequence distribution ($p = 0.19$) were not significantly different between the species.

Overall, temperature and lunar illumination were both associated with the total number of calls being produced during the gliding trials (count model; Table 4.2), with higher temperatures (0.10(±0.04) $p = 0.005$) associated with more vocalizations and the total number of calls peaking for both species around 50% illumination (-5.61(±1.41), $p < 0.001$; Fig. 4.2A). We did not find a significant effect of sex, species, and sequence on the number of calls produced. Even though there were more trials with no vocalizations recorded (n = 23) than trials with any calls (n = 13), none of the variables tested had a significant effect on whether squirrels produced any calls (zero-inflated model; Table 4.2).

Call characteristics were only influenced by the lunar illumination (mean peak frequency: -0.97(±0.30), $p = 0.004$) and superclass, with chirps being significantly lower in frequency (-0.32 (±0.12), $p = 0.02$) and longer (2.56 (±0.30), $p < 0.001$) than high-frequency calls (Fig. 4.2B; Table 4.3). Trills (which we only recorded from one individual on one night (female *G. volans*)) were also significantly longer than high-frequency calls (2.21 (±0.35), $p < 0.001$). Squirrel ID explained most of the variation in the mean peak frequency (Var [2.5%, 97.5%] = 0.07 [0.02, 0.28], % Explained = 97.5%) and the duration (3.04 [0.84, 11.68], 98%) for calls with a known time before glide (n = 12, Table 4.4). Additionally, time before glide was significantly related to both call measurements, with calls becoming shorter (0.02 (±0.001), $p < 0.001$) and lower in frequency (0.07 (±0.003), $p < 0.001$) closer to the time of glide (Fig. 4.3; Table 4.3). Notably, all calls were recorded prior to gliding, and no calls were recorded during any of the glides.

Captive Recordings

We brought 18 flying squirrels temporarily into captivity resulting in 16 individual trials and 12 paired trials; we did not include two individual sessions as the SM4 malfunctioned during the trial resulting in no call data. Our initial dataset had 1707 potential vocalizations across 43 classes, but we filtered our dataset only to include calls classified with high confidence (n = 182). We initially organized these high confidence calls into ten classes (Fig. 4.1A); we further grouped these classifications into three superclasses to improve sample sizes and look at general trends across distinct groups. Barks were relatively low in frequency with longer durations, high-frequency vocalizations were short with variable frequencies, and chirps were mid-frequency vocalizations (Fig. 4.1D). We found that flying squirrels produced chirps and high-frequency vocalizations more often than barks. Unlike the prior two, squirrels only produced barks in social pairings (Fig. 4.1B&C).

We found that the trial type and lunar illumination had a significant effect on the total number of calls recorded, with solitary trials (-0.82(±0.30), $p = 0.01$; Fig. 4.4A) and increased illumination (-1.60 (±0.64), $p = 0.02$; Fig. 4.4B) resulting in fewer calls (Table 4.5). Furthermore, we found that the duration of chirps were influenced by lunar illumination (-0.02(±0.008), $p = 0.005$) and temperature (-0.0008(±0.0002), $p = 0.003$) and that the peak frequency of chirps trended towards increasing with increased lunar illumination, though this relationship was not significant (9.99(±5.30), $p = 0.09$). Additionally, high-frequency vocalizations and barks were not affected by either variable (Fig. 4.5). Finally, we found that frequency did not significantly vary between sex or species (Fig. 4.6B; Table 4.7), though high-frequency vocalizations were significantly associated with exploring compared to other activities ($X^2 = 23.43$, $p = 0.001$; Fig. 4.6A) which reflects general linear models which showed higher-frequency and shorter vocalizations during exploring (Table 4.8).

Discussion

We successfully recorded flying squirrel vocalizations in various behavioural contexts with vocalization characteristics varying between experimental, environmental, and social conditions. In support of our first hypothesis that flying squirrels produce USVs to echonavigate, we found that flying squirrels produce high-frequency vocalizations in some solitary, novel environments. However, we recorded no vocalizations when we sequentially exposed flying squirrels to a T-maze and minimal evidence of calling behaviour prior to and during glides in a natural gliding experiment, which contradicts our main prediction that if flying squirrels echonavigate, they would rely on vocalizations in novel environments and/or while gliding. In support of our second hypothesis that flying squirrels use USVs for social communication, we found that flying squirrels produce significantly more vocalizations when paired with a conspecific, and some vocalizations (i.e. barks) were unique to social contexts. Finally, we found that lunar illumination significantly affected the number, frequency, and duration of calls produced across multiple contexts, with more vocalizations being produced when lunar illumination is lower and low-frequency vocalizations shifting to higher frequencies when lunar illumination was high. This finding supported our third hypothesis that flying squirrels will acoustically camouflage in response to predation risk.

We did find some evidence of echonavigation that was consistent with our predictions. Firstly, when looking at all calls that had matching video, we recorded the highest frequency USVs while squirrels were individually exploring the novel arena (Fig 4.6.); squirrels also produced many vocalizations in both solitary (Fig. 4.4) and pre-gliding contexts (Fig. 4.2&4.3). However, most squirrels didn't produce a single vocalization during the gliding experiment, suggesting that vocalizations were not required for this behaviour. This lack of recordings aligns with previous attempts to observe echonavigation in flying squirrels, where squirrels forced to glide via electric shock did not produce any vocalizations (Chattin, 1969). We had expected that observing flying squirrels during *in situ* gliding behaviour with several minutes of uninterrupted exploration would result in a higher likelihood of squirrels vocalizing before gliding. While we did indeed find evidence of vocalizing in this context, we did not observe any similar call types between or within individuals that preceded gliding. Notably, when we included glide time into our models, individual ID explained all the variation in call frequency (100%) and duration (99%; Table 4.4). This finding suggests that our sample sizes were too small to detect repeatable behaviours, if any were present.

Also, contrary to our first prediction, flying squirrels produced no vocalizations when exposed to a novel T-maze. We predicted that if flying squirrels echonavigate, we would expect them to produce high-frequency calls to aid in navigating this novel environment. Yet not a single T-maze trial resulted in call production. We found that flying squirrels were less likely to make a correct choice when there was less available light, suggesting that (1) squirrels were relying on visual cues in the maze or (2) squirrels were less motivated to choose the open end of the maze during the night. Aside from apparent shadows that the cover may have cast, flying squirrels lack a UV-protective orbital lens (Yolton et al., 1974) meaning that they may be able to detect ultraviolet cues that we cannot see. Acrylic sheets can block ultraviolet light and has been used to protect premature infants from harmful UV light (Siegfried et al., 1992); if flying squirrels can detect UV light, then the covered end would likely appear different to the squirrels, especially when the ambient light was highest during the day. Moving the T-maze to a controlled environment where we can reduce the ambient light as much as possible may improve the likelihood of recording vocalizations in this context.

Flying squirrels were required to walk on the ground to navigate the maze. These are primarily arboreal species, so their perceived vulnerability is likely higher if travelling on the ground, especially at night when owls are active, and predation risk is high. Additionally, flying squirrels socially thermoregulate during the day (Garroway et al., 2013; O'Brien et al., 2021), meaning that flying squirrels may be more motivated to return to their nesting group during natural hours of inactivity. The combination of these factors may lead to increased motivation to exit the maze during the daytime and to hide in the maze during the nighttime. However, we would require additional work on perceived predation risk in these species to explore this idea further.

Our second hypothesis was well supported; we recorded significantly more vocalizations when individuals were paired with a conspecific (Fig. 4.4), and while we still recorded some vocalizations during solitary trials, no call types were unique to the solitary sessions. This finding contradicts repertoires from other echonavigating species that produce specific calls while exploring (Siemers et al., 2009; Yovel et al., 2011). In contrast, we recorded one call type (barks) exclusively in paired trials suggesting that at least a portion of the repertoire in specific to social interactions. Additionally, the call characteristics we measured were not significantly different between trial type and activity (Fig. 4.6) for either chirps or high-frequency calls. All calls made during solitary trials with video were during exploration or while perched - given that we were housing other squirrels in the same aviary as the recording arena, we suspect these solitary calls were social calls directed at conspecifics. Flying squirrels occasionally share resources with large aggregations anecdotally forming at valuable resources such as bird feeders (Layne & Raymond, 1994) and nest cavities (O'Brien et al., 2021). With one squirrel occupying a large arena with abundant food and nesting resources, the individuals may be notifying their conspecifics of the resources available.

Interestingly, aggregations of flying squirrels can regroup in a secondary nest in as little as one day following disturbance to their previous nesting location (Layne & Raymond, 1994). Similarly, flying squirrels leave social nest groups to forage solitarily, and acoustic signalling may be required to locate nestmates among several potential nest locations (Murrant et al., 2014). We suspected that we might record solitary flying squirrels producing a broadcasting call to attract conspecifics before nesting; researchers previously tested for this kind of call at the Kawartha Highlands, where they placed a speaker in a cavity and broadcasted recordings. However, they found none of their recorded calls attracted any conspecifics (Murrant, 2011). Instead, we found during our sessions that flying squirrels produce similar high-frequency calls while in solitary and paired trials, with calls structured similarly between activities and sexes. We further hypothesize that squirrels use these high-frequency calls to facilitate communication with conspecifics even when they are not nearby (e.g., in the same enclosure). These calls could allow individuals to spatially track conspecifics without seeing them and provide an open channel for squirrels to communicate. However, this hypothesis would require housing squirrels in separate arenas, varying the environment in one arena, and then measuring the vocalizations and responses of each individual.

Finally, our third hypothesis was the most strongly supported, with lunar illumination significantly associated with vocalizations in the gliding, solitary, and paired trials. During the temporary captive studies, we found that lunar illumination was the most significant predictor of the total number of vocalizations produced for either trial type and that chirps, the mid-frequency vocalization class, significantly increased in frequency and shortened in duration as lunar illumination increased. These findings are consistent with our assumption that predation risk increases with lunar illumination, and therefore, flying squirrels are shifting their vocalizations to camouflage acoustically. To further support this, we found that the high-frequency vocalizations (which are already similar in frequency to the shifted chirps) remain unchanged in response to lunar illumination. We also found that barks, which we only recorded in paired experiments, were not responsive to lunar illumination. Furthermore, we never recorded barks during solitary trials; therefore, it seems that flying squirrels use barks to facilitate social interactions. In ground squirrels, low-frequency vocalizations are often used in aggressive interactions (Balph & Balph, 1966). We did not observe aggression between squirrels in our trials, but we suggest that the large sample size of barks in our data is due to pairing unfamiliar squirrels in a novel enclosure. We assume flying squirrels are less likely to be aggressive with familiar conspecifics, as demonstrated in marmots who create noisier alarms when paired with unfamiliar partners (Fuong & Blumstein, 2019). It may be that this class of vocalizations is relatively rare in natural settings – limited to encounters with unfamiliar conspecifics. This might imply a brief trade-off between predation risk and addressing potential conflict as the minimum frequency of most barks dip below 10kHz which would be within the hearing range of predators such as owls (Knudsen, 1981).

To account for environmental conditions that may affect vocal activity we also included temperature as a co-variate. We found that the peak frequency of chirps in the social experiments increased with higher temperatures and that the total number of calls increased with temperature during the gliding experiment. Additionally, we found that the duration of chirps decreased with temperature. Interestingly, temperature was only significant when lunar illumination was also significant and temperature had a weaker impact (i.e., smaller F-value) in all models except for the model evaluating the duration of chirps in a social context (F-values: temperature = 14.50, lunar illumination = 9.10). These findings are consistent with vocal production in bats who increase their echolocation frequencies with increased temperatures, likely caused by reduced atmospheric attenuation and increased vocal precision as temperatures increase (Ding et al., 2023; Wu et al., 2021). Furthermore, increased precision and reduced attenuation wouldn't require long durations as the signal can more easily reach conspecifics, further supporting our findings. These results are also consistent with increased movement (Nelson & Sagot, 20198) and conspecific interactions during warmer nights, with predation risk (i.e. lunar illumination) ultimately influencing the likelihood and characteristics of calls when used.

Considering our three main hypotheses, the trills produced during the gliding trials are of particular interest (Fig. 4.3). These cascading trills were all produced by a single female *G. volans* immediately before gliding. These calls are excellent candidates for echonavigating calls as they were loud and within the range of frequencies of calls in other known echolonavigating taxa (4-30kHz; Gould, Negus & Novick, 1964; Yovel et al., 2011); they were also produced in rapid succession, which is common in echolocating and echonavigating species (Gould, Negus & Novick, 1964; Panyutina et al., 2017; Yovel et al., 2011). Additionally, these are the only trills that were recorded throughout all the different recording contexts, suggesting that they might be important in the context of gliding. However, given that these trills were produced during high lunar illumination (0.73), assigning these trills as echonavigating calls directly contradicts two of our assumptions: (1) squirrels are not visually limited during increased lunar illumination and (2) predation risk increases with lunar illumination. Therefore, we would expect that the risk of producing these relatively low-frequency calls should outweigh any echonavigating benefits. Additionally, these calls are good candidates for signals to locate nestmates. A future research step would be to explore this signal in the context of alarm calling, similar to the alarm calls produced by ground squirrels (Wilson & Hare, 2004), though this current dataset cannot accurately address this idea.

In conclusion, we tested three hypotheses exploring the function of USVs in flying squirrels. We did not find strong evidence for echonavigation in these species; while we did find that squirrels produced some calls while in isolation, we hypothesize that that these isolation calls are social calls used to facilitate interactions with nearby conspecifics. We also found that squirrels vocalized significantly more often when paired with a conspecific. Some call types were unique to the paired experiment and associated behaviours, suggesting that flying squirrels use USVs to facilitate close social interactions. Finally, we found that lunar illumination had a significant effect on the number, frequency, and duration of vocalizations, with flying squirrels increasing the frequency and decreasing the duration of chirps (the most common superclass) as moonlight increased. We conclude that flying squirrels acoustically camouflage their vocalizations to facilitate communication with familiar conspecifics.

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References

Arriaga G, Jarvis ED. 2013. Mouse vocal communication system: Are ultrasounds learned or innate? *Brain & Language* 124(1):96-116. DOI: 10.1016/j.bandl.2012.10.002.

Balph DM, Balph DF. (1966). Social communication of Uinta ground squirrels. *Journal of Mammalogy* 47(3):440-450. DOI: 10.3176/eco.2009.2.01.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using *lme4*. *Journal of Statistical Software* 67(1). DOI: 10.18637/jss.v067.i01.

Blumberg MS, Sokoloff G, Kirby RF, Kent KJ. 2000. Distress vocalizations in infant rats: what's all the fuss about? *Psychological Science* 11(1):78-81. DOI: 10.1111/1467-9280.00219.

Boughman JW, Wilkinson GS. 1998. Greater spear-nosed bats discriminate group mates by vocalizations. *Animal Behaviour* 55(6):1717-1732. DOI: 10.1006/anbe.1997.0721.

Brittan-Powell EF, Lohr B, Hahn DC, Dooling RJ. 2005. Auditory brainstem responses in the eastern screech owl: an estimate of auditory thresholds. *The Journal of the Acoustical Society of America* 118(1):314-321. DOI: 10.1121/1.1928767.

Byrnes G, Spence AJ. 2011. Ecological and biomechanical insights into the evolution of gliding in mammals. *Integrative and Comparative Biology* 51(6):991-1001. DOI: 10.1093/icb/icr069.

Castellucci GA, Calbick D, McCormick D. 2018. The temporal organization of mouse ultrasonic vocalizations. *PLoS ONE* 13(10):e0199929. DOI: 10.1371/journal.pone.0199929.

Chattin D. 1969. Acoustical echolocation in flying squirrels. B. A. Thesis, Reed College.

Clarke JA. 1983. Moonlight's influence on predator/prey Interactions between short-eared owls (*Asio flammeus*) and deermice (*Peromyscus maniculatus*). *Behavioural Ecology and Sociobiology* 13(3):205-209.

Clink D. 2023. GibbonR: an R package for the detection and classification of acoustic signals using machine learning. *Available at* [*https://github.com/DenaJGibbon/gibbonR/*](https://github.com/DenaJGibbon/gibbonR/) (accessed 28 April 2023).

Corrigan JG, Flannelly KJ. 1979. Ultrasonic vocalizations of defeated male rats. *Journal of Comparative and Physiological Psychology* 93(1):105-115.

Ding J, Han F, Zhang K, Lin A, Jiang T, Feng J, Liu Y. 2023. Performance of Doppler shift compensation varies with environmental temperature and humidity in bats. *Animal Behaviour* 205(6):35-46. DOI: 10.1016/j.anbehav.2023.08.009.

Fenton MB, Jacobs DS, Richardson EJ, Taylor PJ, White W. 2004. Individual signatures in the frequency-modulated sweep calls of African large-eared, free-tailed bats *Otomops martiensseni* (Chiroptera: Molossidae). *Journal of Zoology* 262(1):11-19. DOI: 10.1017/S095283690300431X.

Fuong H, & Blumstein DT. 2019. Social security: less socially connected marmots produce noisier alarm calls. *Animal Behaviour* 154(1):131-136. DOI: 10.1016/j.anbehav.2019.06.019.

Gambale PG, Bastos RP. 2014. Vocal repertoire and bioacoustic analyses in *Physalaemus cuvieri* (Anura, Leptodactylidae) from southern Brazil. *Herpetological Journal* 24(1):31-39.

García-Navas V, Blumstein DT. 2016. The effect of body size and habitat on the evolution of alarm vocalizations in rodents. *Biological Journal of the Linnean Society* 118(4):745-751. DOI: 10.1111/bij.12789.

Garroway CJ, Bowman J, Wilson PJ. 2013. Complex social structure of southern flying squirrels is related to spatial proximity but not kinship. *Behavioral Ecology and Sociobiology* 67(1):113-122. DOI: 10.1007/s00265-012-1431-3.

Garstang M, Fitzjarrald DR, Fristrup K, Brain C. 2005. The daily cycle of low-frequency elephant calls and near-surface atmospheric conditions. *Earth Interactions* 9(14):1-21. DOI: 10.1175/EI147.

Gilley LM. 2013. Discovery and characterization of high-frequency calls in North American flying squirrels (*Glaucomys sabrinus* and *G. volans*): implications for ecology, behavior, and conservation. D. Phil. Thesis, Auburn University.

Gilley LM, Diggins CA, Pearson SM, Best TL. 2019. Vocal repertoire of captive northern and southern flying squirrels (*Glaucomys sabrinus* and *G. volans*). *Journal of Mammalogy* 100(2):518-530. DOI: 10.1093/jmammal/gyz064.

Gould E, Negus NC, Novick A. 1964. Evidence for echolocation in shrews. *Journal of Experimental Zoology* 156(1):19-37. DOI: 10.1002/jez.1401560103.

Guerra Batista V, Gambale P, Signorelli L. 2012. Acoustic communication in a neotropical frog (*Dendropsophus minutus*): Vocal repertoire, variability and individual discrimination. *Herpetological Journal* 22(4):249-257.

Holy TE, Guo Z. 2005. Ultrasonic songs of male mice. *PLoS Biology* 3(12):e386-e386. DOI: 10.1371/journal.pbio.0030386.

Jackson SM, Schouten P. 2012. *Gliding Mammals of the World*. Melbourne: CSIRO.

Jones G. 2005. Echolocation. *Current Biology* 15(13):R484-R488. DOI: 10.1016/j.cub.2005.06.051.

Ketten DR. 1992. The marine mammal ear: specializations for aquatic audition and echolocation. In: Webster DB, Popper AN, Fay RR, eds. *The Evolutionary Biology of Hearing.*). New York: Springer, 717-750. DOI: 10.1007/978-1-4612-2784-7_44.

Knudsen EI. 1981. The hearing of the barn owl. *Scientific American* 245(6):112-125. DOI: 10.1038/scientificamerican1281-112.

Kronfeld-Schor N, Dominoni D, de la Iglesia H, Levy O, Herzog ED, Dayan T, Helfrich-Forster C. 2013. Chronobiology by moonlight. *Proceedings of the Royal Society B* 280(1765):2013088. DOI: 10.1098/rspb.2012.3088.

Krumm B, Klump G, Köppl C, Langemann U. 2017. Barn owls have ageless ears. *Proceedings of the Royal Society B: Biological Sciences* 284(1863):20171584. DOI: 10.1098/rspb.2017.1584.

Larom D, Garstang M, Payne K, Raspet R, Lindeque M. 1997. The influence of surface atmospheric conditions on the range and area reached by animal vocalizations. *The Journal of Experimental Biology* 200(3):421-431. DOI: 10.1242/jeb.200.3.421.

Layne JN, Raymond MAV. 1994. Communal nesting of southern flying squirrels in Florida. *Journal of Mammalogy* 75(1):110-120. DOI: 10.2307/1382242.

Lazaridis E. 2022. *lunar*: Lunar phase & distance, seasons and other environmental factors. DOI: 10.32614/CRAN.package.lunar.

Liaw A, Wiener M. 2002. Classification and regression by *randomForest*. *R News* 2(3):18-22. DOI: 10.32614/CRAN.package.randomForest.

Ligges U, Krey S, Mersmann O, Schnackenberg S. (2023). *tuneR*: Analysis of Music and Speech. DOI: 10.32614/CRAN.package.tuneR.

Lingnau R, Bastos RP. 2007. Vocalizations of the Brazilian torrent frog *Hylodes heyeri* (Anura: Hylodidae): Repertoire and influence of air temperature on advertisement call variation. *Journal of Natural History* 41(17-20):1227-1235. DOI: 10.1080/00222930701395626.

Masterton B, Heffner H, Ravizza R. 1969. The evolution of human hearing. *The Journal of the Acoustical Society of America* 45(4):966-985. DOI: 10.1121/1.1911574.

Meyer D, Dimitriadou E, Hornik K, Weingessel A, Leisch F. 2023. *e1071*: Misc functions of the Department of Statistics, Probability Theory Group (Formerly: *E1071*). DOI: 10.32614/CRAN.package.e1071.

Murrant MN. 2011. Foraging behaviours of southern flying squirrels (*Glaucomys volans*) in relation to social structure. M. Sc. Thesis, Trent University.

Murrant MN, Bowman J, Garroway CJ, Prinzen B, Mayberry H, Faure PA. 2013. Ultrasonic vocalizations emitted by flying squirrels. *PLoS ONE* 8(8):1-6. DOI: 10.1371/journal.pone.0073045.

Murrant MN, Bowman J, Wilson PJ. 2014. A test of non-kin social foraging in the southern flying squirrel (*Glaucomys volans*). *Biological Journal of the Linnean Society* 113(4):1126-1135. DOI: 10.1111/bij.12385.

Nelson ML, Sagot M. 2018. Effects of temperature and day length on daily movements and home range of Glaucomys volans (southern flying squirrel) in the northeastern United States *Northeastern Naturalist* 25(3):383-390.

O'Brien PP, Bowman J, Coombs A, Newar SL, Garroway CJ. 2021. Winter nest trees of sympatric northern and southern flying squirrels: a test of reinforcement in a hybrid zone. *Canadian Journal of Zoology* 99(10):859-866. DOI: 10.1139/cjz-2021-0086.

Olson MN, Bowman J, Burness G. 2018. Social thermoregulation does not explain heterospecific nesting in North American flying squirrels. *Biological Journal of the Linnean Society* 123(4):805-813. DOI: 10.1093/biolinnean/bly014.

Oswalt GL, Meier GW. 1975. Olfactory, thermal, and tactual influences on infantile ultrasonic vocalization in rats. *Developmental Psychobiology* 8(2):129-135. DOI: 10.1002/dev.420080205.

Panyutina AA, Kuznetsov AN, Volodin IA, Abramov AV, Soldatova IB. 2017. A blind climber: The first evidence of ultrasonic echolocation in arboreal mammals. *Integrative Zoology* 12(2):172-184. DOI: 10.1111/1749-4877.12249.

Penteriani V, Delgado MDM, Campioni L, Lourenço R. 2010. Moonlight makes owls more chatty. *PLoS ONE* 5(1). DOI: 10.1371/journal.pone.0008696.

R Core Team. 2023. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *Available at* https://www.r-project.org/ (accessed 28 April 2023).

Radvanyi A. 1959. Inherent rhythms of activity of the northern flying squirrel in relation to illumination and to lunar and solar photoperiodism. D. Phil. Thesis, University of British Columbia.

Schoeman MC, Goodman SM. 2012. Vocalizations in the Malagasy cave-dwelling fruit bat, *Eidolon dupreanum*: Possible evidence of incipient echolocation? *Acta Chiropterologica* 14(2):409-416. DOI: 10.3161/150811012X661729.

Siegfried EC, Stone MS, Madison KC. 1992. Ultraviolet light burn: A cutaneous complication of visible light phototherapy of neonatal jaundice. *Pediatric Dermatology* 9(3):278-282. DOI: 10.1111/j.1525-1470.1992.tb00348.x.

Siemers M, Schauermann G, Turni H, von Merten S. 2009. Why do shrews twitter? Communication or simple echo-based orientation. *Biology Letters* 5(5):593-596. DOI: 10.1098/rsbl.2009.0378.

Sikes RS, Gannon WL. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* 92(1):235-253. DOI: 10.1644/10-MAMM-F-355.1.

Stead SM, Edwards PD, Persad R, Boonstra R, Teichrob JA, Palme R, Bowman J. 2024. Coping with extreme free cortisol levels: seasonal stress axis changes in sympatric North American flying squirrels. *General and Comparative Endocrinology* 349:114467. DOI: 10.1016/j.ygcen.2024.114467.

Stoffel MA, Nakagawa S, Schielzeth H. 2017. *rptR*: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution* 8(11):1639-1644. DOI: 10.1111/2041-210X.12797.

Tomasi TE. 1979. Echolocation by the short-tailed shrew *Blarina brevicauda*. *Journal of Mammalogy* 60(4):751-759. DOI: 10.1121/10.0020071.

Wilson DR, Hare JF. 2004. Ground squirrel uses ultrasonic alarms. *Nature* 430(6999):523-523. DOI: 10.1038/430523a.

Wilson DR, Hare JF. 2006. The adaptive utility of Richardson's ground squirrel (*Spermophilus richardsonii*) short-range ultrasonic alarm signals. *Canadian Journal of Zoology* 84(9):1322-1330. DOI: 10.1139/Z06-120.

Wu H, Gong L, Jiang T, Feng J, Lin A. 2021. Echolocation call frequencies of bats vary with body temperature and weather conditions. *Animal Behaviour* 180(1):51-61. DOI: 10.1016/j.anbehav.2021.08.005.

Yamazaki Y, Yamada H, Murofushi M, Momose H, Okanoya K. 2004. Estimation of hearing range in raptors using unconditioned responses. *Ornithological Science* 3(1):85-92. DOI: 10.2326/osj.3.85.

Yolton RL, Yolton DP, Renz J, Jacobs GH. 1974. Preretinal absorbance in sciurid eyes. *Journal of Mammalogy* 55(1):14-20. DOI: 10.2307/1379253.

Young BA, Mathevon N, Tang Y. 2013. Reptile auditory neuroethology: what do reptiles do with their hearing? In: Köppl C, Manley G, Popper A, Fay R, eds. *Insights from Comparative Hearing Research.* New York: Springer Handbook of Auditory Research, 323-346. DOI: 10.1007/2506_2013_30.

Yovel Y, Geva-Sagiv M, Ulanovsky N. 2011. Click-based echolocation in bats: not so primitive after all. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 197(5):515-530. DOI: 10.1007/s00359-011-0639-4.

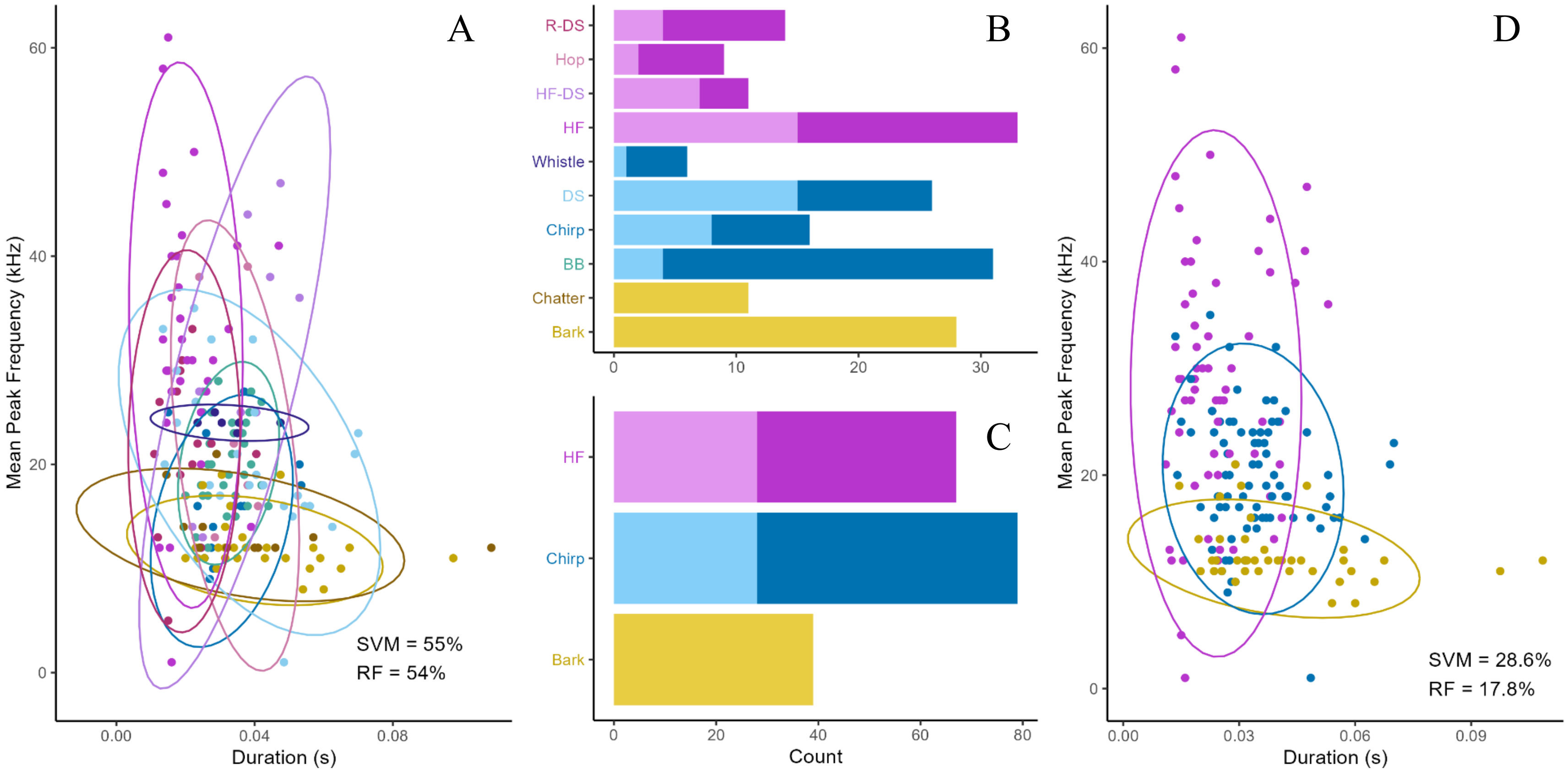

Figure 4.1 High-confidence vocalizations (n = 182) produced by *Glaucomys* spp. during individual and paired recording trials while temporarily in captivity. (A) Calls were classified based on similar frequency, duration, and modulation characteristics (N = 10). (B) Counts for each class (x-axis) during individual (light pink, light blue) and paired (dark blue, dark pink, dark yellow) recording trials; colour groupings correspond to superclasses (HF – pink, chirps – blue, barks – yellow) and apply to all four panels. *Classes:* BB – Broadband, DS – Downsweep, HF – High-Frequency, HF-DS – High-Frequency Downsweep, R-DS – Rapid Downsweep. (C) Classes from A/B were categorized into superclasses (N = 3) based on similar frequency and duration characteristics; colours correspond to individual/paired trials as in B. (D) All vocalizations were categorized into superclasses based on similar frequency and temporal characteristics. Machine learning error estimates (SVM = Support Vector Machine; RF = Random Forest) for classes and superclasses are accordingly given in the bottom right of A and D.

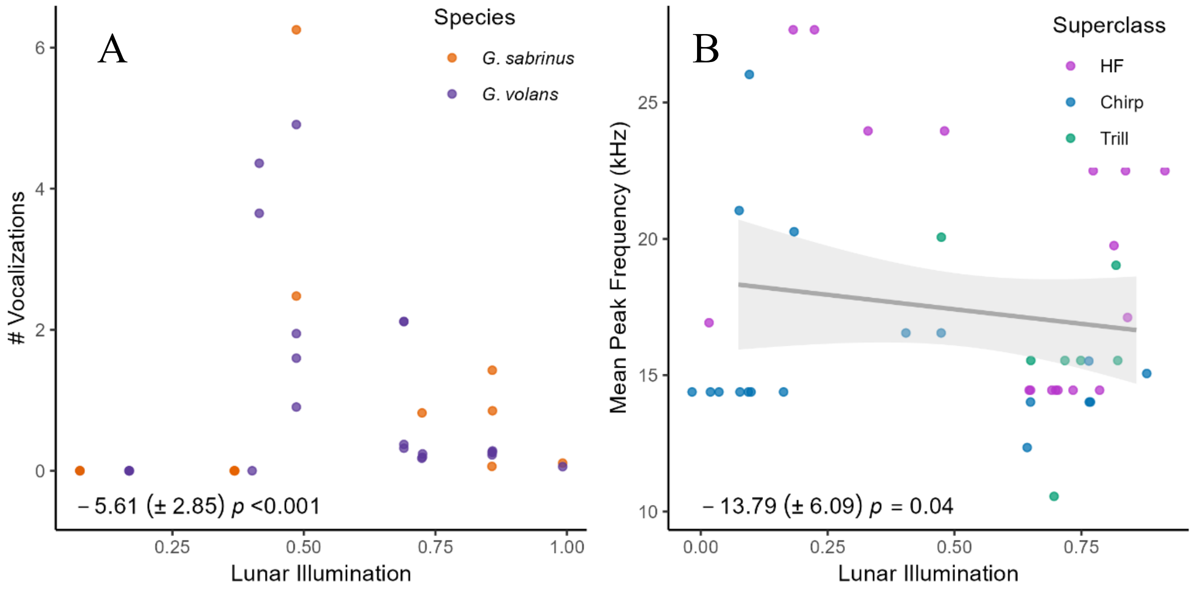

Figure 4.2 Vocal activity of flying squirrels (*Glaucomys*; N = 17) during a natural gliding experiment in the Kawartha Highlands, ON. (A) The total number of vocalizations produced during each trial (n = 36) relative to the lunar illumination (proportion); model estimate for this relationship from the count portion of the zero-inflated Poisson regression is provided in the bottom left. (B) The mean peak frequency of each vocalization (n = 43) recorded during the gliding trials; model estimate for this relationship from the linear mixed effects regression provided in the bottom left. All data points were generated from predicted values from the corresponding models.

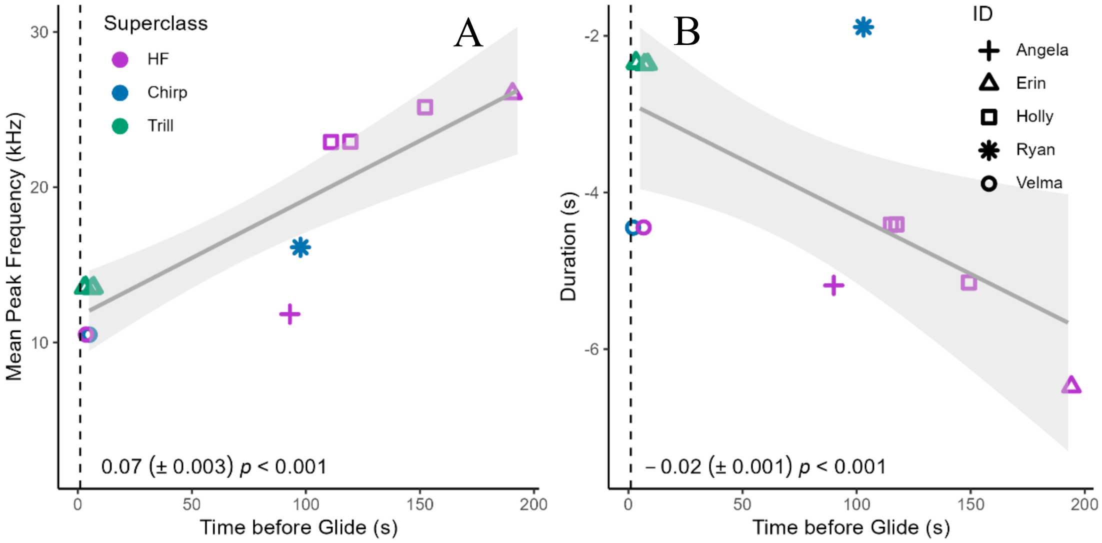

Figure 4.3 Predicted call characteristics of vocalizations (n = 12) produced by flying squirrels (*Glaucomys volans*; N = 5) during a natural gliding experiment in the Kawartha Highlands, ON. Calls are characterized by the mean peak frequency (kHz; A) and duration (s; B) relative to the time before glide (s); the time of glide is indicated as a vertical line. Model estimates for mixed models of the call characteristics relative to time before glide (squirrel ID as a fixed effect) are provided in the bottom left. Colour indicates the superclass and individual ID represented by shape (females: Angela, Erin, Holly, Velma; male: Ryan).

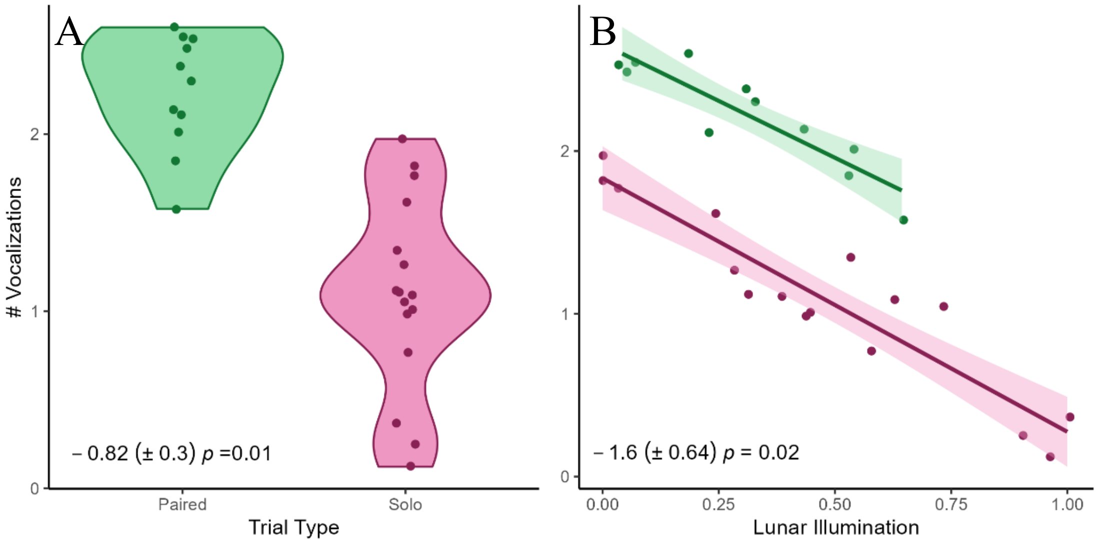

Figure 4.4 Total number of vocalizations (n = 132) produced by flying squirrels (*Glaucomys*; N = 17) during 24hr passive recording sessions at Trent University (Peterborough, ON). (A) The total number of vocalizations recorded during individual (individual, red; n = 16) and paired (green; n = 12) recording sessions and (B) in response to lunar illumination. Model estimates from a generalized linear model are provided in the bottom left; all data points were generated from predicted values from the corresponding models.

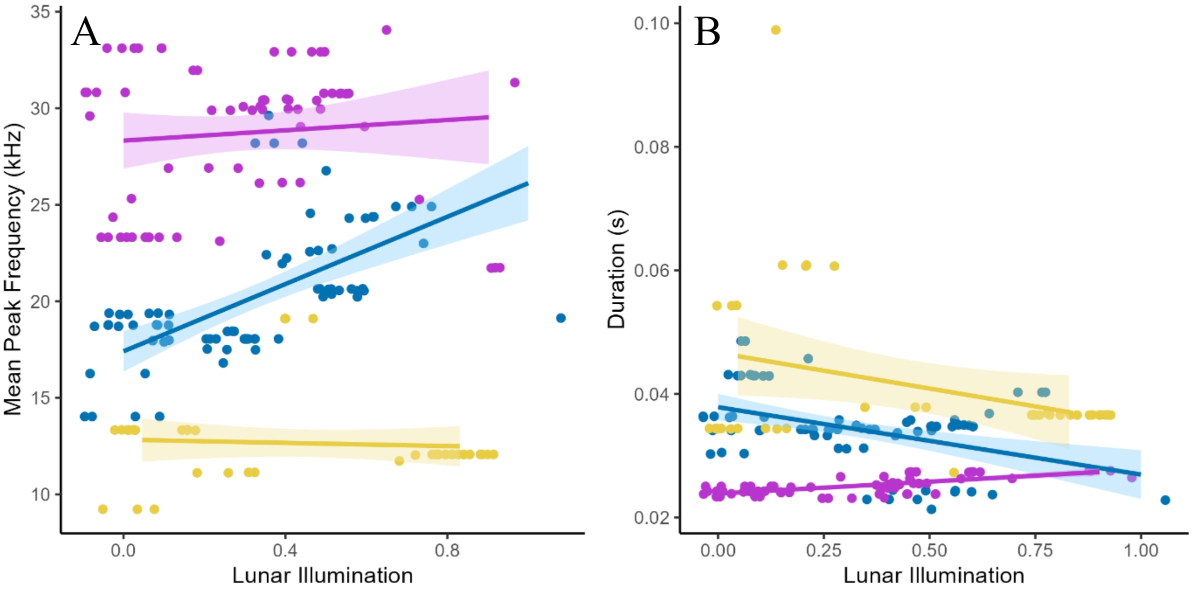

Figure 4.5 Call characteristics relative to lunar illumination for vocalizations produced by flying squirrels (*Glaucomys*) during individual and paired recording sessions at Trent University (Peterborough, ON). Calls are characterized by mean peak frequency (kHz; A) and duration (s; B). Colour indicates the superclasses that calls were categorized into: barks (yellow; n = 39), chirps (blue; n = 75), and high-frequency (pink; n = 66).

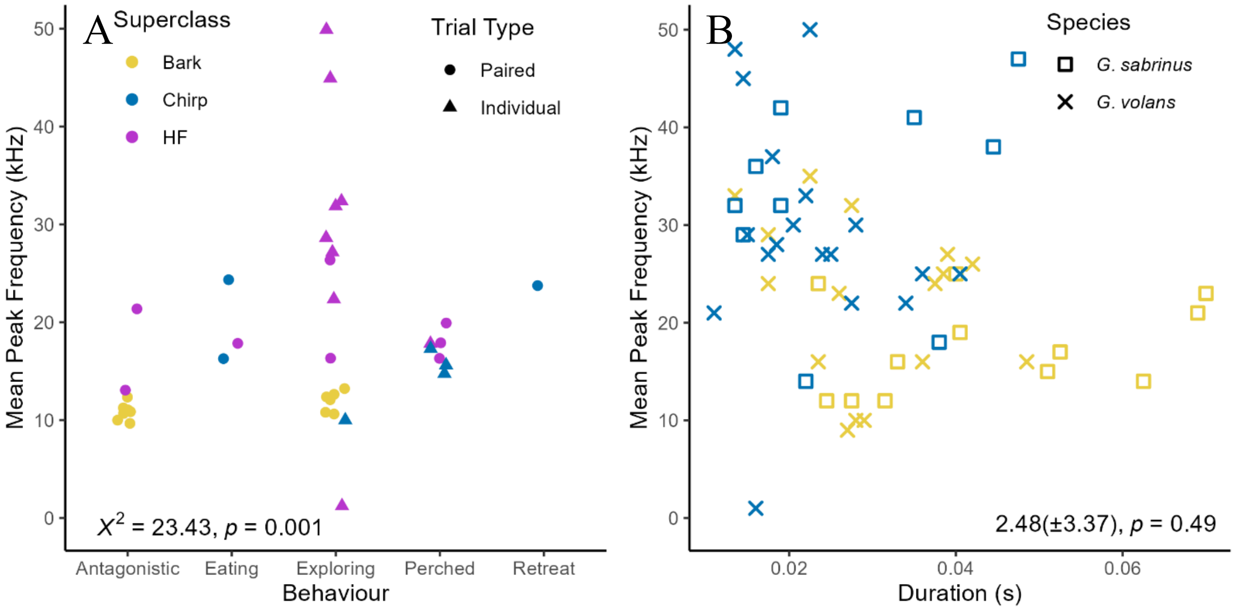

Figure 4.6 Superclass call characteristics relative to behaviour and species for vocalization produced by flying squirrels (*Glaucomys*) while temporarily in captivity. Calls are coloured by superclass (legend provided in A applies to both A and B). (A) Shape indicates whether the vocalization was produced during an individual (n = 13) or paired (n = 24) recording session. Chi-square results for the distribution of superclasses among activities are provided in the bottom left. (B) Shape indicates the species of the individual (*G. sabrinus* = 22; *G. volans* = 34) producing each call during individual trials. Linear mixed effects model results for the relationship between mean peak frequency and species provided in the bottom right.

Table 4.1 Model estimates for the likelihood of a *Glaucomys* individual making the correct choice in a T-maze. Correct choice (1) was measured as choosing the open end ('Treatment', randomized) of the maze first, regardless of whether the squirrel eventually escaped the maze, and modelled in a generalized binomial model. The sequence of the individual as well as the time of day (day/night), the amount of airflow through the covered end (low/high-density hole pattern), and the total time to exit the maze (s) are also included in the model.

	$\bar{x}$ (±*SE*)	***Z*-Value**	***p***
Intercept	2.18 (±1.64)	1.32	0.19
Treatment (Right)	0.07 (±0.52)	0.14	0.89
Sequence	0.03 (±0.31)	-0.10	0.92
Time (Night)	**-1.26 (±0.55)**	**-2.29**	**0.02**
Airflow (Low)	0.84 (±1.17)	0.72	0.47
Time to Exit (s)	**-0.07 (±0.02)**	**-3.54**	**<0.001**

Table 4.2 Model estimates for the zero-inflated Poisson regression of the total number of vocalizations produced by *Glaucomys* individuals (N = 16) during a natural gliding experiment in the Kawartha Highlands, ON. Model estimates are provided for the total number of vocalizations (count model) and the probability of producing any vocalizations (zero-inflation model). Individual sex (female/male), species (*G. sabrinus*/*G. volans*), and the number of times exposed to the experiment (sequence) were included in the model as well as the average ambient temperature and lunar illumination of the entire trial.

	Count Model			**Zero-Inflation Model**		
	$\bar{x}$ (±*SE*)	***Z*-value**	***p***	$\bar{x}$ (±*SE*)	***F*-value**	***p***
Intercept	**2.85 (±0.82)**	**3.47**	**<0.001**	38.13 (±70.58)	0.54	0.59
Sex: Male	-0.77 (±0.81)	-0.94	0.35	8.70 (±61.12)	0.14	0.89
Species: *G.v.*	-0.24 (±0.38)	-0.64	0.52	-7.09 (±39.56)	-0.18	0.89
Sequence	0.31 (±0.22)	1.38	0.17	-1.78 (±12.80)	-0.14	0.89
Temp (°C)	**0.10 (±0.04)**	**2.78**	**0.005**	-2.21 (±6.62)	-0.33	0.74
Lunar Illumination	**-5.61 (±1.41)**	**-3.97**	**<0.001**	-70.95 (±161.36)	-0.44	0.66

Table 4.3 Model estimates for the linear mixed effects models of the mean peak frequency (kHz) and duration (s) of calls (n = 43) produced by *Glaucomys* individuals (N = 10) during a natural gliding experiment in the Kawartha Highlands, ON. Individual sex (female/male), species (*G. sabrinus*/*G. volans*), and the number of times exposed to the experiment (sequence) were included for each squirrel. The lunar illumination at the time of the call and the acoustic recorder that detected the call (Echometer Touch/SM4) were also included in this model. The time before glide (s) was modelled separately on a smaller subset of the data (n = 12). All models include Squirrel ID as a random effect (Table 4.4).

	log(Peak Freq (kHz))			**log(Duration (s))**		
	$\bar{x}$ (±*SE*)	***F*-value**	***p***	**$\bar{x}$ (±*SE*)**	***F*-value**	***p***
Intercept	**30.35 (±6.49)**		**0.003**	-0.03 (±0.05)		0.57
Sex: Male	4.91 (±7.12)	0.48	0.52	-0.001 (±0.06)	0.0005	0.98
Species: *G.v.*	-2.36 (±6.20)	0.14	0.73	0.03 (±0.03)	0.87	0.48
Sequence	-0.25 (±1.25)	0.04	0.84	0.009 (±0.01)	0.59	0.48
Lunar Illumination	**-13.79 (±6.09)**	**5.12**	**0.04**	-0.002 (±0.05)	0.002	0.96
Superclass: Chirp	**-7.40 (±2.75)**	**3.84**	**0.01**	**0.10 (±0.03)**	**5.50**	**0.005**
Superclass: Trill	-3.89 (±2.88)		0.19	0.05 (±0.04)		0.15
Intercept	**10.74 (±1.67)**		**0.003**	**-2.26 (±0.79)**		**0.04**
Time before Glide (s)	**0.07 (±0.003)**	**383.69**	**<0.001**	**-0.02 (±0.001)**	**264.13**	**<0.001**

Table 4.4 Random effect estimates for models estimating call characteristics of *Glaucomys* individuals in varying recording contexts. Random effects (bolded in the models) are estimated as the proportion of variance explained by the random effect and the residuals; the accuracy of the random variance is provided as a confidence interval.

Recording Context	**Model**	**Random Effect Variance**		
		Random: Residual	**% Explained**	**CI**
Gliding	Peak Freq ~ Sex + Species + Seq + Lunar + Superclass + **Squirrel ID**	49.77:27.26	65%	[0, 93.09]
	Duration ~ Sex + Species + Seq + Lunar + Recorder + **Squirrel ID**	0.0004:0.006	6.9%	[0, 0.002]
	Peak Freq ~ Time before Glide + **Squirrel ID**	**0.07:0.0002**	**97.5%**	**[0.02, 0.28]**
	Duration ~ Time before Glide + **Squirrel ID**	**3.04:0.05**	**98%**	**[0.84, 11.68]**
Captivity	*Chirps:* Peak Freq ~ Lunar + Temp + Trial Type + **Trial ID**	19.24:17.86	52%	[0, 36.13]
	Chirps: Duration ~ Lunar + Temp + Trial Type + **Trial ID**	$2.15e^{-5}$:$9.77e^{-5}$	18%	[0, $5.27e^{-5}$]
	HF: Peak Freq ~ Lunar + Temp + Trial Type + **Trial ID**	14.73:115.72	11%	[0, 45.60]
	HF: Duration ~ Lunar + Temp + Trial Type + **Trial ID**	$5.04e^{-6}$:$1.07e^{-4}$	4%	[0, $2.72e^{-5}$]
	Barks: Peak Freq ~ Lunar + Temp + **Trial ID**	7.72:3.81	67%	[0, 16.45]
	Barks: Duration ~ Lunar + Temp + **Trial ID**	**$6.78e^{-4}$:$2.19e^{-4}$**	**76%**	**[$4.79e^{-5}$, 0.001]**
	Peak Freq ~ Sex + Species + Superclass + **Squirrel ID**	20.04:58.32	26%	[0, 26.94]
	log(Duration) ~ Sex + Species + Superclass + **Squirrel ID**	0:0.0001	0%	[0, $3.10e^{-5}$]

Table 4.5 Model estimates for the generalized linear regression of the total number of vocalizations produced by *Glaucomys* individuals (N = 17) during solitary (n = 16) and paired (n = 12) recording sessions at Trent University (Peterborough, ON, Canada). Squirrels from the Kawartha Highlands and Trent Oliver Property were temporarily brought into captivity, and recordings were made in a large two-level arena. The average lunar illumination and ambient temperature (°C) over the recording trial are included in the model as well as the trial type (solitary/paired).

	$\bar{x}$ (±*SE*)	*t*-value	*p*
Intercept	**2.85 (±0.29)**	**9.88**	**<0.001**
Lunar Illumination	**-1.60 (±0.64)**	**-2.50**	**0.02**
Temp (°C)	-0.02 (±0.02)	-1.06	0.30
Type: Solitary	**-0.82 (±0.30)**	**-2.72**	**0.01**

Table 4.6 Model estimates for the linear mixed effects models of the mean peak frequency (kHz) and duration (s; log-transformed) of calls (n = 182) produced by *Glaucomys* individuals (N = 17) during individual (n = 16) and paired (n = 12) recording sessions at Trent University (Peterborough, ON, Canada). Calls were modelled within their superclass, which groups similar classes together. The lunar illumination and ambient temperature (°C), as well as the trial type (individual/paired), were included in the model. Trial ID was included as a random effect; random effect estimates are given in Table 4.4.

		Peak Frequency (kHz)			**log(Duration (s))**		
Call Class		**$\bar{x}$ (±*SE*)**	***F*-value**	***p***	**$\bar{x}$ (±*SE*)**	***F*-value**	***p***
Chirps (n = 75)	**Intercept**	**16.24 (±2.82)**		**<0.001**	**0.05 (±0.004)**		**<0.001**
	Lunar Illumination	9.99 (±5.30)	3.54	0.09	**-0.02 (±0.008)**	**9.69**	**0.005**
	Temp (°C)	0.19 (±0.16)	1.39	0.26	**-0.0008 (±0.0002)**	**11.27**	**0.003**
	Type: Individual	-0.14 (±2.57)	0.003	0.96	0.004 (±0.004)	1.22	0.29
High-Frequency (n = 66)	**Intercept**	**27.43 (±3.50)**		**<0.001**	**0.024 (±0.003)**		**<0.001**
	Lunar Illumination	2.24 (±6.91)	0.11	0.75	0.004 (±0.006)	0.49	0.77
	Temp (°C)	-0.20 (±0.21)	0.95	0.34	$-5.23e^{-5}$ ($\pm 1.80^{-4}$)	0.08	0.50
	Type: Individual	5.71 (±3.59)	2.54	0.13	$-4.81e^{-4}$ (±0.003)	0.02	0.88
Barks (n =39)	**Intercept**	**14.73 (±2.31)**		**0.004**	**0.07 (±0.02)**		**0.03**
	Lunar Illumination	-0.25 (±0.14)	0.60	0.49	-0.03 (±0.04)	3.40	0.49
	Temp (°C)	3.24 (±4.18)	3.15	0.16	-0.001 (±0.001)	3.93	0.49

Table 4.7 Model estimates for the linear mixed effects models of the mean peak frequency (kHz) and duration (s) of calls (n = 55) produced by *Glaucomys* individuals (N = 12) during individual recording sessions at Trent University (Peterborough, ON, Canada). Similar call types were grouped into larger superclasses (chirps and high-frequency [HF] calls). Squirrel ID was included as a random effect; random effect estimates are given in Table 4.4.

	Peak Frequency (kHz)			**log(Duration (s))**		
	$\bar{x}$ (±*SE*)	***F*-value**	***p***	**$\bar{x}$ (±*SE*)**	***F*-value**	***p***
Intercept	**18.35 (±3.52)**		**0.001**	**0.04 (±0.003)**		**<0.001**
Sex: Male	-2.26 (±3.99)	0.32	0.31	-0.004 (±0.004)	0.92	0.34
Species: *G.v.*	3.45 (±4.01)	0.74	0.49	**-0.008 (±0.004)**	**5.14**	**0.03**
Class: HF	**11.34 (±2.24)**	**25.66**	**<0.001**	**-0.01 (±0.003)**	**10.76**	**0.002**

Table 4.8 Model estimates of the general linear models for the mean peak frequency (kHz) and duration (s) of calls (n = 37) produced by *Glaucomys* individuals (N = 9) during paired (n = 24) and individual (n = 13) recording sessions at Trent University (Peterborough, ON, Canada). Calls are categorized into activities (antagonistic, eating, exploring, perched, retreat) recorded on trail cameras mounted in temporary enclosures.

	Peak Frequency (kHz)		**Duration (s)**	
	$\bar{x}$ (±*SE*)	***p***	**$\bar{x}$ (±*SE*)**	***p***
Intercept	**12.22 (±3.21)**	**<0.001**	**0.04 (±0.004)**	**<0.001**
Activity: Eating	7.11 (±6.42)	0.28	0.009 (±0.01)	0.36
Activity: Exploring	**9.07 (±3.97)**	**0.03**	**-0.01 (±0.006)**	**0.04**
Activity: Perched	4.92 (±4.85)	0.32	0.0008 (±0.007)	0.92
Activity: Retreat	11.78 (±10.15)	0.25	-0.006 (±0.02)	0.70

Chapter 5 - General Discussion

The overall objective of my thesis was to explore and identify the ecological and evolutionary traits associated with ultrasonic vocalizations (USVs) in flying squirrels. To achieve this, I compared vocal ranges and ecological traits between flying squirrels and other mammals, contrasted USVs with the recently discovered ultraviolet-induced photoluminescence, and contextualized USVs within flying squirrel behaviour. My work integrated phylogenetic analyses (Chapters 2 and 3), novel vocalizations for a variety of gliding and non-gliding mammals (Chapter 3) and includes the first detailed account of the relationship between flying squirrel behaviour and USV production (Chapter 4).

In Chapter 2, I compared the vocal frequency ranges of flying squirrels to those reported in all other published records within Sciuridae. This was an important first step in addressing whether the production of USVs is uniquely associated with flying squirrel traits. Importantly, flying squirrels are not the only squirrels to use USVs, with the first record of USV-producing squirrels belonging to the Richardson's ground squirrel (Wilson & Hare, 2004). While I did not predict that flying squirrels would be the only squirrels to produce USVs, I hypothesized that some traits associated with flying squirrels would have a significant association with higher frequencies. These traits included some that have been explored previously in the literature, such as body size (Martin et al., 2017) and sociality (Blumstein & Armitage, 1997; Blumstein et al., 2017), but I also included novel factors: nocturnality and recording limits of the research equipment. I compared these traits against several call characteristics to look at the relationship between life history traits and frequency (kHz). This approach allowed me to avoid focusing on the presence of USVs, which are inherently anthropocentrically defined. I found that all the traits I analysed were related to at least one frequency characteristic, but most importantly (in the context of this thesis), nocturnality was positively associated with higher frequencies across several frequency measurements. Given that nocturnality and gliding are inextricably linked traits in Sciuridae, with the only nocturnal squirrels being flying squirrels, I was unable to identify whether gliding or nocturnality was the trait associated with higher frequency use within this chapter.

In Chapter 3, I expanded on the results in Chapter 2, and compared gliding mammals against their non-gliding counterparts with a key objective being to disentangle the relationship between USV production and nocturnality and gliding. This is the first paper to combine vocal ranges across all published gliding mammal calls, including records from gliding marsupials (*Acrobates pygmaeus* (Martin, 2019); *Petaurus australis* (Kavanagh & Rohan-Jones, 1982; Whisson et al., 2021), colugos (*Galeopterus variegatus* (Miard et al., 2019)), and flying squirrels (*Glaucomys sabrinus* (Gilley, 2013; Gilley et al., 2019; Murrant et al., 2013); *Glaucomys volans* (Eisinger et al., 2016; Gilley, 2013; Gilley et al., 2019; Murrant et al., 2013); *Petaurus alborufus* (Shen, 2013); *Petaurus leucogenys* (Ando & Kuramochi, 2008)). I also contributed novel call descriptions for several species of gliding marsupials including high-frequency calls from sugar gliders (*Petaurus breviceps*). I found that higher dominant frequencies were associated with gliding, but not nocturnality, supporting my initial hypothesis that gliding mammal ecology facilitates cryptic, high frequency communication, given predation risk associated with the gliding trait. Crucially, I am not suggesting that only gliding mammals produce USVs as we included many nocturnal species that produce USVs, such as mice (*Mus musculus* (Grimsley et al., 2016; Hoffmann et al., 2012; Lupanova & Egorova, 2015; Musolf et al., 2015; Nyby, 1983; Von Merten et al., 2014; White et al., 1998; Zala et al., 2017)) and tarsiers

(*Tarsier* spp. (Gursky, 2015; Ramsier et al., 2012; Řeháková-Petru et al., 2012)), but that gliding mammals as a group produce higher frequency calls than their closest non-gliding relatives. In this chapter, I also explored the relationship between ultraviolet-induced photoluminescence (UVP) and gliding as another form of crypsis. However, while some gliding mammals do exhibit striking UVP (i.e. flying squirrels (Kohler et al., 2019)), my investigation into UVP in other gliding mammals was inconclusive. Instead, I found that nocturnality was the only trait associated with UVP which is consistent with the hypotheses put forth by other researchers (Kohler et al., 2019; Olson et al., 2021), possibly reflecting the degradation of some luminophores in sunlight (Pine et al., 1985; Schäfer et al., 1997; Toussaint et al., 2023).

In Chapter 4, I contextualized flying squirrel USVs by exposing northern (*Glaucomys sabrinus*) and southern (*G. volans*) flying squirrels to various behavioural tests. This chapter is vital within the context of this thesis as the majority of USV behavioural literature is focused on echolocation in known-echolocators or social interactions in myomorphic rodents. I hypothesized that vocal frequencies could vary within different behavioural (echonavigation and social interactions) or environmental (temperature and lunar illumination) contexts. This is only the second piece of literature to address echonavigation in flying squirrels and, consistent with the previous publication (Chattin, 1969), I did not find convincing evidence for this behaviour in these species, with no vocalizations being recorded during any glides. However, I did find compelling evidence for different USVs being produced in a variety of social and solitary contexts; additionally, squirrels increased their vocal frequencies with increased lunar illumination. While the social aspect of USVs was predicted, recording many USVs during solitary conditions was unexpected. If we consider these calls outside of echonavigation, these calls suggest that flying squirrels are potentially communicating with conspecifics during solitary behaviours, such as foraging and exploring. Additionally, the use of higher frequencies during increased lunar illumination provides novel evidence that flying squirrels are adjusting their vocalizations with increased risk of predation. This finding fits nicely with the results of Chapters 2 and 3 which both suggest that nocturnal gliding mammals are under unique pressures to vocalize at higher frequencies – decreasing predation risk is vital for these mammals as gliding leaves them uniquely vulnerable to predation attempts. Increasing their vocal frequencies would allow flying squirrels to still communicate without compromising their location to potential eavesdroppers.

Limitations and Future Directions

While this dissertation unveils some interesting covariates of vocal range in gliding mammals, there are some key limitations in these experiments. In Chapter 2, I found that the acoustic limitations of the recording devices were one of the main predictors of the perceived vocal range of a given species. This highlights an inherent problem with the way acoustic devices are designed and deployed for acoustic studies. Deployable devices, such as the SM4s used in these studies, are designed to either record the audible (20Hz-20kHz) or ultrasonic ranges (>20kHz), which are defined by the limits of human hearing. This anthropocentric approach means that we require specific intention and methodology to record calls above our hearing range; as a result, ultrasonic calls are perceived as rare when recorded. However, there could be many magnitudes more animals that can use ultrasound to communicate.

To further explore this idea, I opportunistically deployed ultrasonic microphones (SMM-U1 attached to a Song Meter SM4BAT FS recorder; Wildlife Acoustics Inc., Maynard, Massachusets, USA) in various exhibits at the Toronto Zoo (Ontario, Canada) and I found evidence for USVs in several species. Golden lion tamarins (*Leontopithecus rosalia*) and common marmosets (*Callithrix jacchus*) were housed beside each other, and I recorded at least 5 unique high-frequency calls (Fig. 5.1) that ranged from a minimum frequency of 13.85kHz (tonal (±SD = ±1.31); Table 5.1) to a maximum of 33.00kHz (ultrasonic (±0.00); Table 5.1) from these primates. These calls likely originated from the golden lion tamarins as the directional microphone was pointed towards these primates and away from the marmosets and these calls do not match with any documented call in the small ultrasonic repertoire published for common marmosets (Bakker et al., 2014). While much rarer, I also recorded a few high-frequency calls in Vancouver Island marmots (*Marmota vancouverensis*) which were housed in quieter conditions; these calls ranged from 9.50kHz to 39kHz (Fig. 5.2; Table 5.1). The presence of novel high-frequency and ultrasonic vocalizations in these mammals, along with the sugar gliders recorded for Chapter 3, suggest that the lack of sampling for USV has led to an unintentionally biased view of the variety of mammals that produce these calls. Not having a clear idea of which animals produce USVs means that it is difficult to construct and test robust hypotheses to explain vocal frequencies across all mammals.

While the work I have compiled here demonstrates that gliding mammals can produce USVs and are under selective pressures to vocalize within certain ecological constraints, a more comprehensive sampling of their relatives might ultimately reveal that gliding mammals do not produce significantly higher frequencies than their counterparts. Instead, we might find that ultrasound is prolific amongst non-gliding mammals and therefore, new hypotheses could arise in the future. Likely, smaller-bodied mammals will produce USVs more often and in more contexts. However, we could find that drivers such as predator type, social structure, and environment are playing key roles that we do not yet understand. An interesting example of this are subterranean mole voles (*Ellobius talpinus*) who use USVs to communicate (Volodin et al., 2022); previous hypotheses suggest that open habitats are the most conducive to USV production and attenuation of calls in underground, narrow tunnels would prevent the evolution of USVs. Yet, these strictly closed-habitat rodents use USVs to communicate. This contrasts with naked mole rats who have been shown to use calls under 10kHz (Credner et al., 1997), though as far as I am aware, naked mole rats have not been tested for USVs and therefore, cannot be assumed to not produce them.

Truly, a researcher could spend their entire career sampling for USVs in mammals that have not been tested. I strongly recommend to acoustic researchers, especially those focused on smaller mammals, to sample for USVs in their subjects. While understanding a species' vocal range is interesting in and of itself, humans can have direct impacts on the way animals communicate. Anthropogenic noise (i.e. noise pollution) can directly interfere with communication (Kunc & Schmidt, 2019; Slabbekoorn *et al.,* 2018), preventing individuals from properly interacting and increasing the vulnerability of populations that are already at risk. Ultrasound should be of special consideration because of our inability to detect these frequencies which may lead to unintentional impacts. Future research should be done on the impacts of high-frequency noise pollution on mammals that rely on these frequencies.

Conclusions

In conclusion, the nocturnal, gliding nature of flying squirrels plays a key role in the frequencies at which these mammals communicate. Compared to other squirrels, flying squirrels produce significantly higher fundamental, dominant, and maximum frequencies. Furthermore, gliding mammals in general produce significantly higher dominant frequencies than their non-gliding counterparts, despite their extensive variation in phylogenetic relatedness, location, and body sizes. The lack of extant diurnal mammalian gliders suggests that these animals have a unique vulnerability to predation and their communication systems have evolved to minimize detection. This is exemplified in flying squirrels who shift to higher frequencies as lunar illumination increases which corresponds to increased vulnerability. Despite the link between ultrasound production and volancy in other mammals (i.e. bats), I found no evidence for echonavigation in these mammals, further demonstrating that ultrasound production is a form of crypsis, allowing flying squirrels to avoid predation while continuing to communicate with conspecifics. Therefore, I conclude that ultrasonic production is a byproduct of the unique niche occupied by flying squirrels and other gliding mammals.

References

Ando M, Kuramochi Y. 2008. Vocal Communication of the Japanese Giant Flying Squirrel *Petaurista leucogenys*. *The Journal of Agricultural Science* 53(2):176-183.

Bakker J, van Nijnatten TJM, Louwerse AL, Baarends G, Arndt SS, Langermans JAM. 2014. Evaluation of ultrasonic vocalizations in common marmosets (*Callithrix jacchus*) as a potential indicator of welfare. *Lab Animal* 43(9):313-320. DOI: 10.1038/laban.568.

Blumstein DT, Armitage KB. 1997. Does sociality drive the evolution of communicative complexity? A comparative test with ground-dwelling sciurid alarm calls. *The American Naturalist* 150(2):179-200. DOI: 10.1086/286062.

Blumstein DT, Fuong H, Palmer E. 2017. Social security: social relationship strength and connectedness influence how marmots respond to alarm calls. *Behavioral Ecology and Sociobiology*: 71(10). DOI: 10.1007/s00265-017-2374-5

Chattin D. 1969. Acoustical echolocation in flying squirrels. D. Phil. Thesis, Reed College.

Credner S, Burda H, Ludescher F. 1997. Acoustic communication underground: vocalization characteristics in subterranean social mole-rats (*Cryptomys* sp., Bathyergidae). *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 180:245-255. DOI: 10.1007/s003590050045.

Eisinger JW, Scheibe JS, Flaherty EA. 2016. Novel *Glaucomys volans* vocalizations in Indiana and evidence of geographic variation in high frequency communication. *Journal of Mammalogy* 97(4):1219-1227. DOI: 10.1093/jmammal/gyw076.

Gilley LM. 2013. Discovery and characterization of high-frequency calls in North American flying squirrels (Glaucomys sabrinus and G. volans): Implications for ecology, behavior, and conservation. D. Phil. Thesis, Auburn University.

Gilley LM, Diggins CA, Pearson SM, Best TL. 2019. Vocal repertoire of captive northern and southern flying squirrels (*Glaucomys sabrinus* and *G. volans*). *Journal of Mammalogy* 100(2):518-530. DOI: 10.1093/jmammal/gyz064.

Grimsley JMS, Sheth S, Vallabh N, Grimsley CA, Bhattal J, Latsko M, Jasnow A, Wenstrup JJ. 2016. Contextual modulation of vocal behavior in mouse: Newly identified 12 kHz “Mid-frequency” vocalization emitted during restraint. *Frontiers in Behavioral Neuroscience* 10(MAR). DOI: 10.3389/fnbeh.2016.00038.

Gursky S. 2015. Ultrasonic vocalizations by the spectral Tarsier, *Tarsius spectrum*. *Folia Primatologica* 86(3):153-163. DOI: 10.1159/000371885.

Hoffmann F, Musolf K, Penn DJ. 2012. Ultrasonic courtship vocalizations in wild house mice: Spectrographic analyses. *Journal of Ethology* 30(1):173-180. DOI: 10.1007/s10164-011-0312-y.

Kavanagh RP, Rohan-Jones WG. 1982. Calling behaviour of the yellow-bellied glider, *Petaurus australis* Shaw (Marsupialia: Petauridae). *Australian Mammalogy* 5(2):95-111. DOI: 10.1071/AM82011.

Kohler AM, Olson ER, Martin JG, Anich PS. 2019. Ultraviolet fluorescence discovered in New World flying squirrels (*Glaucomys*). *Journal of Mammalogy* 100(1):21-30. DOI: 10.1093/jmammal/gyy177.

Kunc HP, Schmidt R. 2019. The effects of anthropogenic noise on animals: a meta-analysis. *Biology Letters* 15(11):20190649. DOI: https://doi.org/10.1098/rsbl.2019.0649.

Lupanova AS, Egorova MA. 2015. Vocalization of sex partners in the house mouse (*Mus musculus*). *Journal of Evolutionary Biochemistry and Physiology* 51(4):324-331. DOI: 10.1134/S0022093015040080.

Martin K. 2019. The vocal repertoire of feathertail gliders (*Acrobates pygmaeus*) and how macrocomparisons can shape future research on acoustic communication in mammals. D. Phil. Thesis, University of South Whales.

Martin K, Tucker MA, Rogers TL. 2017. Does size matter? Examining the drivers of mammalian vocalizations. *Evolution* 71(2):249-260. DOI: 10.1111/evo.13128.

Miard P, Lim LS, Abdullah NI, Elias NA, Ruppert N. 2019. Ultrasound use by Sunda colugos offers new insights into the communication of these cryptic mammals. *Bioacoustics* 28(5):397-403. DOI: 10.1080/09524622.2018.1463294.

Murrant MN, Bowman J, Garroway CJ, Prinzen B, Mayberry H., Faure PA. 2013. Ultrasonic vocalizations emitted by flying squirrels. *PLoS ONE* 8(8):1-6. DOI: 10.1371/journal.pone.0073045.

Musolf K, Meindl S, Larsen AL, Kalcounis-Rueppell MC, Penn DJ. 2015. Ultrasonic vocalizations of male mice differ among species and females show assortative preferences for male calls. *PLoS ONE* 10(8). DOI: 10.1371/journal.pone.0134123.

Nyby J. 1983. Ultrasonic Vocalizations during Sex Behavior of Male House Mice (*Mus musculus*): A Description. *Behavioral and Neural Biology* 39(1):128-134. DOI: 10.1016/s0163-1047(83)90722-7.

Olson ER, Carlson MR, Ramanujam VMS, Sears L, Anthony SE, Anich PS, Ramon L, Hulstrand A, Jurewicz M, Gunnelson AS, Kohler AM, Martin JG. 2021. Vivid biofluorescence discovered in the nocturnal Springhare (Pedetidae). *Scientific Reports* 11(1):4125-4125. DOI: 10.1038/s41598-021-83588-0.

Pine RH, Rice JE, Bucher JE, Tank DH, Greenhall AM. 1985. Labile pigments and fluorescent pelage in didelphid marsupials. *Mammalia* 49(2):249-256. DOI: 10.1515/mamm.1985.49.2.249.

Ramsier MA, Cunningham AJ, Moritz GL, Finneran JJ, Williams CV, Ong PS, Gursky-Doyen SL, Dominy NJ. 2012. Primate communication in the pure ultrasound. *Biology Letters* 8(4):508-511. DOI: 10.1098/rsbl.2011.1149.

Řeháková-Petru M, Policht R, Peške L. 2012. Acoustic Repertoire of the Philippine Tarsier (*Tarsius syrichta fraterculus*) and Individual Variation of Long-Distance Calls. *International Journal of Zoology* 2012(4). DOI: 10.1155/2012/602401.

Schäfer K, Goddinger D, Höcker H. 1997. Photodegredation of tryptophan in wool. *Journal of the Society of Dyers and Colourists* 113(12):350-355. DOI: 10.1111/j.1478-4408.1997.tb01862.x.

Shen P-S. 2013. Acoustic Behavior of White-faced Flying Squirrel (*Petaurista lena*) in Guanghua Village, Alishan. M. Sc. Thesis, National Sun Yat-sen University.

Slabbekoorn H, Dooling RJ, Popper AN, Fay RR. 2018. *Effects of Anthropogenic Noise on Animals*. New York: Springer.

Toussaint SLD, Ponstein J, Thoury M, Métivier R, Kalthoff DC, Habermeyer B, Guilard R, Bock S, Mortensen P, Sandberg S, Gueriau P, Amson E. 2023. Fur glowing under ultraviolet: in situ analysis of porphyrin accumulation in the skin appendages of mammals. *Integrative Zoology* 18(1):15-26. DOI: 10.1111/1749-4877.12655.

Volodin IA, Dymskaya MM, Smorkatcheva AV, Volodina EV. 2022. Ultrasound from underground: cryptic communication in subterranean wild-living and captive northern mole voles (*Ellobius talpinus*). *Bioacoustics* 31(4):414-434. DOI: 10.1080/09524622.2021.1960191.

Von Merten S, Hoier S, Pfeifle C, Tautz D. 2014. A role for ultrasonic vocalisation in social communication and divergence of natural populations of the house mouse (*Mus musculus domesticus*). *PLoS ONE* 9(5). DOI: 10.1371/journal.pone.0097244.

Whisson DA, McKinnon F, Lefoe M, Rendall AR. 2021. Passive acoustic monitoring for detecting the yellow-bellied glider, a highly vocal arboreal marsupial. *PLoS ONE* 16(5):e0252092. DOI: 10.1371/journal.pone.0252092.

White NR, Prasad M, Barfield RJ, Nyby JG. 1998. 40- and 70-kHz Vocalizations of Mice (*Mus musculus*) during Copulation. *Physiology & Behavior* 63(4):467-473. DOI: 10.1016/S0031-9384(97)00484-8.

Wilson DR, Hare JF. 2004. Ground squirrel uses ultrasonic alarms. *Nature* 430 (6999):523-523. DOI: 10.1038/430523a.

Zala SM, Reitschmidt D, Noll A, Balazs P, Penn DJ. 2017. Sex-dependent modulation of ultrasonic vocalizations in house mice (*Mus musculus musculus*). *PLoS ONE* 12(12):e0188647. DOI: 10.1371/journal.pone.0188647.

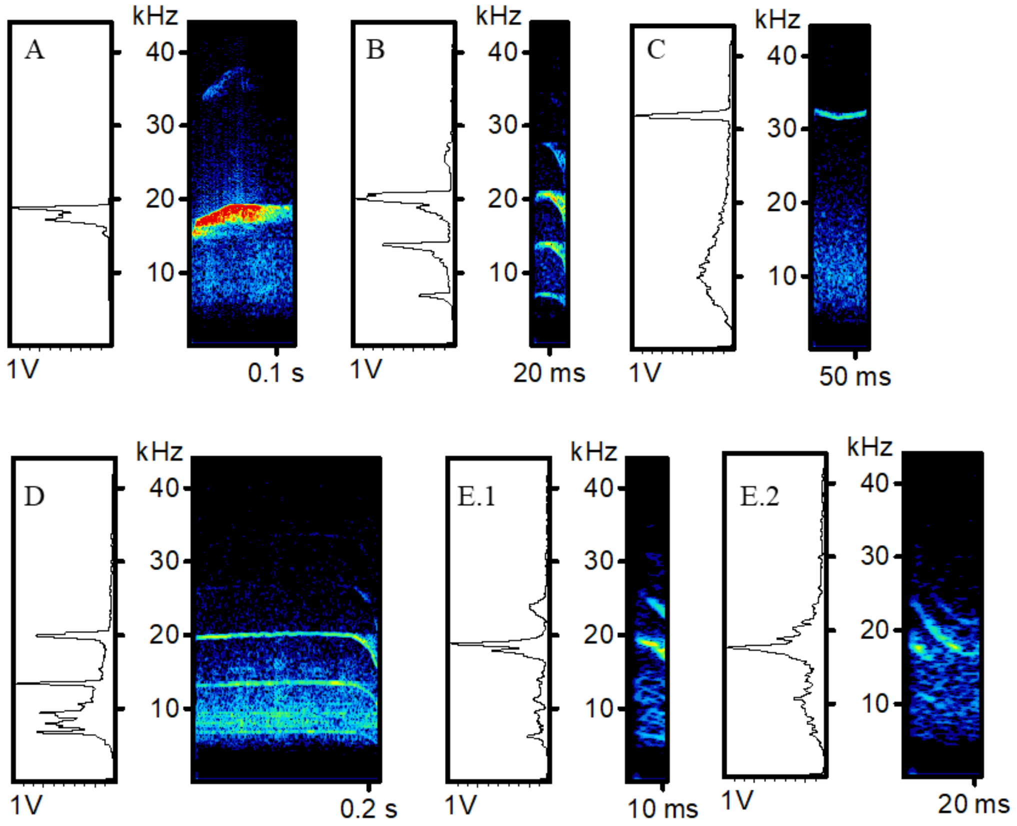

Figure 5.1 Examples of high-frequency vocalizations produced by golden lion tamarins (*Leontopithecus rosalia*). Common marmosets (*Callithrix jacchus*) were housed nearby in connected outdoor enclosures at the Toronto Zoo (ON). (A) Chirp (B) Suppressed Fundamental (c) Ultrasonic (D) Tonal (E & F) Short.

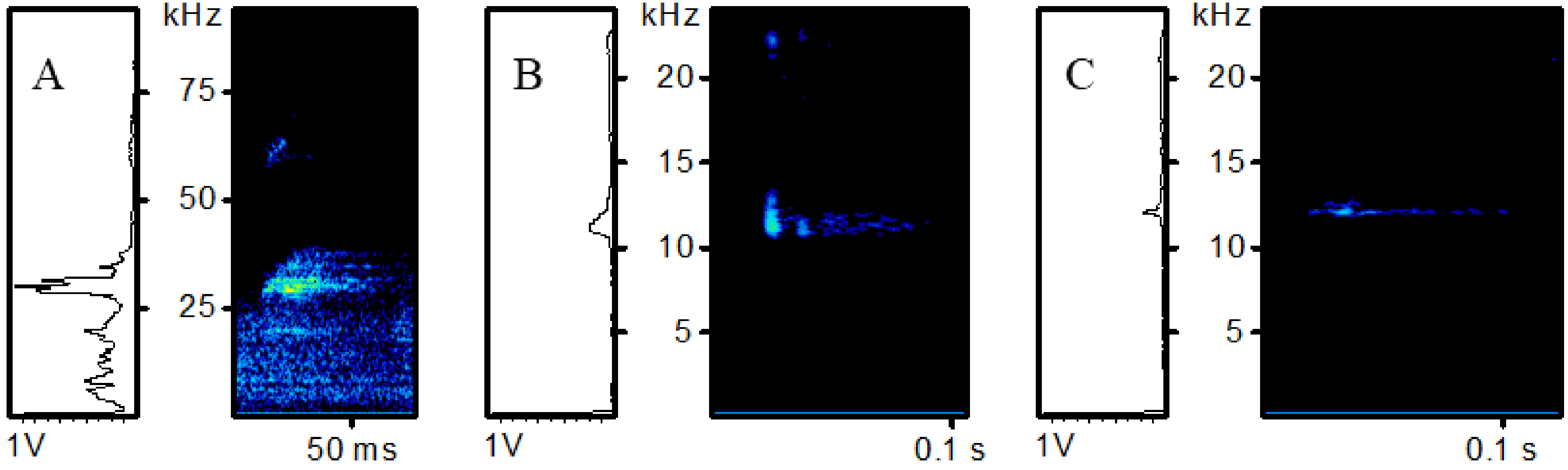

Figure 5.2 Examples of high-frequency vocalizations produced by Vancouver Island marmots (*Marmota vancouverensis*) at the Toronto Zoo (ON). (A) Ultrasonic Chirp (B) Pip (C) Tonal.

Table 5.1: Descriptions of calls from various mammals recorded with ultrasonic microphones at the Toronto Zoo (ON). Duration (s) and frequency (kHz) estimates (x̄ (±SE)) of vocalizations produced by golden lion tamarins (*Leontopithecus rosalia*) (common marmosets (*Callithrix jacchus*) were housed nearby in connected outdoor enclosures) and Vancouver Island marmots (*Marmota vancouverensis*). Frequency estimates were taken from the dominant harmonic if harmonics were present; peak frequencies represent the frequency with the highest energy.

Species	**Call (n)**	**Duration (s)**	**Minimum (kHz)**	**Maximum (kHz)**	**Peak (kHz)**	**Start (kHz)**	**End (kHz)**
Leontopithecus rosalia (*Callithrix jacchus*)	Chirp (11)	0.13 (±0.05) [0.07-0.21]	15.14 (±1.43) [13-17]	20.32 (±0.81) [19-21.5]	18.91 (±0.70) [18-20]	17.09 (±1.58) [14-19.5]	17.77 (±2.18) [13.5-19.5]
	Suppressed Fundamental (15)	0.07 (±0.08) [0.01-0.34]	14.80 (±1.61) [11.5-18.5]	20.53 (±1.54) [18-22.5]	18.73 (±1.57) [16.5-21]	19.07 (±2.27) [14.5-21.5]	15.67 (±2.08) [11.5-20]
	Short (2)	0.02 (±0.01) [0.02-0.03]	15 (±0.71) [14.5-15.5]	22.00 (±2.83) [20-24]	18.00 (±0.71) [17.5-18.5]	21.50 (±3.54) [19-24]	18.75 (±4.60) [15.5-22]
	Tonal (17)	0.19 (±0.08) [0.04-0.33]	13.85 (±1.31) [11.5-16]	19.50 (±1.56) [16-21.5]	17.82 (±1.49) [15.5-20]	18.35 (±1.28) [15-20]	14.41 (±1.50) [11.5-16.5]
	Ultrasonic (4)	0.06 (±0.03) [0.02-0.09]	30.50 (±0.41) [30-31]	33.00 (±0.00) [33-33]	31.50 (±0.41) [31-32]	32.00 (±0.00) [32-32]	32.24 (±0.29) [32-32.5]
Marmota vancouverensis	Pip	0.028	11.8 (±0.93) [10-13]	15.67 (±1.76) [13-19]	14.00 (±1.32) [12-16.5]	13.83 (±1.45) [11.5-16.5]	13.17 (±0.88) [11.5-14.5]
	Tonal	0.08 (±0.02) [0.10-0.03]	9.5	13.5	11.5	11.5	10.5
	Ultrasonic Chirp	0.03	25.5	39	30	29	31

Appendix

Chapter 2 (A1)

Table A1.1 Summary of all references used to build the squirrel vocal range dataset.

Species	Reference	Location	Range (kHz)
Callosciurus caniceps	(Tamura, 1993) (Tamura & Yong, 1993)	Selangor, Malaysia*	1.9 – 4.8 1.4 – 8.0
Callosciurus erythraeus thaiwanensis	(Tamura, 1995)	Kanagawa, Japan	0.09 – 3.3
Callosciurus nigrovittatus	(Tamura & Yong, 1993)	Selangor, Malaysia	1.6 – 8.3
Callosciurus notatus	(Tamura, 1993) (Tamura & Yong, 1993)	Selangor, Malaysia*	0.9 – 8.6 0.8 – 11.1
Callospermophilus lateralis	(Eiler & Banack, 2004)	(California, Nevada), USA	3.7 – 20.1
Callospermophilus saturatus	(Eiler & Banack, 2004)	Washington, USA	6.3 – 21.2
Cynomys gunnisoni	(Waring, 1970)	Colorado, USA	0.9 - 8
Cynomys leucurus	(Waring, 1970)	Colorado, USA	0.1 – 8
Cynomys ludovicianus	(Waring, 1970)	Colorado, USA	0.1 – 8
Glaucomys sabrinus	(Gilley, 2013) (Gilley et al., 2019) (Murrant et al., 2013)	Missouri, USA Missouri, USA Ontario, Canada	8.1 – 24.7 10.9 – 24.9 40 – 65
Glaucomys sabrinus coloratus	(Gilley, 2013)	North Carolina, USA	9.3 – 22.0
Glaucomys volans	(Eisinger, Scheibe & Flaherty, 2016) (Gilley, 2013) (Gilley et al., 2019) (Murrant et al., 2013)	Indiana, USA (Alabama, Missouri, North Carolina), USA North Carolina, USA Ontario, Canada	4 – 42 5.8 – 40.0 15.5 –25.6 19 – 65
Ictidomys mexicanus	(Matocha, 1975)	Texas, USA	2.7 – 5.7
Ictidomys tridecemlineatus	(Matocha, 1975)	Texas, USA	1.4 – 6.0
Marmota bobak	(Nikol'skii, 2007)	(Orenburg, Luhansk, Kharkiv), Ukraine	3.6 – 4.6
Marmota caligata	(Blumstein, 1999) (Taulman, 1973) (Waring, 1966)	Washington, USA Washington, USA Colorado, USA	1 – 6 1 – 3.5 2.5 – 3.5

Table A1.1 *Continued (2/5)*

Species	Reference	Location	Range (kHz)
Marmota flaviventris	(Blumstein & Armitage, 1997)	(Colorado, Utah), USA	1 – 10.5
	(Davis, 1991)	California, USA	2.2 – 4.0
	(Matrosova et al., 2011)	Colorado, USA	2.5 – 4.3
	(Waring, 1966)	Colorado, USA	1.5 – 45
Marmota monax	(Lloyd, 1972)	New York, USA	1.5 – 5
Marmota olympus	(Blumstein, 1999)	Washington, USA	1 – 9
Marmota vancouverensis	(Blumstein, 1999)	British Columbia, USA	1 – 6
Otospermophilus beecheyi	(Leger, Owings & Gelfand, 1980)	California, USA*	3.7 – 14
	(Owings & Leger, 1980)		3.8 – 8.6
	(Owings & Virginia, 1978)		1 – 20
	(Rabin et al., 2003)		3.6 – 6.1
Otospermophilus variegatus	(Krenz, 1977)	Texas, USA	– 7.2
Petaurista lena	(Shen, 2013)	Chiayi, Taiwan, China	1.6 – 12.4
Petaurista leucogenys	(Ando & Kuramochi, 2008)	Japan	0.8 – 12
Sciurus aberti kaibabensis	(Hall, 1981)	Arizona, USA	1 – 4.5
Sciurus carolinensis	(Lishak, 1982)	Alabama, USA*	0.05 – 34
	(Lishak, 1984)		0.2 – 16
Sciurus niger rufiventer	(Zelley, 1971)	Illinois, USA	0.08 – 32
Spermophilus citellus	(Koshev & Pandourski, 2008)	Sofia, Bulgaria	7.1 – 13.7
	(Matrosova et al., 2012)	Prague, Czech Republic	0.4 – 13.7
	(Schneiderová & Policht, 2012)	Prague, Czech Republic	7.7 – 13.5
	(Schneiderová, 2008)	Prague, Czech Republic	0.1 – 77.8
Spermophilus fulvus	(Matrosova et al., 2007)	Saratov, Russia*	2.7 – 6.0
	(Matrosova et al., 2010)		1.9 – 5.5
	(Matrosova et al., 2011)		1.6 – 5.4
	(Matrosova et al., 2012)		0.3 – 5.3

Table A1.1 *Continued (3/5)*

Species	Reference	Location	Range (kHz)
Spermophilus fulvus (Cont.)	(Volodina, Matrosova & Volodin, 2011)	Saratov, Russia	3.6 – 5.4
Spermophilus major	(Brandler, Tukhbatullin & Nikol'skii, 2019)	(Kurgan, Orenburg, Samara, Chelyabinsk), Russia; Republic of Bashkortostan; Republic of Tatarstan; (Aktobe, Kostanay, West K.), Kazakhstan	3.7 – 6.3
Spermophilus pygmaeus	(Nikol'skii, 2007)	Yelansky, Russia	5.0 – 5.6
Spermophilus suslicus	(Matrosova et al., 2007)	Moscow, Russia*	6.3 – 11.5
	(Matrosova et al., 2011)		8.9 – 10.0
	(Matrosova et al., 2012)		0.4 – 8.4
	(Matrosova, Volodin & Volodina, 2006)		0.4 – 11.0
	(Volodin, 2005)		9.0 – 9.8
	(Volodin et al., 2008)		9.3 – 9.7
	(Volodina et al., 2011)		8.7 – 10.0
Spermophilus taurensis	(Schneiderová, 2008)	Antalya, Turkey	4.8 – 8.1
	(Schneiderová & Policht, 2012)	Karaman, Turkey	4.7 – 8.2
Spermophilus xanthoprymnus	(Schneiderová & Policht, 2012)	Kayseri, Turkey	4.0 – 10.7
Tamias alpinus	(Brand, 1976)	California, USA	1.5 – 9.5
Tamias amoenus	(Brand, 1976)	California, USA	1.5 – 11.5
Tamias merriami	(Brand, 1976)	California, USA	1.5 – 15
Tamias minimus	(Bergstrom & Hoffmann, 1991)	Colorado, USA	1.5 – 15
	(Brand, 1976)	California, USA	2 – 10
Tamias ochrogenys	(Gannon & Lawlor, 1989)	California, USA	4.0 – 12.2
Tamias palmeri	(Gannon & Stanley, 1991)	Nevada, USA	2.7 – 11.5
Tamias panamintinus	(Brand, 1976)	California, USA	1.5 – 13.5
Tamias quadrimaculatus	(Brand, 1976)	California, USA	1.5 – 12.5

Table A1.1 *Continued (4/5)*

Species	Reference	Location	Range (kHz)
Tamias quadrivittatus	(Bergstrom & Hoffmann, 1991)	Colorado, USA	1.5 – 16
Tamias senex	(Gannon & Lawlor, 1989)	(Oregon, California), USA	4.6 – 13.0
Tamias siskiyou	(Gannon & Lawlor, 1989)	(Oregon, Washington), USA	3.9 – 14.5
Tamias sonomae	(Brand, 1976)	California, USA	7 – 12.5
Tamias speciosus	(Brand, 1976)	California, USA	1.5 – 13
Tamias striatus	(Burke da Silva, Kramer &Weary, 1994)	Québec, Canada	0.5 – 8.7
	(Dunford, 1970)	New York, USA	1 – 11
	(Elliott, 1978)	New York, USA	1 – 3
Tamias townsendii	(Brand, 1976)	California, USA	1.5 – 14.5
	(Gannon & Lawlor, 1989)	(Oregon, Washington), USA	4.5 – 11.1
Tamias umbrinus	(Bergstrom & Hoffmann, 1991)	Colorado, USA	1 – 15.5
	(Brand, 1976)	California, USA	1.5 – 12.5
Tamiasciurus douglasii	(Smith, 1978)	British Columbia, Canada; (Maine, Maryland), USA	0.5 – 8
Tamiasciurus hudsonicus	(Smith, 1978)	British Columbia, Canada; (Maine, Maryland), USA	0.5 – 8
Urocitellus armatus	(Balph & Balph, 1966)	Utah, USA	2 – 7.5
	(Koeppl, Hoffmann & Nadler, 1978)	Montana, USA	0.5 – 13
Urocitellus beldingi	(Leger, Berney-Key & Sherman, 1984)	California, USA	3.5 – 5.1
	(Robinson, 1980)	Oregon, USA	4 – 7
	(Robinson, 1981)	Oregon, USA	5.9 – 6.9
Urocitellus columbianus	(Betts, 1976)	Montana, USA	~0 – 16
	(Koeppl et al., 1978)	Montana, USA	0.5 – 13
	(Manno et al., 2007)	Alberta, Canada	1.6 – 22.5
Urocitellus elegans	(Fagerstone, 1987)	(Colorado, Wyoming), USA	4.7 – 17
	(Koeppl et al., 1978)	Montana, USA	0.5 – 12
Urocitellus richardsonii	(Fagerstone, 1987)	Alberta, Canada; Montana, USA	4.8 – 16.9
	(Koeppl et al., 1978)	Montana, USA	2 - 12
	(Sloan, Wilson & Hare, 2005)	Manitoba, Canada	3.2 – 6.3
	(Wilson & Hare, 2004)	Manitoba, Canada	22.7– 77.8

Table A1.1 *Continued (5/5)*

Species	Reference	Location	Range (kHz)
Urocitellus richardsonii (Cont.)	(Wilson & Hare, 2006)	Manitoba, Canada	27.2– 56.3
Urocitellus undulatus	(Melchior, 1971)	Alaska, USA	2 – 8
Urocitellus undulatus	(Melchior, 1971)	Alaska, USA	2 – 8
Xerospermophilus spilosoma annectens	(Matocha, 1975)	Texas, USA	3.8 – 6.6
Xerospermophilus spilosoma marginatus	(Matocha, 1975)	Texas, USA	3.7 – 5.4
Xerus inauris	(Furrer & Manser, 2009)	Benede, South Africa	5 - 14

*Indicates that the same location was used in all studies listed.

Table A1.2 Summary of candidate (ΔAICc < 2) secondary phylogenetic generalized least square (PGLS) models of squirrel frequency characteristics (maximum, fundamental, dominant, maximum, and highest harmonic frequencies; kHz). Model parameters are body mass (β_{Mass}), diel pattern of activity (β_{Diel}), sociality (β_{Soc}), habitat openness (β_{Open}), and method detection limits (β_{Lim}). The phylogenetic strength is represented by λ with 95% confidence intervals [lower, upper].

Frequency	Model	ΔAICc	AICc Weight	λ [95% CI]	Effect Size (R^2)
Minimum	$\beta_0 + \beta_{Mass} + \beta_{Lim}$	0	0.20	0.71 [0.21, 0.90]	0.13**
	$\beta_0 + \beta_{Mass} + \beta_{Soc} + \beta_{Lim}$	0.92	0.13	0.67 [0.20, 0.89]	0.14**
	$\beta_0 + \beta_{Mass} + \beta_{Diel} + \beta_{Lim}$	1.65	0.09	0.67 [0, 0.90]	0.13*
	$\beta_0 + \beta_{Mass} + \beta_{Open} + \beta_{Lim}$	1.73	0.08	0.71 [0.24, 0.90]	0.13*
Fundamental	$\beta_0 + \beta_{Mass} + \beta_{Diel} + \beta_{Lim}$	0	0.37	0.28 [0, 0.96]	0.59***
	$\beta_0 + \beta_{Mass} + \beta_{Diel} + \beta_{Open} + \beta_{Lim}$	1.79	0.15	0 [0, 0.95]	0.65***
Dominant	$\beta_0 + \beta_{Mass} + \beta_{Diel} + \beta_{Open} + \beta_{Lim}$	0	0.56	0 [0, 0.32]	0.57***
	$\beta_0 + \beta_{Mass} + \beta_{Diel} + \beta_{Lim}$	1.98	0.21	0.18 [0, 0.72]	0.51***
Maximum	$\beta_0 + \beta_{Mass} + \beta_{Diel} + \beta_{Lim}$	0	0.28	0.30 [0, 0.94]	0.54***
	$\beta_0 + \beta_{Mass} + \beta_{Lim}$	0.49	0.22	0.80 [0.12, 0.96]	0.45***
Highest Harmonic	$\beta_0 + \beta_{Mass} + \beta_{Open} + \beta_{Lim}$	0	0.37	0 [0, 0.52]	0.43***
	$\beta_0 + \beta_{Mass} + \beta_{Diel} + \beta_{Open} + \beta_{Lim}$	1.25	0.20	0 [0, 0.44]	0.42***
	$\beta_0 + \beta_{Mass} + \beta_{Soc} + \beta_{Open} + \beta_{Lim}$	1.89	0.14	0 [0, 0.51]	0.41***
	$\beta_0 + \beta_{Mass} + \beta_{Lim}$	1.97	0.14	0.33 [0, 0.80]	0.38***

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

Table A1.3 An account of licensing permissions for each figure or image used in Figure 1 and 2.

Figure	Original Publisher [Original Author]	Publication [Year]	Original Name	Licensing	Further Details	Link to Original Image
Fig. 2.1a	PLoS ONE [Murrant, M., et al.]	PLoS One [2013]	Figure 1	Open-access journal under the Creative Commons Attribution License	Addition of red labels.	Please refer to (Murrant et al., 2013) in Reference List
Fig. 2.1b	Elsevier [Betts, B.]	Animal Behaviour [1976]	Figure 4	Permissions purchased on [2020-06-17] from Copyright Clearance Center	Original figure cropped to just include 'Soft Chirp' (1 of 6 calls reported); Addition of red labels.	Please refer to (Betts, 1976) in Reference List
Fig. 2.1c	Sofia: Institute of Biodiversity and Ecosystem Research [Koshev, Y. & Pandourski, I]	Acta Zoologica Bulgarica [2008]	Figure 2a	Open-Access Journal from the Bulgarian Academy of Sciences	Original figure cropped slightly to remove border; Addition of red and blue labels.	Please refer to (Koshev & Pandourski, 2008) in Reference List
Fig. 2.1d	Springer Nature [Nikol'skii, A.]	Doklady Biological Sciences [2019]	Figure 1c-d	Permissions purchased on [2020-06-17] from Copyright Clearance Center	Addition of red and blue labels.	Please refer to (Nikol'skii, 2019) in Reference List

Table A1.3 *Continued (2/3)*

Figure	Original Publisher [Original Author]	Publication [Year]	Original Name	Licensing	Further Details	Link to Original Image
Fig. 2.2 *Glaucomys*	Raw Pixel [Audubon, John James]	The Viviparous Quadrupeds of North America [1849]	Plate 28 - Common Flying Squirrel	Creative Commons CC0 1.0 Universal	Originates from Audubon's "The Viviparous Quadrepeds of North America"; Original image cropped.	https://www.rawpixel.com/image/321833/free-illustration-image-john-woodhouse-audubon-squirrel-audubon
Fig. 2.2 *Tamiasciurus*	Getty Images [GlobalIP]	iStock [2008]	Several images of a red squirrel in different poses on white stock photo	Standard license purchased on [2020-06-17]	No further modification of image.	https://www.publicdomainpictures.net/en/view-image.php?image=12181&picture=squirrel-in-snow
Fig. 2.2 *Callosciurus*	Wikimedia Commons [Smit, Joseph]	Zoological Society of London [1971]	*Sciuris quinquestriatus*	Public Domain	No further modification of the image from link provided, but the image has been modified from the original publication[1].	https://commons.wikimedia.org/wiki/File:SciurusQuinquestriatusSmit.jpg
Fig. 2.2 *Marmota*	Wikimedia Commons [De Stefano, Matteo]	MUSE Science Museum [2016]	*Marmota marmota*	Creative Commons 3.0	Image was flipped 180°.	https://commons.wikimedia.org/wiki/File:Marmota_marmota_-_MUSE_2.JPG
Fig. 2.2 *Urocitellus*	PNG ALL [Squirrel PNG Transparent Images]	[2016]	Squirrel Free Download PNG	Creative Commons 4.0 BY-NC	No further modification of image.	http://www.pngall.com/squirrel-png/download/9611

Table A1.3 *Continued (3/3)*

Figure	Original Publisher [Original Author]	Publication [Year]	Original Name	Licensing	Further Details	Link to Original Image
Fig. 2.2 *Tamias*	Getty Images [GlobalIP]	iStock [2010]	Side view of Siberian chipmunk, *Eutamias sibiricus*	Standard license purchased on [2020-06-17]	No further modification of image.	https://www.istockphoto.com/ca/photo/sde-view-of-siberian-chipmunk-euamias-sibiricus-gm119373509-14258937?irgwc=1&esource=AFF_IS_IR_TinEye_77643_&asid=TinEye&cid=IS&utm_medium=affiliate&utm_source=TinEye&utm_content=77643&clickid=35N1iozWkxyOTv1wUx0Mo38WUkiy2LxmXTuaz80
Fig. 2.2 *Xerus*	Wikimedia Commons [Dupont, Bernard]	Flickr [2016]	South African Ground Squirrel (*Xerus inauris*) female	Creative Commons 2.0	Image was cropped to remove background.	https://commons.wikimedia.org/wiki/File:South_African_Ground_Squirrel_(Xerus_inauris)_female_(32469919771).jpg

[1] Anderson J. 1871. On three new species of squirrels from upper Burmah and the Kakhyen Kills, between Burmah and Yunan. In: Longmans, Green, Reader, and Dyer eds. *Proceedings of the Scientific Meetings of the Zoological Society of London.* London: Zoological Society of London, 139-143.

A1 References

Ando M, Kuramochi Y. 2008. Vocal communication of the Japanese giant flying squirrel *Petaurista leucogenys*. *The Journal of Agricultural Science* 53(2):176–83.

Balph DM, Balph DF. 1966. Social communication of Uinta ground squirrels. *Journal of Mammalogy* 47(3):440–50. DOI: 10.2307/1377685.

Bergstrom BJ, Hoffmann RS. 1991. Distribution and diagnosis of three species of chipmunks (*Tamias*) in the front range of Colorado. *The Southwestern Naturalist* 36(1):14–28. DOI: 10.2307/3672112.

Betts BJ. 1976. Behaviour in a population of Columbian ground squirrels *Spermophilus columbianus columbianus*. *Animal Behaviour* 24:652–80.

Blumstein DT. 1999. Alarm calling in three species of marmots. *Behaviour* 6(6):731–57.

Blumstein DT, Armitage KB. 1997. Alarm calling in yellow-bellied marmots: I. The meaning of situationally variable alarm calls. *Animal Behaviour* 53(1):143–71.

Brand L. 1976. The vocal repertoire of chipmunks (genus *Eutamias*) in California. *Animal Behaviour* 24:319–35.

Brandler OV, Tukhbatullin AR, Nikol'skii AA. 2019. Comparative analysis of the alarm call in different age and sexual groups of the russet ground squirrel (*Spermophilus major* Pallas 1778). *Russian Journal of Developmental Biology* 50(4):173–79.

Da Silva KB, Kramer DL, Weary DM. 1994. Context-specific alarm calls of the eastern chipmunk, *Tamias striatus*. *Canadian Journal of Zoology* 72:1087–92.

Davis RO. 1991. Semantical communication in anti-predator alarm calls. In: Hall Jr CA, Doyle-Jones V, Wisawski B, eds. *The Natural History of Eastern California and High Altitude Research.* White Mountain Research Station: University of California, 275-312.

Dunford C. 1970. Behavioral aspects of spatial organization in the chipmunk, *Tamias striatus*. *Behaviour* 36(3):215–31.

Eiler KC, Banack SA. 2004. Variability in the alarm call of golden-mantled ground squirrels (*Spermophilus lateralis* and *S. saturatus*). *Journal of Mammalogy* 85(1):43–50.

Eisinger JW, Scheibe JS, Flaherty EA. 2016. Novel *Glaucomys volans* vocalizations in Indiana and evidence of geographic variation in high frequency communication. *Journal of Mammalogy* 97(4):1219–27.

Elliott L. 1978. Social behavior and foraging ecology of the eastern chipmunk (*Tamias striatus*) in the Adirondack mountains. *Smithsonian Contributions to Zoology* (265):1–107.

Fagerstone KA. 1987. Comparison of vocalizations between and within *Spermophilus elegans elegans* and *S. richardsonii*. *Journal of Mammalogy* 68(4):853–57.

Furrer RD, Manser MB. 2009. The evolution of urgency-based and functionally referential alarm calls in ground-dwelling species. *The American Naturalist* 173(3):400–410.

Gannon WL, Lawlor TE. 1989. variation of the chip vocalizations of the three species of the Townsend chipmunks (genus *Eutamias*).” *Journal of Mammalogy* 70(4):740–53.

Gannon WL, Stanley WT. 1991. Chip vocalization of Palmer’s chipmunk (*Tamias palmeri*). *The Southwestern Naturalist* 36(3):315–17.

Gilley LM. 2013. Discovery and characterization of high-frequency calls in North American flying squirrels (*Glaucomys sabrinus* and *G. volans*): implications for ecology, behavior, and conservation. D. Phil. Thesis, Auburn University.

Gilley LM, Diggins CA, Pearson SM, Best TL. 2019. Vocal repertoire of captive northern and southern flying squirrels (*Glaucomys sabrinus* and *G. volans*). *Journal of Mammalogy* 100(2):518–530 DOI: 10.1093/jmammal/gyz064.

Hall JG. 1981. A field study of Kaibab squirrel in Grand Canyon National Park. *Wildlife Monographs* 75:3–54.

Koeppl JW, Hoffmann RS, Nadler CF. 1978. Pattern analysis of acoustical behavior in four species of ground squirrels. *Journal of Mammalogy* 59(4):677–96.

Koshev Y, Pandourski I. 2008. Structure and variability of alarm calls of European ground squirrel *Spermophilus citellus* L. 1766 (Mammalia: Rodentia) from western Bulgaria. *Acta Zoologica Bulgarica* 60(1):99–105.

Krenz MC. 1977. Vocalizations of the rock squirrel (*Spermophilus variegatus*). M. Sc. Thesis, Texas Tech University.

Leger DW, Berney-Key SD, Sherman PW. 1984. Vocalizations of Belding’s ground squirrels (*Spermophilus beldingi*). *Animal Behaviour* 32(3):753–64.

Leger DW, Owings DH, Gelfand DL. 1980. Single-note vocalizations of California ground squirrels: graded signals and situation-specificity of predator and socially evoked calls. *Zeitschrift Für Tierpsychologie* 52(3):227–46.

Lishak RS. 1982. Gray squirrel mating calls : a spectrographic and ontogenic analysis. *Journal of Mammalogy* 63(4):661–63.

Lishak RS. 1984. Alarm vocalizations of adult gray squirrels. *Journal of Mammalogy* 65(4):681–84.

Lloyd JE. 1972. Vocalization of *Marmota monax*. *Journal of Mammalogy* 53(1):214–16.

Manno TG, Nesterova AP, Debarbieri LM, Kennedy SE, Wright KS, Dobson FS. 2007. Why do male Columbian ground squirrels give a mating call ? *Animal Behaviour* 74:1319–27.

Matocha KG. 1975. Vocal communication in ground squirrels, genus *Spermophilus*. D. Phil. Thesis, Texas Tech University.

Matrosova VA, Blumstein DT, Volodin IA, Volodina EV. 2011. The potential to encode sex, age, and individual identity in the alarm calls of three species of Marmotinae. *Naturwissenschaften* 98(3):181–92.

Matrosova VA, Schneiderová I, Volodin IA, Volodina EV. 2012. Species-specific and shared features in vocal repertoires of three Eurasian ground squirrels (genus *Spermophilus*). *Acta Theriologica* 57:65–78.

Matrosova VA, Volodin IA, Volodina EV. 2006. The diversity of calls produced by live-trapped speckled ground squirrels *Spermophilus suslicus* (Rodentia, Sciuridae) (in Russian). *Bulletin of Moscow Society of Naturalists* 111(5):84–87.

Matrosova VA, Volodin IA, Volodina EV, Vasilieva NA. 2010. Stability of acoustic individuality in the alarm calls of wild yellow ground squirrels *Spermophilus fulvus* and contrasting calls from trapped and free-ranging callers. *Naturwissenschaften* 97(8):707–15.

Matrosova VA, Volodin IA, Volodina EV, Babitsky AF. 2007. Pups crying bass: vocal adaptation for avoidance of age-dependent predation risk in ground squirrels? *Behavioral Ecology and Sociobiology* 62:181–91.

Melchior HR. 1971. Characteristics of arctic ground squirrel alarm calls. *Oecologia* 7(2):184–90.

Murrant MN, Bowman J, Garroway CJ, Prinzen B, Mayberry H, Faure PA. 2013. Ultrasonic vocalizations emitted by flying squirrels. *PLoS ONE* 8(8):1–6.

Nikol'skii AA. 2007. A comparative analysis of the alarm call frequency in different age rodent groups (in Russian). *Zoologicheskii Zhurnal* 86(4):499–504.

Owings DH, Virginia RA. 1978. Alarm calls of California ground squirrels. *Zeitschrift Fur Tierpsychologie* 46:58–70.

Owings DH, Leger DW. 1980. Chatter vocalizations of California ground squirrels: predator- and social-role specificity. *Zeitschrift Für Tierpsychologie* 54(2):163–84.

Rabin LA, Mccowan B, Hooper SL, Owings DH. 2003. Anthropogenic noise and its effect on animal communication: an interface between comparative psychology and conservation Biology. *International Journal of Comparative Psychology* 16(2):172–92.

Robinson SR. 1980. Antipredator behaviour and predator recognition in Belding's ground squirrels. *Animal Behaviour* 28(3):840–52.

Robinson SR. 1981. Alarm communication in Belding's ground squirrels. *Zeitschrift Für Tierpsychologie* 56(2):150–68.

Schneiderová I. 2008. Acoustics communication and alarm calls in two species of ground squirrels *Spermophilus citellus* and *Spermophilus taurensis* (Sciuridae; Rodentia). D. Phil. Thesis, Karlov University in Prague.

Schneiderová I, Policht R. 2012. Acoustic analysis of the alarm call of the Anatolian ground squirrel *Spermophilus xanthoprymnus*: a description and comparison with alarm calls of the taurus *S. taurensis* and European *S. citellus* ground squirrels. *Naturwissenschaften* 99(1):55–64.

Shen P-S. 2013. Acoustic behavior of white-faced flying squirrel (*Petaurista lena*) in Guanghua Village, Alishan. M. Sc. Thesis, National Sun Yat-sen University.

Sloan JL, Wilson DR, Hare JF. 2005. Functional morphology of Richardson's ground squirrel, *Spermophilus richardsonii*, alarm calls: the meaning of chirps, whistles, and chucks. *Animal Behaviour* 70:937–44.

Smith CC. 1978. Structure and function of the vocalizations of tree squirrels (*Tamiasciurus*). *Journal of Mammalogy* 59(4):793–808.

Tamura N. 1993. Role of sound communication in mating Malaysian *Callosciurus*

(Sciuridae). *Journal of Mammalogy* 74(2):468–76.

Tamura N. 1995. Postcopulatory mate guarding by vocalization in the Formosan squirrel. *Behavioral Ecology and Sociobiology* 36(6):377–86.

Tamura N, Yong H-S. 1993. Vocalizations in response to predators in three species of Malaysian *Callosciurus* (Sciuridae). *Journal of Mammalogy* 74(3):703–14.

Taulman JF. 1973. Vocalizations of the hoary marmot, *Marmota caligata*. *Journal of Mammalogy* 58(4):681–83.

Volodin IA, Volodina EV, Matrosova VA, Savinetskaya LE, Shekarova ON, Voytsik VA. 2008. Population density does not affect the alarm call characteristics in the speckled ground squirrel (*Spermophilus suslicus*). *Lynx (Praha), New Series* 39(2):333–42.

Volodin I. 2005. Individuality of alarm calls in the spotted suslik (*Spermophillus suslicus,* Rodentia, Sciuridae). *Zoological Journal* 84(2):228–35.

Volodina EV, Matrosova VA, Volodin IA. 2011. An unusual effect of maturation on the alarm call fundamental frequency in two species of ground squirrels. *Bioacoustics* 20(1):87–98.

Waring GH. 1966. Sounds and communications of the yellow-bellied marmot (*Marmota flaviventris*). *Animal Behaviour* 14:177–83.

Waring GH. 1970. Sound communications of black-tailed, white-tailed, and Gunnison’s prairie dogs. *The American Midland Naturalist* 83(1):167–85.

Wilson DR, Hare JF. 2004. Ground squirrel uses ultrasonic alarms. *Nature* 430:523.

Wilson DR., Hare JF. 2006. The adaptive utility of Richardson’s ground squirrel (*Spermophilus richardsonii*) short-range ultrasonic alarm signals. *Canadian Journal of Zoology* 84:1322–30.

Zelley RA. 1971. The sounds of the fox squirrel, *Sciurus niger rufiventer*. *Journal of Mammalogy* 52(3):597–604.

Chapter 3 (A2)

Methods

Captive Sugar Gliders

Recordings We recorded a group of captive sugar gliders (*Petaurus breviceps*) from March 9-11th, 2021, in Oshawa, Ontario. The group was composed of 20 breeding pairs housed in separate enclosures within the same room; 3 pups were also present at the time of recording (a young male and female with one breeding pair and an older female with another pair). The group is cared for by a breeder (adhering to the Toronto Municipal Code (City of Toronto 2023)) who provided food and water during the recording period. We suspended an omnidirectional SMM-U1 ultrasonic microphone (Wildlife Acoustics) above the enclosures approximately 1-4m from the end of the microphone. We connected the microphone to a Song Meter SM4BAT FS ultrasonic recorder (gain = 12dB, sampling rate = 192kHz, 16-bit resolution, frequency response = 10-140kHz; Wildlife Acoustics); we set the recorder to be armed 24hrs/day and to a minimum trigger frequency of 6kHz over a 3sec window. Our recordings were non-invasive, aligning with the Animals Research Act (Government of Ontario, 1990) and operating as an extension of Trent University animal care protocol 25873.

Analyses The resulting WAV files (10, 871 total) were analyzed using Avisoft SASLab Pro (Specht, 2017). Because of the large volume of files produced, we first classified sounds manually in a small subset of files; we then validated these sound classes and used them to classify the remaining files automatically. For the initial manual classification, 432 recordings were selected from low (1900hrs = 49 files), medium (1700hrs = 82 files, 2000hrs = 65 files, 2100hrs = 66 files), and high (2200hrs = 170 files) activity hours: the sugar gliders were housed in the same room as the building's central air unit, and therefore there was a constant low-amplitude broadband noise between 7.5 and 23.2kHz (dominant frequency = 20.2kHz) with a harmonic between 38.2 and 45.7kHz (dominant harmonic frequency = 42.7kHz) that was continuously recorded by the recording unit resulting in many otherwise empty files. Therefore, we defined activity levels by the proportion of WAV files with sounds detected by Avisoft relative to ambient noise. Low activity hours had <25% WAV files with detectable sounds, high activity hours had >75%, and medium activity levels were between 25% and 75%. We used Avisoft SAS Lab Pro to identify and manually measure sound events (FFT = 512, resolution = 8Hz, overlap = 75%, window = Hamming, resolution 8Hz/32ms). This initial dataset contained 2686 sound events, which we manually classified into three categories: vocalizations, non-vocal animal sounds (sniffing and eating), and environmental noises (wheel, cage noises, unknown). We further categorized vocalizations as bark, breathing, broadband-burst, chirp, high-frequency, ultrasonic, and whistle based on key element differences (duration, peak frequency, etc.; Fig. A2.1). We selected representative calls from each category for templates in the remaining steps.

Captive Springhares

Two springhares (*Pedetes capensis*) were non-invasively recorded at the Prague Zoo over 13 days from January 31st to February 27th, 2013. They were housed with a group of Senegal bush babies (*Galago senegalensis*), the target acoustic species for a previous study (Schneiderová et al., 2016). Both species were recorded with a Marantz PMD 662 recorder (sampling rate = 44.1kHz) protected in wire mesh. An observer would simultaneously observe the animals from the visitor's area, and calls of springhares could be easily identified as the animals occasionally produced them when the researchers entered their enclosure to set up the recorder. We manually identified springhare vocalizations (as described with captive sugar gliders above) and characteristics were automatically measured in Avisoft SAS Lab Pro (Specht, 2017). During our work, we adhered to the "Guidelines for the treatment of animals in behavioural research and teaching" (Morgan and Tromborg, 2007) and the laws of the Czech Republic, the country where the research was conducted.

Free-Ranging Marsupials

Representative recordings of free-ranging marsupials (*Petarus australis, P. breviceps, P. norfolcensis,* and *Pseudocheirus peregrinus*) calls were provided by M. Anderson from Wild Ambience (Anderson, 2022). Recordings were made using two Audio Technica AT4022 omnidirectional microphones attached to a Fostex FR2-LE Field Recorder (sampling rate = 44.1kHz), which was opportunistically deployed in various regions of Australia. *Petaurus australis* and *P. norfolcensis* were recorded in Benarkin State Forest, *P. australis* was also recorded at Twelve Mile Camp. *Petaurus breviceps* and *Pseudocheirus peregrinus* were recorded in Dunn's Swamp in Wollemi State Park. *Petaurus norfolcensis* were recorded in the September 2018 and the other species were recorded in November 2018. Anderson non-invasively records animals by using drop rigs that are left out overnight over several recording days; he opportunistically takes only the clearest calls corresponding to animals being closer to the rig. All of Anderson's recordings adhere to the 2014 Australian Nature Conservation Act (Australian Capital Territory 2022). Calls were manually identified (as described with captive sugar gliders above) and manually measured in Avisoft SAS Lab Pro (Specht, 2017).

Photoshop Methods

Images captured under ultraviolet (UV) conditions with the gel filter were colour-corrected to reduce the impact of visible light caused by the UV flashlight (blue-purple) and tinted gel filter (yellow). However, the combined effect was an increase in artificial cyan colouration that was not visible in normal UV conditions and was an artifact of the photo methodology. To remove this superficial cyan, we used Photoshop (Adobe Inc., 2019) to manually colour-correct the photos; we developed a simple method that was replicable with over 100 pictures in this publication and could be implemented as a standard in future studies focused on macro-analyses on UVP (i.e., without spectroscopy or multispectral imaging (Toussaint et al., 2023)):

1. Remove Cyan: We used the Hue/Saturation function to remove all cyan by selecting ‘Cyans’ from the Default menu and sliding the Saturation bar to the far left.*
2. White Balance: The resulting image from step one has an over-saturation of greys. We used the Levels function to manually correct this by selecting the grey dropper and clicking on the gray colour card that we placed in all of the photographs (included on the scale bar, printable at: https://smallpond.ca/jim/scale/).**

*We acknowledge that this also removes some blue from species exhibiting blue/green fluorescence under normal conditions; however, species with strong blue/green photoluminescence still retain green colouration in the final image (see *Sicista subtilis*). Therefore, the resulting four photographs (white light, UV, UV + yellow, edited) should be considered as a set to minimize the bias of other visible colours generated in this process.

Results

Captive Sugar Gliders

We recorded 275 vocalizations over three recording days; we identified six novel vocalization types (Fig. A2.1, Table A2.3), one of which was purely ultrasonic (peak frequency (kHz; ±SE) = 37.15 (±2.76); Fig. A2.1D). Barks (16.01 (±1.77); Fig. SA2.1A) and broadband calls (23.74 (±4.73); Fig. A2.1B) also partially extended into the ultrasonic range while high-frequency calls (19.02 (±2.33); Fig. A2.1C), whistles (15.30; Fig. A2.1E), and sniffing (13.10 (±2.12); Fig. A2.1F) were sonic calls. Given that the ultrasonic microphones can distort calls under 20kHz, the data for high-frequency calls, whistles, and sniffing may not reflect reality and therefore, their true frequency ranges should be confirmed with sonic microphones. Additionally, the minimum frequency for barks and broadband bursts should be confirmed with sonic microphones. We did not include ultrasonic calls in further analyses as we could not remove pups from the recording space and many mammalian young can produce ultrasonic isolation calls that are lost, or modified, later in life. However, the high amplitude of barks and broadband suggest that these were produced by adults that were not confined to the nests as the pups were (hence, likely resulting in the very soft amplitudes of the ultrasonic calls).

Captive Springhares

We identified 105 vocalizations produced by the pair of springhares (Table A2.4). All calls were similar in duration (1.32s (±0.47)) and peak frequency (0.19 (±0.04)); we assigned this call type as ‘growl’ (Fig. A2.2E). Springhares were observed producing growls during mild arousal and growls were often paired with stomping of their hindlimbs.

Free-Ranging Marsupials

We identified 8 novel call types across these four species (Table A2.4), including yap vocalization in the sugar glider (*Petaurus breviceps*), which was not detected using ultrasonic recording equipment with captive individuals (Fig. A2.2B; Table A2.3). All calls described have low peak frequencies (<3kHz) and vary from the relatively short belch produced by *Petaurus norfolcensis* (0.16s (±0.04), Fig. A2.2D) to the long cry of *Petaurus australis* (4.02s (±0.51), Fig. A2.2E).

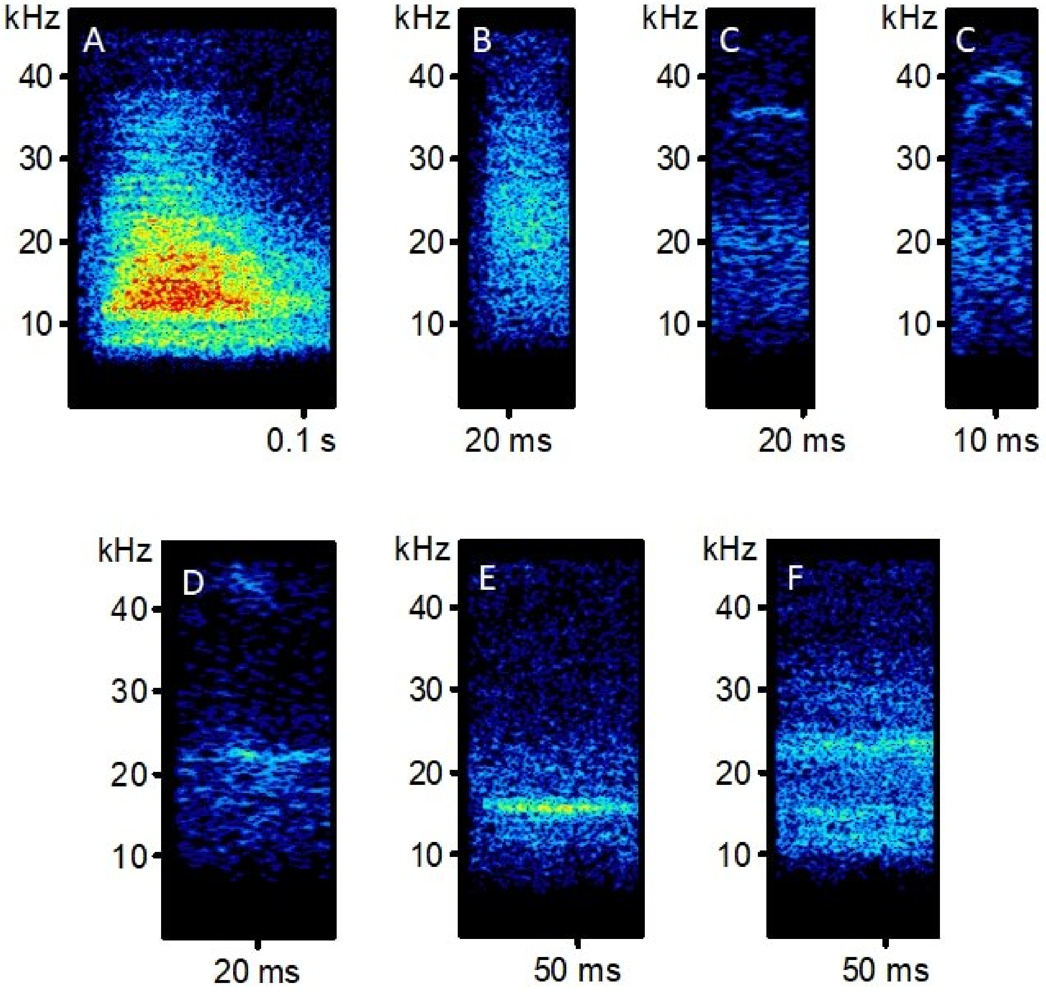

Figure A2.1 Examples of captive sugar glider (*Petaurus breviceps*) call types recorded in Oshawa, ON, during March 2021. 20 breeding pairs and 3 juveniles were recorded on a Song Meter SM4BAT FS. (A) Bark (B) Broadband Burst (C) Ultrasonic (D) High Frequency (E) Whistle (F) Sniffing.

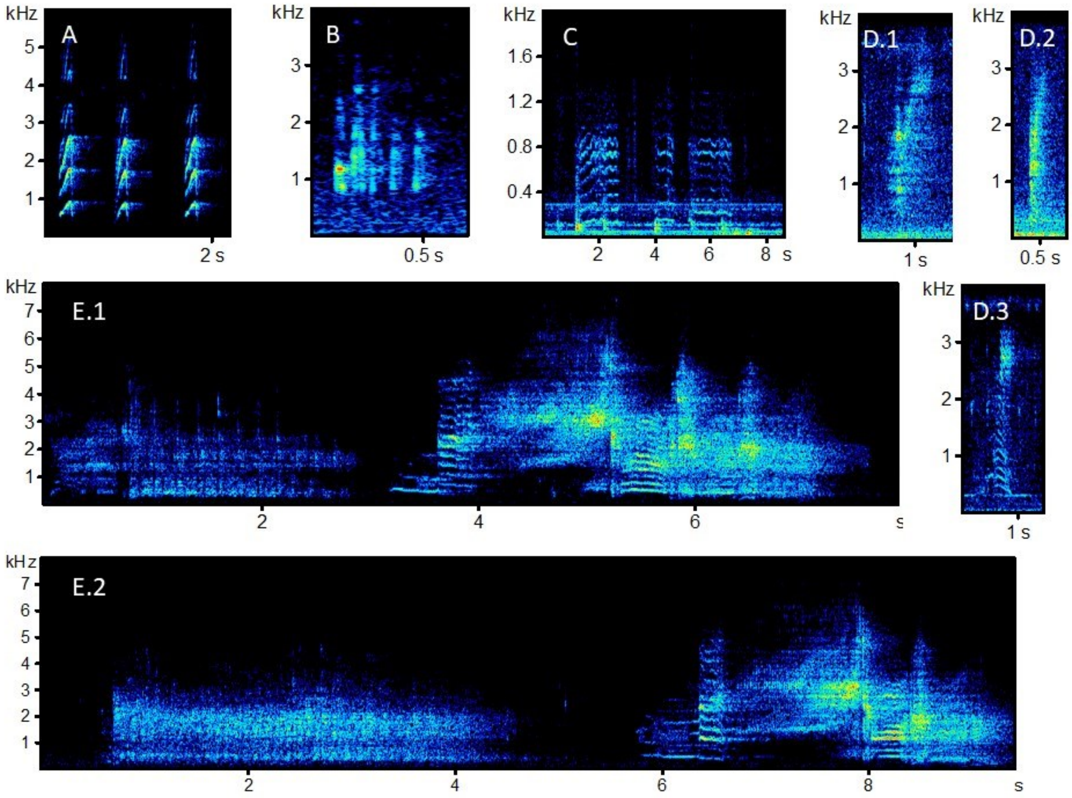

Figure A2.2 Spectrogram examples of calls from various mammals recorded with sonic microphones. Captive springhares (*Pedetes capensis*) were recorded at the Prague Zoo; all other mammals are free-ranging marsupials recorded in different regions of Australia via drop-rig microphones. (A) *Petaurus breviceps* (Yap). (B) *Pseudocheirus peregrinus* (Twitter). (C) *Pedetes capensis* (Growl). (D) *Petaurus norfolcensis* (1: Nasal Grunt, 2: Belch, 3: Higher Nasal Grunt). (E) *Petaurus australis* (1: Gurgle and Cry, 2: Rattle and Cry).

Table A2.1 Summary of references used for the frequency range, ultraviolet-induced photoluminescence, and other traits. Summary of references for the frequency ranges (minimum (kHz; the minimum frequency of the dominant harmonic) – maximum (kHz; the maximum frequency of the dominant harmonic)) from novel species (n = 4; methods and complete results in Info. S1) and literature (n = 69), presence of ultraviolet-induced photoluminescence (UVP; colours provided when possible) from our novel investigation (n = 57; methods provided in Info. S1 (Incon. = Inconclusive, sample sizes provided in brackets)) and previous literature (n = 25), and ecological traits (body mass (g), sociality (social: stable or cyclical social structures of multiple individuals (colonial, congregated single-burrow systems, monogamous, etc.); solitary: stable solitary lifestyle), diel activity patten (Diel A.P.; diurnal: active during the day; nocturnal: active during the night), and habitat openness (openness of dominant habitat type; closed: closed canopy, taller vegetation; open: open canopy, shorter vegetation) of gliding mammals (n = 15) and their close relatives (n = 77). Bolded species indicate those that were opportunistically sampled from museum collections.

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
Marsupials								
Acrobates pygmaeus	(Martin, 2019) (Reinhold, 2023)	0.9-39	Pink	13	Yes	Social	Nocturnal	Closed
Ailurops ursinus	(Bool et al., 2021)	0.19-13.5		7000	No	Social	Diurnal	Closed
Bettongia leuseur			No (1)[1]	1100	No	Social	Nocturnal	Open
Dactylopsila trivirgata	(Volodin, 2002) (Reinhold, 2021) (Reinhold, 2023)	2-3.5	Blue/White Blue/White	407.5	No	Social	Nocturnal	Closed
Dasyurus geoffroyi			White (1)[1]	1100	No	Solitary	Nocturnal	Closed
Dasyrus hallucatus	(Dempster, 1994) (Reinhold, 2023)	0.4-2	Pink/Orange/Yellow/Green	580	No	Solitary	Nocturnal	Closed
Didelphis virginiana			Pink/Blue (2)[1]	3950	No	Solitary	Nocturnal	Closed

Table A2.1 *Continued (2/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
			Marsupials (Continued)					
***Didelphis virginiana* (Cont.)**	(Meisner, 1983)		Yes					
	(Pine et al., 1985)		No					
	(Tumlison & Tumlison, 2021)		Pink/Blue					
Marmosa mexicana			Pink (1)[1]	67	No	Solitary	Nocturnal	Closed
	(Pine et al., 1985)		Orange					
Marmosa murina			Pink (1)[1]	53.86[2]	No	Solitary	Nocturnal	Closed
	(Toussaint et al., 2023)		Pink/Red					
Micoureus demerarae			Pink (1)[1]	108.21	No	Solitary	Nocturnal	Closed
Perameles nasuta	(Reinhold, 2021)		Pink	801.5[2]	No	Solitary	Nocturnal	Closed
	(Reinhold, 2023)		Pink/ Yellow/ Blue/ White					
Petaroides volans	(Reinhold, 2023)		White/Grey	1250	Yes	Solitary	Nocturnal	Closed
Petaurus australis	**Supp. B**	0.25-4.63	No (1)[1]	572.5[2]	Yes	Social	Nocturnal	Closed
	(Kavanagh & Rohan-Jones, 1982)	0.7-6.5						
	(Whisson et al., 2021)	0.5-4.5						
Petaurus breviceps	**Supp. B**	1.18-43.76		123.38	Yes	Social	Nocturnal	Closed
Petaurus norfolcensis	**Supp. B**	0.18-3.05		245	Yes	Social	Nocturnal	Closed
Petaurus notatus			Incon. (1)[1]	128.5	Yes	Social	Nocturnal	Closed
	(Reinhold, 2021)		Blue/White					

Table A2.1 *Continued (3/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
Marsupials (Continued)								
Petaurus notatus (Cont.)	(Reinhold, 2023)		Pink/Blue/ White					
Philander opossum			No (1)[1]	437	No	Solitary	Nocturnal	Closed
	(Pine et al., 1985)		Pink/ Lavender/ Yellow					
Pseudocheirus peregrinus	**Supp. B**	0.46-3.25		700	No	Social	Nocturnal	Closed
	(Reinhold, 2023)		Green/White					
Trichosurus vulpecula	(Signal, Foster & Temple, 2001)	0.5-12	Incon. (2)[1]	2850	No	Solitary	Nocturnal	Closed
	(Bolliger, 1944)		Pink/Blue					
	(Reinhold, 2023)		Pink/Orange/ Blue					
Primates and Relatives								
Aotus trivirgatus	(Moynihan, 1964)	0.2-4.2*	No (1)[1]	1100[2]	No	Social	Nocturnal	Closed
Arctocebus calabarensis			White (1)[1]	365.5	No	Solitary	Nocturnal	Closed
Avahi laniger	(Stranger & Macedonia, 1994)	3.8-4	No (1)[1]	950	No	Social	Nocturnal	Closed
Callithrix jacchus	(Epple, 1968)	0.5-15*	No (1)[1]	330	No	Social	Diurnal	Closed
	(Kato et al., 2014)	2.28-10.37						
	(Bezerra & Souto, 2008)	0.25-21.96						
	(Winter, 1978)	5.5-10.5						
Callithrix penicillata	(Santos et al., 2017)	5.5-10	No (2)[1]	380.5	No	Social	Diurnal	Closed

Table A2.1 *Continued (4/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
			Primates and Relatives (Continued)					
Euoticus elegantulus			No (1)[1]	315	No	Social	Nocturnal	Closed
Galago moholi	(Anderson et al., 2000)	0.86-2.41		196.5[2]	No	Social	Nocturnal	Closed
Galago senegalensis	(Zimmermann, 1985)[3]	0.26-13.8	No (1)[1]	197.5	No	Social	Nocturnal	Closed
Galeopterus variegatus	(Miard et al., 2019)	37.4-39.2	Incon. (1)[1]	1450	Yes	Solitary	Nocturnal	Closed
Lagothrix lagothricha	(Casamitjana, 2002) (León et al., 2014)[3]	4.45-5.41 0.5-5.5	No (1)[1]	7375[2]	No	Social	Diurnal	Closed
Leontocebus fuscicollis	(Moody & Menzel, 1976) (Zimmermann et al., 2000)	1.5-5.5 11.7-17.5	No (1)[1]	332[2]	No	Social	Diurnal	Closed
Microcebus murinus	(Scheumann, Linn & Zimmermann, 2017) (Zimmermann et al., 2000)	10-30 12-15.6*	Pink (1)[1]	60	No	Social	Nocturnal	Closed
Microcebus rufus	(Zimmermann et al., 2000)	14.5-32.1		50	No	Social	Nocturnal	Closed
Microcebus sambiranensis	(Hending et al., 2017)	0.93-24.5		33.25	No	Social	Nocturnal	Closed
Otolemur garnettii	(Rosti et al., 2020)[3]	0.23-1.71	Incon. (1)[1]	764[2]	No	Social	Nocturnal	Closed
Paragalago cocos	(Butynski et al., 2006)	0.77-1.2		144[2]	No	Social	Nocturnal	Closed

Table A2.1 *Continued (5/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
				Primates and Relatives (Continued)				
Paragalago granti	(Butynski et al., 2006)	0.75		150.5	No	Social	Nocturnal	Closed
Paragalago zanzibaricus	(Butynski et al., 2006)	0.75-1		145	No	Social	Nocturnal	Closed
Plecturoebus moloch	(Moynihan, 1966) (Robinson, 1979)	0.2-7* 0.14-6.15	No (1)[1]	804	No	Social	Diurnal	Closed
Saimiri sciureus	(Boinski & Mitchell, 1995) (Boinski & Mitchell, 1997) (Winter, Ploog & Latta, 1966)	0.5-2.5 0.5-9.52 0.2-18	No (1)[1]	925	No	Social	Diurnal	Closed
Tarsius syrichta	(Ramsier et al., 2012) (Řeháková-Petru et al., 2012)[3]	67-79 1.31-22.44		125	No	Social	Nocturnal	Closed
Tarsius tarsier	(Burton & Nietsch, 2010) (Gursky, 2015) (Nietsch, 1999)	1.2-16.6 20-80 3.4-16.9	No (1)[1]	116[2]	No	Social	Nocturnal	Closed
Tupaia belangeri	(Binz & Zimmermann, 1989)	0.2-14.4	No (1)[1]	150	No	Social	Diurnal	Closed
				Rodents				
Anomalurus derbianus			No (2)[1]	700	Yes	Social	Nocturnal	Closed
Callosciurus caniceps	(Tamura, 1993)	1.71-5.44	No (1)[1]	221.1[2]	No	Solitary	Diurnal	Closed

Table A2.1 *Continued (6/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
				Rodents (Continued)				
Callosciurus caniceps (Cont.)	(Tamura & Yong, 1993)	1.43-7.96						
Callospermophilus lateralis	(Eiler & Banack, 2004)	4.37-10.63	No (2)[1,3]	168.8[2]	No	Social	Diurnal	Closed
Cynomys gunnisoni	(Ackers & Slobodchikoff, 1999)	0.70-6.23	No (2)[1,3]	730[2]	No	Social	Diurnal	Open
	(Loughry et al., 2019)	0.38-1.19						
	(Loughry, Oeser & Hoogland, 2019)	0.39-1.18						
	(Slobodchikoff et al., 2012)	0.5-4						
	(Perla & Slobodchikoff, 2022)	3.6-4.6						
	(Waring, 1970)	0.1-4						
Cynomys ludovicianus	(Shannon et al., 2020)	1.15-1.98	Incon. (3)[1,3]	862[2]	No	Social	Diurnal	Open
	(Waring, 1970)	0.1-4						
	(Wilson-Henjum et al., 2019)	2.00-4.09						
Eliomys quercinus	(Nummert et al., 2023)		Pink/Blue	82.5	No	Social	Nocturnal	Closed

Table A2.1 *Continued (7/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
			Rodents (Continued)					
Glaucomys sabrinus	(Gilley, 2013)[3]	8.12-28.72	Pink (4)[1,3], Blue (>10)[4]	141.6[2]	Yes	Social	Nocturnal	Closed
	(Gilley et al., 2019)	10.89-24.89						
	(Murrant et al., 2013)	20-80						
	(Kohler et al., 2019)		Pink/Red, Blue					
Glaucomys volans	(Eisinger, Scheibe & Flaherty, 2016)	4-42	Pink (4)[1,3], Blue (>10)[4]	55.4[2]	Yes	Social	Nocturnal	Closed
	(Gilley, 2013)	5.84-40.05						
	(Gilley et al., 2019)	15.55-25.62						
	(Murrant et al., 2013)	19-80						
	(Kohler et al., 2019)		Pink/Red, Blue					
Hylopetes spadiceus	(Touissant et al., 2023)		Pink	65	Yes	Solitary	Nocturnal	Closed
Ictidomys tridecemlineatus	(Matocha, 1975)	1.40-5.93	No (2)[1,3]	270	No	Solitary	Diurnal	Open
	(Matocha, 1977)	4.16-5.92						
Marmota flaviventris	(Blumstein & Armitage, 1997)	1-4.25*	White (2)[1]	3350[2]	No	Social	Diurnal	Open
	(Davis, 1991)	2.24-4.03						

Table A2.1 *Continued (8/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
			Rodents (Continued)					
Marmota flaviventris (Cont.)	(Matrosova et al., 2011)	2.49-4.3						
	(Waring, 1966)	1.5-5						
Marmota vancouverensis	(Blumstein, 1999)	1-3.8	Incon. (1)[1]	6250[2]	No	Social	Diurnal	Open
Melomys cervinipes	(Reinhold, 2021)		Blue/White	72.9	No	Solitary	Nocturnal	Closed
Mus musculus	(Grimsley et al., 2016)	12-64.6	No (5)[1]	21	No	Social	Nocturnal	Open
	(Hoffmann, Musolf & Penn, 2012)[3]	45.60-109.79						
	(Lupanova & Egorova, 2015)	1.8-88						
	(Musolf et al., 2015)[3]	69.75-74.69						
	(Nyby, 1983)	70						
	(Von Merten et al., 2015)[3]	65.3-83.6						
	(White et al., 1998)	40-70						
	(Zala et al., 2017)[3]	51.22-69.81						
	(Tumlison & Tumlison, 2021)		No					
Onychomys leucogaster	(Hafner & Hafner, 1979)	10.9-11.8	Pink (5)[1]	32.5	No	Solitary	Nocturnal	Open

Table A2.1 *Continued (9/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
				Rodents (Continued)				
Onychomys torridus	(Miller & Engstrom, 2012)	10.0-11.8						
	(Hafner & Hafner, 1979)	12.8	Pink/White (3)^{1}	22	No	Solitary	Nocturnal	Open
Otospermophilus beecheyi	(Leger, Owings & Gelfand, 1980)	3.29-4.11	White (2)^{1,3}	605.1^{2}	No	Social	Diurnal	Open
	(Owings & Leger, 1980)	2-13						
	(Owings & Virginia, 1978)	2-12						
	(Rabin et al., 2003)	3.36-6.15						
Otospermophilus variegatus	(Krenz, 1977)	0.05-7	White (1)^{1}	835.5^{2}	No	Social	Diurnal	Open
Pedetes capensis	**Supp. B**	0.16-0.21		3500	No	Social	Nocturnal	Open
	(Olson et al., 2021)		Pink/Blue					
Peromyscus californicus	(Miller & Engstrom, 2012)	10.8-20.2	White (3)^{1}	43.8	No	Social	Nocturnal	Closed
	(Riede et al., 2022)	11.8-81						
Peromyscus eremicus	(Miller & Engstrom, 2012)	18.3-33.5	Pink (3)^{1}	25	No	Solitary	Nocturnal	Open
Peromyscus leucopus	(Miller & Engstrom, 2012)	18.9-27.4	No (4)^{1,3}	23	No	Solitary	Nocturnal	Closed
Peromyscus maniculatus	(Riede et al., 2022)	6.2-79	No (1)^{1}	17	No	Social	Nocturnal	Closed
	(Sales, 1972)	35-60						

Table A2.1 *Continued (10/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
				Rodents (Continued)				
Peromyscus polionotus	(Miller & Engstrom, 2012)	20.4-27.9	Incon. (2)[1]	12.5	No	Social	Nocturnal	Open
Petaurista alborufus	(Shen, 2013)[3]	1.51-10.3	No (1)[1]	1491.6[2]	Yes	Solitary	Nocturnal	Closed
Petaurista leucogenys	(Ando & Kuramochi, 2008)	0.8-12	Incon. (1)[1]	1178	Yes	Solitary	Nocturnal	Closed
Petaurista petaurista			No (1)[1]	1750	Yes	Solitary	Nocturnal	Closed
Pteromyscus pulverulentus	(Touissant et al., 2023)		Pink	193	Yes	Solitary	Nocturnal	Closed
Rattus fuscipes	(Barnett & Stewart, 1975)	2.5-24		130[2]	No	Social	Nocturnal	Closed
	(Reinhold, 2023)		Blue/White					
Rattus rattus	(Kaltwasser, 1990)	0.01-70		185	No	Social	Nocturnal	Closed
	(Reinhold, 2021)		Blue/Green					
	(Udall et al., 1964)		Blue/Green					
Reithrodontomys mexicanus	(Miller & Engstrom, 2010)	8.24-20.12	Pink/White (1)[1]	14	No	Solitary	Nocturnal	Closed
Sciurus aberti	(Hall, 1981)[3]	1-4.5	Incon. (2)[1]	606.2[2]	No	Social	Diurnal	Closed
Sciurus carolinensis	(Lishak, 1982)	0.05-14		505	No	Social	Diurnal	Closed
	(Lishak, 1984)	0.25-16						
	(Kohler et al., 2019)		No					
	(Tumlison & Tumlison, 2021)		No					
Sciurus niger	(Zelley, 1971)[3]	0.01-5		1361	No	Social	Diurnal	Closed
	(Kohler et al., 2019)		No					

Table A2.1 *Continued (11/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
			Rodents (Continued)					
Sciurus niger (Cont.)	(Tumlison & Tumlison, 2021)		No					
Sicista subtilis	(Volodin et al., 2019)	6.21-9.86	Blue/ Green (1)[1]	9.1	No	Social	Nocturnal	Open
Spermophilus citellus	(Koshev & Pandourski, 2008)	7.1-15.33	No (1)[1]	229[2]	No	Solitary	Diurnal	Open
	(Matrosova et al., 2012)	0.28-15.76						
	(Schneiderová, 2008)	0.13-14.81						
	(Schneiderová, 2012)	7-13						
	(Schneiderová & Policht, 2012a)	6.97-15.15						
	(Schneiderová & Policht, 2012b)	5.85-15.24						
	(Schneiderová et al., 2015)	7.6-12						
	(Schneiderová et al., 2017)	8.5-14						
	(Schneiderová, Štefanská & Kratochvíl, 2019)	7.4-13.5						
Spermophilus suslicus	(Matrosova et al., 2007)	6.32-11.8	White (2)[1]	220	No	Social	Diurnal	Open

Table A2.1 *Continued (12/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
				Rodents (Continued)				
Spermophilus suslicus (Cont.)	(Matrosova et al., 2011)	8.91-10.02						
	(Matrosova et al., 2012)	0.18-84						
	(Matrosova et al., 2016)	7.76-9.86						
	(Matrosova, Volodin & Volodina, 2006)	0.01-14						
	(Matrosova, Volodin & Volodina, 2009)	9.04-9.73						
	(Volodin, 2005)	8.97-9.76						
	(Volodin et al., 2008)	9.29-9.70						
	(Volodina, Matrosova & Volodin, 2010)	8.72-10.37						
Tamias amoenus	(Brand, 1976)	1.5-11.5	No (2)[1,3]	50.20[2]	No	Solitary	Diurnal	Open
Tamias merriami	(Brand, 1976)	1.5-15	No (1)[1]	75	No	Social	Diurnal	Closed
Tamias minimus	(Bergstrom & Hoffmann, 1991)	1-13	No (1)[1]	42.59[2]	No	Solitary	Diurnal	Closed
	(Brand, 1976)	2-10						
Tamias ruficaudus			No (1)[1]	60	No	Solitary	Diurnal	Closed
Tamias sibiricus	(Blake, 1992)	1.2-8.5	No (1)[1]	94.85[2]	No	Solitary	Diurnal	Closed

Table A2.1 *Continued (13/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
				Rodents (Continued)				
Tamias sibiricus (Cont.)	(Lissovsky, Obolenskaya & Emelyanova, 2006)	1-11.77						
Tamiasciurus douglasii	(Smith, 1978)	0.5-8	No (1)[1]	203.1[2]	No	Solitary	Diurnal	Closed
Tamiasciurus hudsonicus	(Greene & Meagher, 1998)	2.4-10.1		203.5[2]	No	Solitary	Diurnal	Closed
	(Smith, 1978)	0.5-7						
	(Kohler et al., 2019)		No					
Urocitellus armatus	(Balph & Balph, 1966)	2-7.3	No (2)[1]	313	No	Social	Diurnal	Open
	(Koeppl, Hoffmann & Nadler, 1978)	0.05-11						
Urocitellus beldingi	(Leger, Berney-Key & Sherman, 1984)	3.5-5.6	No (2)[1]	246.9[2]	No	Social	Diurnal	Open
	(Robinson, 1980)	4-7						
	(Robinson, 1981)	5.9-7.1						
Urocitellus columianus	(Betts, 1976)	0.1-10	Incon. (3)[1,3]	465.7[2]	No	Social	Diurnal	Open
	(Koeppl, Hoffmann & Nadler, 1978)	0.01-7						
	(Manno et al., 2007)	1.6-5.8						
Urocitellus richardsonii	(Davis, 1984)	3.5-11	No (1)[1]	667.5[2]	No	Social	Diurnal	Open
	(Fagerstone, 1987)	0.01-8.96						

Table A2.1 *Continued (14/14)*

Species	Reference	Min–Max Freq	UVP	Body Mass (g)	Gliding	Sociality	Diel A.P.	Habitat Openness
		Rodents (Continued)						
Urocitellus richardsonii (Cont.)	(Koeppl, Hoffmann & Nadler, 1978)	0.05-8.5						
	(Sloan, Wilson & Hare, 2005)	3.18-6.31						
	(Wilson & Hare, 2004)	31.7-55						
	(Wilson & Hare, 2006)	24.4-56.3						
Urocitellus undulatus	(Goncharov et al., 2021)	0.05-9.8	No (1)[1]	742.9	No	Social	Diurnal	Open
	(Melchior, 1971)	1-8						
Xerospermophilus spilosoma	(Matocha, 1975)[3]	3.70-6.57	No (2)[1]	89	No	Solitary	Diurnal	Open

[1] Novel UVP observations; methods and results in Information S1

[2] Indicates sexual dimorphism; mass averaged between sexes

[3] Data taken from subspecies

[4] UVP observation confirmed with observation of live individuals in the Kawartha Highlands, ON

*Inferred from spectrograms

Table A2.2 Binary phylogenetic generalized linear mixed (PGLM) model results for ultraviolet-induced photoluminescence (UVP) of gliding Mammalia and their relatives (n = 83) for all reported colours (white included). Slope estimated ($\bar{x}$ (±SE)) are given for each variable: gliding (Y/N), diel activity pattern (Diel A.P.; nocturnal/diurnal), habitat openness (Habitat; closed/open), and sociality (solitary/social). The phylogenetic signal (s^2; estimated from 1000 trees) and model fit (R^2) estimations are provided. Significant effects are given in bold.

	Variables						**Model Fit**	
	Intercept	log(Body Mass (g))	Gliding: *Y*	**Diel A.P.: *Nocturnal***	Habitat: *Open*	Sociality: *Solitary*	s^2	R^2
$\bar{x}$ (±SE)	-2.44 (±1.40)	-0.09 (±0.39)	-0.89 (±0.95)	**3.28** **(±1.10)**	0.76 (±0.83)	0.36 (±0.64)	**0.33**	0.51
P	0.08	0.82	0.35	**0.003**	0.36	0.57	**0.01**	

Table A2.3 Descriptions of calls produced by captive sugar gliders (*Petaurus breviceps*) recorded with an ultrasonic microphone. Duration (s) and frequency (kHz) estimates (x̄ (±SE)) of vocalizations produced by captive sugar gliders located in Oshawa, ON, during March 2021. Frequency estimates taken from the dominant harmonic, if harmonics present; peak frequencies represent the frequency with the highest energy.

Call Type	n	Duration (s)	Minimum (kHz)	Maximum (kHz)	Peak (kHz)
Bark	12	0.13 (±0.04) [0.062-0.19]	9.40 (±2.38) [6.30-13.80]	24.73 (±2.76) [18.50-28.50]	16.01 (±1.77) [13.50-18.50]
Broadband Burst	7	0.04 (±0.00) [0.034-0.045]	9.33 (±0.70) [8.80-10.80]	43.76 (± 3.96) [35.60-47.80]	23.74 (±4.73) [19.10-30.50]
High Frequency	5	0.03 (±0.00) [0.032-0.037]	17.62 (±2.52) [15.00-21.00]	20.20 (±2.51) [17.20-23.20]	19.02 (±2.33) [16.50-22.10]
Sniffing	2	0.11 (±0.05) [0.017-0.14]	9.50 (±0.28) [9.30-9.70]	16.25 (±0.78) [15.70-16.80]	13.10 (±2.12) [11.60-14.60]
Ultrasonic	2	0.02 (±0.00) [0.016-0.022]	33.80 (±0.14) [33.70-33.90]	38.15 (±3.04) [36.00-40.30]	37.15 (±2.76) [35.20-39.10]
Whistle	1	0.06	14.80	16.60	15.30

Table A2.4 Descriptions of calls from various mammals recorded with sonic microphones. Duration (s) and frequency (kHz) estimates (x̄ (±SE)) of vocalizations produced by captive springhares *(Pedetes capensis*) and various free-ranging marsupials. Frequency estimates taken from the dominant harmonic if harmonics present; peak frequencies represent the frequency with the highest energy.

Species	Call (n)	Duration (s)	Minimum (kHz)	Maximum (kHz)	Peak (kHz)
Pedetes capensis	Growl (105)	1.32 (±0.47) [0.44-2.81]	0.16 (±0.04) [0.058-0.22]	0.21 (±0.04) [0.13-0.31]	0.19 (±0.04) [0.085-0.27]
Petaurus australis	Cry (7)	4.02 (±0.51) [3.33 – 4.84]	0.25 (±0.27) [0.09 – 0.27]	4.63 (±0.69) [3.37 – 5.53]	2.07 (±0.90) [1.21 – 3.56]
	Gurgle (2)	4.02 (±0.18) [3.90 – 4.14]	0.28 (±0.00) [0.28 – 0.28]	3.60 (±0.21) [3.46 – 3.75]	1.77 (±0.13) [1.68 – 1.87]
	Rattle (1)	2.48	0.28	4.21	0.37
Petaurus breviceps	Yap (23)	0.22 (±0.04) [0.13 – 0.29]	1.18 (±0.36) [0.37 – 1.59]	2.20 (±0.50) [0.93 – 2.71]	2.01 (±0.51) [0.75 – 2.53]
Petaurus norfolcensis	Belch (57)	0.16 (±0.04) [0.021 – 0.25]	0.18 (±0.0) [0.18 – 0.18]	2.59 (±0.28) [1.92 – 3.51]	1.29 (±0.59) [0.18 – 2.20]
	Higher Nasal Grunt (26)	0.19 (±0.03) [0.12 – 0.27]	2.39 (±0.41) [0.46 – 2.62]	3.05 (±0.045) [3.00 – 3.09]	2.65 (±0.26) [1.40 – 2.81]
	Nasal Grunt (107)	0.49 (±0.18) [0.058 – 0.81]	0.51 (±0.52) [0.18 – 1.78]	2.42 (±0.23) [1.59 – 3.00]	1.74 (±0.47) [0.18 – 2.71]
Pseudocheirus peregrinus	Twitter (9)	0.37 (±0.05) [0.30 – 0.43]	0.46 (±0.13) [0.18 – 0.65]	3.25 (±0.56) [2.53 – 4.50]	1.31 (±0.32) [0.84 – 1.78]

A2 References

Ackers S, Slobodchikoff C. 1999. Communication of stimulus size and shape in alarm calls of Gunnison's prairie dogs, *Cynomys gunnisoni. Ethology* 105:149–162. DOI: 10.1046/j.1439-0310.1999.00381.x.

Adobe Inc. 2019. Adobe Photoshop. Adobe Inc., San Jose, California, USA. *Available at https://www.adobe.com/products/photoshop.html* (accessed 28 April 2023).

Anderson M. 2022. Wild Ambiance. *Available at https://wildambience.com* (accessed 28 April 2023).

Anderson MJ, Ambrose L, Bearder SK, Dixson AF, Pullen S. 2000. Intraspecific variation in the vocalizations and hand pad morphology of southern lesser bush babies (*Galago moholi*): a comparison with *G. senegalensis*. *International Journal of Primatology* 21:537–555. DOI: 10.1023/A:1005400205038.

Ando M, Kuramochi Y. 2008. Vocal communication of the Japanese giant flying squirrel *Petaurista leucogenys*. *The Journal of Agricultural Science* 53:176–183.

Australian Capital Territory Government. 2022. Nature Conservation Act 2014. *Available at https://www.legislation.act.gov.au/a/2014-59/default.asp* (accessed 03 August 2023).

Balph D, Balph D. 1966. Social communication of Uinta ground squirrels. *Journal of Mammalogy* 47:440–450. DOI: 10.2307/1377685.

Barnett S, Stewart A. 1975. Audible signals during intolerant behaviour of *Rattus fuscipes*. *Australian Journal of Zoology* 23:103–112. DOI: 10.1071/zo9750103.

Bergstrom B, Hoffmann R. 1991. Distribution and diagnosis of three species of chipmunks (*Tamias*) in the front range of Colorado. *The Southwestern Naturalist* 36:14–28. DOI: 10.2307/3672112.

Betts B. 1976. Behaviour in a population of Columbian ground squirrels *Spermophilus columbianus columbianus*. *Animal Behaviour* 24:652–680. DOI: 10.1016/s0003-3472(76)80079-6.

Bezerra BM, Souto A. 2008. Structure and usage of the vocal repertoire of *Callithrix jacchus*. *International Journal of Primatology* 29:671–701. DOI: 10.1007/s10764-008-9250-0.

Binz H, Zimmermann E. 1989. The vocal repertoire of adult tree shrews (*Tupaia belangeri*). *Behaviour* 109:142–162. DOI: 10.1163/156853989x00196.

Blake B. 1992. Estrous calls in captive Asian chipmunks, *Tamias sibiricus*. *Journal of Mammalogy* 73:597–603. DOI: 10.2307/1382031.

Blumstein D. 1999. Alarm calling in three species of marmots. *Behaviour* 136:731–757. DOI: 10.1163/156853999501540.

Blumstein D, Armitage K. 1997. Does sociality drive the evolution of communicative complexity? A comparative test with ground-dwelling sciurid alarm calls. *The American Naturalist* 150:179–200. DOI: 10.1086/286062.

Boinski S, Mitchell CL. 1995. Wild squirrel monkey (*Saimiri sciureus*) “caregiver” calls: contexts and acoustic structure. *American Journal of Primatology* 35:129–137. DOI: 10.1002/ajp.1350350205.

Boinski S, Mitchell CL. 1997. Chuck vocalizations of wild female squirrel monkeys (*Saimiri sciureus*) contain information on caller identity and foraging activity. *International Journal of Primatology* 18:975–993. DOI: 10.1023/A:1026300314739.

Bool I, Slaymaker M, Magrath R.D, Arfian A, Karya A, Analuddin K, Courtney Jones SK. 2021. First record of acoustic behaviour in Sulawesi bear cuscus (*Ailurops ursinus*). *Austral Ecology* 46:507–512. DOI: 10.1111/aec.12993.

Brand L. 1976. The vocal repertoire of chipmunk (genus *Eutamias*) in California. *Animal Behaviour* 24:319–335. DOI: 10.1016/s0003-3472(76)80040-1.

Burton JA, Nietsch A. 2010. Geographical variation in duet songs of Sulawesi tarsiers: evidence for new cryptic species in south and southeast Sulawesi. *International Journal of Primatology* 31:1123–1146. DOI: 10.1007/s10764-010-9449-8.

Butynski TM, De Jong YA, Perkin AW, Bearder SK, Honess PE. 2006. Taxonomy, distribution, and conservation status of three species of dwarf galagos (*Galagoides*) in eastern Africa. *Primate Conservation* 21:63–79. DOI: 10.1896/0898-6207.21.1.63.

Casamitjana J. 2002. The vocal repertoire of the woolly monkey *Lagothrix lagothricha*. *Bioacoustics* 13:1–19. DOI: 10.1080/09524622.2002.9753483.

Charlton BD. 2021. Marsupial vocal communication: a review of vocal signal production, form, and function. In: Rosenfeld CS, Hoffmann F, eds. *Neuroendocrine regulation of animal vocalization*. Academic Press; p. 297–312. DOI: 10.1016/b978-0-12-815160-0.00002-5.

City of Toronto. 2023. Toronto Municipal Code: Chapter 349, Animals. *Available at* https://www.toronto.ca/legdocs/municode/1184_349.pdf (accessed 03 August 2023).

Clink D. 2023. GibbonR: an R package for the detection and classification of acoustic signals using machine learning. *Available at* https://github.com/DenaJGibbon/gibbonR/ (accessed 28 April 2023).

Davis L. 1984. Alarm calling in Richardon’s ground squirrels (*Spermophilus richardsonii*). *Zeitschrift für Tierpsychologie* 66:152–164. DOI: 10.1111/j.1439-0310.1984.tb01362.x.

Davis R. 1991. Semantical communication in anti-predator alarm calls. In: Hall CA, Doyle-Jones V, Widawski B, eds. *The Natural History of Eastern California and High-Altitude Research*. San Fransisco: University of California, 257–312.

Dempster ER. 1994. Vocalizations of adult Northern Quolls, *Dasyurus hallucatus*. *Australian Mammalogy* 17:43–49. DOI: 10.1071/am94005.

Eiler K, Banack S. 2004. Variability in the alarm call of golden-mantled ground squirrels *Spermophilus lateralis* and *S. saturatus*. *Journal of Mammalogy* 85:43–50. DOI: 10.1644/1545-1542(2004)085<0043:vitaco>2.0.co;2.

Eisinger J, Scheibe J, Flaherty E. 2016. Novel *Glaucomys volans* vocalizations in Indiana and evidence of geographic variation in high frequency communication. *Journal of Mammalogy* 97:1219–1227. DOI: 10.1093/jmammal/gyw076.

Epple G. 1968. Comparative studies on vocalization in marmoset monkeys (Hapalidae). *Folia Primata* 8:1–40. DOI: 10.1159/000155129.

Fagerstone K. 1987. Comparison of vocalizations between and within *Spermophilus elegans elegans* and *S. richardsonii*. *Journal of Mammalogy* 68:853–857. DOI: 10.2307/1381564.

Gilley L. 2013. Discovery and characterization of high-frequency calls in North American flying squirrels (*Glaucomys sabrinus* and *G. volans*): implications for ecology, behavior, and conservation. D. Phil. Thesis, Auburn University.

Gilley L, Diggins C, Pearson S, Best T. 2019. Vocal repertoire of captive northern and southern flying squirrels (*Glaucomys sabrinus* and *G. volans*). *Journal of Mammalogy* 100:518–530. DOI: 10.1093/jmammal/gyz064.

Goncharov D, Policht R, Hambálková L, Salovarov V, Hart, V. 2021. Individual-based acoustic variation of the alarm calls in the long-tailed ground squirrel. *Royal Society Open Science* 8:200147. DOI: 10.1098/rsos.200147.

Government of Ontario. 2019. Animals Research Act, R.S.O. 1990, c.A.22. *Available at https://www.ontario.ca/laws/statute/90a22/v8* (accessed 03 August 2023).

Greene E, Meagher T. 1998. Red squirrels, *Tamiasciurus hudsonicus*, produce predator-class specific alarm calls. *Animal Behaviour* 55:511–518. DOI: 10.1006/anbe.1997.0620.

Grimsley JMS., Sheth S, Vallabh N, Grimsley CA, Bhattal J, Latsko M, Jasnow A, Wenstrup JJ. 2016. Contextual modulation of vocal behavior in mouse: newly identified 12 khz “mid-frequency” vocalization emitted during restraint. *Frontiers in Behavioral Neuroscience* 10. DOI: 10.3389/fnbeh.2016.00038.

Gursky S. 2015. Ultrasonic vocalizations by the spectral tarsier, *Tarsius spectrum*. *Folia Primatologica* 86:153–163. DOI: 10.1159/000371885.

Hafner MS, Hafner DS. 1979. Vocalizations of grasshopper mice (genus *Onychomys*). *Journal of Mammalogy* 60:85–94. DOI: 10.2307/1379761.

Hall J. 1981. A field study of Kaibab squirrel in Grand Canyon National Park. *Wildlife Monographs* 75:3–54.

Hending D, Holderied M, Mccabe G. 2017. The use of vocalizations of the sambirano mouse lemur (*Microcebus sambiranensis*) in an acoustic survey of habitat preference. *International Journal of Primatology* 38:732–750. DOI: 10.1007/s10764-017-9977-6.

Hoffmann F, Musolf K, Penn DJ. 2012. Ultrasonic courtship vocalizations in wild house mice: spectrographic analyses. Journal of Ethology 30:173–180. DOI: 10.1007/s10164-011-0312-y.

Kaltwasser M-T. 1990. Acoustic signaling in the black rat (*Rattus rattus*). Journal of Comparative Psychology 104:227–232. DOI: 10.1037/0735-7036.104.3.227.

Kato Y, Gokan H, Oh-Nishi A, Suhara T, Watanabe S, Minamimoto T. 2014. Vocalizations associated with anxiety and fear in the common marmoset

(*Callithrix jacchus*). Behavioural Brain Research 275:43–52. DOI: 10.1016/j.bbr.2014.08.047.

Kavanagh RP, Rohan-Jones WG. 1982. Calling behaviour of the yellow-bellied glider, *Petaurus australis* Shaw (Marsupialia: Petauridae). *Australian Mammalogy* 59:95–111. DOI: 10.1071/AM82011.

Koeppl J, Hoffmann R, Nadler C. 1978. Pattern analysis of acoustical behavior in four species of ground squirrels. *Journal of Mammalogy* 59:677–696. DOI: 10.2307/1380133.

Kohler AM, Olson ER, Martin JG, Anich PS. 2019. Ultraviolet fluorescence discovered in new world flying squirrels (*Glaucomys*). *Journal of Mammalogy* 100:21–30. DOI: 10.1093/jmammal/gyy177.

Koshev Y, Pandourski I. 2008. Structure and variability of alarm calls of European ground squirrel *Spermophilus citellus* L. 1766 (Mammalia: Rodentia) from Western Bulgaria. *Acta Zoologica Bulgarica* 60:99–105.

Krenz M. 1977. Vocalizations of the rock squirrel (*Spermophilus variegatus*). Master Thesis, Texas Tech University.

Leger D, Berney-Key S, Shermant P. 1984. Vocalizations of Belding's ground squirrels (*Spermophilus beldingi*). *Animal Behaviour* 32:753–764.

Leger D, Owings D, Gelfand D. 1980. Single-note vocalizations of California ground squirrels: graded signals and situation-specifity of predator and socially evoked calls. *Zeitschrift für Tierpsychologie* 52:227–246. DOI: 10.1111/j.1439-0310.1980.tb00714.x.

León JJ, Vargas SA, Ramírez MA, Galvis NF, Cifuentes EF, Stevenson PR. 2014. Vocal communication in woolly monkeys (*Lagothrix lagothricha lugens*) in Cueva de los Guacharos National Park, Colombia. In: Defler TR, Stevenson PR, eds. *The woolly monkey: behavior, ecology, systematics, and captive research*. Springer, New York; p. 187–205. DOI: 10.1007/978-1-4939-0697-0_11

Lishak R. 1982. Gray squirrel mating calls: a spectrographic and ontogenic analysis. *Journal of Mammalogy* 63:661–663. DOI: 10.2307/1380274.

Lishak R. 1984. Alarm vocalizations of adult gray squirrels. *Journal of Mammalogy* 65:681–684. DOI: 10.2307/1380852.

Lissovsky A, Obolenskaya E, Emelyanova L. 2006. The structure of voice signals of Siberian chipmunk (*Tamias sibiricus* Laxmann 1769; Rodentia: Sciuridae). *Russian Journal of Theriology* 5:93–98. DOI: 10.15298/rusjtheriol.05.2.07.

Long CV. 2007. Vocalisations of the degu *Octodon degus*, a social caviomorph rodent. *Bioacoustics: The International Journal of Animal Sound and its Recording* 16:223–244. DOI: 10.1080/09524622.2007.9753579.

Loughry W, Oeser M, Anderson C, Hoogland J. 2019. The importance of individual variation in the alarm calls of Gunnison's prairie dogs. *Animal Behaviour* 150:59–68. DOI: 10.1016/j.anbehav.2019.01.019.

Loughry W, Oeser M, Hoogland J. 2019. Alarm calls of the same individual vary during a response to the same predator in Gunnison's prairie dogs (*Cynomys* *gunnisoni*). *Canadian Journal of Zoology* 97:1092–1100. DOI: 10.1139/cjz-2019-0064.

Lupanova AS, Egorova MA. 2015. Vocalization of sex partners in the house mouse (*Mus musculus*). *Journal of Evolutionary Biochemistry and Physiology* 51:324–331. DOI: 10.1134/s0022093015040080.

Manno T, Nesterova A, Debarbieri L, Kennedy S, Wright K, Dobson F. 2007. Why do male Columbian ground squirrels give a mating call? *Animal Behaviour* 74:1319–1327. DOI: 10.1016/j.anbehav.2007.02.033.

Martin K. 2019. The vocal repertoire of feathertail gliders (*Acrobates pygmaeus*) and how macrocomparisons can shape future research on acoustic communication in mammals. D. Phil. Thesis, University of New South Whales.

Matocha K. 1975. Vocal communication in ground squirrels, genus *Spermophilus.* D. Phil. Thesis, Texas Tech University.

Matocha K. 1977. The vocal repertoire of *Spermophilus tridecemlineatus*. *The American Midland Naturalist* 98:482–487. DOI: 10.2307/2424997.

Matrosova V, Blumstein D, Volodin I, Volodina E. 2011. The potential to encode sex, age, and individual identity in the alarm calls of three species of Marmotinae. *Naturwissenschaften* 98:181–192. DOI: 10.1007/s00114-010-0757-9.

Matrosova V, Rusin M, Volodina E, Proyavka S, Savinetskaya L, Shekarova O, Rashevska H, Volodin I. 2016. Genetic and alarm call diversity across scattered populations of speckled ground squirrels (*Spermophilus suslicus*). *Mammalian Biology* 81:255–265. DOI: 10.1016/j.mambio.2016.01.001.

Matrosova V, Schneiderová I, Volodin I, Volodina E. 2012. Species-specific and shared featured in vocal repertoires of three Eurasian ground squirrels (genus *Spermophilus*). *Acta Theriologica* 57:65–78. DOI: 10.1007/s13364-011-0046-9.

Matrosova V, Volodin I, Volodina E. 2006. The diversity of calls produced by live-trapped speckled ground squirrels *Spermophilus suslicus* (Rodentia, Sciuridae) (in Russian). *Bulletin of Moscow Society of Naturalists* 111:84–87.

Matrosova V, Volodin I, Volodina E. 2009. Short-term and long-term individuality in speckled ground squirrel alarm calls. *Journal of Mammalogy* 90:158–166. DOI: 10.1644/08-mamm-a-032.1.

Matrosova V, Volodin I, Volodina E, Babitsky A. 2007. Pups crying bass: vocal adaptation for avoidance of age-dependent predation risk in ground squirrels? *Behavioural Ecology and Sociobiology* 62:181–191. DOI: 10.1007/s00265-007-0452-9.

Meisner DH. 1983. Psychedelic opossums-fluorescence of the skin and fur of *Didelphis virginiana kerr*. *Ohio Journal of Science* 83:4.

Melchior H. 1971. Characteristics of arctic ground squirrel alarm calls. *Oecologia* 7:184–190. DOI: 10.1007/bf00346360.

Miard P, Lim LS, Abdullah NI, Elias NA, Ruppert N. 2019. Ultrasound use by sunda colugos offers new insights into the communication of these cryptic mammals. *Bioacoustics* 28:397–403. DOI: 10.1080/09524622.2018.1463294.

Miller JR, Engstrom MD. 2010. Stereotypic vocalizations in harvest mice (*Reithrodontomys*): harmonic structure contains prominent and distinctive audible, ultrasonic, and non-linear elements. *The Journal of the Acoustical Society of America* 128:1510. DOI: 10.1121/1.3455855.

Miller JR, Engstrom MD. 2012. Vocal stereotypy in the rodent genera *Peromyscus* and *Onychomys* (Neotominae): taxonomic signature and call design. *Bioacoustics* 21:193–213. DOI: 10.1080/09524622.2012.675176.

Moody MI, Menzel EW Jr. 1976. Vocalizations and their behavioral contexts in the tamarin *Saguinis fuscicollis. Folia Primata* 25:73–94. DOI: 10.1159/000155708.

Morgan KN, Tromborg CT. 2007. Sources of stress in captivity. *Applied Animal Behaviour Science* 102:262–302. DOI: 10.1016/j.applanim.2006.05.032.

Moynihan M. 1964. Some behavior patterns of the platyrrhine monkeys I. The night monkey (*Aotus trivirgatus*). *Smithsonian Miscellaneous Collections* 146:1–84.

Moynihan M. 1966. Communication in the Titi monkey, *Callicebus*. *Journal of Zoology* 150:77–127. DOI: 10.1111/j.1469-7998.1966.tb02999.x.

Murrant M, Bowman J, Garroway C, Prinzen B, Mayberry H, Faure P. 2013. Ultrasonic vocalizations emitted by flying squirrels. *PLOS ONE* 8:1–6. DOI: 10.1371/journal.pone.0073045.

Musolf K, Meindl S, Larsen AL, Kalcounis-Rueppell MC, Penn DJ. 2015. Ultrasonic vocalizations of male mice differ among species and females show assortative preferences for male calls. *PLOS ONE* 10:e0134123. DOI: 10.1371/journal.pone.0134123.

Nietsch A. 1999. Duet vocalizations among different populations of Sulawesi tarsiers. *International Journal of Primatology* 20:567–583.

Nummert G, Ritson K, Nemvalts K. 2023. Photoluminescence in the garden dormouse (*Eliomys quercinus*). *Zoology* 157. DOI: 10.1016/j.zool.2023.126075.

Nyby J. 1983. Ultrasonic vocalizations during sex behavior of male house mice (*Mus musculus*): a description. *Behavioral and Neural Biology* 39(1):128–134. DOI: 10.1016/s0163-1047(83)90722-7.

Olson ER, Carlson M, Ramanujam V, Sears L, Anthony S, Anich P, Ramon L, Hulstrand A, Jurewicz M, Gunnelson A, Kohler A, Martin J. 2021. Vivid biofluorescence discovered in the nocturnal springhare (Pedetidae). *Scientific Reports* 11:4125. DOI: 10.1038/s41598-021-83588-0.

Owings D, Leger D. Chatter vocalizations of California ground squirrels: predator- and social-role specificity. *Zeitschrift für Tierpsychologie* 54:163–184. DOI: 10.1111/j.1439-0310.1980.tb01070.x.

Owings D, Virginia R. Alarm calls of California ground squirrels (*Spermophilus beecheyi*). *Zeitschrift für Tierpsychologie* 46:58–70. DOI: 10.1111/j.1439-0310.1978.tb01438.x.

Perla BS, Slobodchikoff CN. 2002. Habitat structure and alarm call dialects in Gunnison’s prairie dog (*Cynomys gunnisoni*). *Behavioral Ecology* 13:844–850. DOI: 10.1093/beheco/13.6.844.

Pine RH, Rice JE, Bucher JE, Tank Jr DH, Greenhall AM. 1985. Labile pigments and fluorescent pelage in didelphid marsupials. *Mammalia* 49:249–256. DOI: 10.1515/mamm.1985.49.2.249.

R Development Core Team. 2022. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *Available at* *https://www.r-project.org/* (accessed 28 April 2023).

Rabin L, McCowan B, Hooper S, Owings D. 2003. Anthropogenic noise and its effect on animal communication: an interface between comparative psychology and conservation biology. *International Journal of Comparative Psychology* 16(2):172–192. DOI: 10.46867/c4f59p.

Ramsier MA, Cunningham AJ, Moritz GL, Finneran JJ, Williams CV, Ong PS, Gursky-Doyen SL, Dominy NJ. 2012. Primate communication in the pure ultrasound. *Biology Letters* 8(4):508–511. DOI: 10.1098/rsbl.2011.1149.

Řeháková-Petru M, Policht R, Peške L. 2012. Acoustic repertoire of the Philippine tarsier (*Tarsius syrichta fraterculus*) and individual variation of long-distance calls. *International Journal of Zoology* 2012(4):602401. DOI: 10.1155/2012/602401.

Reinhold L. 2021. Mammals with fluorescent fur: observations from the wet tropics. *North Queensland Naturalist* 51:1–8. DOI: 10.53060/prsq23.5.

Reinhold L. 2023. Photoluminescence in Fur. M. Phil. Thesis, James Cook University.

Riede T, Kobrina A, Bone L, Darwaiz T, Pasch B. 2022. Mechanisms of sound production in deer mice (*Peromyscus* spp.). *Journal of Experimenal Biology* 225:jeb243695. DOI: 10.1242/jeb.243695.

Robinson JG. 1979. An analysis of the organization of vocal communication in the Titi monkey *Callicebus moloch. Zeitschrift für Tierpsychologie* 49:381–405. DOI: 10.1111/j.1439-0310.1979.tb00300.x.

Robinson S. 1980. Antipredator behaviour and predator recognition in Belding's ground squirrels. *Animal Behaviour* 28:840–852. DOI: 10.1016/s0003-3472(80)80144-8.

Robinson S. 1981. Alarm communication in Belding's ground squirrels. *Zeitschrift für Tierpsychologie* 56:150–168. DOI: 10.1111/j.1439-0310.1981.tb01293.x.

Rosti H, Pihlström H, Bearder S, Pellikka P, Rikkinen J. 2020. Vocalization analyses of nocturnal arboreal mammals of the Taita Hills, Kenya. *Diversity* 12:1–23. DOI: 10.3390/d12120473.

Sales GD. 1972. Ultrasound and mating behaviour in rodents with some observation on other behavioural situations. *Journal of Zoology* 168:149–164. DOI: 10.1111/j.1469-7998.1972.tb01345.x.

Santos SG, Duarte MHL, Sousa-Lima RS, Young RJ. 2017. Comparing contact calling between black tufted-ear marmosets (*Callithrix penicillata*) in a noisy urban environment and in a quiet forest: noise effects on contact calls in black tufted-ear marmosets. *International Journal of Primatology* 38:1130–1137. DOI: 10.1007/s10764-017-0002-x.

Scheumann M, Linn S, Zimmermann E. 2017. Vocal greeting during mother-infant reunions in a nocturnal primate, the gray mouse lemur (*Microcebus murinus*). *Scientific Reports* 7:1–7. DOI: 10.1038/s41598-017-10417-8.

Schneiderová I. 2008. Acoustics communication and alarm calls in two species of ground squirrels *Spermophilus citellus* and *Spermophilus taurensis* (Sciuridae; Rodentia). D. Phil. Thesis, Charles University.

Schneiderová I. 2012. Frequency-modulated second elements of two-element alarm calls do not enhance discrimination of callers in three Eurasian ground squirrels. *Current Zoology* 58:749–757. DOI: 10.1093/czoolo/58.5.749.

Schneiderová I, Policht R. 2012a. Acoustic analysis of alarm calls of the European ground squirrel (*Spermophilus citellus*) and the Taurus ground squirrel (*S. taurensis*) (Mammalia: Sciuridae). *Zoologischer Anzeiger* 251:139–146. DOI: 10.1016/j.jcz.2011.07.005.

Schneiderová I, Policht R. 2012b. Acoustic analysis of the alarm calls of the Anatolian ground squirrel *Spermophilus xanthoprymnus*: a description and comparison with alarm calls of the Taurus *S. taurensis* and European *S. citellus* ground squirrels. *Naturwissenschaften* 99:55–64. DOI: 10.1007/s00114-011-0870-4.

Schneiderová I, Schnitzerová P, Uhlíková J, Brandl P, Zouhar J, Matějů J. 2015. Differences in alarm calls of juvenile and adult European ground squirrels (*Spermophilus citellus*): findings on permanently marked animals from a semi-natural enclosure. *Zoo Biology* 34(6):503–512. DOI: 10.1002/zoo.21233.

Schneiderová I, Štefanská L, Kratochvíl L. 2019. Geographic variability in the alarm calls of the European ground squirrel. *Current Zoology* November:1–9. DOI: 10.1093/cz/zoz055.

Schneiderová I, Volodina E, Matrosova V, Volodin I. 2017. One plus one: binary alarm calls retain individual signature for longer periods than single-note alarms in the European ground squirrel (*Spermophilus citellus*). *Behavioural Processes* 138:73–81. DOI: 10.1016/j.beproc.2017.02.014.

Schneiderová I, Zouhar J, Štefanská L, Bolfíková BČ, Lhota S, Brandl P. 2016. Vocal activity of lesser galagos (*Galago* spp.) at zoos. *Zoo Biology* 35:147–156. DOI: 10.1002/zoo.21261. DOI: 10.1002/zoo.21261.

Shannon G, McKenna M, Wilson-Henjum G, Angeloni L, Crooks K, Wittemyer G. 2020. Vocal characteristics of prairie dog alarm calls across an urban noise gradient. *Behavioral Ecology* 31:393–400. DOI: 10.1093/beheco/arz200.

Shen P. 2013. Acoustic behavior of white-faced flying squirrel (*Petaurista lena*) in Guanghua Village, Alishan. Master Thesis, National Sun Yat-sen University.

Signal T, Foster TM, Temple W. 2001. Determination of auditory thresholds in the brushtail possum (*Trichosurus vulpecula*). *Physiology & Behavior* 23:195–200. DOI: 10.1016/s0031-9384(01)00446-2.

Sloan JL, Wilson DR, Hare JF. 2005. Functional morphology of Richardson's ground squirrel, *Spermophilus richardsonii*, alarm calls: the meaning of chirps, whistles and chucks. *Animal Behaviour* 70:937–944. DOI: 10.1016/j.anbehav.2005.01.013.

Slobodchikoff C, Briggs W, Dennis P, Hodge A. 2012. Size and shape information serve as labels in the alarm calls of Gunnison's prairie dogs *Cynomys gunnisoni. Current Zoology* 58:741–748. DOI: 10.1093/czoolo/58.5.741.

Smith C. 1978. Structure and function of the vocalizations of tree squirrels (*Tamiasciurus*). *Journal of Mammalogy* 59:793–808. DOI: 10.2307/1380144.

Specht R. 2017. Avisoft-SASLab Pro: sound analysis and synthesis laboratory. Avisoft Bioacoustics E.K.

Stranger KF, Macedonia JM. 1994. Vocalizations of aye-ayes (*Daubentonia madagascariensis*) in captivity. *Folia Primatologica* 62:160–169. DOI: 10.1159/000156773.

Tamura N. 1993. Role of sound communication in mating Malaysian *Callosciurus* (Sciuridae). *Journal of Mammalogy* 74:468–476. DOI: 10.2307/1382404.

Tamura N, Yong H. 1993. Vocalizations in response to predators in three species of Malaysian *Callosciurus* (Sciuridae). *Journal of Mammalogy* 74:703–714. DOI: 10.2307/1382292.

Toussaint SLD, Ponstein J, Thoury M, Métivier R, Kalthoff DC, Habermeyer B, Guilard R, Bock S, Mortensen P, Sandberg S. 2023. Fur glowing under ultraviolet: in situ analysis of porphyrin accumulation in the skin appendages of mammals. *Integrative Zoology* 18:15–26. DOI: 10.21203/rs.3.rs-499384/v3.

Tumlison R, Tumlison TL. 2021. Investigation of fluorescence in selected mammals of Arkansas. *Journal of the Arkansas Academy of Science* 75:29–35. DOI: 10.54119/jaas.2021.7515.

Udall SL, Briggs FP, Pautzke CF, Janzen DH. 1964. Fluorescence studies. In *Wildlife research: problems, programs, progress.* United States Department of the Interior, Fish and Wildlife Service and Bureau of Sport Fisheries and Wildlife, Washington; p. 64.

Volodin I. 2002. Uninterrupted vocalization in the striped possum *Dactylopsila trivirgata* (Marsupialia, Petauridae) during the whole respiratory cycle. *Zoologicheskiĭ Zhurnal* 81:1526–1529.

Volodin I. 2005. Individuality of alarm calls in the spotted suslik (*Spermophilus suslicus*, Rodentia, Sciuridae) (in Russian). *Zoological Journal* 84:228–235.

Volodin IA, Klenova AV, Ilchenko OG, Volodina EV. 2019. High frequency audible calls in northern birch mice *Sicista betulina* in response to handling: effects of individuality, sex and body mass on the acoustics. *BMC Research Notes* 12:677. DOI: 10.1186/s13104-019-4719-9.

Volodin I, Volodina E, Matrasova V, Savinetskaya L, Shekarova O, Voytsik V. 2008. Population density does not affect the alarm call characteristics in the speckled ground squirrel (*Spermophilus suslicus*). *Lynx (Praha)* 39:333–342.

Volodina E, Matrosova V, Volodin I. 2010. An unusual effect of maturation on the alarm call fundamental frequency in two species of ground squirrels. *Bioacoustics* 20:87–98. DOI: 10.1080/09524622.2011.9753634.

Von Merten S, Hoier S, Pfeifle C, Tautz D. 2015. A role for ultrasonic vocalisation in social communication and divergence of natural populations of the house mouse (*Mus musculus domesticus*). *PLOS ONE* 10:e0118130. DOI: 10.1371/journal.pone.0097244.

Waring G. 1966. Sounds and communications of the yellow-bellied marmot (*Marmota flaviventris*). *Animal Behaviour* 14:177–183. DOI: 10.1016/s0003-3472(66)80028-3.

Waring G. 1970. Sound communications of black-tailed, white-tailed, and Gunnison's prairie dogs. *The American Midland Naturalist* 83:167–185. DOI: 10.2307/2424014.

Whisson DA, McKinnon F, Lefoe M, Rendall AR. 2021. Passive acoustic monitoring for detecting the yellow-bellied glider, a highly vocal arboreal marsupial. *PLOS ONE* 16:e0252092. DOI: 10.1371/journal.pone.0252092.

White NR, Prasad M, Barfield RJ, Nyby JG. 1998. 40- and 70-khz vocalizations of mice (*Mus musculus*) during copulation. *Physiology & Behavior* 63:467–473. DOI: 10.1016/s0031-9384(97)00484-8.

Wilson DR, Hare JF. 2004. Ground squirrel uses ultrasonic alarms. *Nature* 430:523. DOI: 10.1038/430523a.

Wilson DR, Hare JF. 2006. The adaptive utility of Richardson's ground squirrel (*Spermophilus richardsonii*) short-range ultrasonic alarm signals. *Canadian Journal of Zoology* 84:1322–1330. DOI: 10.1139/z06-120.

Wilson-Henjum G, Job J, McKenna M, Shannon G, Wittemyer G. 2019. Alarm call modification by prairie dogs in the presence of juveniles. *Journal of Ethology* 37:167–174. DOI: 10.1007/s10164-018-0582-8.

Winter M. 1978. Some aspects of the ontogeny of vocalizations of hand-reared common marmosets. In Roth H, Wolters H-J, Hearn JP, eds. *Biology and Behaviour of Marmosets*. Göttingen: Eigenverlag Rothe, 127–139.

Winter P, Ploog D, Latta J. 1966. Vocal repertoire of the squirrel monkey (*Saimiri sciureus*), its analysis and significance. *Experimental Brain Research* 1:359–384. DOI: 10.1007/bf00237707.

Zala SM, Reitschmidt D, Noll A, Balazs P, Penn DJ. 2017. Sex-dependent modulation of ultrasonic vocalizations in house mice (*Mus musculus musculus*). *PLOS ONE* 12(12):e0188647. DOI: 10.1371/journal.pone.0188647.

Zelley R. The sounds of the fox squirrel, *Sciurus niger rufiventer*. *Journal of Mammalogy* 52:597–604. DOI: 10.2307/1378595.

Zimmermann E. 1985. The vocal repertoire of the adult Senegal bushbaby (*Galago senegalensis senegalensis*). *Behaviour* 94:212–233. DOI: 10.1163/156853985x00190.

Zimmermann E, Vorobieva E, Wrogemann D, Hafen T. 2000. Use of vocal fingerprinting for specific discrimination of gray (*Microcebus murinus*) and rufous mouse lemurs (*Microcebus rufus*). *International Journal of Primatology* 21:837–852.

Chapter 4 (A3)

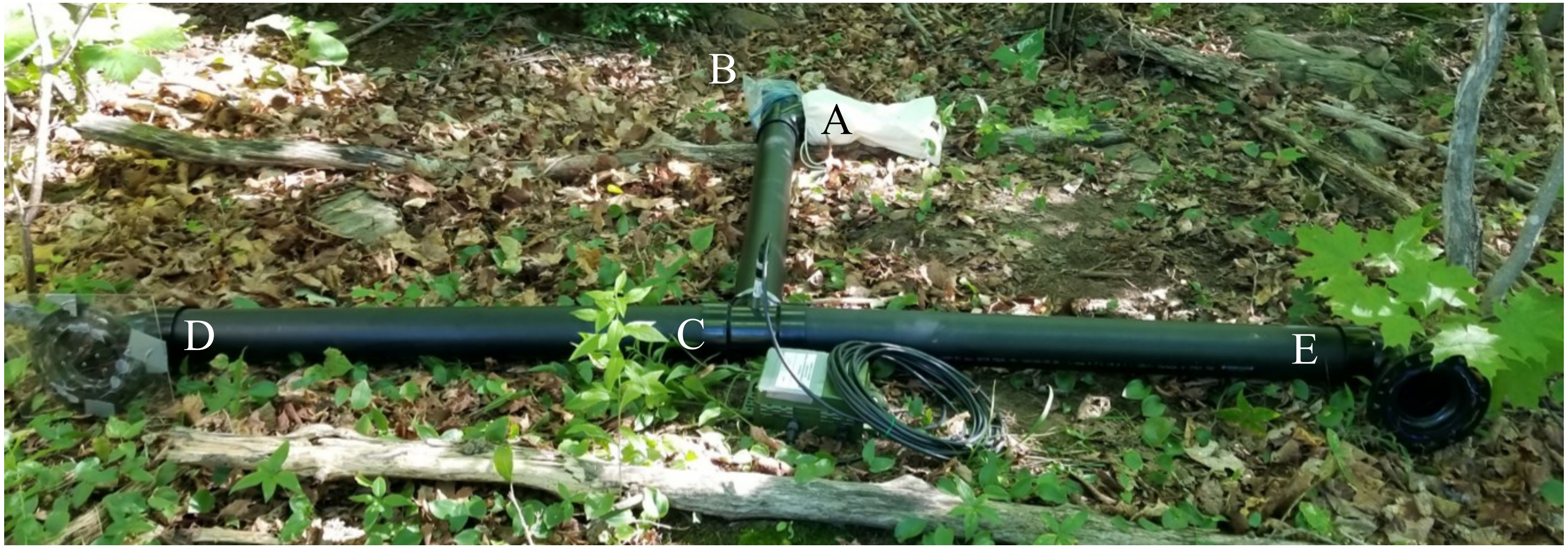

Figure A3.1 Field T-maze used to investigate echonavigation in flying squirrels (*Glaucomys* spp.). (A) Squirrels would enter at the curved T-junction (either from a trap or bag that could be attached to the junction). (B) A trail camera (here covered in plastic for weatherproofing) was attached to the straight portion of the curved T-junction to record squirrel behaviour as they entered A and traveled to C. (C) Squirrels would decide which arm to travel to from the T-junction; a hole was drilled into the top of the junction so that an ultrasonic microphone could be inserted into the maze. (D & E) Each arm had a 90° elbow to obscure the exits from the squirrel while at C; a plexiglass cover (pictured here on arm D) with holes drilled into it was randomly assigned to an arm (with two possible covers, low or high hole density, that could be assigned).

Table A3.1 Summary of call characteristics for 80 vocalizations emitted by *Glaucomys sabrinus* (*G.s.*; individuals = 5) and *G. volans* (*G.v.*; individuals = 12) during a gliding experiment in the Kawartha Highlands, ON (2020). All calls (n) were recorded with an Echo Meter Touch Pro for Android except for one peep recorded on a Song Meter SM4BAT Ultrasonic Recorder (equipped with an omnidirectional SMM-U1 Ultrasonic Microphone).

Superclass	**Class**	**Species**	**n**	**Duration (ms; ±SD) [Min – Max]**	**Peak Frequency (kHz; ±SD) [Min – Max]**
Chirp	Broadband	*G.v.*	1	44.5	24.4
	Chirp	*G.s.*	2	51.5 (±18.4) [38.5 – 64.5]	22.3 (±0.7) [21.9 – 22.8]
		G.v.	16	122.3 (±115.7) [11.5 – 472.5]	13.8 (±1.5) [10.5 – 16.1]
	Warble	*G.v.*	1	73.0	20.0
Screech		*G.v.*	35	216.6 (±59.7) [82.0 – 405.5]	13.7 (±1.9) [10.1 – 17.7]
High-Frequency		*G.s.*	8	4.7 (±2.1) [3.0 – 8.5]	14.5 (±2.8) [12.3 – 21.0]
		G.v.	10	7.1 (±4.0) [1.5 – 12.0]	23.3 (±10.7) [10.5 – 44]
Trill		*G.s.*	2	19.0 (±4.9) [15.5 – 22.5]	17.3 (±5.0) [13.8 – 20.9]
		G.v.	5	84.7 (±40.6) [27.0 – 123.0]	15.4 (±4.3) [13.1 – 23.1]

Table A3.2 Summary of call characteristics for 55 vocalizations emitted by *Glaucomys sabrinus* (*G.s.*; N = 3) and *G. volans* (*G.v.*; N = 13) during individual aviary trials. All calls were recorded on a Song Meter SM4BAT Ultrasonic Recorder (equipped with an omnidirectional SMM-U1 Ultrasonic Microphone), and squirrels were recorded continuously for 24 hours.

Superclass	**Class**	**Species**	**n**	**Duration (ms; ±SD) [Min – Max]**	**Peak Frequency (kHz; ±SD) [Min – Max]**
Chirp	Broadband	*G.v.*	4	39.2 (±1.9) [37.5 – 42]	25.5 (±1.3) [24 – 27]
	Chirp	*G.s.*	2	26 (±2.1) [24.5 – 27.5]	12.0 (±0) [12 – 12]
		G.v.	6	28.2 (±4.2) [23.5 – 36]	14.0 (±5.4) [9 – 23]
	Downsweep	*G.s.*	9	50 (±14.8) [31.5 – 70]	18 (±4.3) [12 – 25]
		G.v.	6	24.5 (±12.7) [13.5 – 48.5]	28.2 (±7.1) [16 – 35]
	Whistle	*G.s.*	1	23.5	24
HF[1]	HF	*G.s.*	6	19.5 (±7.9) [13.5 – 35]	35.3 (±5.3) [29 – 42]
		G.v.	8	18.8 (±4.8) [13.5 – 28]	37.1 (±9.2) [28 – 50]
	HF Downsweep	*G.s.*	3	38.0 (±13.9) [22 – 47.5]	33 (±17.1) [14 – 47]
		G.v.	4	31.4 (±8.2) [24 – 40.5]	26 (±1.2) [25 – 27]
	Hop	*G.s.*	1	38	18
		G.v.	1	34	22
	Rapid Downsweep	*G.v.*	4	19.5 (±7.0) [11 – 27.5]	25.7 (±5.5) [21 – 33]

[1] High-frequency

Table A3.3 Summary of call characteristics for 128 vocalizations emitted by *Glaucomys sabrinus* (*G.s.*) and *G. volans* (*G.v.*) during paired aviary trials (*G.s./G.v.* = 3; *G.v./G.v.* = 9). All calls were recorded on a Song Meter SM4BAT Ultrasonic Recorder (equipped with an omnidirectional SMM-U1 Ultrasonic Microphone), and squirrels were recorded continuously for 24 hours.

Superclass	**Class**	**Species Pairing**	**N**	**Duration (ms; ±SD) [Min – Max]**	**Peak Frequency (kHz; ±SD) [Min – Max]**
Bark	Bark	*G.s./G.v.*	2	39.0 (±12.0) [30.5 – 47.5]	19.0 (±0) [19 – 19]
		G.v./G.v.	26	42.2 (±18.0) [20 – 97.5]	11.6 (±2.1) [8 – 18]
	Chatter	*G.s./G.v.*	2	68.7 (±56.2) [29 – 108.5]	16.5 (±6.4) [12 – 21]
		G.v./G.v.	9	33.2 (±13.8) [14.5 – 57]	13.3 (±2.3) [12 – 19]
Chirp	Broadband	*G.s./G.v.*	10	34.8 (±5.5) [26.5 – 44]	20.9 (±3.6) [16 – 28]
		G.v./G.v.	17	29.4 (±5.5) [20 – 38]	18.3 (±3.8) [12 – 25]
	Chirp	*G.v./G.v.*	8	35.4 (±13.2) [15 – 53.5]	18.4 (±5.4) [11 – 27]
	Downsweep	*G.s./G.v.*	5	39.8 (±10.7) [27 – 56]	18.2 (±1.8) [16 – 21]
		G.v./G.v.	5	32.2 (±15.6) [14 – 54.5]	22.2 (±6.7) [16 – 32]
	Whistle	*G.s./G.v.*	4	36.6 (±7.9) [28.5 – 47.5]	24 (±0.8) [23 – 25]
		G.v./G.v.	1	30.5	24
HF[1]	HF	*G.s./G.v.*	7	23 (±11.4) [13.5 – 47]	36.9 (±10.8) [27 – 58]
		G.v./G.v.	11	23.0 (±9.0) [12.5 – 39]	27.4 (±14.4) [12 – 61]
	HF Downsweep	*G.s./G.v.*	2	41.0 (±17.0) [29 – 53]	28.5 (±10.6) [21 – 36]
		G.v./G.v.	2	31.5 (±9.2) [25 – 38]	28.5 (±21.9) [13 – 44]
	Hop	*G.s./G.v.*	1	38	39
		G.v./G.v.	6	32.1 (±8.4) [23 – 42.5]	22.0 (±9.0) [13 – 38]
	Rapid Downsweep	*G.s./G.v.*	5	16.1 (±3.6) [12 – 19]	23.4 (±7.2) [13 – 30]
		G.v./G.v.	5	25.1 (±9.4) [15 – 40.5]	16 (±7.3) [5 – 22]

[1] High-frequency