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Whence and Whither: Acoustic Variability and Biogeography of Tarsiers in North Sulawesi

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Acoustic Variability and Biogeography of Tarsiers

in North Sulawesi

by

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ABSTRACT

I recorded and analyzed the morning duet calls of eastern tarsiers (*Tarsius* spp.) in North Sulawesi to examine the effects of geography and geologic history on their call structure. *Tarsius* species exhibit interspecifically variable duet calls shown to correlate with species differentiation and distribution. They are distributed across Sulawesi, a biogeographically complex island in the Indonesian archipelago, where tectonic activity and multiple glaciations during the Pleistocene generated and modified barriers to their dispersal and gene flow.

Recordings were made at ten locations from November of 2012 through June of 2014. Two locations were categorized as mainland, while eight island locations were categorized as either shallow or deep, according to the distance and bathymetric depth separating them from the mainland. My first hypothesis was that tarsier calls on islands separated by depths of less than 130 meters would be more strongly correlated to calls found on the mainland than would the calls from islands separated by deeper water, due to dispersal and possible hybridizations during glaciations. There was a higher degree of similarity between the mainland locations and the shallow water islands than was found between the deep water islands and either shallow water islands or the mainland.

My second hypothesis was that a stepping stone pattern of colonization would be evidenced in the acoustic structure of tarsiers from the Sangihe Arc, with each island showing vocalizations more similar to its immediate neighbors than to other islands. Since tarsiers were not found to be present on two of the islands, I was unable to trace the

entire arc as planned. It was found, however, that Sangihe (the largest island and the farthest north of the islands) was the most acoustically unique, as expected.

Both genetic drift and environmental factors pay a role in evolving animal communication, but I hypothesize that it is more likely the former at work in this case, as the habitats are similar, and I found no strong evidence of short term habitat adaptations or frequency partitioning. The spectral and temporal structure of the duet calls on the mainland and shallow water islands showed no clear geographical bias or patterns, suggesting that panmixia and hybridization during recurring glaciations may function in preventing subdivisions among the populations.

In loving memory of my mother,
Jil Cole Kulander

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INTRODUCTION

Sulawesi, Indonesia, is a biogeographically complex island, where shifting tectonic "microplates" of Asian, Australopapuan, and ophiolithic origin have sutured together over the past 50 million years, each contributing its own distinct biota to what now is a single landmass (De Boer & Duffels, 1996; Driller, et al., 2009; Evans et al., 2003; Hall 1996; Krause 1966; Stelbrink et al., 2012). These converging land masses, along with successive and reiterated glaciations during the Miocene and Pliocene, generated and modified barriers to dispersal and gene flow, offering a continually shifting landscape of vicariance and hybridization events throughout the proto–Sulawesi archipelago (Driller et al., 2015; Hall, 1996; Merker et al., 2009; Stelbrink et al., 2012). Sulawesi and its surrounding islands accordingly provide a unique laboratory in which to examine and assess distinct biogeographic patterns of island distributions and colonizations.

Tarsiers are small nocturnal primates endemic to Indonesia, Malaysia, and the Philippines, with the three largest regions of endemism (Borneo, Sulawesi, and the South Philippines) each exhibiting distinct phenotypes (Groves & Shekelle, 2010; Merker et al 2009; Wright et al. 2003). Of these, the Sulawesi species are the only tarsier group known to exhibit the interspecifically variable "duet calls." These calls have been shown to correlate with species differentiation and distribution (Gursky–Doyen 2013; MacKinnon & McKinnon, 1980; Nietsch 2003; Nietsch 1999). Several Sulawesi tarsier species have now been described based initially on the acoustic structure of their duet calls, whose value as diagnostic tools has been well documented in both the tarsier family and in many

of its primate cousins (Ambrose 2003; Burton, & Nietsch, 2010; Gursky–Doyen, 2013; Haimoff, 1984; Nietsch, 1999; Shekelle, 2008).

In this chapter, I first will address Sulawesi's unique biogeography, and the geologic history that have made it one of the world's biogeographic "hotspots." I will then examine the tarsiers' biological history as the lineage has evolved, diversified, and survived in this landscape. Next, I'll discuss the research and significance of acoustic form in both tarsiers and other primates, and my aims in conducting this research. Finally, I will present the purpose and significance of this research as it relates to taxonomy, conservation, and our understanding of primate evolution.

Geography

At about 180,681 Km^2, Sulawesi is the world's 11th largest island, and Indonesia's 2nd largest, and sits front and center in Wallacea, one of the world's most biogeographically complex and tectonically active regions. The island itself is comprised of allochthonous fragments of four different tectonic systems, including the west Sulawesi volcano–plutonic Arc, the central Sulawesi metamorphic belt, the east Sulawesi ophiolite belt, and a fourth containing several smaller continental fragments (Hall, 1996). Tectonic subduction and collision throughout the last 50 million years have brought together the above belts to form this unique island (Burton & Nietsch, 2010; De Boer & Duffels, 1996; Esselstyn et al., 2009; Esselstyn & Brown, 2009; Gursky, 2010; Hall, 1996; Stelbrink et al., 2012).

Sulawesi's southwestern peninsula is Asian in origin, believed to have fragmented

from the Sunda shelf during the Eocene (DeBoer & Duffels, 1996; Evans et al., 2003;

Hall, 1996). Many of the island's endemic fauna were colonists from this original

Figure 1. Map of Sulawesi showing the geologic origins of the island.
The W. Sulawesi Plutono-Volcanic Arc fragmented from the Sunda shelf, contributing Asian organisms,
while the Banggai-Sula Block contributed Australopapuan flora and fauna (Watkinson, I.M., 2011).
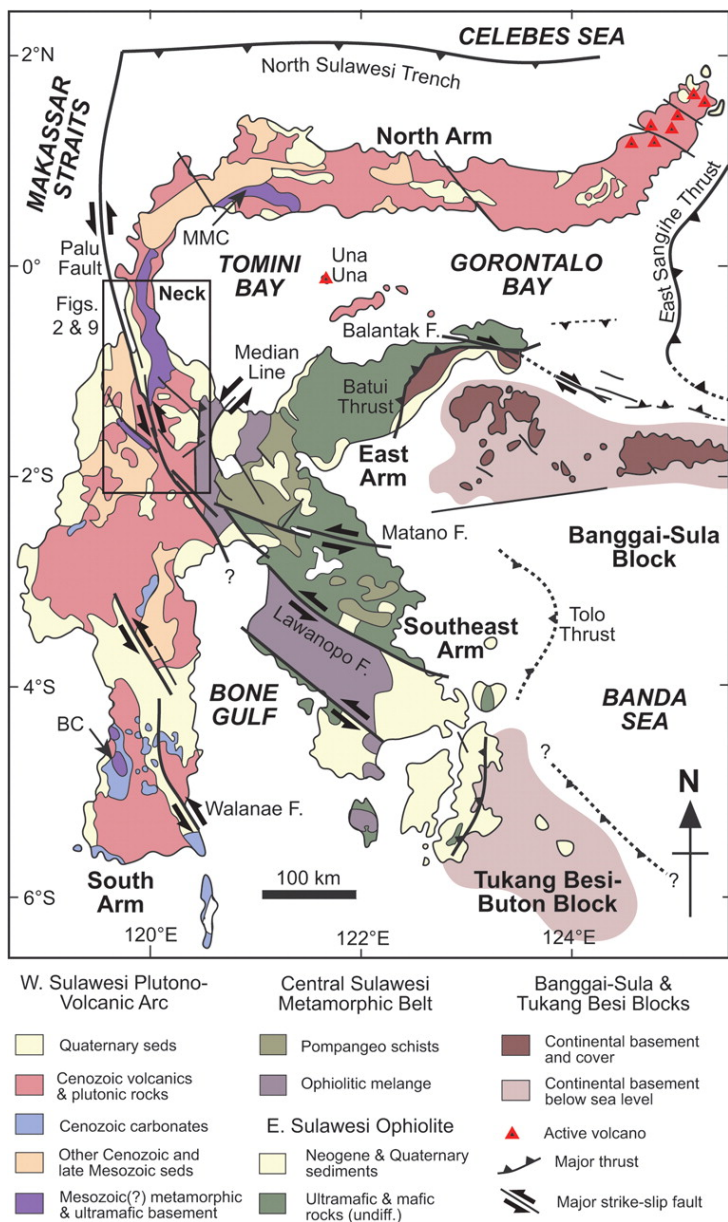
Tectonic movement during the early Miocene joined a newly formed ophiolithic archipelago with the Sula Spur – a landmass on the edge of the Australian continental margin. The Sula Spur brought its own Australopapuan organisms to add to the proto–Sulawesi melting pot. Researchers have shown that some of these ancient tectonic boundaries coincide closely with species boundaries of a variety of fauna, including frogs, macaques, toads, and tarsiers (Driller et al., 2015; Evans et al., 2003; Ruedas & Morales, 2005).

Glaciations during the Miocene and Pleistocene further contributed to diversification and speciation of the Sulawesi biota as the oscillating changes in sea levels led to the emergence and submergence of land bridges—the former allowing dispersal and the latter creating vicariant events. During glaciations, low sea levels (120–130m below present, during the last glacial maximum) would have connected many of the now–isolated islands, allowing otherwise landlocked species repeated opportunities to disperse. The punctuating interglacial periods would subsequently cause the seas to engulf connecting corridors, potentially leading to allopatric speciation and high levels of species diversification (Driller et al., 2015; Lambeck et al., 2002; Yokoyama, 2000;).

Past fluctuations in sea level were instrumental not just for Sulawesi tarsiers, but for their counterparts in Borneo and the Philippines as well. Sundaland, home to the endemic *Tarsius bancanus,* is composed of a large continental shelf, whose islands (most significantly Borneo and Sumatra) were connected during glaciations, providing tarsiers easy dispersal opportunities. Meanwhile, *T. syrichta*'s habitat held similar dispersal opportunities when oceans fell, as the now–isolated southern Philippine islands became the contiguous Greater Mindanao (Brown et al., 2010; Hall, 1996).

Plate tectonics and glacial events left indelible marks on the flora and fauna of Sulawesi, where 98% of the terrestrial mammals are endemic, and there is strong evidence that those movements and sea level changes influenced the tarsier radiation (Driller et al., 2015; Evans et al., 2003; Merker et al., 2009).

Tasiers in Time

Tarsiiformes rank among the most ancient mammal families, with an independent lineage that last saw an ancestor in common with remaining primates at least 58 Mya (Merker et al., 2009). Their genesis lies shrouded in mystery, as researchers struggle to elucidate phylogenies from a meager fossil record.

Primates, a mammalian order with over 600 extant species, are believed to have begun their radiation and diversification between the Late Paleocene and Early Eocene (Pozzi et al., 2014). The rich tropical forests that then covered much of the earth and extended well beyond the confines of today's rainforests would have helped to develop the primate visual systems and unique appendages, while the lack of seasonality is hypothesized to have allowed them to develop longer life histories and more complex brains (Jablonski, 2005). The Early Eocene ancestor we shared with the tarsiiforms would likely have been very similar to the tarsier – less than 1 kg, insectivorous or frugivorous, and using exclusively vertical leaping and clinging, an almost unique form of saltatorial locomotion among primates (Jablonski, 2005).

The identity of that ancient ancestor remains up for debate, however, as divergent theories on early primate phylogeny struggle to identify not just Primates' earliest origins, but to inform our understanding of the current phylogenetic tree. The earliest

known crown primates emerged in the Early Eocene, when superfamilies Omomyidea and Adapoidea appeared on the scene, spanning Asia, Europe, North America, and Africa. The large–scale faunal extinction concurrent with the angiosperm radiation at the Cretaceous–Paleogene boundary had recently opened up an array of niches that opportunistic mammals—among them the well–represented euprimates—quickly moved to occupy. The tropical forests of the Eocene that covered much of the Holarctic, proved to be an ideal ecosystem to nurture the unique physiology and life history of early primates (Pozzi et al., 2014).

The exact configuration of early primate lineages as they diversified is hotly contested, and hypotheses regarding Tarsiiformes' emergence provoke different configurations of the primate phylogeny. The split between tarsiiforms and the rest of the primates lies in such antiquity that long branch attraction makes pinpointing the divergence and subsequent phylogeny extremely difficult even using molecular techniques (Wright et al., 2003).

Two dominant theories of tarsier phylogeny have developed over the years, with a growing aggregate of evidence supporting the existence of a Tarsier and anthropoid clade, the suborder Haplorrhini, alongside a sister group Strepsirrhini—containing lemurs, lorises, pottos, and galagos. Increasingly, molecular data (including evidence from retrotransposons, nuclear and mitochondrial DNA, and macromutations in nuclear DNA) have reinforced the positioning of tarsiers as a sister group to anthropoids, representing the oldest haplorrhine lineage (Groves & Shekelle, 2010; Gursky–Doyen 2011; Merker et al 2009; Poux, 2004; Wright et al., 2003).

A more traditional view, dating back to the 18th century (Groves, 2010) and derived primarily from morphological data, holds that the tarsiers belong in the "prosimian" group alongside their Strepsirrhine cousins. Tarsiers have historically been relegated to this "prosimian" group, which includes all of the Lemuriformes, with Tarsiiformes putting in a guest appearance. Classified as lemurs when they were discovered, Tarsiers were first identified as an independent lineage by Storr in 1780 (Pozzi et al., 2014). While "prosimian," has been changed from a clade to grade, it is still used as a term for morphological and ecological comparisons among the members. A minority of molecular studies support the prosimian distinction (Huang, 2012). When examining mouse lemurs or galagos, it is superficially easy to see how tempting it would be to embed the tarsier within the Strepsirrhines (Poux & Douzery, 2004).

Fossil evidence of early primates shows an early division between the omomyid and adapid lineages. These early clades split around 50 Mya, though the exact timing and relation to other primate lineages remains debated. The haplorrhine/strepsirrhine division posits that the crown clade Haplorrhini's common ancestor may lie within the omomyid lineage, or that the split occurred prior, leaving tarsiers the lone descendants of the omomyids, with anthropoids stemming from the Eosimiidae. It is also commonly hypothesized that omomyids are a sister clade to the haplorrhines, and do not have extant descendants (Bajpai et al., 2008.). A second early lineage, the adapids, are similarly extinct, though their clade is solidly nested within the strepsirrhines (Bajpai et al., 2008; Nijman & Nekaris, 2010; Ross, 2000).

Tarsiers began to emerge concurrently with adapids and omomyids as these latter made their appearance in the Early Eocene fossil record. Beginning in the latter part of

the Early Eocene, there is a sparse but informative fossil record, which suggests early origins of tarsiers in Asia, and an exceptionally conserved morphology, ecological niche, and by extension, ecosystem (Jablonski, 2005). †*Tarsius eocaenus* was found in China in 1994, and remains the earliest known tarsier fossil – dating to around 40 MYA (Nijman & Nekaris, 2010). The controversial †*Afrotarsius*, found in Egypt, is the only tarsiid fossil found outside of Asia (Ducrocq, 2001).

While these fossils are few and far between, they show the extremely conserved morphology and conserved habitat of the taxon. †*Xanthorysis tabrumi,* also from China, dates to the Late Middle Eocene, and was identified as a sister group to the tarsiids – which suggests that the tarsiid radiation would have begun by the Early Paleogene (Nijman & Nekaris, 2010.) The paucity of fossils has led to drastically differing timelines for the origin and radiations of primates, as no crown primate fossils exceeding 56 Mya have been found, but molecular estimates place the origins of primates into the Late Cretaceous (Campanian, 74.1 Mya, 95% CI: 68.2–81.2 Mya; Pozzi et al., 2014).

In the Middle Eocene, the number of primate clades began to wane in North America, but continued holding in Asia and Africa, where the biome remained tropical and forested (Jablonski, 2005). At some point in the anthropoids' early origins, there was a shift to diurnality. Much of the tarsier's ocular morphology is homologous to the anthropoids'—including diurnal adaptations such as a lack of a tapetum lucidum, and presence of a fovea centralis. If that shift did indeed happen in the tarsiid–anthropoid stem lineage, then tarsiers' adaptations to nocturnality would be secondarily acquired (Ross 1996, 2000).

In the Early Eocene, angiosperm forests had spread across the Holarctic, providing full tropical rainforests, multistratal vegetation, and large quantities of insects. For tarsiers, this primeval ecosystem has remained stable to the present day, albeit in ever decreasing area (Jablonski, 2005; Wright et al., 2003). It was in these early forests that the tarsier's specialized locomotion was likely honed. Leaping gave them an advantage as they traversed the understory in search of protein–rich insects. As tarsiids moved with the continually shrinking rainforests, there was little need to change their mode of transportation, whereas the anthropoids may have been forced to evolve novel modes of locomotion—such as bipedality—in their changing ecosystems (Jablonski, 2005; Wright, 2003).

During the Miocene, when the tarsiids' biome covered much of the planet, they could be found throughout the Holarctic, and it is probably during this time that the initial split between the modern tarsier groups was initiated. The Philippine tarsiers colonized Greater Mindanao and the Western tarsiers took up on Sundaland. Molecular clock estimates for this split suggest that it had occurred by the Oligocene (Groves & Shekelle, 2010). Recent phylogenetic work by Driller et al (2015) used autosomal markers and the sex determining region of the Y chromosome to estimate that Sulawesi tarsiers separated from the other crown tarsiids in the mid Miocene—an epoch that saw great opportunities for dispersal as the Sunda shelf and the Sula Spur collided, while low seas caused even more land to emerge (Evans et al., 2003; Hall, 1996).

Once on the proto–Sulawesi peninsula, it was at least 13 Mya before tarsiers further split (Driller et al., 2015). Repeated sea level drops during the Pliocene introduced land bridges, allowing local fauna to colonize islands surrounding the principal landmass

of Sulawesi during the rest of the epoch and in the subsequent fluctuations of Pleistocene

sea levels (Burton & Nietsch, 2010, Merker et al., 2009).

Tarsiers Today

      While dispersal and colonization in the Miocene established tarsiers throughout

Asia, retreating rainforests and tectonic movement resulted in the isolation of the

Tarsiidae in three distinct zoogeographic regions, wherein allopatric speciation led to

three major regions of endemism. *Tarsius bancanus,* the sole recognized species in the

western tarsier group, remained on the Sunda Shelf, colonizing the now unconnected

islands of Borneo and Sumatra. The *"syrichta"* group colonized the Philippines, where

they dispersed throughout Greater Mindanao, a region that would have been a contiguous

landmass during glacial periods, but is today comprised of a series of isolated islands.

The eastern tarsier group, once represented only by *T. spectrum*, is now believed to be the

most taxonomically diverse of the groups, and is the focus of this study (Groves &

Shekelle, 2010; Gursky, 2010; Merker et al., 2009; Wright et al., 2003). The Eastern

tarsiers are on and around Sulawesi, where both tectonic movement and changing ocean

levels continuously changed the geography of their world. Extant tarsiers occupy only a

fraction of their once expansive range, and these three zoogeographic regions are prime

real estate, harboring rainforests similar to those dominating the region in the Eocene

(Merker et al., 2009).

      The tarsiids (Merker et al., 2009) are a group of nocturnal, morphologically

cryptic species, with a highly conserved morphology that can appear deceptively simple.

Estimates of the divergence of the three distinct lineages ranges from 5.6 MYA to 27.4

MYA (Hasegawa & Horai, 2009; Matsui et al., 2010; Wright et al., 2003). Given the age

of these lineages, there is room for debate over the exact number of species, and even

distinct genera, within the family. Groves and Shekelle (2010) have outlined a new

taxonomy, naming the Western, Eastern, and Philippine tarsiers each to its own genus:

Cephalopachus (one species with 4 subspecies on Sumatra, Borneo, and outlying

islands), *Tarsius* (9 recognized species and 2 in the process of description, all restricted to

Sulawesi and outlying islands), and *Carlito* (1 species and 3 subspecies but possibly as

many as three species [Brown et al. 2014], restricted to the Philippine Archipelago),

respectively. Within the Sulawesi endemic *Tarsius,* the species count has grown rapidly

as researchers look beyond morphology, studying acoustics and genetics to help identify

new species (MacKinnon & MacKinnon, 1980; Merker et al., 2009; Nietsch, 2003).

Reconstructing the phylogeography that resulted in the tarsiers' current pattern of

distribution helps to elucidate not just patterns of current distribution, but also their

conservation status and evolution of morphological traits. For these tiny, leaping

insectivores, the angiosperm rainforests that once dominated the earth have not

disappeared entirely, and much of Southeast Asia continues to provide its smallest

primate denizens with the non–seasonal tropical forests in which insects abound, as do

the understory, small diameter trees, and high quality sleeping sites that supported their

ancient ancestors (MacKinnon, 1980; Nijman, 2010).

Tarsiers today still weigh in at only 100–140 grams, and are the only purely

faunivorous extant primates (Driller et al., 2009). Tarsier locomotion has been likewise

conserved: vertical clinging and leaping, perfectly adapted for the understory–rich second

growth forests (Driller et al., 2009; Reason, 1978). Tarsiers of both sexes are territorial,

and their frequent family vocal displays, olfactory marking, and aggressive patrol of their

home area result in little overlap among families. One study found that males have

slightly larger territories on average (2.12–3.47 Ha) than females (1.46–3.36 Ha)

(Gursky–Doyen, 2010, 2011). Most territories have between one and three sleeping sites,

which are either dense thickets of grass, bamboo, pandanus, dense tangles of vines, tree

hollows with multiple exits, or the aerial roots of strangling figs (MacKinnon &

MacKinnon, 1980). Families tend to use one site preferentially, and the quality of

sleeping sites are believed to be a good indicator of the type of mating system within each

family, as a site with sufficient resources to successfully support a growing tarsier family

may attract multiple adult females to the territory–holding male (Gursky–Doyen, 2010).

Most observed family groups consist of a mated pair and their immature offspring,

though at those sites considered high quality (large, protected sleeping trees,) researchers

have consistently found polygyny (Gursky, 2010; Gursky–Doyen, 2010.) Unusually,

tarsiers exhibit intrapopulation variation in their mating system (Gursky, 2010). In

polygynous groupings (which were found to comprise 16–18% of families), a single adult

male lives with multiple adult females and the immature offspring of all the pairings

(Gursky–Doyen, 2010). While tarsiers are not known to mate for life, they may exhibit

site and mate fidelity for years at a time – albeit with the occasional extra–pair copulation

to mitigate inbreeding (Driller et al., 2009, Gursky, 2010, Gursky–Doyen, 2010).

    In mammalian polygynous mating systems, groups tend to experience male biased

dispersal, whereas it is generally the young female who leaves home in monogamous

groups (Gursky–Doyen, 2010). Tarsier dispersal patterns are more or less equal, with no

difference in dispersal rates between the sexes—or between offspring produced by

polygynous or monogamous matings. Males and females are both likely to disperse, though the males tend to outdistance their sisters (Gursky–Doyen, 2010.)

Both males and females scent mark the borders of their territories with urine, while males also employ an epigastric gland on their abdomen to mark not only territorial boundaries, but their mates' fur as well. Females mark their mates and territories with their genitals during estrus (MacKinnon & MacKinnon, 1980; Nijman & Nekaris, 2010).

Tarsiers have an unusually long gestation for their size, with pregnancies lasting six months – more comparable to larger primates such as baboons and macaques (6 and 5.5 months, respectively) than to similarly–sized primates such as the mouse lemur, which gestate an average of only 2 months (Altmann et al, 1977; Gursky–Doyen, 2011; Hadidian & Bernstein 1979; Nijman & Nekaris, 2010; Schmid & Speakman, 2000). Biannual mating seasons in May and November lead to births in April though May and November through December (Nijman & Nekaris, 2010; MacKinnon, 1980). Newborn infants weigh in at almost a third of their mother's weight – which perhaps contributes to their early development milestones, such as their ability to travel independently in less than a month, and their weaning at just over two months (Gursky–Doyen, 2011; MacKinnon & MacKinnon, 1980; Nijman & Nekaris, 2010).

With such heavy offspring, tarsier mothers have a system whereby they "park" or "cache and carry" (Gursky, 2010; Nijman & Nekaris, 2010) their young – carrying them by mouth and leaving them in trees within their foraging territory. Tarsiers hunt in proximity to other family members, so there often are immature females present around to check up on related infants. Adults forage as a loose group, which may lower the yields of their hunt; though it is thought that the benefits of a group against predation

pressures outweigh the costs involved in competition (Ambrose, 2003; Gursky, 2002, 2005).

Seasonal changes in the forest bring changes in resources, and tarsiers spend more of their time foraging in the dry season, increasing territorial disputes, intragroup encounters, and predation (Gursky, 2000). Tarsiers have been seen to exhibit predator mobbing of snakes, one of their most ubiquitous predators. Monitor lizards, snakes, civets, birds, and domestic cats are all known predators, and tarsiers' role in the bush meat trade has been suggested, but not observed by researchers (Gursky, 2005; MacKinnon & MacKinnon, 1980.) Humans do, however, routinely catch them as pets, most often causing the tarsiers to die in captivity.

Acoustics

Animals across the kingdom use acoustics to communicate with conspecifics and the world at large. Apes, baboons, gibbons, colubus, tamarins, guenons, lemurs, and tarsiers, have all shown taxonomic differentiation based on their vocalizations (Nietsch, 1999; Reason, 1978). In nocturnal animals particularly, vocalizations are a key component in conspecific communication, and cryptic animals, species that look superficially similar despite being otherwise genetically isolated (Zimmerman & Radespiel, 2014), often show more distinctive vocalizations than morphology (Ambrose, 2003). For animals traversing a dark world, textured acoustics and pungent scent markers constitute information–rich messages. For researchers, vocalizations are an important tool in locating, identifying, and monitoring cryptic species, as well as a supplemental

taxonomic key to help elucidate phylogenetic relationships (Burton & Nietsch, 2010;

Nietsch, 1999).

The genus *Tarsius* is the only tarsier group in which audible calls have been

recorded. As the species in this genus are more gregarious than in their sister genera

(Gursky, 2002), it would not be surprising that they exhibit more social behaviors,

including more variance in their acoustic communication. It was recently discovered,

however, that the Philippine genus, *Carlito,* emits ultrasonic alarm calls, demonstrating

that it communicates acoustically—though to what extent remains largely unknown

(Gursky–Doyen, 2013).

Most primates have a repertoire of acoustics, and the duet calls examined here are

categorized as "advertising calls" (used to defend territory or call a mate). Advertising

calls are typically loud, meant to be heard over long distances, stereotypic, and, in many

taxa, species–specific—featuring some of the most prominent differences in acoustic

structure between species (Burton & Nietsch, 2010; Nietsch, 1999; Zimmerman et al.,

2000). These unique calls are often indicators of species boundaries, and their assessment

is commonly used to noninvasively examine specific diversity and patterns, in order to

begin to assess phylogenetic relationships (Burton & Nietsch, 2010; Zimmerman et al.,

2000; Ambrose, 2003).

Duet calls are conspicuous advertising calls wherein males and females

coordinate acoustic phrases, making adjustments to the timing and frequency of their

vocalizations in response to each other (Brumm & Naguib, 2009). Duetting occurs in a

variety of animal species, a phenomenom attributed to functional convergence, since it is

found in a wide range of taxa. Among primates it is found almost exclusively in

monogamously stable, territorial species (Haimoff 1986; Marshall–Ball et al., 2006). For the evolution of duet coordination to occur, participation must theoretically be beneficial to individuals of both sexes, and it is hypothesized that these benefits come in the form of joint territory defense, pair bond formation and reinforcement, mate guarding, and maintaining spatial organization among intraspecific neighbors (Haimoff 1986; Marshall–Ball et al., 2006; Méndez–Cárdenas & Zimmermann, 2009; Naguib et al., 2009). Tarsier duet calls also have been shown to function in mate recognition and attraction (Burton & Nietsch, 2010; Nietsch, 1999). These functions ensure that vocalization forms have significant impacts on reproductive isolation. Differences in signaling and recognition are an effective isolating mechanism in sexual selection, and contribute to the differentiation of species according to the Recognition Concept of Species (Ambrose, 2003; Zimmermann & Radespiel, 2014).

Tarsier duets are most often heard around dawn, as the family returns to its sleeping spot for the day. They last an average of 4 minutes, and can be heard almost half a kilometer away, with most groups calling multiple times as they approach the sleeping site. Calls are sexually dimorphic, with all adult and often even juvenile members lending their voices to the chorus. Duet calls are occasionally heard at dusk or during the night as well (Burton & Nietsch, 2010; Gursky, 2010; Gursky–Doyen, 2011; MacKinnon & MacKinnon, 1980; Nietsch, 1999).

There are no data on whether tarsier vocalizations are learned or genetically programmed. Without invasive cross–fostering experiments or hybrid individuals to examine, this question may not be answered in the near future. Studies on gibbons suggest that their songs are most likely genetically determined (Nietsch, 1999), though

gibbon duets are considered convergent with tarsier duets, and thus not necessarily comparable (Haimoff, 1986).

Mackinnon and Mackinnon (1980) were the first to describe three geographic variants of tarsier calls based on their "acoustic form." Their description of "Manado form" describes the most prominent duet call in North Sulawesi, extending from the tip of the Northern peninsula down to Gorontalo – a geographic region found to have low genetic diversity by Driller et al. (2015).

Subsequent studies found that the differences in acoustic form of the duet call could be mapped not only onto the geography of the area, but also onto specific differentiation within the genus *Tarsius* (Ambrose, 2003; Burton & Nietsch, 2010; MacKinnon & MacKinnon, 1980;). *Tarsius spectrum* and *T. dianae* were among the first species to be differentiated based initially on the differences in their acoustic forms, and geographic communities across Sulawesi show population–wide variations in duet calls (Burton & Nietsch, 2010; Nietsch, 1999; Wright, 2003).

Testing the Acoustic Variability of Tarsiers in North Sulawesi

Because the duet call has been used to successfully differentiate species in Sulawesi tarsiers, we mapped the acoustic structure of this ritualized call to the known geologic history of North Sulawesi and its surrounding islands. We chose ten islands on which to record the tarsier populations, as well as two mainland locations.

These locations were chosen and categorized into three distinct groups. The "mainland" grouping features two distinct locations on the North Sulawesi mainland (Tangkoko and Klabat,); the "deep" group contains islands separated by long distances

and deep water channels, namely: Biaro, Tagulandang, Sangihe, and Siau. These ophiolithic islands have never been contiguous with the mainland; colonization is expected to have occurred through rafting alone. The final, "shallow," grouping is comprised of the six islands directly surrounding the mainland (Bangka, Bunaken, Lembeh, Mantehage, Manado Tua, and Talisei). Initially, these were further categorized by the depth of sea channels separating them, as local diving lore holds that the islands of Bunaken, Mantehage, and Manado Tua are cut off from mainland Sulawesi by trenches plummeting up to three miles under sea level (Jackson, 2003; Bunaken National Park, 2016; Greenwald, 2015). GIS data obtained from GEBCO, NOAA, and GoogleEarth, however, do not support evidence of a deep water channel isolating these islands; therefore, they were all grouped together as "shallow" islands. All "shallow" islands are separated from the mainland by depths of less than 100 meters, and would thus have been accessible by land during periodic glaciations.

Our hypothesis was that the calls of tarsier populations on the "shallow" islands would be more similar to the calls of the mainland populations than would those inhabiting "deep" islands, due to dispersal and possible hybridization during glaciations.

The "shallow" islands surround the tip of the North Sulawesi Peninsula, where they sit on a submerged shelf connecting them to Sulawesi proper. During glaciations, when sea levels dropped over 130 meters, this shelf would have emerged to form terrestrial corridors allowing for either initial colonization events or subsequent panmixia amongst populations already on their way to allopatric speciation. The Last Glacial Maximum was only about 26,000 years ago (Lambeck et al 2002; Yokoyama et al.,

2000). "Deep" islands, on the other hand, have always had deep–water barrier separating

them from the mainland, necessitating colonization of terrestrial species by rafting.

We also attempted to test the congruence between tarsier vocalization structures

and the expected stepping stone pattern of colonization of the Sangihe Arc, which

stretches from Sulawesi to Sangihe, via Talisei, Bangka, Biaro, Tagulandang, and Siau.

In examining this arc, we wanted to see if the call structure of each island's population

was more similar to its neighboring islands' call structure than to any others, suggesting

stepping stone colonization (Gilpin, 1980).

Aside from changes due to genetic drift, there were several other variables to

account for, including acoustic adaptation to local environmental conditions. Habitat

structure, predation considerations, and ambient noise, all may have had more influence

on song structure in either present or ancestral populations than had genetic distance. It is

also important to consider that inferred patterns of colonization may be incorrect, and

recent rafting events (during the Pleistocene or even later) or lack of gene flow between

shallow–water islands may be responsible for unexpected results (see Figure 1).

Conclusion and Significance

The exceptional biogeography and geological history of the islands of North

Sulawesi provide an unparalleled backdrop for the evolution of today's tarsiers, and thus

my investigation into its expected phylogeography and acoustic variance. Colonization

patterns of dispersal and allopatric speciation across this area have resulted in isolated

regions of endemism, and with each speciation a new song is composed. Since tarsiers

are one of the only extant primate taxa known to have dispersed and diversified on a

volcanic arc, recording their ritualized calls has offered a unique opportunity to analyze

primate acoustic structure while mapping it against a rich phylogeographic backdrop

woven by the events of the last fifty million years, including tectonic activity and shifting

continents, rafting, allopatric speciation, panmixia, and the changing landscapes of a

climate in flux.

Humans and macaques are the only other extant primate species to successfully

colonize volcanic arcs, and we share many processes and mechanisms with our tiny

tarsiid relatives. Examining tarsier adaptation, diversification, and evolution allows us to

draw parallels with other extant primates, and perhaps most importantly, with the

shadows of our own history (Zimmerman & Radespiel, 2014).

Identified as one of the world's top conservation "hotspots," Sulawesi's

biodiversity is under critical anthropogenic pressure. With 98% of its mammal species

categorized as endemic, this means losing not only local populations, but unique species

(Palacios et al., 2012). Bangka island, analyzed in 2012, has since been almost

completely decimated by unauthorized mining, while the rest of our field sites are losing

acreage by the day. Some 83% of Indonesian primates are endangered, a statistic difficult

to fathom not just for the biodiversity of our order, but also because primates are a

prominent indicator species of this epidemic habitat destruction, and data on their

biogeography and ecology are important in the context of environmental destruction and

extinction (Kappeler, 2002; Palacios et al., 2012). Examining questions of

phylogeography and speciation in this area, and their application to conservation, is of

pressing urgency.

MATERIALS AND METHODS

Acoustic Data Collection

For our purposes, "site" refers to an individual tree or vegetative shelter, home to a single tarsier family group (monogamous or polygynous pairs and their progeny). Tarsiers are territorial and families will defend their sleeping sites; thus we can assert fairly confidently that recordings from a single site belong to only one family group (Gursky, 2003). A "location," by contrast, should encompass a tarsier population with many territories. Each island represents one location, with two locations on mainland Sulawesi separated by 20 km and significant anthropogenic disturbance.

Our initial strategy was to record each of our 12 locations for a total of 2 weeks apiece, enabling us to record multiple iterations of several different family groups' duet calls. In practice, it often took longer than expected to locate sleeping trees, two of our locations proved devoid of tarsiers, and heavy rains often made recording impossible (due to equipment function, a prohibitive signal–to–noise ratio, and behavioral changes), especially during the rainy season between November and April. My team of 1–4 local parabiologists and I made all recordings. These included Yunus Masala and Erdenivan Tundu, rangers from Tangkoko National Park, and students Maryati Abiduna and Muhammad Rizki, from Universitas Negeri Manado.

When our team arrived at each location, we first checked in with the Kepala Desa, or the local head, and asked permission to either camp or stay with local family and friends of the team. Locals offered invaluable information on the nearest tarsier

whereabouts, and the first evening in any location was spent attempting to track tarsiers from their sleeping trees. Tarsiers were located acoustically and through searching for their olfactory markers.

If tarsiers were seen to enter a sleeping site, or we suspected a sleeping site due to word of mouth and/or olfactory cues, we returned to it the following morning. Since tarsier duets typically occur between 5am and 6am, equipment was assembled by 4:30am, and usually dismantled by 7:30am.

The recorder was turned on at the first audible tarsier vocalization, and left running until the calls had stopped for one minute. This was done to preserve battery life, which became a limiting factor at remote recording sites. Start and stop time were noted, as were the types of vegetation serving as a sleeping site, the weather, latitude and longitude, and elevation.

To reduce extraneous noise, tripods were erected beside the suspected sleeping site and microphones were shock mounted. We positioned microphones as closely as possible to the site, since the surrounding rainforest quickly degrades the tarsiers' high frequency calls (Marten & Marler, 1977). An XLR cable was then run from the microphone to the field recorder, which was routinely placed five to fifteen meters from the suspected sleeping site to mitigate the presence of field researchers. Tarsiers are notoriously unperturbed by the presence of humans, though we found families in more remote areas to be slightly less habituated, and thus more likely to choose a different sleeping site if our presence was overly intrusive. Distance and direction from the microphone to the calling individual affected the quality of the recordings, as did ambient noise levels.

On average, it took 2–3 days at any new location to focus in on an inhabited sleeping tree and successfully record its inhabitants. We recorded at each inhabited site until we had at least two successful recordings. While doing so, members of our team scouted out the subsequent site. Once we had satisfactory recordings and a new target, we moved on to the new site. Evenings were spent surveying for new potential sleeping trees.

Table 1. Recording dates and total number of sites recorded for each location sampled. Tarsiers were not found on Biaro or Tagulandang.

Location	Number of Sites Recorded	Date of Recordings
Bangka	4	2013, April 04-19
Biaro	0	2013, March 03-10
Bunaken	2	2013, July19-August 1 2014, July 05-07
Klabat	4	2013, May 25-30 2013, June 07-16
Lembeh	4	2013, January 08-22
Manado Tua	5	2013, June 18-July-01
Mantehage	3	2013, July 05-19 2013, August 31
Sangihe	2	2013, February 26-March 05
Siau	3	2013, February 06-20
Tagulandang	0	2013, March 11-20
Talisei	3	2013, April 26-May 09
Tangkoko	4	2012, November 17 2012, December 09 2012, December 16-20 2012, December 28-30 2013, January 02 2013, March 21-26 2013, May 26 2013, June 13

Locations and Location–Specific Methods

    Mainland Locations: Tangkoko and Klabat

    Tangkoko.

Tangkoko Nature Reserve was where the concept of tarsier recordings for taxonomic study originated. MacKinnon and MacKinnon (1980) recorded dawn duet calls in the forest outside the village of Batu Putih in 1979 – while working to get the area designated as a nature reserve. Tangkoko tarsier vocalizations would become the "Manado Form" in the MacKinnons' work, and it was against these vocalizations that other forms were subsequently compared. Tangkoko Nature Reserve itself claims a 4,450 ha footprint, which when added to the adjoining Batu Angus/Dua Saudara Nature Reserve, gives a combined area of 8,867 ha (Palacios et al., 2012). The twin reserves feature three volcanoes, with the highest peak reaching 1,350 m (Palacios et al., 2012). It is bordered by the sea to the west, and a network of villages, roads, and a small city on all other sides. Illegal logging and poaching represent the biggest threat to local conservation. Within the Tangkoko Nature Reserve, a smaller 600 ha patch near the village of Batu Putih has been designated a Recreational Park, and is the focus of tourism, management, and protective measures.

Vegetation here is classified as lowland tropical rainforest with seasonal rainfall variation (Duboscq et al., 2013; MacKinnon & MacKinnon, 1980; Palacios et al., 2012) although a large percentage of the land is primary and secondary forest, with some burned areas and surreptitious coconut plantations. The wet season lasts from October to May, when most of its 1550–2,400 mm of rain falls (Palacios et al., 2012).

Recordings were taken in the Tangkoko Recreational Park in November and December of 2012, as well as January, March, May, and June of 2013. Four different family groups were recorded during this period. All duetting vocalizations began between 5:13am and 5:27am, and all families recorded in Tangkoko were residents of the ubiquitous and large strangler figs found in the park. Weather was varied, and the session was abandoned if the rain presented high noise interference or danger to the equipment.

Klabat.

Gunung Klabat was our second recording site on the island of Sulawesi. It sits roughly 20 km inland from Tangkoko, with a mountain that ascends to an elevation of 1,995 m (Limbong et al., 2003). An active stratovolcano, it has not erupted in modern history, although it is suspected to have possibly done so in the 17th century (Siahan et al., 2005). Vegetation here is similar to Tangkoko, though with less management and supervision. Coconut plantations are much more ubiquitous.

Dawn duets were recorded between 5:02 am and 5:20 am in May and June of 2013, at elevations between 566–604 meters. All the recorded families inhabited large strangler figs, and four different locations were sampled. Weather was consistently either overcast or raining, and several attempts were aborted due to rain.

Western Shallow Water Islands: Bunaken Marine Park

Three of our "shallow" islands sit within Bunaken National park, a marine park with an area of 5,265 Ha (Sidangoli et al., 2013). The park includes coral reefs, sea grass beds, mangroves, and five islands – all about 15 km off the western coast of North Sulawesi

(Fava et al., 2009; Sidangoli et al., 2013). Bunaken Marine Park was established in 1991, and its marine areas are some of the most diverse in the world, with new species being discovered regularly.

About 30,000 people live within the park boundaries, most on the three islands where we recorded (Bunaken, Manado Tua, and Mantehage), but also on the smaller islands of Nain and Siladen (Sievanen, 2011). Tourism attracts an estimated 25,000 more people each year, generating an average of US$32M a year in the early 2000's, and it is likely that that number has increased in recent years (Fava et al., 2009; Sidangoli et al., 2013). The islands of Bunaken National park are distinct for having large individual trees and complex mangrove ecosystems (Whitten, 2001).

Bunaken

Situated 13 km NE of Manado harbor, but only 3 km from the mainland, Bunaken Island rises in its western part to a rounded hill only 139 m in elevation. The shoreline is ringed by a coral reef (Erwin & Sweetkind–Singer, 2009). Though volcanic in origin, most of the island consists of uplifted fossil coral. While coconut, cassava, banana, and mango plantations take up much of the island's 8 km^2 surface area, weedy scrub also occupies the island, with mangroves and small beaches edging the shoreline (Yorke, 2014).

 In the 1970s, the Indonesian government promoted the growth of tourism on the island and the population has since doubled to around 5,000 in the three small villages on the island. With the creation of the national park in 1991, a tourism economy has supplanted the traditional livelihoods of farming and fishing (Sievanen, 2011). Tourism is

focused largely on the marine preserve offshore, leaving the interior of the island mostly

to agriculture. Unfortunately, this also means that all resources engaged in protecting the

park also focus offshore, while little energy is spent on the terrestrial parts of the park.

Figure 2. Map of Bunaken Island showing the location of sites recorded in July, 2014.

While resorts tend to offer acceptable habitat – guests like lush vegetation

surrounding their amenities - they are also more likely than local homes and villages to

have cats. Cats are known predators of tarsiers (Gursky, 2005; MacKinnon &

MacKinnon, 1980; and personal observation), and the only two places we found tarsiers

on the island were resorts that kept several dogs on site.

Our first excursion to Bunaken, from July 19th to August 1st, 2013, was

unsuccessful. We spent every morning from 4:15am until 7am, and every evening from

4:30pm until 8pm searching the island with a team of five. One evening we found a scent

marking, and a second evening there was a probable tarsier sighting, but vocalizations were never heard.

We returned to Bunaken the following June for four days. During this trip, tarsiers were recorded on June 5th and June 7th, 2014. Our first successful site was within the Cha Cha Resort, near "cottage 4," where the calling began at 5:45. According to the manager, the tarsiers had been there for several years. The second site came as a surprise after an unsuccessful morning. We had broken down the recording equipment and were beginning breakfast, when tarsier calls erupted at 7:54am in a grove of secondary forest and liana beside the Panorama Resort. Weather was warm and sunny in both instances.

Manado Tua

This circular island encompasses 10 km^2, much of it taken up by an inactive volcanic cone rising at a 27 to 45 degree slope to 600 m above sea level, the highest point of the five islands comprising Bunaken Marine Park, and often used as a key navigation landmark (Erwin & Sweetkind–Singer, 2009). Manado Tua supported a kingdom in the 17th century, but its inhabitants fled to the mainland due to piracy threats, lack of fresh water, and an "invasion of black macaques" (Sievanen, 2011). They took the name with them, and Manado is now the principal port in the region. Unlike Bunaken Island's robust growth, Manado Tua's population has actually decreased (currently around 3,200 inhabitants), and primarily restricted to the shore (Sievanen, 2011).

While the human population lives on the island's circumference, their gardens and coconut plantations extend well inland, up the sides of the mountain. Woody scrubland and bamboo cover much of the remaining area of the island. During our two weeks of

recording, from June 18th to July 1st, the weather ranged from sunny to cool and overcast to heavy showers. We recorded at five sites, including three sites along the north and northeast coast, one site half a kilometer inland at an elevation of 100 m, and one site on the upper slopes of the volcanic cone, half a kilometer from the summit at an elevation of about 500 meters. The uppermost sleeping site was in a bamboo grove, while the rest were in a combination of secondary forest and liana.

Figure 3. Map of Manado Tua Island, showing the location of sites recorded in June and July of 2013.
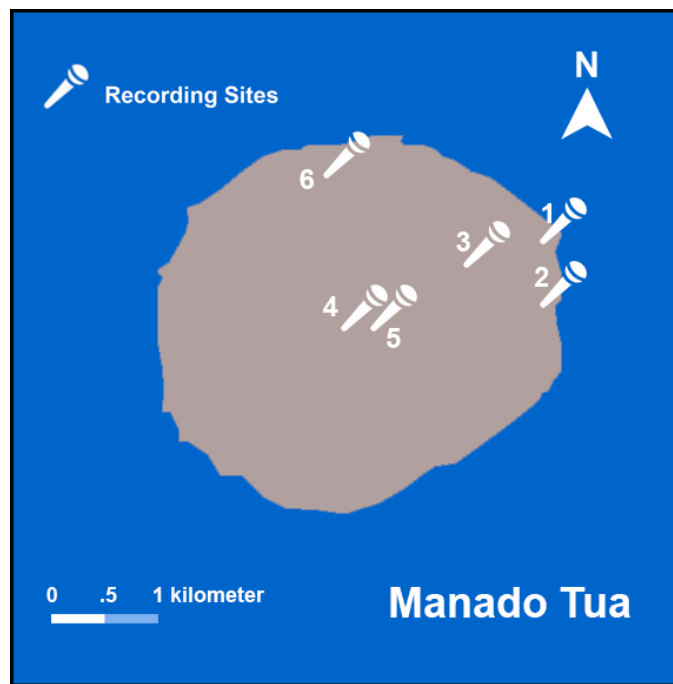
Manado Tua has one of the last (and smallest), wild populations in the world of crested black macaques (*Macaca nigra*), and is also home to dwindling populations of the marsupial bear cuscus (*Ailurops ursinus*) and several species of fruit bats. Despite being a part of the Bunaken National Marine Park, there is little oversight, and during our

fifteen–day stay we twice saw poachers heading down the volcano with up to seven dead

cuscus they intended to sell in markets on the mainland.

Mantehage

Mantehage Island, 13 kilometers off the coast of Sulawesi and 7 km northeast of

Bunaken and Manado Tua, is relatively flat, rising no more than 100 feet from the sea,

and is composed of 53 km^2 of extensive mangrove forest, alang–alang (*Imperata*

cylindrica) grasslands, coconut palms, and farmland. The island's 1,500 inhabitants live

mostly in three villages, and use the mangrove forests for construction material, fish

traps, and charcoal manufacturing for Manado (Murdiyarso et al., 2009). Consequently,

there is significant degradation of the mangrove forests that cover half the island.

(Djamaluddin, 2004).

Habitat degradation may be a factor in the relative scarcity of tarsiers encountered

during the two weeks spent on the island: only 5 of 16 days resulted in successful

recordings). Our team spent July 5th through July 19th, 2013, on Mantehage, and during

this time tarsiers were recorded at three sites—almost entirely in extremely degraded

areas, including overgrown gardens and in mangroves that had experienced severe

slashback. Conditions ranged from clear to periods of heavy rains during our stay, but

tarsiers were only recorded on cloudy days with no precipitation.

Figure 4. Map of Mantehage Island showing the location of sites recorded in July and August, 2013.
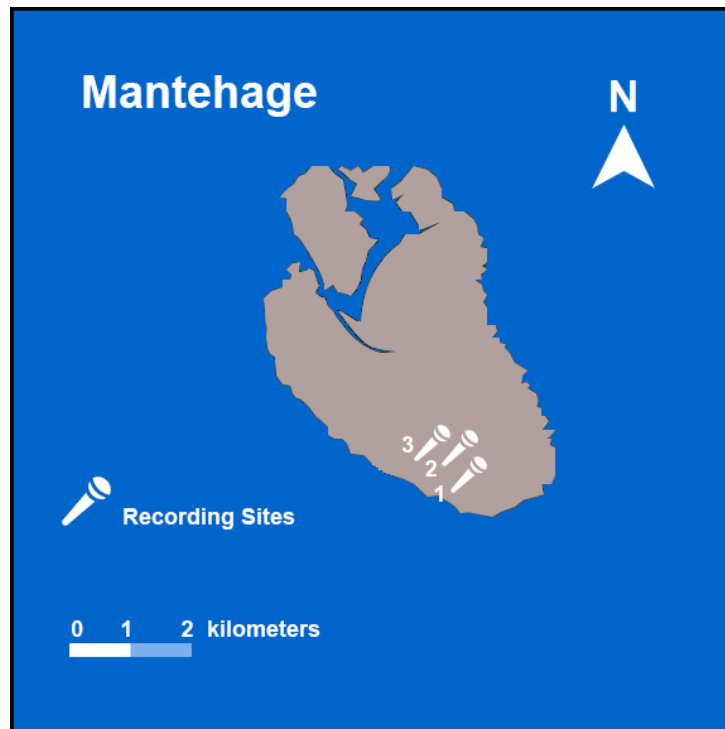
Northern and Eastern Shallow Water Islands: Bangka, Talisei, and Lembeh

Bangka

Bangka Island (not to be confused with the larger, more popular island with the same name off the coast of Sumatra) is a small island located 5.2 km off of the northeastern tip of Sulawesi. Around 2,500 people live on this 40 km^2 island, mostly in the three coastal villages. A nascent diving industry and a traditional fishing economy are currently threatened by illegal mining, as an international mining company embroiled in permit controversy has already begun the use of open pit extraction techniques to mine

iron ore and build a refining smelter. This will directly affect 20 km^2, or half the island

(Satriastanti, 2011), which has already been cleared of primary growth and coastal

coconut plantations. It will no doubt indirectly affect the ecosystem of the entire island

and its neighbors.. Shrubs and secondary forest cover just 14% of the island. Javanese

deer (*Rusa timorensis*), bear cuscus, water monitor lizards (*Varanus s. salvator*) and wild

boar (*Sus scrofa*) share the island with tarsiers. Mangroves and beaches line the southern

end of the island, while the northern shoreline is primarily rocky (Andaria et al., 2013;

and personal observation).

Figure 5. Map of Bangka Island showing the location of sites recorded in April, 2013.

Recording took place from April 4th to April 19th, 2013, during which time

thunderstorms, showers, and overcast skies happened daily. Work was concentrated in

four general sites—two in the mangrove, one in a fig/liana grove, and another in a copse

of bamboo. Calls were recorded from 4:58am to 5:14am.

Talisei

Situated 8.5 kilometers off the coast of Sulawesi, this spindle–shaped island is

only 2 km wide but 10 km in length (20 km^2), and trends in a northeast–southwest

direction. Its coastline is fringed by sand beaches and mangrove forests, while hills run

down the island's spine, rising to 359 m in elevation and creating a slightly higher profile

than the neighboring Bangka, which is easily seen 3.5 km to the east. (Erwin &

Sweetkind–Singer, 2009).

Though Talisei has suffered heavy anthropogenic habitat loss and the Sulawesi

wild pig population has been decimated, bird diversity is high, and the island's small

community of crested black macaques is only one of three purportedly native populations

left in the wild (Lee & Kussoy, 1999). Recordings took place from April 26th to May 9th,

2013, in the island's southeastern quadrant during moderate weather—partly cloudy to

overcast—though recordings were abandoned twice due to heavy showers, and once due

to equipment malfunction. We recorded at three distinct sleeping sites, with small

strangler figs, bamboo copse, and mangrove, each represented.

Figure 6. Map of Talisei Island showing the location of sites recorded in April and May, 2013.

Lembeh

The narrow 22–km–long, rifle–shaped island hugs the eastern end of the North Sulawesi peninsula, protecting the harbor of Bitung just 500 m across the shallow (11m deep), strait (Erwin & Sweetkind–Singer, 2009). The island's highest point sits at 477 m, though our recordings were all near sea level. We spent 15 days (from January 8th to the 22nd, 2013) on the northern tip of the island, recording five sites in varied terrain—similar to what we encountered across the channel: liana and bamboo, as well as the small strangler figs. All habitat had been disturbed to some extent, with coconut palms planted

throughout. The weather ranged from dry and sunny to torrential rains that cancelled

recordings on four of the mornings and washed out our camp on our final day.

Figure 7. Map of Lembeh Island showing the location of sites recorded in January, 2013.
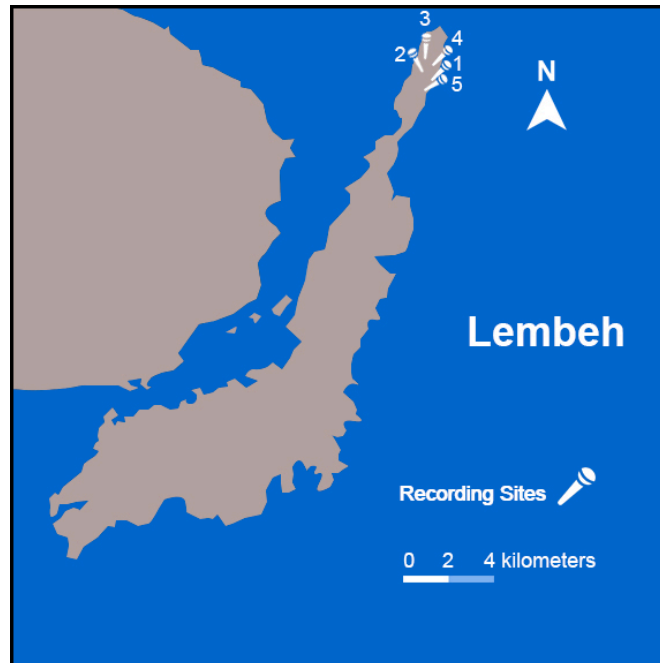
The Sangihe Islands: Biaro, Tagulandang, Siau, and Sangihe

The Sangihe Islands, which include Biaro, Tagulandang, Siau and Sangihe,

constitute an ophiolithic archipelago stretching from Indonesia to the Philippines, with

intervening depths of no less than 300 m. These islands are the result of volcanic activity

and arose from a narrow submarine ridge no earlier than the Cretaceous (Krause, 1966).

Of the 37 known mammal species on the Sangihe islands, 30 are endemic (22 of which

are Chiroptera; Riley, 2002). But habitat loss (only 800 hectares of primary forest

remains on all of the Sangihe islands combined) and hunting have resulted in eight

species being listed as globally threatened on the IUCN's Red List of Threatened Species,

including *Tarsius sangirensis* (IUCN 2000; Riley 2002; Shekelle & Salim, 2013).

Biaro

The southernmost of the Sangihe Islands, Biaro is a small island with rolling

terrain and severely degraded habitat. It covers only 26 km^2. We surveyed Biaro from

March 3rd through March 10th, 2013, finding no evidence of tarsiers. Several local reports

were found to be squirrels (*Prosciurillus* spp.).

Tagulandang

Seventy km north of Sulawesi and 27 km southeast of Siau Island, Tagulandang is

a 55 km^2 mountainous island with an extinct volcano rising 805 m to a caldera. A

collapsed wall of the caldera forms a large indentation on the western shore of this

otherwise circular island (Erwin & Sweetkind–Singer, 2009). A nearby volcanic eruption

in 1871 triggered a tsunami that struck Tagulandang, killing over 400 people including

the island's king (Rampengan et al., 2014). Tagulandang was surveyed from March 11th

through March 20th of 2013; no sign of tarsiers were found. Locals did not report ever

having seen tarsiers.

Siau

Situated 110 km north of Sulawesi and 65 km south of Sangihe Island, Siau is a

densely populated volcanic isle that presents a dramatic profile, rising 1,784 m at its

northern end to Gunung Karangetang, an active, and constantly smoking, volcano that last erupted in 2011. The island's 115 km^2 are ringed by steep coasts and limited beaches, yet it is home to 40,758 inhabitants, most of whom subsist on farming and fishing. Indonesia is the world's largest producer of nutmeg, with one–third to one–half of the nation's production coming from Siau (Rampengan et al., 2014). The tarsiers on Siau are considered a separate species (*Tarsius tumpara*) from their counterparts on both Sulawesi and Sangihe, putting them at peril due their small numbers and habitat loss (Shekelle, 2008).

Figure 8. Map of Siau Island showing the location of sites recorded in February, 2013.
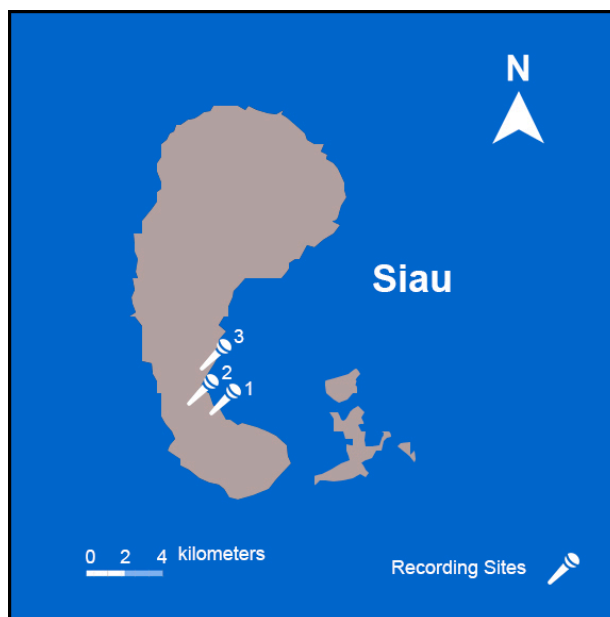
We spent 16 days on the island, from February 6th through the 20th of 2013, during which time torrential downpours alternated with cloudy days. Duet calls proved much more elusive than on the islands closer to Sulawesi, and very few people knew what tarsiers were, identifying our photos as squirrels and our recordings as bird song

(*Nectarinia* species, or sunbirds, have a call that is very similar to male *Tarsius* contact calls, though with a slightly lower frequency range). We recorded 3 sleeping sites in liana tangles, bamboo, and small fig trees, with calls beginning between 5:09 and 5:29.

Sangihe

Situated 187 km north of Sulawesi, Sangihe is the largest island in the Sangihe archipelago, comprising almost 600 km^2. It has one of the area's most active volcanos, Gunung Awu, whose last eruption was in 2004. An eruption in 1856 claimed an estimated, 2,000 to 6,000 human lives (Rampengen et al., 2014). The island's shoreline is steep and rocky in places, and low and marshy in others, rising to 1,359 m near its northern end (Erwin & Sweetkind–Singer, 2009).

Figure 9. Map of Sangihe Island showing the location of sites recorded in February and March, 2013.

Widespread deforestation has affected many species on Sangihe, which has more threatened single–island endemic species than any other Indonesian island (Riley, 2002), including the endemic *Tarsius sangirensis* (Shekelle & Salim, 2009). Calls were recorded from February 26th through March 3rd of 2013, and weather was consistently overcast without precipitation. Two sites offered recordable duet calls—both in or near large bamboo groves, beginning between 5:21am and 5:27am.

Equipment

All but one of the vocalizations were recorded to CF–cards using the Tascam HD–P2 Portable Stereo CF Recorder. The HD–P2 is a 2–channel stereo recorder that uses the Broadcast WAVE File (BWF) format to write audio files. The recording bit rate was 24–bit, and sampling frequency was 192 kHz. Total Harmonic Distortion was 0.01% at 1 kHz. The microphone was connected via 3–pin XLR cables, one of which was 3 m, and the other 25 m.

A Sennheiser ME66 super–cardioid short shotgun condenser microphone was used for the vast majority of the recordings. All of the recorded sites had high ambient noise, both anthropogenic and otherwise. The ME66 is directional, and thus when pointed at the vocalizing tarsiers, it mitigated the cacophony of sound from motors, birds (domestic and wild), and cicadas (*Dilobopyga spp.*; De Boer and Duffels, 1996). My microphone captured frequencies ranging from 40–20,000Hz, and was powered by a Sennheiser K6 Microphone System Powering Module. The ME66 has a dynamic range of 115db, and a signal–to–noise ratio of 84 db. A WindTech SG–1 Windscreen was consistently used, along with an Auray DUSM–1 Universal Shock Mount.
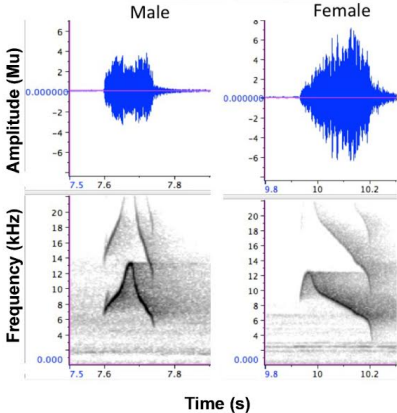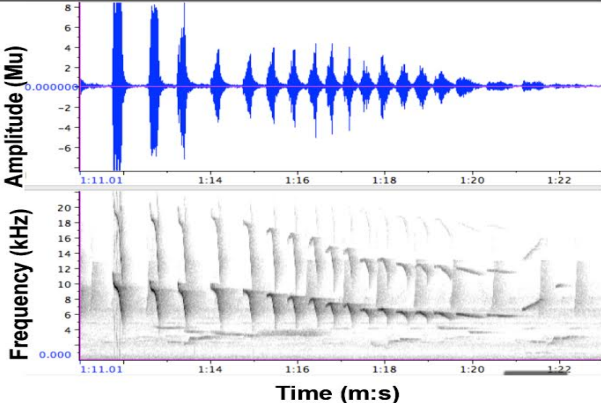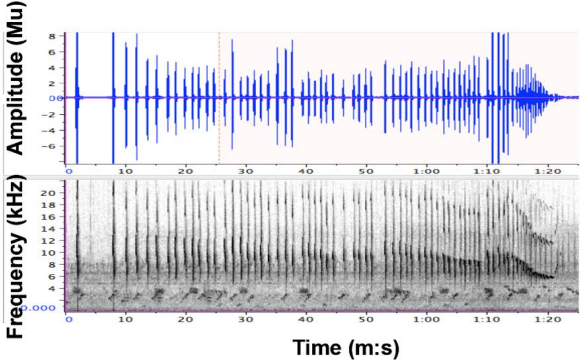
A single recording was captured via a Zoom H1 Ultra–Portable Digital Audio Recorder, which is a hand–held stereo digital audio recorder with a recording bit rate of 24–bit and a sampling frequency of 96 kHz. This recorder used a built–in unidirectional condenser microphone, and wrote conventional WAV files to a microSD card.

Laboratory Analyses

Sound Analysis

We used the Cornell Lab of Ornithology's sound analysis software, RavenPro 1.4, to visually interpret the temporal and spectral qualities of the calls, using their spectrogram view to show how the frequency (on the y axis), varies over time (on the x axis). RavenPro divides the entire sound into a series of short records and calculates a single spectrum for each, using Discrete Fourier Transform, or DFT. The spectrum of each record is then arranged by time (Charif, Strickman, & Waack, 2010). WAV files were imported from CF–cards into RavenPro using the Hann window function, and each record is then arranged by time (Charif, Strickman, & Waack, 2010).

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Figure 10. Acoustic terms and definitions of units of sound analyzed for *Tarsius* vocalizations involved in dawn duet calls. Not to scale. (Haimoff 1984; Nietsch 2003).

Call type	Spectrogram	Definition
Note		A temporally uninterrupted vocalization of any distinct frequency or frequency modulation.
Phrase		A single vocal activity consisting of a collection of distinctive notes separated by short intervals.
Song		The complex of notes and phrases given by a family group (composed of at least one male and one female) separated by intervals of no more than 60 seconds.

window size was set to 800 samples, as this provided the best resolution for visualization, and the high sample rate allowed for improved frequency measurements. Filter bandwidth was set to 248 dB. Brightness and contrast were adjusted as necessary for optimal visual interpretation of the spectrogram – these values do not change the measurement values.

Once loaded into RavenPro, male and female notes within each song were counted independently, as were the duet phrases, which have a unique phonology whereby a gradual change in the female's note phonology and shortened intervals form a stereotyped vocalization (see Figure 10). Male and female tarsier advertising calls are easily distinguishable from one another by both phonology and temporal patterns. In 1999, Nietsch confirmed earlier researchers' suggestions that Sulawesi tarsiers' duet calls were sexually dimorphic by observing both captive and wild tarsiers (Nietsch 1999; MacKinnon and MacKinnon 1980; Niemitz 1991).

To refrain from selection bias in choosing which notes to analyze, we randomized our samples using online website Random.org, which generates true random numbers. For males, the total number of male notes within a song was entered, ten random numbers were generated, and those numbers were then applied to the notes' positions within the song. Females' note counts were likewise entered, and five random notes were chosen. Most songs showed a strong male to female ratio, thus obtaining ten clear male notes was possible in all the usable songs, whereas clear female notes were scarcer. For both sexes, a new number was generated when a note was too unclear for analysis. For females, a new number was generated if one of the notes fell within the duet phrase.

Table 2. Definition of RavenPro measurements used in in this study to analyze sound parameters of *Tarsius* vocalizations (adapted from Charif, 2010).

Measurement	Definition
Begin Time	Time at which selection begins.
End Time	The time at which the selection ends.
Delta Time	The difference between Begin and End Times, or the duration of the selection.
High Frequency	The highest frequency within a selection.
Low Frequency	The lowest frequency within a selection.
Delta Frequency	The difference between high and low frequency selections.
Peak Frequency	The frequency of maximum power.
Center Frequency	The frequency at which the selection is divided into two intervals of equal energy.
Frequency 5%	The frequency at which the selection is divided into energy intervals of 5% and 95%.
Frequency 95%	The frequency at which the selection is divided into energy intervals of 95% and 5%.
Bandwidth 90%	The difference between Frequency 5% and Frequency 95%.
1st Quartile Frequency	The frequency dividing the selection energy into intervals of 24% and 75%.
3rd Quartile Frequency	The frequency dividing the selection energy into intervals of 75% and 25%.
IQR Bandwidth	Inter–quartile bandwidth; the IQR measures the difference between the 1st and 3rd Quartiles.
Gap Frequency	The difference in frequencies between the beginning and the ending of a note.

Female notes were, on average, more elaborate than the male notes, so in addition to the standard measurements done on both sexes, we used pitch tracking to analyze female note complexity. The duet phrase was treated both as an element in its own right, and as a categorization for the female notes it encompassed, whose measurements were averaged for a female duet note reading.

Figure 11. An example of robust measurements on a single contact note from a male *T. spectrum.* Selection parameters are indicated by blue shading.
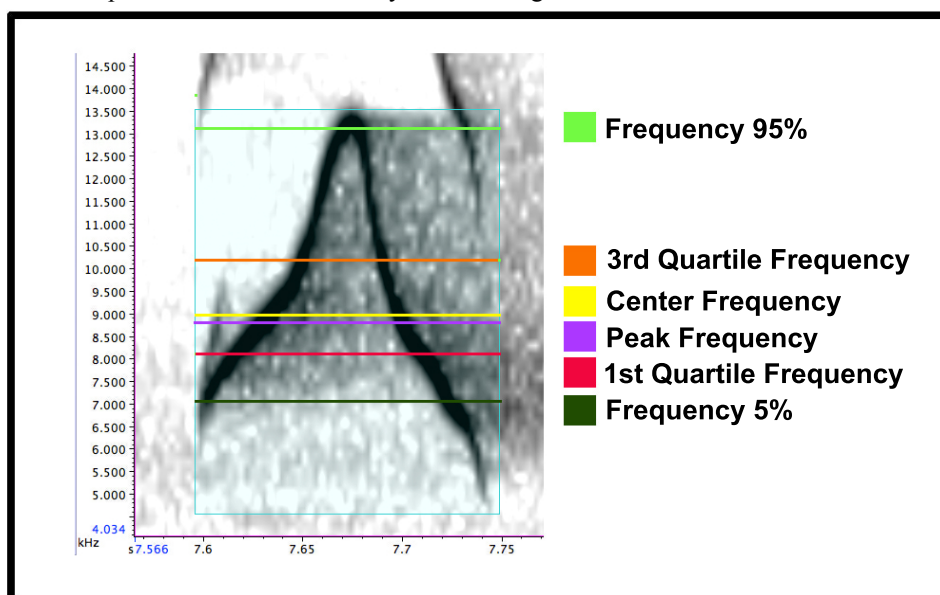
"Robust signal measurements", as described by the RavenPro manual, analyze the energy within a selection, and thus do not depend excessively on the selection borders. These measurements include the 1st and 3rd quartile frequencies and inter–quartile range, the frequencies at the center, 5% interval, 95% interval, and in the 90% bandwidth, and the maximum frequency (figure 11). Selection–based measurements may be subjective, as they are based solely on the borders of the manual selection box. Begin and end time (and thus delta time), are based solely on where the selection is drawn, as are high and

low frequency, delta frequency, and the gap in frequencies. We also measured the

presence and frequencies of significant biological noise, including other birds and insects

(see Figure 11).

Statistics

Measurements from RavenPro were imported into Excel, and spreadsheets were

converted to CSV (comma separated values) files. R (version 3.1.1 GUI 1.65 Snow

Leopard build) was then used for all analyses. Three datasets were analyzed, including

random male notes, random female notes, and duet phrases. Duet phrase parameters, as

well as average female note values were recorded within the duet phrase datasheet. Our

data did not meet assumptions of normality, requiring non–parametric methods of

analysis.

Principal Component Analysis (PCAs) were carried out on the data sets to look

for patterns of covariation in sleeping tree vegetation type or weather conditions. We then

ran the datasets against location and location classification ("mainland," "shallow," and

"deep"). We did screeplots for each dataset, to examine the relative eigenvector

magnitudes. Based on the eigenvalues, we chose to analyze the four principal

components of the average female notes within a duet phrase, the five principal

components for the parameters of the duet phrase itself, and the six principal components

for both the random male and the random female notes. We analyzed only three principal

components when comparing weather and vegetation.

Those selected components were then plotted against one another within each of

the four datasets. Score plots were generated and examined for cohesive groups, while

coordinates of the data points were scrutinized in their relationships with one another. Principal components that helped to discriminate groupings of locations or categories were noted, as were their standard deviations and the variable contributing the most to their variance.

Those variables were then subjected to a Tukey's honest significance test (Tukey's HSD), in which the selected measurements were compared to the same measurements taken at other locations as well as other location categories. These were plotted and examined for statistical differences (p–values < 0.05). Variables showing significant differences were mapped.

RESULTS

In the field, it was possible to audibly differentiate the duetting calls of *Tarsius spectrum* species (found at all of our locations not classified as "deep") from the calls of *T. sangirensis,* and *T. tumpara* (recorded on Sangihe and Siau, respectively*)*. Female calls took two distinct acoustic forms, both distinguishable by ear and when visually represented by a spectrogram. Male contact calls were very similar to each other at all locations—although spectrographic analysis showed the Sangihe and Siau's males called at consistently lower frequencies than their mainland counterparts. In this section, I will first examine the different acoustic forms we encountered, following it up with summaries of the measurements and the statistically significant variables that emerged on a location–by–location basis, in addition to site–by–site analyses at two of our locations.

Note Structure

Female note structure within a song could be easily divided into two acoustic forms. Female notes on the mainland, and "shallow" islands all exhibit a moderately conserved structure, changing slightly when leading up to a duet phrase, with drastic changes within the phrase itself. This acoustic form, first identified by MacKinnon and MacKinnon (1980), as "Manado Form," was identified at 8 of our ten tarsier–inhabited locations: Tangkoko, Klabat, Lembeh, Manado Tua, Mantehage, Bunaken, Talisei, and Bangka. In this form, females characteristically produce a long descending whistle, beginning with a sharp rise and descent. The frequency band of this initial hook reaches from a low of 5 kHz up to around 16 kHz (see Figure 12). This is typically followed by a

further decline in pitch, which may fall sharply, decline gradually, or even rise minimally

before descending to finish at the note's lowest frequency.

In the Sangihe form, the female note structure remained remarkably conserved

throughout the song, as we were unable to detect a duet phrase with distinct vocal

stereotypes in any of our recordings. The Sangihe form, first described by Shekelle

(2008), calls the female note a "two–unit," call, and spectrograms reveal a short phrase

with two clear components (Shekelle et al., 2008). The first unit is a long, low, note, with

a very slight initial rise in frequency, and occasionally an equally slight downturn at the

Figure 12. Female notes as they appear when not in a duet phrase. Shown are distinct forms recorded at the following locations: a) Tangkoko Nature Reserve, b) Siau Island, and c) Sangihe Island.
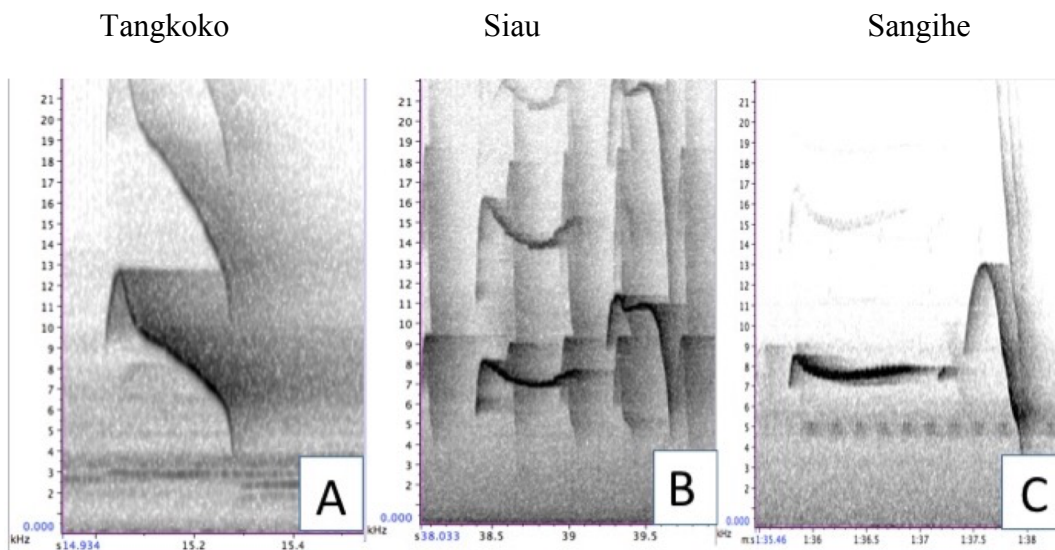
end. The second unit begins almost as soon as the first ends, though always at a higher

frequency than the first unit (occasionally the two connect). The second unit quickly rises

and then descends a few kilohertz below the frequency of the first unit.

The Siau form provides a dynamic structural intermediary between the Manado and Sangihe forms. Calls here exhibited the same two–unit note complex seen on Sangihe, though the structures of the first and second units were both visibly less conserved than Sangihe's, changing significantly from note to note. While we were unable to discern any recurring patterns, Siau's female notes were much more modulated than Sangihe's, with one to five changes in pitch direction within a given unit. For much of the duet phrase, it was difficult to even tell the difference between male and female notes, as female notes often shortened in both frequency range and duration, appearing very similar to the type 3 male note described below (See Figure XX)

We differentiated male tarsier calls into three separate categories based on the structures we saw across all of the locations. It is important to note, however, that these categories were extracted from a continuum, and there were numerous intermediate forms that were categorized as closely as possible. Male "type 1" consists of a single broadband chirp, with high fundamental frequencies ranging from 10 kHz to 15 kHz, and low frequencies between 2 kHz and 5 kHz. These chirps show no modulation, and all measured examples were under 0.4 seconds. Maximum frequency changed frequently among type 1 notes, even when their structure did not otherwise change. Type 1 notes were the most frequently recorded, making up 48.5% of the male notes, with Male type 2 notes the second most numerous, at 30%. Type 2 notes exhibited a single pitch change.

These notes incorporated a quick rise and sharp descent, and were likewise under 0.4

seconds. Type 2 notes were audibly indistinct from the type 1 (as were type 3 notes). We

Figure 13. Male chirps, showing typical forms of notes identified as a) type 1, b) type 2, c) and d) are both examples of type 3, with multiple modulations.
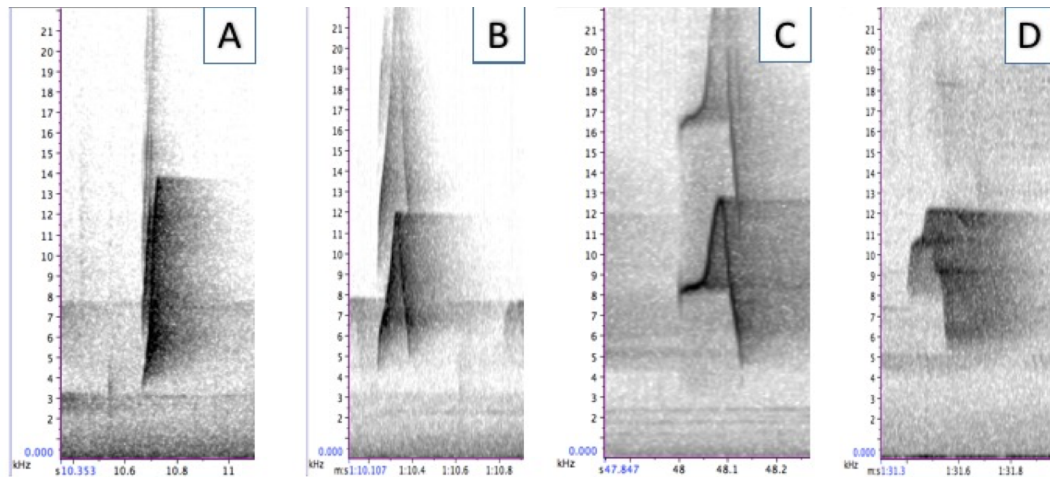
classified notes with multiple modulations as type 3. These notes, like type 2, had a rapid

and distinct rise and fall in pitch, though notes were frequently upturned at the start, or

showed a short modulation in either the rise or the descent of the tone. Approximately

21.5% of the male notes recorded were classified as type 3 (see Figure 13).

Duet Structure

We found three distinct duetting forms, in which the females' notes, in particular,

shaped the duet phrase. In the "Manado Form" (MacKinnon & MacKinnon, 1980) that

Figure 14. Spectrograms showing distinct duet phrase forms, including a) Manado Form, recorded at Tangkoko Reserve, b) Siau Form, from Siau island, and c) a recording from Sangihe Island, in which the conserved structure and timing of the female notes may be observed
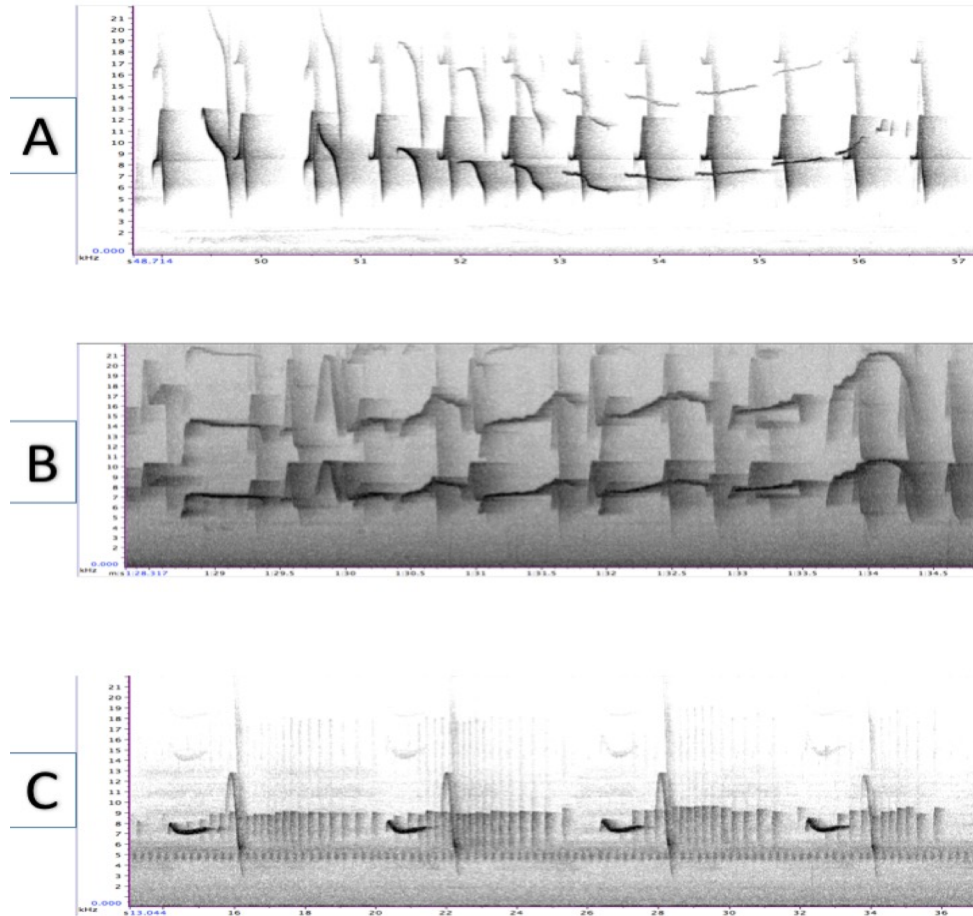
we encountered on all but Sangihe and Siau, both females and males were heard initiating the duet. Within the song, the female's characteristic descending whistle and the male's wide–band chirps increase in frequency and intensity, running together into the duet phrase itself, where the ratio of female to male notes ranged from a mean of 0.64 on Bunaken, to Tangkoko's 1.48. The duet phrase begins when the female's notes begin to change in structure. Notes begin at an increasingly lowered frequency while maintaining a somewhat conserved low frequency and duration. Meanwhile, inter–note intervals

increasingly shorten until they become difficult to discern, making the lowest part of the

duet phrase audible only as a long low whistle. Finally, those intervals increase, while

initial frequencies ascend – though the degree to which they do so is variable. In most

instances, females then paused for at least a few seconds before returning to their pre–

duet–phrase structured calls (see Figure 14)

On Sangihe, we were unable to measure a distinct "duet phrase" within the duet

song. Both female and male notes remained highly conserved throughout, with the

female's characteristic two–note phrase repeated at regular intervals, and the male's

reiterated broadband chirping throughout. Male and female both contact called as they

approached each other and the sleeping spot, and the calls increased slightly in intensity

and speed; however, we did not witness or record any change in note structure to either

male or female notes during the morning duet call.

Finally, the Siau duet call provided a unique look at a duet phrase with a structure

intermediate to the Sangihe and Manado forms. Female notes throughout the song most

clearly resembled Sangihe's double unit form, though within the duet phrase itself, the

characteristic swoop of the Manado form duet phrase is recognizable. To begin the duet

phrase, the female's notes become more and more erratic, with increasing modulations,

the loss of the second unit, and a highly variable structure (both note to note, and

individual to individual), that nevertheless flattens its frequency band and reduces inter–

note intervals to approximate a long descending then ascending whistle at the end of the

phrase.

Principal Component Analysis

For randomized female notes, we retained 6 of the 13 principal components (PC) based upon visual interpretation of the scree plots. The proportions of variance for PC1 through PC6 were 0.425 (SD $\pm$2.350), 0.311 (SD $\pm$2.010), 0.099 (SD $\pm$1.133), 0.070 (SD $\pm$0.956), 0.042 (SD $\pm$0.738), and 0.023 (SD $\pm$0.544), respectively. For randomized males, we also chose to analyze the first 6 (of 16) principal components, with proportions of variance as follows: PC1=0.282 (SD $\pm$2.126), PC2=0.204 (SD $\pm$1.805), PC3=0.129 (SD $\pm$1.436), PC4=0.104 (SD $\pm$0.1.288), PC5=0.082 (SD $\pm$1.149) and PC6=0.059 (SD $\pm$0.973).

With smaller sample sizes and fewer variables, fewer principal components from duet phrase measurements were chosen. When the entire phrase was measured, only 6 principal components were extracted, and from those we chose to use five, as the final one was effectively 0. Proportions of variance for PC1 through PC5 were 0.405 (SD $\pm$1.559), 0.244 (SD $\pm$1.209), 0.161 (SD $\pm$0.984), 0.150 (SD $\pm$0.949), and 0.040 (SD $\pm$0.490). When female notes within a duet phrase were averaged, only 5 principal components emerged, and of those we eliminated the last again. Proportion of variance for PC1 was 0.408 (SD $\pm$1.429), PC2's variance was 0.301 (SD $\pm$1.226), PC3 was 0.202 (SD $\pm$1.006), and finally, PC4 was 0.089 (SD $\pm$0.667).

Each principal component was then plotted against every other principal component within the dataset, while data points were colored and shaped according to location and location type – "mainland," "shallow," "deep", or "distant". We used the same methods to run PCAs according to the vegetation type found at the sleeping sites

and for weather. Neither showed any significant correlations. For the measurements taken of the entire duet phrase, the first and second principal components best grouped the variables by location and location type. We obtained the loadings of these (fundamental frequency range was the highest contributing variable of PC1 at 0.566, while duet duration was also a large contributor to PC2 at –0.45). The same two principal components were used in averaged female notes within a duet phrase (where the loadings were similar, with fundamental frequency range contributed to PC1 at 0.679, while duration contributed to PC2 at 0.513).

For male note measurements, we chose the first three principal components, whose main contributing variables were center frequency (0.436), BW90% (0.436), and maximum frequency (0.354), respectively. For non–duet–phrase female notes, we focused on the first three principal components: PC1 had the 3rd quartile frequency as the largest contributing factor (–0.410), while the fundamental frequency range was the largest factor of PC2 (–0.413), and duration proved most important to PC3 (0.441).

The Tukey's Honest Significant Difference test (Tukey's HSD) was run on the high–contribution variables mentioned above, and the results are below in a location–by–location analysis.

Analyses by Location

Tangkoko

We recorded 15 duet calls at 4 different sites within Tangkoko National Park. Of these recordings, 8 resulted in usable duet phrases. Two of our sites provided two

recordings each, one site was recorded once, and a fourth site was recorded three times. All of the recordings were done at large strangler figs, between 5:08 am and 5:23 am. Duet songs contained between 1 and 7 duet phrases, with a mean of 3.5 phrases per song.

All of the duet phrases contained between 11 and 23 female notes and 7 to 23 male notes, giving an average female to male ratio of 1.48 with a standard deviation (SD) of $\pm$0.40. Duet phrases averaged 9.29 seconds (SD $\pm$3.02), with a first quartile (Q1) of 7.76 seconds, and a third quartile (Q3) of 9.67 seconds. The fundamental frequency of the duet phrase spanned from an average low frequency of 5,344 Hz (SD $\pm$664.76, Q1=5,219 Hz, Q3=5419 Hz), to an average high frequency of 10,945 Hz (SD $\pm$963.32, Q1=10,163 Hz, Q3=11,482 Hz), giving it a range averaging 5,602 Hz (SD $\pm$1158.58, Q1=4,805 Hz, Q3=5,960 Hz) (see Table 3).

For each duet phrase, we also measured all of the female notes contained within it. During this phrase, the female emits notes much more frequently than in any other part of the song, with notes accelerating and lowering in pitch – as well as shortening in both duration and frequency range, towards the middle of the phrase. When all the female notes were averaged, Tangkoko exhibited a mean fundamental frequency range of 2,424 Hz (SD $\pm$506.66, Q1=2,085 Hz, Q3=2,776 Hz), ranging from a low of 5,697 Hz (SD $\pm$769.98, Q1=5,238 Hz, Q3=5,917 Hz), to a high of 8,121 Hz (SD $\pm$386.22, Q1=7,861 Hz Q3=8,355 Hz). Mean note duration was 0.451 seconds (SD $\pm$0.05, Q1=0.408 s, Q3=0.493 s). In addition to the duet phrases, 10 male notes were selected randomly from each song, while 5 non–duet–phrase female notes were selected.

Ten of our recordings proved clear enough to extract measurements from these notes (three recordings each from our first three sites, and a single location from the last).

Table 3. Mean values of key characters of vocalizations from Tangkoko, ± standard deviation, and 1st and 3rd quartile for each variable.

TANGKOKO	Duet Phrase	Duetting Females	Randomized females	Randomized males
Number of Sites	4	4	4	4
Number of Duet Phrases per Song	1 – 7			
Mean $\pm$SD	3.5$\pm$2.33			
Sex Ratio $\pm$SD	1.478$\pm$0.40			
Q1,Q3	1.29,1.58			
Number of Samples	8	124	50	100
Low Frequency (Hz) $\pm$SD	5344$\pm$664.76	5697$\pm$769.98	4361$\pm$914.10	4799$\pm$659.39
Q1, Q3	5219, 5419	5238, 5917	3695, 4754	4262, 5251
High Frequency (Hz) $\pm$SD	10945$\pm$963.32	8121$\pm$386.22	12706$\pm$916.83	12576$\pm$539.84
Q1, Q3	10163, 11482	7861, 8355	12197, 13232	12269, 12837
Frequency Range (Hz) $\pm$SD	5602$\pm$1158.58	2424$\pm$506.66	8345$\pm$1440.12	7777$\pm$723.07
Q1, Q3	4805, 5960	2085, 2776	7324, 9398	7292, 8046
Duration (s) $\pm$SD	9.287$\pm$3.02	0.4512$\pm$0.05	0.3929$\pm$0.07	0.2571$\pm$0.09
Q1, Q3	7.76, 9.668	0.4075, 0.4925	0.357, 0.439	0.1918, 0.3098
Rate of Change (Hz/s) $\pm$SD	644$\pm$195.92	5498$\pm$1539.45	21585$\pm$3642.60	34458$\pm$13508.19
Q1, Q3	518.7, 794.2	4488, 6141	18622, 24529	23961, 40796
Peak Frequency (Hz) $\pm$SD			9123$\pm$1369.81	8426$\pm$1169.63
Q1, Q3			8269, 9938	7924, 8786
Interquartile bandwidth (Hz) $\pm$SD			664.1$\pm$507.01	1772.6$\pm$737.05
Q1, Q3			1378.1, 1970.3	1367.3, 2110.3
1st Quartile (Hz) $\pm$SD			8262$\pm$864.88	7709$\pm$577.11
Q1, Q3			7644, 8603	7440, 8053
3rd Quartile (Hz) $\pm$SD			9926$\pm$723.59	9481$\pm$822.17
Q1, Q3			9388, 10153	8947, 9916
Center Frequency (Hz) $\pm$SD			9194$\pm$788.62	8481$\pm$526.74
Q1, Q3			8613, 9421	8269, 8753
Bandwidth 90% (Hz) $\pm$SD			4027$\pm$963.52	4990$\pm$899.37
Q1, Q3			3445, 4468	4479, 5642
5% Frequency (Hz) $\pm$SD			6952$\pm$680.72	6575$\pm$642.26
Q1, Q3			6686, 7149	6202, 6891
95% Frequency (Hz) $\pm$SD			10978$\pm$902.62	11565+902.618
Q1, Q3			10336, 11359	11154, 12059

Thus sample sizes are *n*=100 for males and *n*=50 for females. Female notes lasted an

average of 0.393 seconds (SD ±0.07, Q1=0.357 s, Q3=0.439 s) and exhibited a mean

fundamental frequency range of 8342 Hz (SD ±1440.12, Q1=7324 Hz, Q3=9398), with a

low frequency mean of 4,361 Hz (SD ±914.10, Q1=3,695 Hz, Q3=4,754 Hz), and a high

frequency mean of 12,706 Hz (SD ±916.83, Q1=12,197 Hz, Q3=13,232 Hz). Male notes

averaged 0.257 seconds (SD ±0.09, Q1=0.1918 s, Q3=0.310 s), with a mean fundamental

frequency range of 7,777 Hz (SD ±723.07, Q1=7,292 Hz, Q3=8,046), a low frequency

mean of 4,799 Hz (SD ±659.39, Q1=4,262 Hz, Q3=5,251 Hz), and a high frequency

mean of 12,576 Hz (SD ±539.84, Q1=12,269 Hz, Q3=12,837 Hz).

For these randomized individual notes, we analyzed additional measurements that

rely on the energy within a selection rather than the borders of the selection itself (Charif

et al., 2010). The maximum frequency, or frequency at which the note is the most

powerful, averaged 9,123 Hz (SD ±1369.81, Q1=8,269 Hz, Q3=9,938 Hz) for females

and 8,426 Hz (SD ±1169.63, Q1=7,924 Hz, Q3=8,786 Hz) for males. Female center

frequency was 9,194 Hz (SD ±788.62, Q1=8,613 Hz, Q3=9,421 Hz), while male center

frequency had a mean of 8,481 Hz (SD ±526.74, Q1=8,269 Hz,Q3=8,753 Hz). 1st quartile

frequency, 3rd quartile frequency, and the resulting inter–quartile range bandwidth (IQR

BW) were measured, as were the frequencies dividing the selections into intervals with

5% and 95% of the note's energy, and the bandwidth between the two (BW 90%). Mean

IQR bandwidth for Tangkoko females was 1,664 Hz (SD ±507.01, Q1=1,378 Hz,

Q3=1,970 Hz), while males measured a mean 1,772 Hz (SD ±737.05, Q1=1,367 Hz,

Q3=2,110 Hz). Mean BW 90% for females was 4,027 Hz (SD ±963.52, Q1=3,445 Hz,

Q3=4,468 Hz), and for males a mean 4,990 Hz (SD ±899.37, Q1=4,479 Hz, Q3=5,642 Hz).

Tukey's HSD was run on high–contribution variables, as determined by PCA. For both the duet phrase as a whole, as well as the averaged female notes within the duet phrases, Tukey HSD found significant differences in the fundamental frequency range between Tangkoko and Lembeh (p = 0.030 and 0.001, respectively). The duration of Tangkoko's average duet phrase note was significantly different than Siau's (p = 0), and the duet phrase duration was significantly different from that on Bunaken (p = 0), Klabat (p = 0.022), and Mantehage (p = 0.002; see Table 4).

Individual Tangkoko male notes exhibited the most significant differences in the power distribution within a note. For center frequency measurements, there were significant differences between Tangkoko and Bangka, Bunaken, Lembeh, and Talisei (all at p = 0). For 90% Bandwidth, Tangkoko differed from Manado Tua (p = 0.025), Mantehage (p = 0.001), Talisei (p = 0), Sangihe (p = 0), and Siau (p = 0). High frequency measurements showed significant differences between Tangkoko and Manado Tua and Mantehage (p = 0 and 0.001, respectively) (see Table 3).

Females showed even more significant differences, including 3rd Quartile frequency (p = 0 for Bangka, Klabat, Lembeh, Manado Tua, and Siau, and Sangihe; p = 0.022 for Mantehage), fundamental frequency range (p = 0 for Lembeh, Manado Tua, and Mantehage, 0.017 for Bunaken, 0.004 for Talisei, and 0.003 for Sangihe), center frequency (p = 0 for Bangka, Bunaken, Klabat, Manado Tua, Mantehage, Sangihe, and Siau, p = 0.015 for Lembeh), and duration (p = 0 for distant islands Sangihe and Siau; p = 0.010, 0.016, and 0.002 for Klabat, Manado Tua, and Talisei, respectively).

Table 4. Results of Tukey's HSD test of high–contribution variables. P–values indicated in bold represent significant differences between Tangkoko and other locations.

TANGKOKO	Bangka	Bunaken	Klabat	Lembeh	Manado Tua	Mantehage	Talisei	Sangihe	Siau
Female Duet Notes									
Frequency range	0.000	1.000	0.805	**0.030**	0.987	0.939	.000		0.997
Note duration	0.000	1.000	0.876	0.000	1.000	1.000	0.944		**0.000**
Full Duet									
Frequency range	1.000	0.720	0.806	**0.001**	0.778	1.000	0.988		1.000
Duet duration	0.061	**0.000**	**0.022**	0.746	**0.002**	0.060	0.975		1.000
Male Notes									
Center frequency	**0.000**	**0.000**	0.377	**0.000**	**0.000**	1.000	**0.000**	0.992	1.000
Maximum frequency	**0.033**	**0.001**	0.249	**0.000**	0.073	0.999	**0.000**	0.511	0.488
Bandwidth 90%	1.000	**0.016**	0.411	0.068	**0.025**	**0.001**	**0.000**	**0.000**	**0.000**
High frequency	0.844	0.076	1.000	0.948	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**
Female Notes									
3rd quartile	**0.000**	0.079	**0.000**	**0.000**	**0.000**	**0.022**	1.000	**0.000**	**0.000**
Frequency range	0.297	**0.017**	0.565	**0.000**	**0.000**	**0.000**	**0.004**	**0.003**	0.847
Center frequency	**0.000**	**0.000**	**0.000**	**0.015**	**0.000**	**0.000**	1.000	**0.000**	**0.000**
Note duration	0.897	0.296	**0.010**	1.000	**0.016**	1.000	**0.002**	**0.000**	**0.000**

Klabat

On Klabat, we successfully recorded 8 mornings at four different sites. We recorded twice at two of our sites, while one site was recorded once, and the fourth site three times. As in Tangkoko, all recordings were done at large strangler figs, though here duets were performed between 5:02 am and 5:20 am, and contained many more duet phrases than in Tangkoko national park. Songs contained between 3 to 28 duet phrases, with a mean of 13.9 phrases per song.

All of the duet phrases contained 10 – 19 female notes and 8 – 22 male notes, giving an average female to male ratio of 1.06 $\pm$ 0.21. Duet phrases averaged 10.04 seconds (SD $\pm$3.5, Q1=8.75, Q3=10.47). The fundamental frequency of the duet phrase spanned from an average low frequency of 6,476 Hz (SD $\pm$867.14, Q1=5,926 Hz, Q3=6,993 Hz), to an average high frequency of 12,982 Hz (SD $\pm$614.46, Q1=12,696 Hz, Q3=13,296 Hz), giving it a range averaging 6,505 Hz (SD $\pm$879.02, Q1=6,168 Hz, Q3=6,935 Hz)(see Table 5).

When all the female notes within the duet phrase were averaged, Klabat exhibited a mean fundamental frequency range of 3,152 Hz (SD $\pm$744.02, Q1=2,594 Hz, Q3=3,665 Hz), with a low frequency of 6,470 Hz (SD $\pm$1011.15, Q1=5,764 Hz, Q3=7,152 Hz), to a high of 9,622 Hz (SD $\pm$426.55, Q1=9,515 Hz, Q3=9,829 Hz). Mean female note duration was 0.63 seconds (SD $\pm$0.21, Q1=0.53 s, Q3=0.61 s).

For the randomized notes, in which 10 male notes and 5 non–duet–phrase female notes were selected from each song, we were able to use all 8 recordings, for a sample size of *n*=40 for females and *n*=80 for males. Female notes lasted an average of 0.46 seconds (SD $\pm$0.09, Q1=0.40 s, Q3=0.51 s) and exhibited a mean fundamental frequency

range of 8,926 Hz (SD ±1,437.32, Q1=8,260 Hz, Q3=9,827), with a low frequency mean

of 4,752 Hz (SD ±1,286, Q1=3,738 Hz, Q3=5,581 Hz), and a high frequency mean of

13,677 Hz (SD ±853.92, Q1=13,431 Hz, Q3=14,000 Hz). Male notes averaged 0.35

seconds (SD ±0.07, Q1=0.30 s, Q3=0.39 s), with a mean fundamental frequency range of

7,758 Hz (SD ±897.16, Q1=7,317 Hz, Q3=8,379), a low frequency mean of 4,805 Hz

(SD ±594.03, Q1=4,421 Hz, Q3=5,236 Hz), and a high frequency mean of 12,564 Hz

(SD ±636.92, Q1=12,297 Hz, Q3=12,929 Hz).

Robust measurements for each of the randomized notes gave us additional

information, including the peak frequency, $\bar{x} =$10,754 Hz (SD ±1,214.91, Q1= 9,948 Hz,

Q3=11,757 Hz). Female center frequency averaged 10,413 Hz (SD ±744.22, Q1=9,948

Hz, Q3=10,853 Hz), while male center frequency had a mean of 8,204 Hz (SD ±924.89,

Q1=8,010 Hz,Q3=8,613 Hz). Mean IQR bandwidth for Klabat females was 2,067.2 Hz

(SD ±901.47, Q1=1,679 Hz, Q3=2,584 Hz), while males $\bar{x} =$ 1,763.6 Hz (SD ±872.15,

Q1=1,206 Hz, Q3=2,412 Hz). Mean BW 90% for females was 4,910 Hz (SD ±1,202.4,

Q1=4,264 Hz, Q3=5,857 Hz), and for males $\bar{x} =$ 5,295 Hz (SD ±964.79, Q1=4,716 Hz,

Q3=6,051 Hz).

When the THSD test was run on the variables chosen based on principal

component analyses, we found the duet phrases recorded on Klabat to be remarkably

conserved, differing only in duration between two other locations. Average female note

duration within a duet was significantly different from Siau (which differed from all

locations except the very similar Sangihe,) at p = 0. Duration of the entire duet phrase

was significantly different from that of Tangkoko, p = 0.022 (see Table 6).

Table 5. Mean measurements of vocalizations from from Klabat, ± standard deviation, and 1st and 3rd quartile statistics for each variable.

KLABAT	Duet Phrase	Duetting Females	Randomized females	Randomized males
Numer of Sites	4	4	4	4
Number of Duet Phrases per Song	3–38			
Mean +SD	13.88 +9.19			
Sex Ratio +SD	1.06+0.21			
Q1,Q3	1.05, 1.26			
Number of Samples	8	109	40	80
Low Frequency (Hz) +SD	6476+867.14	6470+1011.15	4752+1286.00	4805+594.03
Q1, Q3	5926, 6993	5764, 7152	3738, 5581	4421, 5236
High Frequency (Hz) +SD	12982+614.46	9622+426.55	13677+853.915	12564+636.92
Q1, Q3	12696, 13296	9515, 9839	13431, 14000	12297, 12929
Fundamental Frequency Range (Hz) +SD	6506+879.02	3152+744.02	8926+1437.32	7758+897.16
Q1, Q3	6168, 6935	2594, 3665	8260, 9827	7317, 8379
Duration (s) +SD	10.04+3.50	0.63+0.21	0.46+0.09	0.35+0.07
Q1, Q3	8.75, 10.47	0.53, 0.61	0.40, 0.51	0.30, 0.39
Rate of Change (Hz/s) +SD	707.9+211.95	5207+1257.63	20138+4970.95	23502+6516.80
Q1, Q3	621.0, 822.2	4621, 5754	17257, 23010	19365, 26262
Peak Frequency (Hz) +SD			10754+1214.91	7925+1390.71
Q1, Q3			9948, 11757	7451, 8527
Interquartile bandwidth (Hz) +SD			2067.2+901.47	1763.6+872.15
Q1, Q3			1679.6, 2584.0	1205.9, 2411.7
1st Quartile (Hz) +SD			9233+805.92	7419+968.56
Q1, Q3			8613, 9991	6460, 8096
3rd Quartile (Hz) +SD			11301+711.66	9183+871.47
Q1, Q3			10680, 11886	8699, 9582
Center Frequency (Hz) +SD			10413+744.22	8204+924.89
Q1, Q3			9948, 10853	8010, 8613
Bandwidth 90% (Hz) +SD			4910+1202.39	5295+964.79
Q1, Q3			4264, 5857	4716, 6051
5% Frequency (Hz) +SD			7300+947.31	6191+793.74
Q1, Q3			6503, 8096	5685, 6654
95% Frequency (Hz) +SD			12209+847.69	11486+849.71
Q1, Q3			11714, 12791	11003, 12145

Table 6. Results of Tukey's HSD test of high–contribution variables. P values indicated in bold represent significant differences between Klabat and remaining locations

KLABAT	Bangka	Bunaken	Lembeh	Manado Tua	Mantehage	Talisei	Tangkoko	Sangihe	Siau
Female Duet Notes									
Frequency range	0.755	0.963	0.588	0.260	0.241	0.832	0.805		1.000
Note duration	0.824	0.998	0.972	0.994	0.976	1.000	0.876		**0.000**
Full Duet									
Frequency range	0.711	0.166	0.055	0.064	0.823	0.303	0.806		0.968
Duet duration	1.000	0.193	0.695	0.976	1.000	0.390	**0.022**		0.364
Male Notes									
Center frequency	0.362	**0.000**	**0.000**	0.485	0.802	**0.002**	0.377	0.339	0.977
Maximum frequency	0.998	**0.000**	**0.006**	1.000	0.128	0.352	0.249	**0.012**	**0.005**
Bandwidth 90%	0.162	**0.000**	0.999	**0.000**	**0.000**	**0.000**	0.411	**0.000**	**0.000**
High frequency	0.923	0.110	0.934	**0.000**	**0.004**	**0.000**	1.000	**0.000**	**0.000**
Female Notes									
3rd quartile	0.775	0.908	0.774	0.938	0.348	**0.000**	**0.000**	**0.000**	**0.000**
Frequency range	**0.001**	**0.000**	**0.044**	**0.000**	**0.000**	**0.000**	0.565	0.163	0.087
Center frequency	0.976	0.999	0.094	0.895	1.000	**0.000**	**0.000**	**0.000**	**0.000**
Note duration	0.628	1.000	**0.002**	1.000	**0.022**	0.999	**0.010**	**0.000**	**0.000**

As with all of our locations, Klabat's individual non–duet–phrase notes showed more variance than did their duet notes. We found significant differences between the males' center frequencies from those on Bunaken, Lembeh, and Talisei (p = 0 for the first two, and 0.002 for Talisei.) Males also showed significant differences in peak frequencies from Bunaken, Lembeh, Sangihe, and Siau (p = 0.000, 0.006, 0.012, and 0.005, respectively). P–values were all 0 for 90% Bandwidth between males of Klabat and Bunaken, Manado Tua, Mantehage, Talisei, Tangkoko, Sangihe, and Siau, while high frequencies between Klabat and Manado Tua, Talisei, Sangihe, Siau, and Mantehage likewise showed significant differences (p = 0 for all but Mantehage, p = 0.004). Non–duet–phrase females also showed significant differences using the Tukey HSD test, including 3rd Quartile frequency (p = 0 for Talisei, Tangkoko, Sangihe, and Siau), fundamental frequency range (p = 0 for Bunaken, Manado Tua, Talisei, and Mantehage, p = 0.001 for Bangka, and p = 0.044 for Lembeh), center frequency (p = 0 for Talisei, Tangkoko, and distant islands Sangihe and Siau), and duration (p = 0 for distant islands Sangihe and Siau, and p = 0.002, 0.022, and 0.010 for Lembeh, Mantehage, and Tangkoko, respectively).

Shallow Water Islands

Lembeh

On Lembeh, we took 12 recordings at 5 sites, though only 7 of the recordings were clear enough to be used, primarily due to recurrent rains. Two sites yielded one recording apiece, a third yielded two, and a fourth gave us three recordings. All calls ranged between 4:58 and 5:36 in the morning, and all but one of our sites (recorded at a

strangler fig), were characterized by thick bamboo copses. Songs contained between 1 and 8 duet phrases, with a mean of 4.1.

Duets on Lembeh lasted an average of 7.22 seconds (SD $\pm$1.91), with females contributing between 10 and 14 notes per duet phrase, $\bar{x} = 12.4$, while males contributed 7 to 14 notes per phrase, $\bar{x} = 11.14$, resulting in a sex ratio $\bar{x} = 1.14$ (SD $\pm$0.20, Q1=1.04, Q3=1.14). The fundamental frequency range of the duet phrase was 8,416 Hz (SD $\pm$974.42, Q1=7,780 Hz, Q3=9,189 Hz), with a low average frequency of 5,896 Hz (SD $\pm$355.57, Q1=5,734 Hz, Q3=5,946 Hz) and a high average frequency of 14,313 Hz (SD $\pm$985.70, Q1=13,682 Hz, Q3=15,135 Hz)(see Table 7).

We measured 87 female notes within the seven recorded duet phrases from Lembeh. When these notes were averaged, they exhibited a fundamental frequency range of 4,074 Hz (SD $\pm$1,104.5, Q1=3,101 Hz, Q3=4,850 Hz), ranging from an average low of 6,210 Hz (SD $\pm$432.98, Q1=5,881 Hz, Q3=6,419 Hz) to an average high of 10,284 Hz (SD $\pm$1,146.59, Q1=9,301 Hz, Q3=11,193 Hz). Note length averaged 0.49 seconds (SD $\pm$0.08, Q1=0.42 s, Q3=0.55 s).

For our non–duet–phrase notes, we were able to extract measurements from an 8th recording, made at a liana tangle in secondary forest. Our sample size was thus $n = 40$ for females (five randomized notes per song), and $n = 80$ for males (ten randomized notes per song). Female notes averaged 0.38 seconds (SD $\pm$0.08, Q1=0.32 s, Q3=0.43 s), while male notes lasted an average of 0.29 seconds (SD $\pm$0.10, Q1=0.20, Q3=0.37.) The fundamental frequency of female notes exhibited a mean low frequency of 4,507 Hz (SD $\pm$827.24, Q1=4,029 Hz, Q3=5,176 Hz) and a mean high frequency of 14,398 Hz (SD $\pm$662.98, Q1=13,960 Hz, Q3=14,815 Hz) giving them a range of 9,891 Hz (SD

+1,274.73, Q1=9,052 Hz, Q3=10,592 Hz). Males had a slightly narrower fundamental frequency range of 8,443 Hz (SD +1,160.68, Q1=7,647, Q3=9,405), with a mean low frequency of 4,256 Hz (SD +727.04, Q1=3,706 Hz, Q3=4,747 Hz) and a mean high of 12,698 Hz (SD +701.08, Q1=12,136 Hz, Q3=13,190 Hz). Male notes lasted an average of 0.29 seconds (SD +0.10, Q1=0.20 s, Q3=0.37 s), while female note duration averaged 0.38 seconds (SD +0.08, Q1=0.32 s, Q3=0.43 s).

Additional measurements were again undertaken on these randomized notes. Male notes peaked in intensity at 7,237 Hz (SD +1,173.32, Q1=6,374 Hz, Q3=8,183 Hz), with 90% of their energy between 5,875 Hz (SD +654.78, Q1=5,512 Hz, Q3=6,374 Hz) and 11,277 Hz (SD +935.68, Q1=10,928 Hz, Q3=11,736 Hz) giving them a mean 90% bandwidth of 5,403 Hz (SD +980.44, Q1=4,910 Hz, Q3=6,029 Hz).

Additional robust measurements were again carried out on these randomized notes. Male notes peaked in intensity at 7,237 Hz (SD +1,173.32, Q1=6,374 Hz, Q3=8,183 Hz), with 90% of their energy between 5,875 Hz (SD +654.78, Q1=5,512 Hz, Q3=6,374 Hz) and 11,277 Hz (SD +935.68, Q1=10,928 Hz, Q3=11,736 Hz) giving them a mean 90% bandwidth of 5403 Hz (SD +980.44, Q1=4,910 Hz, Q3=6,029 Hz). All male notes recorded on Lembeh were type 1. Female notes had a mean 90% bandwidth of 6,052 Hz (SD +1,304.05, Q1=5,340 Hz, Q3=6,718 Hz) ranging from 6,844 Hz (SD +491.65, Q1=6,546 Hz, Q3=7063 Hz) to 12,896 Hz (SD +1,292.51, Q1=12,252, Q3=13,695) and peaking at 9,964 Hz (SD +1,560.44, Q1=9,130 Hz, Q3=10,680 Hz). Center frequencies averaged 9,844 Hz (SD +896.92, Q1=9,130 Hz, Q3=10,379 Hz) for females and 7,503 Hz (SD +848.71, Q1=6,718 Hz, Q3=8,204 Hz) for males. Lembeh

females had an IQR bandwidth of 2,376 Hz (SD $\pm$745.98, Q1=1,895 Hz, Q3=2,972 Hz), while males' notes averaged 1,912 Hz (SD $\pm$695.42, Q1=1,464.3 Hz, Q3=2,239.5 Hz).

Table 7. Mean measurements from Lembeh, $\pm$ standard deviation, and 1st and 3rd quartile statistics for each variable.

LEMBEH	Duet Phrase	Duetting Females	Randomized females	Randomized males
Numer of Sites	4	4	4	4
Number of Duet Phrases per Song	1–8			
Mean $\pm$SD	4.14$\pm$2.12			
Sex Ratio $\pm$SD	1.14$\pm$0.20			
Q1,Q3	1.04, 1.14			
Number of Samples	7	87	40	80
Low Frequency (Hz) $\pm$SD	5896$\pm$355.57	6210$\pm$432.98	4507$\pm$827.24	4256$\pm$727.04
Q1, Q3	5734, 5946	5881, 6419	4029, 5176	3706, 4747
High Frequency (Hz) $\pm$SD	14313$\pm$985.70	10284$\pm$1146.59	14398$\pm$662.98	12698$\pm$701.08
Q1, Q3	13682, 15135	9301, 11193	13960, 14815	12136, 13190
Fundamental Frequency Range (Hz) $\pm$SD	8416$\pm$974.42	4074$\pm$1104.50	9891$\pm$1274.73	8442$\pm$1160.68
Q1, Q3	7780, 9189	3101, 4850	9051, 10592	7647, 9405
Duration (s) $\pm$SD	7.22$\pm$1.91	0.49$\pm$0.08	0.38$\pm$0.08	0.29$\pm$0.10
Q1, Q3	5.49, 8.86	0.42, 0.55	0.32, 0.43	0.20, 0.37
Rate of Change (Hz/s) $\pm$SD	1252.7$\pm$412.57	8804$\pm$3677.05	26992$\pm$6221.80	33492$\pm$16077.87
Q1, Q3	948.8, 1539.1	5648, 10574	22621, 29530	21985, 39313
Peak Frequency (Hz) $\pm$SD			9964$\pm$1560.44	7137$\pm$1173.32
Q1, Q3			9130, 10680	6374, 8183
Interquartile bandwidth (Hz) $\pm$SD			2376$\pm$745.98	1912.7$\pm$695.42
Q1, Q3			1895, 2972	1464.3, 2239.5
1st Quartile (Hz) $\pm$SD			8583$\pm$806.00	6725$\pm$773.39
Q1, Q3			8269, 9130	6202, 7321
3rd Quartile (Hz) $\pm$SD			10959$\pm$1088.02	8638$\pm$846.47
Q1, Q3			10153, 11413	7989, 9216
Center Frequency (Hz) $\pm$SD			9844$\pm$896.92	7503$\pm$848.71
Q1, Q3			9130, 10379	6718, 8204
Bandwidth 90% (Hz) $\pm$SD			6052$\pm$1304.05	5403$\pm$980.44
Q1, Q3			5340, 6718	4910, 6029
5% Frequency (Hz) $\pm$SD			6844$\pm$491.65	5875$\pm$654.78

Q1, Q3	6546, 7063	5512, 6374
95% Frequency (Hz) +SD	12896+1292.51	11277+935.68
Q1, Q3	12252, 13695	10928, 11736
Gap Frequency (Hz) +SD		−182.0+889.13
Q1, Q3		−10.9, 0
Gap Duration (s) +SD		0.04+0.05
Q1, Q3		0, 0.08
Gap Ratio (Hz/s) +SD		-2117.8+9658.75
Q1, Q3		−136.2, 0

When the Tukey's HSD test was run on the high–contribution variables, Lembeh was found to be significantly different than other locations in 62 of 104 comparisons— thus presenting more instances of differentiation than any of the other islands but Sangihe and Siau, the distant islands. When duet phrases were tested, Lembeh's fundamental frequency was significantly longer than all of the other locations except for Klabat, for which it still averaged longer, though not significantly so (p = 0.002 for Bangka, p = 0.001 for Bunaken, p = 0.055 for Klabat, p = 0 for Manado Tua, p = 0.004 for Mantehage, p = 0 for Talisei, p = 0.001 for Tangkoko, and p = 0.027 for Siau. The duration of the duet phrases showed significant differences only to the longer Siau call, with a p–value of 0.014

Bunaken, Klabat, Mantehage, Tangkoko, Sangihe, and Siau (all at p = 0), as well as from Manado Tua (p = 0.024). Lembeh's peak frequency similarly had a p–value of 0 as compared to Bunaken, Mangehage, Tangkoko, Sangihe, and Siau, with statistical differences when compared to Klabat (p = 0.006) and Manado Tua (p = 0.033) as well. BW90% and high frequency were significantly different against Manado Tua, Mantehage, Talisei, Sangihe, and Siau (all p = 0), and BW90% was different than

Table 8. Results of Tukey's HSD test of high–contribution variables. P–values indicated in bold represent significant differences between Lembeh and other locations.

LEMBEH	Bangka	Bunaken	Klabat	Manado Tua	Mantehage	Talisei	Tangkoko	Sangihe	Siau
Female Duet Notes									
Frequency range	0.054	0.319	0.588	**0.003**	**0.006**	**0.047**	**0.030**		0.650
Note duration	0.999	1.000	0.972	1.000	1.000	0.990	1.000		**0.000**
Full Duet									
Frequency range	**0.002**	**0.001**	0.055	**0.000**	**0.004**	**0.000**	**0.001**		**0.027**
Duet duration	0.729	**0.014**	0.695	0.163	0.727	1.000	0.746		0.990
Male Notes									
Center frequency	0.073	**0.000**	**0.000**	**0.024**	**0.000**	0.987	**0.000**	**0.000**	**0.000**
Maximum frequency	0.126	**0.000**	**0.006**	**0.033**	**0.000**	0.979	**0.000**	**0.000**	**0.000**
Bandwidth 90%	**0.020**	**0.000**	0.999	**0.000**	**0.000**	**0.000**	0.068	**0.000**	**0.000**
High frequency	0.160	**0.007**	0.934	**0.000**	**0.000**	**0.000**	0.948	**0.000**	**0.000**
Female Notes									
3rd quartile Frequency	1.000	1.000	0.774	1.000	0.994	**0.000**	**0.000**	**0.000**	**0.000**
Frequency range	**0.000**	**0.000**	**0.044**	**0.000**	**0.000**	**0.000**	**0.000**	1.000	**0.000**
Center frequency	0.802	0.231	0.094	**0.000**	0.093	**0.023**	**0.015**	**0.000**	**0.000**
Note duration	0.563	0.133	**0.002**	**0.003**	1.000	**0.000**	1.000	**0.000**	**0.000**

Bunaken (p = 0) and Bangka (p = 0.020). High frequency was significantly different for Bunaken as well (p = 0.020)(see Table 8).

Lembeh females had 3rd quartile values significantly different than Talisei, Tangkoko, Sangihe or Siau (p = 0), frequency ranges significantly higher than Bangka, Bunaken, Klabat, Manado Tua, Mantehage, Talisei, Tangkoko, and Siau (p = 0 for all but Klabat, where p = 0.044). Center frequencies were slightly more conserved, with significant p–values differentiating Lembeh from Manado Tua, Talisei, Tangkoko, Sangihe, and Siau (p = 0, 0.023, 0.015, 0, and 0, respectively). Note duration here was differentiated from Talisei, Sangihe, and Siau (p = 0), and from Klabat and Manado Tua (p = 0.002 and 0.003, respectively).

Bangka

We made eight recordings on Bangka, only one of which turned out to be unusable. We recorded at four sites, twice each, though our fourth site only yielded one usable recording. In three out of the seven recordings, the tarsiers did not use a duet phrase during their song, though both males and females were vocalizing. Thus we had a single duetting recording at two of our sites, and two recordings with duet phrases at a third site.

We recorded in a bamboo grove, in fig and liana tangles, and in mangroves. Weather was consistently overcast with occasional showers, with no clear mornings. Duet calls on Bangka began between 4:58 am and 5:15 am, and contained 0 to 8 duet phrases (mean=4), with an average female calling 18.75 times to the male's average of 16 (female/male ratio of 1.30 ± 0.45).

Duet phrases (*n=4)* lasted an average of 10.41 seconds (SD $\pm$2.43, Q1=8.58 s,

Q3=12.46 s) and spanned an average of 5,282 Hz (SD $\pm$984.06 Hz, Q1=4,474 Hz,

Q3=5,951 Hz). The mean low frequency of Bangka's duet phrases was 6,577 Hz (SD

$\pm$589.05, Q1= 4,474 Hz, Q3= 5,951 Hz), and the mean high frequency was 11,859 Hz

(SD $\pm$1,241.00, Q1=11,209 Hz, Q3=12,163 Hz). Within the duet phrases, individual

female notes (*n*=75) had an average low frequency of 6,977 Hz (SD $\pm$509.32, Q1=6,638

Table 9. Mean measurements from Bangka, ± standard deviation, and 1st and 3rd quartile statistics for each variable.

BANGKA	Duet Phrase	Duetting Females	Randomized females	Randomized males
Number of Sites	3	3	4	4
Number of Duet Phrases per Song	2–8			
Mean $\pm$SD	4$\pm$2.83			
Sex Ratio $\pm$SD	1.3$\pm$0.45			
Q1,Q3				
Number of Samples	4	75	35	70
Low Frequency (Hz) $\pm$SD	6577$\pm$589.05	6977$\pm$509.32	5159$\pm$669.19	4748$\pm$686.84
Q1, Q3	6229, 6953	6638, 7152	4814, 5364	4333, 5174
High Frequency (Hz) $\pm$SD	11859$\pm$1241.00	9186$\pm$744.06	12780$\pm$1072.94	12421$\pm$579.39
Q1, Q3	11209, 12163	8724, 9412	12270, 13540	11962, 12925
Fundamental Frequency Range(Hz) $\pm$SD	5282$\pm$984.06	2209$\pm$1079.88	7621$\pm$1459.76	7673$\pm$829.11
Q1, Q3	4474, 5951	1721, 2539	7144, 8444	6980, 8414
Duration (s) $\pm$SD	10.41$\pm$2.43	0.4$\pm$0.08	0.42$\pm$0.07	0.3$\pm$0.08
Q1, Q3	8.58, 12.46	0.34, 0.44	0.37, 0.46	0.25, 0.36
Rate of Change (Hz/s) $\pm$SD	512.9$\pm$42.02	5440$\pm$1895.94	18491$\pm$4052.54	27658$\pm$9577.78
Q1, Q3	496.8, 528.1	4296, 6709	16406, 21244	20558, 33725
Peak Frequency (Hz) $\pm$SD			10708$\pm$1423.88	7747$\pm$1403.21
Q1, Q3			10422, 11477	6718, 8872
Interquartile bandwidth (Hz) $\pm$SD			1952.8$\pm$1001.86	1841.4$\pm$603.87
Q1, Q3			1292.0, 2519.4	1399.7, 2298.7
1st Quartile (Hz) $\pm$SD			8995$\pm$1180.3	6954$\pm$804.21
Q1, Q3			8699, 9819	6212, 7580
3rd Quartile (Hz) $\pm$SD			10947$\pm$896.08	8795$\pm$721.83

Q1, Q3	10745, 11413	8253, 9388
Center Frequency (Hz) +SD	10180+1144.50	7899+780.27
Q1, Q3	9927, 10724	7343, 8613
Bandwidth 90% (Hz) +SD	4630+1157.17	4894+841.43
Q1, Q3	3833, 5319	4414, 5426
5% Frequency (Hz) +SD	6903+806.01	6071+630.91
Q1, Q3	6589, 7300	5534, 6546
95% Frequency (Hz) +SD	11533+769.53	10964+912.93
Q1, Q3	11219, 11908	10341, 11714
Gap Frequency (Hz) +SD		−170.34+676.62
Q1, Q3		−99.12, 0
Gap Duration (s) +SD		0.03+0.05
Q1, Q3		0, 0.08
Gap Ratio (Hz/s) +SD		5277+282414.6
Q1, Q3		−2850, 0

Hz, Q3=7,152 Hz) and an average high frequency of 9,187 Hz (SD ±744.06, Q1=8,724 Hz, Q3=9,412 Hz), giving them a range of 2,209 Hz (SD ±1,079.88, Q1=1,721 Hz, Q3=1,079 Hz). Duet–phrase female notes had a mean duration of 0.04 s (SD ±0.08, Q1=1,721 Hz, Q3=2,539 Hz)(see Table 9).

Non–duet phrase females' notes (*n*=35) were only slightly longer on average at 0.42 seconds (SD ±0.07, Q1=0.37, Q3=0.46), and had a broader frequency range spanning 7,621 Hz (SD ±1,459.76, Q1=7,144, Q3=8,444) from a low mean of 5,159 Hz (SD ±669.19, Q1=4,814 Hz, Q3=5,364 Hz) to a high mean of 12,780 Hz (SD ±1,072.94, Q1=12,270 Hz, Q3=13,540 Hz). Peak frequency in female notes occurred at an average of 10,708 Hz (SD ±1,423.88, Q1=10,422 Hz, Q3=11,477 Hz), and most of the energy occurred between the 5% frequency average of 6,903 Hz (SD ±806.01, Q1=6,589 Hz, Q3=7,300 Hz) and the 95% frequency average of 11,533 Hz (SD ±769.53, Q1=11,219 Hz, Q3=11,908 Hz). IQR Bandwidth was 1,952.8 Hz (SD ±1,001.86, Q1=1,292.8 Hz,

Q3=2,419.4 Hz), and center frequency was 10,180 Hz (SD $\pm$1,144.5, Q1=9,927 Hz,

Q3=10,724 Hz).

Male calls on Bangka (*n*=70) stretched from a low frequency mean of 4,768 Hz

(SD $\pm$686.84, Q1=4,333 Hz, Q3=5,174 Hz) to a high frequency mean of 12,421 Hz (SD

$\pm$579.39, Q1=11,962 Hz, Q3=12,925 Hz), spanning a range of 7,673 Hz (SD $\pm$829.11,

Q1=6,980 Hz, Q3=8,414 Hz) and lasting an average of 0.30 seconds (SD $\pm$0.08,

Q1=0.25, Q3=0.36). Peak frequency averaged 7,747 Hz (SD $\pm$1,403.21, Q1=6,718 Hz,

Q3=8,872 Hz) and center frequency averaged 7,899 Hz (SD $\pm$780.27, Q1=7,343 Hz,

Q3=8,613 Hz). 90% of the energy in the male notes was contained by the 4,894 Hz (SD

$\pm$841.43, Q1=4,414 Hz, Q3=5,426 Hz) between the 5% frequency at 6,071 Hz (SD

$\pm$630.91, Q1=5,534 Hz, Q3=6,546 Hz), and the 95% frequency at 10,964 Hz (SD

$\pm$912.93, Q1=10,341 Hz, Q3=11,714 Hz). IQR bandwidth for Bangka males averaged

1,841.4 Hz (SD $\pm$603.87, Q1=1,399.7 Hz, Q3=7,580 Hz). All three male note types were

represented on Bangka, though the majority (57%) were type 1.

Male notes on Bangka showed more significant differentiation from the other

locations than did female notes or duet phrases. When the Tukey HSD test was run, the

center frequency of the male notes was statistically separable from Bunaken, Tangkoko

($p = 0$), Mantehage ($p = 0.008$), and Sangihe ($p = 0.005$).

Maximum frequency was distinguishable from Bunaken, Siau ($p = 0$), Mantehage

($p = 0.019$), Tangkoko ($p = 0.033$), and Sangihe ($p = 0.002$). When comparing high

frequency, Bangka was significantly different from Manado Tua, Talisei, Sangihe, and

Siau ($p = 0$). BW90% was statistically different between Bangka and Lembeh ($p =$

Table 10. Results of Tukey's HSD test of high–contribution variables. P–values indicated in bold represent significant differences between Bangka and other locations.

BANGKA	Bunaken	Klabat	Lembeh	Manado Tua	Mantehage	Talisei	Tangkoko	Sangihe	Siau
Female Duet Notes									
Frequency range	1.000	0.755	0.054	1.000	0.998	1.000	1.000		0.984
Note duration	1.000	0.824	0.999	0.996	1.000	0.895	1.000		**0.000**
Full Duet									
Frequency range	0.936	0.711	**0.002**	0.991	1.000	1.000	1.000		1.000
Duet duration	0.396	1.000	0.729	0.999	1.000	0.464	0.061		0.395
Male Notes									
Center frequency	**0.000**	0.362	0.073	1.000	**0.008**	0.745	**0.000**	**0.005**	0.108
Maximum frequency	**0.000**	0.998	0.126	1.000	**0.019**	0.883	**0.033**	**0.002**	**0.000**
Bandwidth 90%	0.086	0.162	**0.020**	0.295	**0.022**	**0.001**	1.000	**0.000**	**0.000**
High frequency	0.641	0.923	0.160	**0.000**	0.241	**0.000**	0.844	**0.000**	**0.000**
Female Notes									
3rd quartile	1.000	0.775	1.000	1.000	0.997	**0.000**	**0.000**	**0.000**	**0.000**
Frequency range	0.670	**0.001**	**0.000**	**0.000**	**0.000**	0.892	0.297	**0.000**	1.000
Center frequency	0.908	0.976	0.802	0.202	0.889	**0.000**	**0.000**	0.889	**0.000**
Note duration	0.906	0.628	0.563	0.745	0.763	0.270	0.897	**0.000**	**0.000**

0.020), Mantehage (p = 0.022), Talisei (p = 0.001), and Sangihe and Siau (p = 0)(see

Table 10).

The third quartile frequency of Bangka's female notes was statistically different

than that of Talisei, Tangkoko, Sangihe, and Siau (p = 0), and the frequency range was

statistically different from that of Lembeh, Manado Tua, Mantehage, Sangihe, and Klabat

(the latter at p = 0.001, and the rest at p = 0). Center frequencies fairly were similar to all

the other location, with only Talisei and Tangkoko diverging at p = 0, and note duration

diverged only from the distant islands of Sangihe and Siau (p = 0).

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Talisei

We recorded 8 morning duets over the course of two weeks on Talisei Island. Of

those, two were discarded due to high ambient noise from rain; the remaining six were

used in our analysis of random male and female notes as well as duet phrases. We

recorded at three sites, including a bamboo copse (one recording,) a medium strangler fig

(three recordings), and in mangrove (two recordings). All calls began between 5:06 am

and 5:20am, and each morning there were between 1 and 13 duet phases ($\bar{x} = 5$).

Males and females contributed fairly equally to the duet phrases, with each phrase

containing 7 to 15 female notes (mean=10.67,) and 7 to 16 male notes ($\bar{x} = 11.33$) for a

mean female to male ratio of 0.95 (SD±0.16). Duet phrases lasted an average of 9.87

seconds (SD ±2.94, Q1=7.86 s, Q3=10.55 s). Their fundamental frequency ranged from

an average low frequency of 5,708 Hz (SD ±495.78, Q1=5,472 Hz, Q3=5,689 Hz) to an

average high frequency of 10,725 Hz (SD ±1,557.84, Q1=9,679 Hz, Q3=12,031 Hz),

giving it a range $\bar{x} = 5,017$ Hz (SD ±1,714.70, Q1=3,763 Hz, Q3=6,342 Hz).

Table 11. Mean measurements from Talisei, followed standard deviation, and 1st and 3rd quartile statistics for each variable.

TALISEI	Duet Phrase	Duetting Females	Randomized females	Randomized males
Numer of Sites	3	3	3	3
Number of Duet Phrases per Song	1–13			
Mean $\pm$SD	5.0$\pm$5.83			
Sex Ratio $\pm$SD	0.95$\pm$0.16			
Q1,Q3	0.84, 1.08			
Number of Samples	6	75	30	60
Low Frequency (Hz) $\pm$SD	5708$\pm$495.78	6126$\pm$363.48	4944$\pm$919.80	4897$\pm$839.84
Q1, Q3	5472, 5689	5880, 6323	4330, 5324	4219, 5752
High Frequency (Hz) $\pm$SD	10725$\pm$1557.84	8518$\pm$829.29	12067$\pm$1512.19	11883$\pm$613.17
Q1, Q3	9679, 12031	7870, 8872	10757, 13442	11569, 12309
Fundamental Frequency Range (Hz) $\pm$SD	5017$\pm$1714.70	2392$\pm$937.38	7123$\pm$1537.36	6986$\pm$797.29
Q1, Q3	3763, 6342	1907, 2319	5939, 7795	6339, 7698
Duration (s) $\pm$SD	9.87$\pm$2.94	0.61$\pm$0.09	0.47$\pm$0.09	0.3981$\pm$0.09
Q1, Q3	7.86, 10.55	0.51, 0.68	0.39, 0.53	0.34, 0.47
Rate of Change (Hz/s) $\pm$SD	522.7$\pm$199.38	3945$\pm$1365.89	15639$\pm$4693.99	18421$\pm$4718.485
Q1, Q3	426.1, 479.5	2914, 4266	11503, 19509	15035, 20635
Peak Frequency (Hz) $\pm$SD			9064$\pm$1251.80	7395$\pm$1539.40
Q1, Q3			8441, 9981	6148, 8441
Interquartile bandwidth (Hz) $\pm$SD			1435.5$\pm$1068.42	1861.9$\pm$577.66
Q1, Q3			861.3, 1475.0	1378.1, 2336.4
1st Quartile (Hz) $\pm$SD			8419$\pm$1141.54	6562$\pm$962.25
Q1, Q3			7623, 9195	5825, 7235
3rd Quartile (Hz) $\pm$SD			9855$\pm$966.65	8424$\pm$730.39
Q1, Q3			9130, 10508	7935, 8893
Center Frequency (Hz) $\pm$SD			9130$\pm$1050.47	7649$\pm$808.89
Q1, Q3			8484, 10088	6966, 8075
Bandwidth 90% (Hz) $\pm$SD			3748$\pm$1254.39	4215$\pm$667.34
Q1, Q3			2799, 4371	3747, 4662
5% Frequency (Hz) $\pm$SD			7146$\pm$740.42	5736$\pm$685.86
Q1, Q3			6718, 7386	5060, 6255
95% Frequency (Hz) $\pm$SD			10894$\pm$1370.76	9952$\pm$1047.95
Q1, Q3			9615, 11972	9453, 10734
Gap Frequency (Hz) $\pm$SD				−446.9$\pm$732.27

Q1, Q3	−901.3, 0
Gap Duration (s) +SD	0.03+0.05
Q1, Q3	0, 0.10
Gap Ratio (Hz/s) +SD	−4369+7150.69
Q1, Q3	0, 0

When the female notes within the duet phrase (*n*=75,) were averaged, Talisei ranged from a low of 6,126 Hz (SD ±363.48, Q1=5,880 Hz, Q3=6,323 Hz) to a high of 8,518 Hz (SD ±829.29, Q1=7,870 Hz, Q3=8,872 Hz). Mean note duration was 0.61 (SD ±0.09, Q1=0.51, Q3=0.68) (see Table 11).

Talisei's non–duet–phrase female notes (*n*=30,) had a mean duration of 0.47 seconds (SD ±0.09, Q1=0.39, Q3=0.53) and a mean fundamental frequency range of 7,123 Hz (SD ±1,537.36, Q1=5,939 Hz, Q3=7,795 Hz). Low frequency averaged 4,944 Hz (SD ±919.80, Q1=4,330 Hz, Q3=5,324 Hz), and high frequency averaged 12,067 Hz (SD ±12,067, Q1=10,757 Hz, Q3=13,442 Hz), with the peak hitting at 9,064 Hz (SD ±1,251.80, Q1=8,441 Hz, Q3=9,981 Hz). IQR bandwidth was 1,435.5 Hz (SD ±1,068.42 Hz, Q1=861.3 Hz, Q3=1,068.42 Hz), from a 1st quartile of 8,419 Hz (SD ±1,141.54, Q1=7,623 Hz, Q3=9,195 Hz) to a 3rd quartile of 9,855 Hz (SD ±966.65, Q1=9,130 Hz, Q3=10,508 Hz). 5% frequency was 7,146 Hz (SD ±740.42, Q1=6,718 Hz, Q3=7,386 Hz) and 95% frequency was 10,894 Hz (SD ±1,370.76, Q1=9,615 Hz, Q3=11,972 Hz), leaving 90% of the energy within a 3,748 Hz band (SD ±1,254.39, Q1=2,799 Hz, Q3=4,371 Hz).

All three types of male notes were represented on Talisei, with type 1 representing 68.3%. Notes had a mean duration of 0.40 seconds (SD ±0.09, Q1=0.34, Q3=0.47), and a mean fundamental frequency range of 6,986 Hz (SD ±797.29, Q1=6,339 Hz, Q3=7,698

Hz). The low frequency averaged 4,897 Hz (SD $\pm$839.84, Q1=4,219 Hz, Q3=5,752 Hz),

while the high frequency averaged 11,883 Hz (SD $\pm$613.17, Q1=11,569 Hz, Q3=12,309

Hz). 90% of the energy within the male notes was contained within a bandwidth of 4,215

Hz (SD $\pm$667.34, Q1=3,747 Hz, Q3=4,662 Hz), falling between the 5% frequency mark

of 5,736 Hz (SD $\pm$685.86, Q1=5,060, Q3=6,255) and the 90% frequency mark of 9,952

Hz (SD $\pm$1,047.95, Q1=9,453 Hz, Q3=10,734 Hz). Frequency peaked at a mean 7,395 Hz

(SD $\pm$1,539.4, Q1=6,148 Hz, Q3=8,441 Hz), while the center point averaged 7,649 Hz

(SD $\pm$808.89, Q1=6,966 Hz, Q3=8,075 Hz).

Talisei's duet phrases showed few differences from the duet phrases of other

locations. The frequency range for the duet phrase was statistically different from that on

Lembeh (p = 0.047), and the average female note length within the phrase was

statistically different from that of Siau (p = 0).

Running Tukey's HSD on the randomized male and female notes did result in

uncovering substantial differences between Talisei and other locations. For males, center

frequency was significantly different from Klabat (p = 0.002), and Siau (p = 0.001), as

well as Bunaken, Mantehage, Tangkoko, and Sangihe (all p = 0.0). Comparing peak

frequencies amongst locations showed significant differences between Talisei and

Bunaken, Mantehage, Tangkoko, Sangihe, and Siau (p = 0). BW90% showed differences

between Talisei and Bangka (p = 0.001), Siau (p = 0.006), Klabat, Lembeh, Tangkoko,

and Sangihe (all p = 0). High frequency averages for most of the locations (Bangka,

Klabat, Lembeh, Manado Tua, Tangkoko, Sangihe, and Siau), were significantly different

from Talisei (p = 0) (see Table 12).

Table 12. Results of Tukey's HSD test of high–contribution variables. P–values indicated in bold represent significant differences between Talisei and other locations.

TALISEI	Bangka	Bunaken	Klabat	Lembeh	Manado Tua	Mantehage	Tangkoko	Sangihe	Siau
Female Duet Notes									
Frequency range	1.000	1.000	0.832	**0.047**	0.995	0.965	1.000		0.997
Note duration	0.895	0.999	1.000	0.990	0.998	0.990	0.944		**0.000**
Full Duet									
Frequency range	0.895	0.999	1.000	0.990	0.998	0.990	0.944		**0.000**
Duet duration	1.000	0.977	0.303	**0.000**	0.999	1.000	0.988		0.997
Male Notes									
Center frequency	0.745	**0.000**	**0.002**	0.987	0.540	**0.000**	**0.000**	**0.000**	**0.001**
Maximum frequency	0.883	**0.000**	0.352	0.979	0.657	**0.000**	**0.000**	**0.000**	**0.000**
Bandwidth 90%	**0.001**	1.000	**0.000**	**0.000**	0.542	1.000	**0.000**	**0.000**	**0.006**
High frequency	**0.000**	0.906	**0.000**	**0.000**	**0.000**	0.550	**0.000**	**0.000**	**0.000**
Female Notes									
3rd quartile	**0.000**	0.066	**0.000**	**0.000**	**0.000**	**0.023**	1.000	**0.000**	**0.000**
Frequency range	0.892	0.998	**0.000**	**0.000**	**0.013**	**0.001**	**0.004**	**0.000**	0.926
Center frequency	**0.000**	**0.000**	**0.000**	**0.023**	**0.000**	**0.000**	1.000	**0.000**	**0.000**
Note duration	0.270	1.000	0.999	**0.000**	0.995	**0.005**	**0.002**	**0.000**	**0.000**

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For Talisei's non–duet–phrase female notes, 3rd quartile measurements were significantly different from those on Mantehage (p = 0.02,) as well as Bangka, Klabat, Lembeh, Mando Tua, Sangihe, and Siau (p = 0). The fundamental frequency range of the notes was significantly different than Klabat, Lambeh, Sangihe (p = 0), Manado Tua (p = 0.013), and Mantehage (p = 0.004). Center frequency was significantly lower than on Bangka, Bunaken, Klabat, Manado Tua, Mantehage (p = 0) and Lembeh (p = 0.02), but higher than on Sangihe or Siau (p = 0). Finally, note duration was significantly longer than on Lembeh or Mantehage (p = 0 and 0.005, respectively), and shorter than that on Sangihe and Siau (p = 0).

Bunaken

Bunaken Island presents a problem in that we had only two successful recordings to work from; our sample sizes thus are somewhat low. On the first trip Bunaken, we had six researchers searching for two weeks, and while two tarsiers were believed to have been seen one evening, and a scent mark was found on a different evening, they were not heard. On the second excursion to the island, in June of 2014, we approached a resort where Tarsiers were purported to inhabit, and obtained permission to record on the premises (we had previously searched fruitlessly for tarsiers directly outside the property).

The first recording began at 5:45 am, from a liana tangle within the resort bounds. This call contained 6 duet phrases, with an average of 23 female notes and 18 male notes within each phrase. A second recording at this location failed due to high ambient noise and excessive distance. Our third recording was taken at 7:45 am after we had abandoned

recording for the day and returned to camp. Despite having stayed at that site multiple times throughout a two–year period, this was the only instance during which we heard tarsier contact calling. This call was also unique in that it was the only instance where we observed a solo female attempting to duet. She sang three duet phrases, though the first was not recorded. Neither recorded duet phrases included male notes.

Duet phrases had a low mean frequency of 78,03 Hz (SD $\pm$219.27, Q1=7,726 Hz, Q3=7,881 Hz) and a high of 11,838 Hz (SD $\pm$536.20, Q1=11,648 Hz, Q3=12,027 Hz), with a range of 4,035 Hz (SD $\pm$755.54, Q1=3,768 Hz, Q3=4,302 Hz). Duet phrases had a mean duration of 5.61 seconds (SD $\pm$5.61, Q1=4.90 s, Q3=6.31 s), with individual female notes lasting an average of 0.49 seconds each (SD $\pm$0.49, Q1=0.48 s, Q3=0.51 s). Female notes within the duet phrases had a mean low frequency of 7,990 Hz (SD $\pm$914.18, Q1=7,667 Hz, Q3=8,313 Hz), a mean high frequency of 10,311 Hz (SD $\pm$527.37, Q1=10,125 Hz, Q3=10,498 Hz), and a frequency range of 2,321 Hz (SD $\pm$1,441.54, Q1=1,811 Hz, Q3=2,831 Hz) (see Table 13).

Table 13. Mean measurements from Bunaken, followed by standard deviation, and 1st and 3rd quartile statistics for each variable.

BUNAKEN	Duet Phrase	Duetting Females	Randomized females	Randomized males
Numer of Sites	2	2	2	2
Number of Duet Phrases per Song	2–6			
Mean $\pm$SD	4$\pm$2.83			
Sex Ratio $\pm$SD	0.64$\pm$0.91			
Q1,Q3	0.32, 0.96			
Number of Samples	2	31	10	20
Low Frequency (Hz) $\pm$SD	7803$\pm$219.27	7990$\pm$914.18	5692$\pm$1174.99	6469$\pm$1369.24

Q1, Q3	7726, 7881	7667, 8313	5495, 6295	5428, 7676
High Frequency (Hz) ±SD	11838±536.20	10311±527.37	12403±607.47	12117±698.35
Q1, Q3	11648, 12027	10125, 10498	12125, 12600	11895, 12557
Fundamental Frequency Range (Hz) ±SD	4035±755.54	2321±1441.54	6711±1022.20	5648±1124.33
Q1, Q3	3768, 4302	1811, 2831	5995, 7324	3911, 6308
Duration (s) ±SD	5.61±2.00	0.49±0.04	0.46±0.07	0.28±0.06
Q1, Q3	4.90, 6.31	0.48, 0.51	0.44, 0.50	0.25, 0.31
Rate of Change (Hz/s) ±SD	793.9±417.83	4606±2544.15	14819±3691.87	21312±7183.89
Q1, Q3	646.2, 941.7	3707, 5506	12195, 15572	16712, 24057
Peak Frequency (Hz) ±SD			10663±650.99	9836±1671.77
Q1, Q3			10228, 11219	8473, 11348
Interquartile bandwidth (Hz) ±SD			758±223.52	1612.8±480.07
Q1, Q3			624.5, 818.3	1335.0, 1938.0
1st Quartile (Hz) ±SD			10090±496.49	8837±1161.87
Q1, Q3			9765, 10433	7720, 9582
3rd Quartile (Hz) ±SD			10848±628.83	10450±1143.3
Q1, Q3			10444, 11305	9776, 11477
Center Frequency (Hz) ±SD			10629±642.89	9783±1230.31
Q1, Q3			10261, 11219	8969, 10917
Bandwidth 90% (Hz) ±SD			2817±613.15	4216±1214.57
Q1, Q3			2379, 3338	3251, 4716
5% Frequency (Hz) ±SD			8544±501.91	7334±1413.86
Q1, Q3			8290, 8839	6632, 8376
95% Frequency (Hz) ±SD			11361±505.83	11550±624.19
Q1, Q3			11133, 11520	11122, 12016
Gap Frequency (Hz) ±SD				−605.3±878.93
Q1, Q3				−1459.2, 0
Gap Duration (s) ±SD				0.03±0.04
Q1, Q3				0, 0.05
Gap Ratio (Hz/s) ±SD				−9452±15180.34
Q1, Q3				−16823, 0

The female notes that were not part of the duet phrases had a much larger range of 6,711 Hz (SD $\pm$1,022.20, Q1=5,995 Hz, Q3=7,324 Hz), ranging from a low frequency of 5,692 Hz (SD $\pm$1174.99, Q1=5,495 Hz, Q3=6,295 Hz) to a high of 12,403 Hz (SD $\pm$607.47, Q1=12,125 Hz, Q3=12,600 Hz). Peak frequency occurred at 10,663 Hz (SD $\pm$650.99, Q1=10,228 Hz, Q3=11,219 Hz), and BW90% occurred at 2,817 Hz (SD $\pm$613.15, Q1=2,379 Hz, Q3=3,338 Hz) between the 5% frequency at 8,544 Hz (SD $\pm$501.91, Q1=8,290 Hz, Q3=8,839 Hz) and the 95% frequency, at 11,361 Hz (SD $\pm$505.83, Q1=11,133Hz, Q3=11,520 Hz). 1st quartile frequency was 10,090 Hz (SD $\pm$496.49, Q1=9,765 Hz, Q3=10,433 Hz), while 3rd quartile frequency was 10,848 Hz (SD $\pm$628.83, Q1=10,444 Hz, Q3=11,305 Hz), leaving an IQR of 758 Hz (SD $\pm$223.52, Q1=624.5 Hz, Q3=818.3 Hz). Center frequency was 10,629 Hz (SD $\pm$642.89, Q1=10,261 Hz, Q3=11,219Hz).

All male notes recorded were type 1 notes lasting an average of 0.28 seconds (SD $\pm$0.06, Q1=0.25, Q3=0.06). Average low frequency was 6,469 Hz (SD $\pm$1,369.24, Q1=5,428 Hz, Q3=7,676 Hz), and average high frequency was 12,117 Hz (SD $\pm$698.35, Q1=11,895 Hz, Q3=12,557 Hz), range 5,648 Hz (SD $\pm$1,124.33, Q1=3,911 Hz, Q3=6,308 Hz). Frequency peaked at 9,836 Hz (SD $\pm$1,671.77, Q1=8,473 Hz, Q3=11,348 Hz), and centered at 9,783 Hz (SD $\pm$1,230.31, Q1=8,969 Hz, Q3=10,917 Hz). 5% frequency had a mean of 7,334 Hz (SD $\pm$1,413.86, Q1=6,632 Hz, Q3=8,376 Hz), 1st quartile frequency averaged 8,837 Hz (SD $\pm$1,161.87, Q1=7,720 Hz, Q3=9,582 Hz), 3rd quartile averaged 10,450 Hz (SD $\pm$1,143.3, Q1=9,776 Hz, Q3=11,477 Hz), and 95% frequency averaged 11,550 Hz (SD $\pm$624.19, Q1=11,122 Hz, Q3=12,016 Hz).

Interquartile bandwidth averaged 1,612.8 Hz (SD ±480.07, Q1=1,335 Hz, Q3=1,938 Hz) and 90% fell within a 4,216 Hz band (SD ±1,214.57, Q1=3,251 Hz, Q3=4,716 Hz).

When Tukey's HSD test was done on PCA high–contribution Bunaken characters, male notes showed the most differentiation. All values were significantly different from Sangihe (center frequency, $p = 0.001$; max frequency, $p = 0.012$; BW90%, $p = 0.002$; and high frequency, $p = 0$), and all except BW90% were different than Siau (max frequency, $p = 0.005$; center and high frequencies, $p = 0$). BW90% did, however, exhibit significant differences between Bunaken and Klabat and Lembeh ($p = 0$), and with Tangkoko ($p = 0.016$). Center frequency for Bunaken was significantly different than all other sites ($p = 0$). In addition to differentiating Sangihe and Siau from Bunaken, maximum frequency also differentiated Bunaken from Lembeh ($p = 0.006$). Finally, high frequency measurements showed significant differences between Bunaken and Lembeh ($p = 0.007$), and Bunaken and Manado Tua ($p = 0$). It is important to note, however, that male notes all came from a single morning's recording.

Bunaken females were significantly different from Sangihe females in every category we tested ($p = 0$) and significantly different from Siau in all but frequency range ($p = 0$). Frequency range distinguished Bunaken from Klabat and Lembeh ($p = 0$), and Tangkoko ($p = 0.017$), while center frequency differentiated Bunaken from Talisei and Tangkoko ($p = 0$) (see Table 14).

Table 14. Results of Tukey's HSD test of high–contribution variables. P–values indicated in bold represent significant differences between Bunaken and other locations.

BUNAKEN	Bangka	Klabat	Lembeh	Manado Tua	Mantehage	Talisei	Tangkoko	Sangihe	Siau
Female Duet Notes									
Frequency range	1.000	0.963	0.319	1.000	0.997	1.000	1.000		0.999
Note duration	1.000	0.998	1.000	1.000	1.000	0.999	1.000		**0.001**
Full Duet									
Frequency range	0.936	0.166	**0.001**	0.999	0.890	0.977	0.720		0.827
Duet duration	0.396	0.193	**0.014**	0.591	0.398	**0.006**	**0.000**		**0.007**
Male Notes									
Center frequency	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**
Maximum frequency	0.998	1.000	**0.006**	1.000	0.128	0.352	0.249	**0.012**	**0.005**
Bandwidth 90%	0.086	**0.000**	**0.000**	0.921	1.000	1.000	**0.016**	**0.002**	0.091
High frequency	0.641	0.110	**0.007**	**0.000**	1.000	0.906	0.076	**0.000**	**0.000**
Female Notes									
3rd quartile Frequency range	1.000	0.908	1.000	1.000	1.000	0.066	0.079	**0.000**	**0.000**
Center frequency	0.670	**0.000**	**0.000**	0.870	0.366	0.998	**0.017**	**0.000**	0.709
Note frequency	0.908	0.999	0.231	1.000	1.000	**0.000**	**0.000**	**0.000**	**0.000**
duration	0.906	1.000	0.133	1.000	0.212	1.000	0.296	**0.000**	**0.000**

Female notes within a duet phrase showed little differentiation, with only note duration on Siau showing any significant difference from Bunaken (p = 0.001). Duration of the full duet showed differences as well, separating Bangka from Lembeh (p = 0.014), Talisei, (p = 0.006), Tangkoko (p = 0), and Siau (p = 0.007). Duet phrase frequency range was different only between Bunaken and Lembeh (p = 0.001).

Manado Tua

We made nine recordings on Manado Tua; one recording was not clear enough to measure, and a second recording had no clear duet phrases. We recorded at five different sites from the eastern shore of the island to the near the top of the caldera. Three of the sites provided two recordings each, and two provided single recordings, for a total of 8 recordings. Calls began between 5:03 and 5:21 in the morning, all in secondary forest and liana tangles. On one recording there were no measureable duet phrases, remaining mornings yielded 4 – 10 duet phrases, with $\bar{x}$ =16.14 female notes and $\bar{x} = 15.71$ male notes, a female/male ratio of 1.05±0.20 per phrase. Duet phrases had an average duration of 9.96 seconds (SD ±1.35, Q1=9.34 s, Q3=10.88 s) and an average frequency range of 4,634 Hz (SD ±1,042.18, Q1=3,866 Hz, Q3=5,535 Hz), from an average low frequency of 6,822 Hz (SD ±314.33, Q1=6,550 Hz, Q3=7,035 Hz) to an average high frequency of 11,457 Hz (SD ±1,030, Q1=10,878 Hz, Q3=12,359 Hz) (see Table 15).

Female notes within the duet phrase lasted $\bar{x} = 0.52$ seconds (SD ±0.05, Q1=0.49 s, Q3=0.56 s), had a low frequency of $\bar{x} = 7,238$ Hz (SD ±374.51, Q1=7,093 Hz, Q3=7,414 Hz) and a high frequency of $\bar{x} = 9,202$ Hz (SD ±634.18, Q1=8,774 Hz, Q3=9,577 Hz), giving them a frequency range of $\bar{x} = 1,964$ Hz (SD ±415.19, Q1=1,774

Hz, Q3=2,104 Hz). The average non–duet note, by contrast, was slightly shorter at $\bar{x} =$ 0.45 seconds (SD $\pm$0.08, Q1=0.39 s, Q3=0.50 s), but with a much greater frequency range, spanning a band of 5,987 Hz (SD $\pm$1,422.19, Q1=4,842 Hz, Q3=6,745 Hz), from a low of 6,248 Hz (SD $\pm$1,434.96, Q1=5,399 Hz, Q3=7,287 Hz) to a high of 12,235 Hz (SD $\pm$1,010.43, Q1=11,381 Hz, Q3=12,841 Hz). Peak frequency for these non–duet female notes had a mean of 10,709 Hz (SD $\pm$1,210.32, Q1=10,336 Hz, Q3=11,197 Hz), a center frequency of 10,692 Hz (SD $\pm$853.43, Q1=10,164 Hz, Q3=11,025 Hz). 90% of the energy was in the 3239 Hz (SD $\pm$1,055.93, Q1=2,412 Hz, Q3=3,618 Hz) between the 5% frequency at 8115 Hz (SD $\pm$1,041.49, Q1=7,580 Hz, Q3=8,786 Hz) and the 95% frequency at 11,353 Hz (SD $\pm$853.62, Q1=10,680 Hz, Q3=11,886 HZ). Mean interquartile range was 1,135 Hz (SD $\pm$972.93, Q1=516.8 Hz, Q3=1,205 Hz), and stretched from the 1st quartile at 9,905 Hz (SD $\pm$1,190.19, Q1=9,475 Hz, Q3=10,508 Hz) to the 3rd quartile at 11,040 Hz (SD $\pm$758.77, Q1=10,508 Hz, Q3=11,370 Hz).

Male notes of all three types were present on Manado Tua, with 50% represented by type 3 notes, 35% represented by type 1, and the remaining 15% by type 2. Mean frequency range for males was larger than for females at $\bar{x} =$ 6,381 Hz (SD $\pm$1,050.27, Q1=5,610 Hz, Q3=6,872 Hz), and ranged from a low of 4,980 Hz (SD $\pm$799.01, Q1=4,574 Hz, Q3=5,480 Hz) to a high of 11,361 Hz (SD $\pm$554.18, Q1=11,090 Hz, Q3=11,705 Hz). Notes lasted only $\bar{x} =$ 0.29 seconds (SD $\pm$0.08, Q1=0.23 s, Q3=0.35 s), and hit high peak frequency at $\bar{x} =$ 7,826 Hz (SD $\pm$1,366.88, Q1=6,891 Hz, Q3=8,969 Hz). Center frequency was located at $\bar{x} =$7,932 Hz (SD $\pm$785.37, Q1=7,407 Hz, Q3=8,484 Hz), while Q1 was at $\bar{x} =$ 6,986 Hz (SD $\pm$727.80, Q1=6,503 Hz, Q3=7,580 Hz), Q3 was at 8,997 Hz (SD $\pm$764.47, Q1=8,484 Hz, Q3=9,518 Hz), and the IQR

Table 15. Mean measurements from Manado Tua, ± standard deviation, followed by 1ˢᵗ and 3ʳᵈ quartile statistics for each variable.

MANADO TUA	Duet Phrase	Duetting Females	Randomized females	Randomized males
Numer of Sites	5	5	6	5
Number of Duet Phrases per Song	4–10			
Mean $\pm$SD	6.71$\pm$2.75			
Sex Ratio $\pm$SD	1.05$\pm$0.20			
Q1,Q3	0.92, 1.17			
Number of Samples	7	113	45	80
Low Frequency (Hz) $\pm$SD	6822$\pm$314.33	7238$\pm$374.51	6248$\pm$1434.96	4980$\pm$799.01
Q1, Q3	6550, 7035	7093, 7414	5399, 7287	4574, 5480
High Frequency (Hz) $\pm$SD	11457$\pm$1030.00	9202$\pm$634.18	12235$\pm$1010.43	11361$\pm$554.18
Q1, Q3	10878, 12359	8774, 9577	11381, 12841	11090, 11705
Fundamental Frequency Range (Hz) $\pm$SD	4634$\pm$1042.18	1964$\pm$415.19	5987$\pm$1422.19	6381$\pm$1050.27
Q1, Q3	3866, 5535	1774, 2104	4842, 6745	5610, 6872
Duration (s) $\pm$SD	9.96$\pm$1.35	0.52$\pm$0.05	0.45$\pm$0.08	0.29$\pm$0.08
Q1, Q3	9.34, 10.88	0.49, 0.56	0.39, 0.50	0.23, 0.35
Rate of Change (Hz/s) $\pm$SD	473.4$\pm$123.12	7437$\pm$9924.5	13396$\pm$3342.12	24339$\pm$9599.33
Q1, Q3	399.00, 556.00	3544, 4331	11430, 15745	17216, 27748
Peak Frequency (Hz) $\pm$SD			10709$\pm$1210.32	7826$\pm$1366.88
Q1, Q3			10336, 11197	6891, 8969
Interquartile bandwidth (Hz) $\pm$SD			1135$\pm$972.93	2011.2$\pm$901.63
Q1, Q3			516.8, 1205.9	1162.8, 2680.9
1st Quartile (Hz) $\pm$SD			9905$\pm$1190.19	6986$\pm$727.80
Q1, Q3			9475, 10508	6503, 7580
3rd Quartile (Hz) $\pm$SD			11040$\pm$758.77	8997$\pm$764.47
Q1, Q3			10508, 11370	8484, 9518
Center Frequency (Hz) $\pm$SD			10692$\pm$853.43	7932$\pm$785.37
Q1, Q3			10164, 11025	7407, 8484
Bandwidth 90% (Hz) $\pm$SD			3239$\pm$1055.93	4534$\pm$797.35
Q1, Q3			2412, 3618	4048, 5179
5% Frequency (Hz) $\pm$SD			8115$\pm$1041.49	5942$\pm$528.63
Q1, Q3			7580, 8786	5556, 6298
95% Frequency (Hz) $\pm$SD			11353$\pm$853.62	10475$\pm$530.04
Q1, Q3			10680, 11886	10250, 10820
Gap Frequency (Hz) $\pm$SD				−1577$\pm$1231.36
Q1, Q3				−2442.0, 0
Gap Duration (s) $\pm$SD				0.06$\pm$0.04

Q1, Q3	0, 0.08
Gap Ratio (Hz/s) $\pm$SD	$-$ 20039$\pm$16757.03
Q1, Q3	$-$31945,0

bandwidth stretched 2,011.2 Hz (SD $\pm$901.63, Q1=1,162.8 Hz, Q3=2,680.9Hz). 90% of the energy was in the 4,534 Hz (SD $\pm$797.35, Q1=4,048 Hz, Q3=5,179 Hz) between 5,942 Hz (SD $\pm$528.63, Q1=5,556 Hz, Q3=6,298 Hz) and 10,475 Hz (SD $\pm$530.04, Q1=10,250 Hz, Q3=10,820 Hz).

After running Tukey's HSD on the PCA high contribution variables, Manado Tua's duet phrases were found to differ significantly only from those of Lembeh, on the basis of frequency range ($p = 0$), and from Tangkoko on the basis of duration ($p = 0.002$). Female notes within the duet phrases also differed from Lembeh on the basis of frequency range ($p = 0.003$) while note duration differed significantly from Siau's longer notes ($p = 0$). Female non–duet–phrase notes differed significantly from Tangkoko, Sangihe, and Siau in all high–contribution categories (note duration versus Tangkoko, $p = 0.016$, all others $p = 0$). Talisei differed from Manado Tua in 3rd quartile frequency, frequency range, and center frequency ($p = 0$). Lembeh was statistically different in note duration ($p = 0.003$), frequency range, and center frequency ($p = 0$). Note duration was the only statistical differentiation between females of Manado Tua and Mantehage ($p = 0.033$).

Males showed more differentiation, with a unique high frequency that was statistically higher than Sangihe or Siau, and lower than remaining locations ($p = 0$). Bunaken showed differences in center and peak frequency, while Klabat exhibited differences in 90% bandwidth ($p = 0$) (see Table 16).

Table 16. Results of Tukey's HSD test of high–contribution variables. P–values indicated in bold represent significant differences between Manado Tua and other locations.

MANDO TUA	Bangka	Bunaken	Klabat	Lembeh	Mantehage	Talisei	Tangkoko	Sangihe	Siau
Female Duet Notes									
Frequency range	1.000	1.000	0.260	**0.003**	1.000	0.995	0.987		0.855
Note duration	0.996	1.000	0.994	1.000	1.000	0.998	1.000		**0.000**
Full Duet									
Frequency range	0.991	0.999	0.064	**0.000**	0.971	0.999	0.778		0.931
Duet duration	0.999	0.591	0.976	0.163	0.999	0.063	**0.002**		0.084
Male Notes									
Center frequency	1.000	**0.000**	0.485	**0.024**	**0.013**	0.540	**0.000**	**0.007**	0.153
Maximum frequency	1.000	**0.000**	1.000	**0.033**	**0.040**	0.657	0.073	**0.004**	**0.001**
Bandwidth 90%	0.295	0.921	**0.000**	**0.000**	0.955	0.542	**0.025**	**0.000**	**0.000**
High frequency	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Female Notes									
3rd quartile Frequency range	1.000	1.000	0.938	1.000	0.950	**0.000**	**0.000**	**0.000**	**0.000**
Center frequency	0.202	1.000	0.895	**0.000**	1.000	**0.000**	**0.000**	**0.000**	**0.000**
Note duration	0.745	1.000	1.000	**0.003**	**0.033**	0.995	**0.016**	**0.000**	**0.000**

Manado Tua differed from Lembeh in all the tested variables, as well as from Sangihe in center frequency (p = 0.007), maximum frequency (p = 0.004), and BW 90% (p = 0). Mantehage exhibited differences from Manado Tua in center frequency (p = 0.013) and maximum frequency (p = 0.040). Tangkoko males differed from Manado Tua males in center frequency as well (p = 0) as well as in BW90% (p = 0.025). Siau differed in maximum frequency (p = 0.001) and BW90% (p = 0).

Mantehage

Only four successful recordings were acquired over the course of two weeks on Mantehage. All four recordings were taken in overcast weather at three different sites, beginning between 4:58 am and 5:30 am. All sites were in secondary forest and overgrown gardens. Extensive sampling in the mangroves did not result in any successful recordings, as we were never close enough to the animals to be able to successfully filter their calls from the ambient noise, and did not even hear them at all on many mornings. In addition, tarsiers on Mantehage were more elusive than in other locations, and seemed to actively avoid people, making recording more difficult. Returning to sites where we had previously recorded was more difficult here as well, as tarsiers generally would not return to the same sleeping site if we had been there the previous day.

Each morning, pairs emitted 2 to 8 duet phrases lasting an average of 12.1 seconds (SD $\pm$1.92, Q1=11.66 s, Q3=12.83 s), with an average female to male ratio of 1.68$\pm$3. The frequency range of the phrase was $\bar{x} = 5,420$ Hz (SD $\pm$823.16, Q1=5,362 Hz, Q3=5,849 Hz), ranging from a low of 6,578 Hz (SD $\pm$209.24, Q1=6,473 Hz, Q3=6,717 Hz) to a high of 11,998 Hz (SD $\pm$1,004.54, Q1=11,954 Hz, Q3=12,488 Hz). Duet phrases had an average of 20 female notes (*n*=80), each accounting for a mean 0.47

seconds (SD $\pm$0.02, Q1=0.45 s, Q3=0.48 s) and reaching across a mean frequency band

of 1,722 Hz (SD $\pm$357.69, Q1=1,606 Hz, Q3=1,847 Hz). Mean low frequency for female

notes within a duet phrase was $\bar{x} = $ 7,267 Hz (SD $\pm$293.99, Q1=7,035 Hz, Q3=7,509 Hz)

and mean high frequency was 8,989 Hz (SD $\pm$591.01, Q1=8,622 Hz, Q3=9,334 Hz) (see

Table 17).

Table 17. Mean measurements from Mantehage, ± standard deviation, followed by 1st and 3rd quartile statistics for each variable.

MANTEHAGE	Duet Phrase	Duetting Females	Randomized females	Randomized males
Numer of Sites	3	3	3	3
Number of Duet Phrases per Song	2–8			
Mean $\pm$SD	5.5$\pm$3			
Sex Ratio $\pm$SD	1.68$\pm$1.42			
Q1,Q3	0.99, 1.78			
Number of Samples	4	80	20	50
Low Frequency (Hz) $\pm$SD	6578$\pm$209.24	7267$\pm$293.99	6587$\pm$690.33	5508$\pm$863.98
Q1, Q3	6473, 6717	7035, 7509	6106, 6911	4770, 6030
High Frequency (Hz) $\pm$SD	11998$\pm$1004.54	8989$\pm$591.01	12086$\pm$909.66	12128$\pm$652.67
Q1, Q3	11954, 12488	8622, 9334	11741, 12675	11843, 12557
Fundamental Frequency Range (Hz) $\pm$SD	5420$\pm$823.16	1722$\pm$357.69	5499$\pm$907.95	6620$\pm$931.48
Q1, Q3	5362, 5849	1606, 1847	4991, 6005	6052, 7249
Duration (s) $\pm$SD	12.1$\pm$1.92	0.47$\pm$0.02	0.38$\pm$0.10	0.28$\pm$0.08
Q1, Q3	11.66, 12.83	0.45, 0.48	0.29, 0.46	0.23, 0.33
Rate of Change (Hz/s) $\pm$SD	449.2$\pm$31.53	3684$\pm$658.25	15407$\pm$4662.46	24791$\pm$5881.55
Q1, Q3	433.3, 471.7	3409, 4015	11820, 16692	20878, 29198
Peak Frequency (Hz) $\pm$SD			10637$\pm$565.68	8597$\pm$1308.03
Q1, Q3			10164, 11100	7634, 9475
Interquartile bandwidth (Hz) $\pm$SD			633.1$\pm$631.11	1517.7$\pm$1002.64
Q1, Q3			333.8, 689.1	829, 1927.2
1st Quartile (Hz) $\pm$SD			10097$\pm$819.44	7805$\pm$849.26
Q1, Q3			9604, 10551	7278, 8441
3rd Quartile (Hz) $\pm$SD			10730$\pm$567.62	9323$\pm$907.85
Q1, Q3			10336, 11197	8613, 9991

Center Frequency (Hz) +SD	10543+576.20	8446+915.17
Q1, Q3	10164, 11025	7924, 8990
Bandwidth 90% (Hz) +SD	2605.5+1207.03	4325+1021.59
Q1, Q3	1851.8, 2799.3	3618, 5103
5% Frequency (Hz) +SD	8646+1227.27	6756+683.14
Q1, Q3	7924, 9345	6212, 7375
95% Frequency (Hz) +SD	11251+716.97	11081+802.23
Q1, Q3	10680, 11800	10648, 11639
Gap Frequency (Hz) +SD		−693+996.26
Q1, Q3		−1230, 0
Gap Duration (s) +SD		0.04+0.05
Q1, Q3		0, 0.09
Gap Ratio (Hz/s) +SD		−7437+10758.73
Q1, Q3		−13460, 0

Non–duet–phrase female notes (n=20) exhibited the usual broader frequency range, $\bar{x} = 5{,}499$ Hz (SD $\pm$907.95, Q1=4,991 Hz, Q3=6,005 Hz), from a low of 6,587 Hz (SD $\pm$690.33, Q1=6,106 Hz, Q3=6,911 Hz) to a high of 12,086 Hz (SD $\pm$909.66, Q1=11,741 Hz, Q3=12,675 Hz). These notes were also shorter than the average duet–phrase note, lasting an average of only 0.38 seconds (SD $\pm$0.10, Q1=0.29 s, Q3=0.46 s). Frequency peaked at 10,637 Hz (SD $\pm$565.68, Q1=10,164 Hz, Q3=11,100 Hz) and centered at 10,543 Hz (SD $\pm$576.20, Q1=10,164 Hz, Q3=11,025 Hz), with half of all the note's energy concentrated in the IQR bandwidth, the 633.1 Hz (SD $\pm$631.11, Q1=333.8 Hz, Q3=689.1 Hz) between the 1st quartile of 10,097 Hz (SD $\pm$819.44, Q1=9,604 Hz, Q3=10,551 Hz) and the 3rd quartile of 10,730 Hz (SD $\pm$567.62, Q1=10,336 Hz, Q3=11,197 Hz). 90% of all the energy was concentrated in the 2,605 Hz (SD $\pm$1,207.03, Q1=1,851.8 Hz, Q3=2,799.3 Hz) between the 5% frequency of $\bar{x} = 8{,}646$ Hz (SD $\pm$1,227.27, Q1=7,924 Hz, Q3=9,345 Hz) and the 95% frequency of $\bar{x} = 11{,}251$ Hz (SD $\pm$716.97, Q1=10,680 Hz, Q3=11,800 Hz).

Male notes (n=40) were slightly shorter, $\bar{x} = 0.28$ seconds (SD ± 0.08, Q1=0.23 s,

Q3=0.33 s), but had a broader mean frequency range of $\bar{x} = 6,620$ Hz (SD ± 931.48,

Q1=6,052 Hz, Q3=7,249 Hz). Mean low frequency was $\bar{x} = 5,508$ Hz (SD ± 863.98,

Q1=4,770 Hz, Q3=6,030 Hz), while mean high frequency was $\bar{x} = 12,128$ Hz (SD

± 652.67, Q1=11,843 Hz, Q3=12,557 Hz). Both maximum and center frequencies were

lower than in female notes, at $\bar{x} = 8,597$ Hz (SD $\pm 1,308.03$, Q1=7,634 Hz, Q3=9,475 Hz)

and $\bar{x} = 8,446$ Hz (SD ± 915.17, Q1=7,924 Hz, Q3=8,990 Hz) respectively. 90% of the

note's energy sat in a 4,325 Hz (SD $\pm 1,021.59$, Q1=3,618 Hz, Q3=5,103 Hz) band

between the 5% frequency of $\bar{x} = 6,756$ Hz (SD ± 683.14, Q1=6,212 Hz, Q3=7,375 Hz)

and the 95% frequency of $\bar{x} = 11,081$ Hz (SD ± 802.23, Q1=10,648 Hz, Q3=11,639 Hz),

while half of all the energy sat in the 1,517.7 Hz (SD $\pm 1,002.64$, Q1=829 Hz, Q3=1,927.2

Hz) band between the 1st and 3rd quartiles of $\bar{x} = 7,805$ Hz (SD ± 849.26, Q1=7,278 Hz,

Q3=8,441 Hz) and $\bar{x} = 9,323$ Hz (SD ± 907.85, Q1=8,613 Hz, Q3=9,991 Hz). 32% of the

male notes were classified as type 1; the remaining 68% of the notes were split evenly

between types 2 and 3.

We ran Tukey's HSD on the PCA high contribution variables, and only Lembeh

showed any significant differences from Mantehage when the duet phrase was examined.

Frequency range was the distinguishing factor, both the duet phrase as a whole ($p = 0.006$), as well as for the female notes within the duet phrase ($p = 0.004$) (see Table 18).

Table 18. Results of Tukey's HSD test of high–contribution variables. P–values indicated in bold represent significant differences between Mantehage and other locations.

MANTEHAGE	Bangka	Bunaken	Klabat	Lembeh	Manado Tua	Talisei	Tangkoko	Sangihe	Siau
Female Duet Notes									
Frequency range	0.998	0.997	0.241	**0.006**	1.000	0.965	0.939		0.749
Note duration	1.000	1.000	0.976	1.000	1.000	0.990	1.000		**0.000**
Full Duet									
Frequency range	1.000	0.890	0.823	**0.004**	0.971	1.000	1.000		1.000
Duet duration	1.000	0.398	1.000	0.727	0.999	0.463	0.060		0.393
Male Notes									
Center frequency	**0.008**	**0.000**	0.802	**0.000**	**0.013**	**0.000**	1.000	0.985	1.000
Maximum frequency	**0.019**	**0.014**	0.128	**0.000**	**0.040**	**0.000**	0.999	0.901	0.932
Bandwidth 90%	**0.022**	1.000	**0.000**	**0.000**	0.955	1.000	**0.001**	**0.000**	**0.001**
High frequency	0.241	1.000	**0.004**	**0.000**	**0.000**	0.550	**0.001**	**0.000**	**0.000**
Female Notes									
3rd quartile	0.997	1.000	0.348	0.994	0.950	**0.023**	**0.022**	**0.000**	**0.000**
Frequency range	**0.000**	0.366	**0.000**	**0.000**	0.939	**0.001**	**0.000**	**0.000**	**0.000**
Center frequency	0.889	1.000	1.000	0.093	1.000	**0.000**	**0.000**	**0.000**	**0.000**
Note duration	0.763	0.212	**0.022**	1.000	**0.033**	**0.005**	1.000	**0.000**	**0.000**

Non–duet female notes were different from Sangihe and Siau in all the high–contribution variables tested (all at p = 0), as was Talisei (p = 0.023 for Q3, p = 0.001 for frequency range, p = 0 for center frequency, and p = 0.005 for note duration). Tangkoko was likewise highly distinct, with differences in Q3 (p = 0.022), frequency range, and center frequency (p = 0). Frequency ranges for Bangka, Klabat, and Lembeh were all significantly different than Mantehage's (p = 0), while Klabat and Manado Tua differed in note duration (p = 0.022 and 0.033, respectively).

For male notes, Lembeh showed the greatest number of significant differences, with p–values of 0 for each high–contribution variable tested. Center frequencies on Mantehage were different than those on Bangka, Bunaken, Manado Tua, and Talisei (p–values=0.008, 0, 0.013, 0, respectively), while maximum frequencies tested significantly different from those on Bangka, Bunaken, Manado Tua, and Talisei (p–values=0.019, 0.014, 0.040, and 0, respectively). BW 90% contributed to significant differences for Bangka, Klabat, Tangkoko, Sangihe, and Siau (p–values=0.022, 0, 0.001, 0, 0.001) and finally, high frequency was significantly different for Klabat, Manado Tua, Tangkoko, Sangihe, and Siau (p = 0.004, 0, 0.001, 0, 0, respectively).

Siau

Six duet calls were recorded on Siau, only three were analyzeable in RavenPro. Calls began between 5:09 and 5:28 in the morning, and each usable recording was at a unique site, with sleeping trees located in a bamboo grove, a liana tangle, and a small strangler fig. Each morning we counted 1 to 3 duet phrases, with an average of 8.33 female notes and 32.33 male notes. Duet phrases lasted $\bar{x}$ =11.69 seconds (SD $\pm$9.14,

Q1=7.59 s, Q3=16.62 s) and spanned a fundamental frequency range of $\bar{x}$ = 5,644 Hz

(SD ±1,544, Q1=4,872 Hz, Q3=6,417 Hz) from a low of $\bar{x}$ = 5,494 Hz (SD ±1,363.57,

Q1= 5,081 Hz Q3=6,281 Hz) to a high of $\bar{x}$ = 11,137 Hz (SD ±738.4, Q1=10,760 Hz,

Q3=11,498 Hz) (see Tabl 19). Siau tarsier's duet phrases did not show a clear pattern as

did the other locations; instead, phrases had a high degree of entropy, with even the

difference between male and female notes much more indistinct than elsewhere.

Within the duet phrases, female notes did not follow a temporal or structural

pattern. We averaged them all, however, and found a mean duration of $\bar{x}$ = 1.52 seconds

(SD ±0.99, Q1=0.97, Q3=0.99), spanning a mean fundamental frequency range of $\bar{x}$ =

 2,904 Hz (SD ±2,144.13, Q1=1,699 Hz, Q3=3,750 Hz). Low frequencies averaged 6,084

Hz (SD ±1,085.61, Q1=5,689 Hz, Q3=6,703 Hz), while high frequencies averaged 8,989

Hz (SD ±1,063.85, Q1=8,402 Hz, Q3=9,439 Hz).

Non–duet phrase female notes were much more conserved, showing significantly

less variation in tone or timing. Female notes lasted $\bar{x}$ = 1.24 seconds (SD ±0.09,

Q1=1.17 s, Q3=1.31 s), with a low frequency of $\bar{x}$ = 3,804 Hz (SD ±776.36, Q1=3,205

Hz, Q3=4,509 Hz) and a high frequency of $\bar{x}$ = 11,519 Hz (SD ±361, Q1=11,245 Hz,

Q3=11,810 Hz). The average fundamental frequency range of non–duet–phrase female

notes was $\bar{x}$ = 7,715 Hz (SD ±642.88, Q1=7,446 Hz, Q3=8,048 Hz). Frequencies peaked

at $\bar{x}$ = 6,337 Hz (SD ±1,404.68, Q1=5,620 Hz, Q3=6,546 Hz), and centered at $\bar{x}$ = 6,104

Hz (SD ±704.74, Q1=5,599 Hz, Q3=6,589 Hz). 90% of the energy had a mean bandwidth

of $\bar{x}$ = 3,629.1 Hz (SD ±1,645.51, Q1=2,411 Hz, Q3=4,888.1 Hz), while 50% of the

energy was within a 953.2 Hz (SD ±1,149.94, Q1=172.3 Hz, Q3=1,162.8 Hz) band. 5%

frequency was $\bar{x}$ = 5,820 Hz (SD ±519.33, Q1=5,469 Hz, Q3=6,051 Hz); 95% frequency

was $\bar{x}$ = 9,449 Hz (SD $\pm$1,966.94, Q1=7,881 Hz, Q3=10,939 Hz); 1st quartile frequency

was $\bar{x}$ = 5,955 Hz (SD $\pm$552.59, Q1=5,577 Hz, Q3=6,288 Hz); and 3rd quartile frequency

was $\bar{x}$ = 7,730 Hz (SD $\pm$1,637.71, Q1=5,706 Hz, Q3=7,730 Hz).

Table 19. Mean measurements from Siau, ± standard deviation, followed by 1st and 3rd quartile statistics for each variable.

SIAU	Duet Phrase	Duetting Females	Randomized females	Randomized-males
Numer of Sites	3	3	3	3
Number of Duet Phrases per Song	1–3			
Mean $\pm$SD	2$\pm$1			
Sex Ratio $\pm$SD	0.29$\pm$0.12			
Q1,Q3	0.24, 0.35			
Number of Samples	3	25	15	30
Low Frequency (Hz) $\pm$SD	5494$\pm$1363.57	6084$\pm$1085.61	3804$\pm$776.36	3334$\pm$911.43
Q1, Q3	5081, 6281	5689, 6703	3205, 4509	2546, 3948
High Frequency (Hz) $\pm$SD	11137$\pm$738.40	8989$\pm$1063.85	11519$\pm$361.00	9886$\pm$725.46
Q1, Q3	10760, 11498	8402, 9439	11245, 11810	9580, 10367
Fundamental Frequency Range (Hz) $\pm$SD	5644$\pm$1544	2904$\pm$2144.13	7715$\pm$642.88	6552$\pm$1054.18
Q1, Q3	4872, 6417	1699, 3750	7446, 8048	6127, 6990
Duration (s) $\pm$SD	11.687$\pm$9.14	1.52$\pm$0.99	1.24$\pm$0.09	0.27$\pm$0.05
Q1, Q3	7.59, 16.62	0.97, 1.91	1.17, 1.31	0.24, 0.31
Rate of Change (Hz/s) $\pm$SD	1508.1$\pm$2100.29	1828$\pm$203.56	6250$\pm$638.53	25209$\pm$6372.40
Q1, Q3	295.5, 2120.2	1734, 1936	5767, 6668	20763, 28964
Peak Frequency (Hz) $\pm$SD			6337$\pm$1404.68	9017$\pm$1028.54
Q1, Q3			5620, 6546	8570, 9711
Interquartile bandwidth (Hz) $\pm$SD			953.2$\pm$1149.94	1554.7$\pm$590.33
Q1, Q3			172.3, 1162.8	1130.5, 2067.2
1st Quartile (Hz) $\pm$SD			5955$\pm$552.59	7489$\pm$518.2
Q1, Q3			5577, 6288	7020, 7752
3rd Quartile (Hz) $\pm$SD			7730$\pm$1637.71	9044$\pm$582.97
Q1, Q3			5706, 7730	8839, 9302
Center Frequency (Hz) $\pm$SD			6104$\pm$704.74	8402$\pm$512.12

Q1, Q3	5599, 6589	8312, 8721
Bandwidth 90% (Hz) $\pm$SD	3629.1$\pm$1645.51	3450$\pm$865.94
Q1, Q3	2411.7, 4888.1	2950, 4091
5% Frequency (Hz) $\pm$SD	5820$\pm$519.33	6121$\pm$633.63
Q1, Q3	5469, 6051	5857, 6406
95% Frequency (Hz) $\pm$SD	9449$\pm$1966.94	9571$\pm$707.73
Q1, Q3	7881, 10939	9356, 10024
Gap Frequency (Hz) $\pm$SD		−2403$\pm$1335.23
Q1, Q3		−3351, −1171
Gap Duration (s) $\pm$SD		0.04$\pm$0.01
Q1, Q3		0.03,0.04
Gap Ratio (Hz/s) $\pm$SD		65804$\pm$44915.76
Q1, Q3		−79698, −30019

Male notes on Siau averaged 0.27 seconds (SD $\pm$0.05, Q1=0.24 s, Q3=0.31 s), and while they had a fairly mean standard frequency range of 6,552 Hz (SD $\pm$1,054.18, Q1=6,127 Hz, Q3=6,990 Hz), they were significantly lower than any of the other locations, with low frequencies of $\bar{x} = 3,334$ Hz (SD $\pm$911.43, Q1=2,546 Hz, Q3=3,948 Hz) and high frequencies of $\bar{x} = 9,886$ Hz (SD $\pm$725.46, Q1=9,580 Hz, Q3=10,367 Hz). 5% frequencies had a mean of $\bar{x} = 6,121$ Hz (SD $\pm$633.63, Q1=5,857 Hz, Q3=6,406 Hz); 95% frequencies had a mean of $\bar{x} = 9,571$ Hz (SD $\pm$707.73, Q1=9,356 Hz, Q3=10,024 Hz), leaving 90% of their energy in a 3,450 Hz (SD $\pm$865.94, Q1=2,950 Hz, Q3=4,091 Hz) bandwidth. The IQR bandwidth was $\bar{x} = 1,554.7$ Hz (SD $\pm$590.33, Q1=1,130.5 Hz, Q3=2,067.2 Hz), between the 1st quartile mean of 7,489 Hz (SD $\pm$518.2, Q1=7,020 Hz, Q3=7,752 Hz) and the 3rd quartile mean of 9,044 Hz (SD $\pm$582.97, Q1=8,839 Hz, Q3=9,302 Hz). Male notes on Siau were, on average, more complex than at other locations, with significantly more modulations.

Table 20. Results of Tukey's HSD test of high–contribution variables. P–values indicated in bold represent significant differences between Siau and other locations.

SIAU	Bangka	Bunaken	Klabat	Lembeh	Manado Tua	Mantehage	Talisei	Tangkoko	Sangihe
Female Duet Notes									
Frequency range	0.984	0.999	1.000	0.650	0.855	0.749	0.997	0.997	0.997
Note duration	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	
Full Duet									
Frequency range	1.000	0.827	0.968	**0.027**	0.931	1.000	0.997	1.000	
Duet duration	0.395	**0.007**	0.364	0.990	0.084	0.393	1.000	1.000	
Male Notes									
Center frequency	0.108	**0.000**	0.977	**0.000**	0.153	1.000	**0.001**	1.000	0.972
Maximum frequency	**0.000**	0.486	**0.005**	**0.000**	**0.001**	0.932	**0.000**	0.488	1.000
Bandwidth 90%	**0.000**	0.091	**0.000**	**0.000**	**0.000**	**0.001**	**0.006**	**0.000**	0.902
High frequency	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.977
Female Notes									
3rd quartile Frequency range	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.462
Center frequency range	1.000	0.709	0.087	**0.000**	**0.001**	**0.000**	0.926	0.847	**0.000**
Center frequency	0.889	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.004**
Note duration	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

For non–duet–phase notes, both males and females showed numerous significant differences. Male note center frequency was significantly different from Bunaken and Lambeh (p = 0) as well as from Talisei (p = 0.001). Maximum frequency was significantly different from Bangka, Lembeh, Talisei (p = 0), Klabat (p = 0.005), and Manado Tua (p = 0.001). BW90% differed from Bangka, Klabat, Lembeh, Manado Tua, and Tangkoko (p = 0), as well as from Mantehage (p = 0.001) and Talisei (p = 0.006). Male high frequency was significantly different than all of the other locations except Sangihe, (p = 0) (see Table 20).

For females, 3rd quartile measurements were different from all locations bu Sangihe (p = 0). Frequency range was different from Manado Tua (p = 0.001), Lembeh, Mantehage, and Sangihe (p = 0). Center frequency was significantly different from Bunaken, Klabat, Lembeh, Manado Tua, Mantehage, Talisei, Tangkoko (p = 0), and Sangihe (p = 0.004). Finally, female note duration was significantly shorter than on Sangihe, but significantly longer than any at of the other locations (p = 0).

Sangihe

On Sangihe, two of five recording sessions were successful. Songs began at 5:21 am and at 5:27 am, each from a different bamboo copse. Neither of the recordings exhibited anything that we could identify as a duet phrase. Female notes stayed consistent in structure, frequency, and spacing throughout the song, thus no section was labeled as a duet phrase. Females had a mean low frequency of $\bar{x} = 2,871$ Hz (SD $\pm$324.51, Q1=2,688 Hz, Q3=3,126 Hz), and a high frequency of $\bar{x} = 13,091$ Hz (SD $\pm$677.83, Q1=12,623 Hz,

Q3=13,557 Hz), giving them a mean range of 10,220 Hz (SD $\pm$542.5, Q1=9,620 Hz,

Q3=10,699 Hz). Female notes on Siau were the longest of any location, with an average

length of 2.21 seconds (SD $\pm$0.12, Q1=2.12 s, Q3=2.31 s). Peak frequency was exhibited

at $\bar{x} = 6,878$ Hz (SD $\pm$939.39, Q1=6,018 Hz, Q3=7,698 Hz), and center frequency $\bar{x} = $

7,494 Hz (SD $\pm$423.91, Q1=7,235 Hz, Q3=7,838 Hz). The 90% bandwidth was 2,588 Hz

(SD $\pm$660.80, Q1=2,240 Hz, Q3=3,015 Hz), extending from the 5% frequency of $\bar{x} = $

5,982 Hz (SD $\pm$424.62, Q1=5,728 Hz, Q3=6,051 Hz) to the 95% frequency, $\bar{x} = 8,570$

Hz (SD $\pm$678.81, Q1=8,096 Hz, Q3=9,076 Hz). 1st quartile frequency was $\bar{x} = 7,390$ Hz,

(SD $\pm$376.64, Q1=7,106 Hz, Q3=7,698 Hz), and 3rd quartile $\bar{x} = 7,696$ Hz (SD $\pm$453.08,

Q1=7,429 Hz, Q3=8,097 Hz); and the interquartile bandwidth between the two was 305.8

Hz (SD $\pm$124.23, Q1=172.3 Hz, Q3=419.9 Hz) (see Table 21).

Table 21. Mean measurements from Sangihe, ± standard deviation, followed by 1st and 3rd quartile statistics for each variable

SANGIHE	Duet Phrase	Duetting Females	Randomized females	Randomized males
Numer of Sites	0	0	2	2
Number of Duet Phrases per Song	NA			
Mean $\pm$SD	NA			
Sex Ratio $\pm$SD	NA			
Q1,Q3	NA			
Number of Samples	0		10	20
Low Frequency (Hz) $\pm$SD			2871$\pm$324.51	4631$\pm$1251.48
Q1, Q3			2688, 3126	4056, 5369
High Frequency (Hz) $\pm$SD			13091$\pm$677.83	9677$\pm$608.71
Q1, Q3			12623, 7698	9318, 10122
Fundamental Frequency Range (Hz)$\pm$SD			10220$\pm$542.50	5046$\pm$1322.52
Q1, Q3			9620, 10699	4152, 5735
Duration (s) $\pm$SD			2.21$\pm$0.12	0.13$\pm$0.03
Q1, Q3			2.12, 2.31	0.10, 0.15
Rate of Change (Hz/s) $\pm$SD			4622$\pm$258.37	42102$\pm$15019.69

Q1, Q3	4530, 4809	34239, 50858
Peak Frequency (Hz) $\pm$SD	6878$\pm$939.39	9111$\pm$886.17
Q1, Q3	6018, 7698	8635, 9755
Interquartile bandwidth (Hz) $\pm$SD	305.8$\pm$124.23	1358.8$\pm$604.95
Q1, Q3	172.3, 419.9	1044.4, 1830.3
1st Quartile (Hz) $\pm$SD	7390$\pm$376.64	7870$\pm$612.54
Q1, Q3	7106, 7698	7440, 8506
3rd Quartile (Hz) $\pm$SD	7696$\pm$453.08	9229$\pm$614.97
Q1, Q3	7429, 8097	8764, 9647
Center Frequency (Hz) $\pm$SD	7494$\pm$423.91	8678$\pm$662.99
Q1, Q3	7235, 7838	8247, 8678
Bandwidth 90% (Hz) $\pm$SD	2588$\pm$660.80	3068$\pm$705.28
Q1, Q3	2240, 3015	2401, 3618
5% Frequency (Hz) $\pm$SD	5982$\pm$424.62	6376$\pm$537.62
Q1, Q3	5728, 6051	5857, 6718
95% Frequency (Hz) $\pm$SD	8570$\pm$678.81	9444$\pm$652.86
Q1, Q3	8096, 9076	9022, 9959
Gap Frequency (Hz) $\pm$SD		−1204.9$\pm$1492.87
Q1, Q3		−1678.5, 0
Gap Duration (s) $\pm$SD		0.02$\pm$0.02
Q1, Q3		0, 0.04
Gap Ratio (Hz/s) $\pm$SD		−36156$\pm$48176
Q1, Q3		−51469, 0

Most (70%) of the male notes on Sangihe were type 2; the remainder were all type 1. Male notes lasted an average of 0.13 seconds (SD $\pm$0.03, Q1= 0.10 s, Q3=0.15 s), and encompassed a frequency range of 5,046 Hz (SD $\pm$1,322.52, Q1=4,152 Hz, Q3=5,735 Hz), between the low frequency of $\bar{x} = 4,631$ Hz (SD $\pm$1,251.48, Q1=4,056 Hz, Q3=5,369 Hz) and the high frequency, $\bar{x} = 9,677$ Hz (SD $\pm$608.71, Q1=9,318 Hz, Q3=10,122 Hz). Frequency peaked at $\bar{x} = 9,111$ Hz (SD $\pm$886.17, Q1=8,635 Hz, Q3=9,755 Hz) and centered at $\bar{x} = 8,678$ Hz (SD $\pm$662.99, Q1=8,247 Hz, Q3=8,678 Hz).

Table 22. Results of Tukey's HSD test of high–contribution variables. P–values indicated in bold represent significant differences between Siau and other locations.

SANGIHE	Bangka	Bunaken	Klabat	Lembeh	Manado Tua	Mantehage	Talisei	Tangkoko	Siau
Male Notes									
Center frequency	**0.005**	**0.001**	0.339	**0.000**	**0.007**	0.985	**0.000**	0.992	0.972
Maximum frequency	**0.002**	0.769	**0.012**	**0.000**	**0.004**	0.901	**0.000**	0.511	1.000
Bandwidth 90%	**0.000**	**0.002**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.902
High frequency	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.977
Female Notes									
3rd quartile	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.462
Frequency range	**0.000**	**0.000**	0.163	1.000	**0.000**	**0.000**	**0.000**	**0.003**	**0.000**
Center frequency	0.889	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.004**
Note duration	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

Half of the energy in each note sat in the IQR bandwidth spanning 1,358.8 Hz (SD

$\pm$604.95, Q1=1,044.4 Hz, Q3=1,830.3 Hz) between the 1st quartile, $\bar{x} = 7,870$ Hz (SD

$\pm$612.54, Q1=7,440 Hz, Q3=8,506 Hz) and the 3rd quartile, $\bar{x} = 9,229$ Hz (SD $\pm$614.97,

Q1=8,764 Hz, Q3=9,647 Hz). 90% of the energy was in the bandwidth of 3,068 Hz (SD

$\pm$705.28, Q1=2,401 Hz, Q3=3,618 Hz) between the 5% frequency, $\bar{x} = 6376$ Hz (SD

$\pm$537.62, Q1=5,857 Hz, Q3=6,718 Hz) and the 95% frequency $\bar{x} = 9,444$ Hz (SD

$\pm$652.86, Q1=9,022 Hz, Q3=9,959 Hz).

Because we identified no duet phrases within the Sangihe duet calls, we classified

them as different from all the other locations in regard to duet phrase measurements and

female notes within a duet phrase.

For female non–duet–phrase notes, Sangihe showed very few similarities to any

of the other locations when high–contribution variables from the PCA were compared

using a Tukey HSD test. Bangka differed in 3rd quartile measurements, frequency range,

and note duration (p = 0). Klabat and Lembeh differed from Sangihe in 3rd quartile

measurements, center frequency, and note duration (p = 0); and Bunaken, Manado Tua,

Mantehage, Talisei, and Tangkoko all differed across every variable (Tangkoko

frequency range at p = 0.003, remaining all p = 0). Even Siau showed significant

differences in frequency range (p = 0), center frequency (p = 0.004), and note duration (p

= 0) (see Table 22).

Male's notes also showed more differences than similarities with the other

locations, with Siau being the notable exception with no statistical differences. Bangka

was significantly different in all characters (center frequency, p = 0.005; maximum

frequency, p = 0.002; BW90% and high frequency, p = 0. Bunaken differed in center

frequency (p = 0.001), BW90% (p = 0.002), and high frequency (p = 0). Both Lembeh and Talisei differed in all variables (p = 0). For Manado Tua, center frequency was different (p = 0.007), as was maximum frequency (p = 0.004). BW90% and high frequency were also significantly different from Mandado Tua and Tangkoko (p = 0).

Locations by category

Biaro and Tagulandang (islands on which we found no evidence of tarsiers), were categorized as "deep", along with Sangihe and Siau. The latter two are the only locations in which females' spectrograms are visually unique from remaining recording sites, and the calls themselves acoustically distinguishable. Klabat and Tangkoko were categorized as "mainland," while Lembeh, Talisei, Bangka, Bunaken, Mangehage, and Manato Tua were classified as "shallow."

We analyzed all categories using the same high contribution measurements as in the island to island comparisons, based on PCA values, including 90% bandwidth, center, maximum, and high frequency for male notes; and duration, 3rd quartile, fundamental and center frequencies for female notes. We also tested note duration and fundamental frequency for duets and female duet notes within the mainland, shallow, and deep categories. We ran Tukey's HSD test on each comparison to elucidate significant differences between the groupings.

Deepwater Islands

"Deep" islands yielded the least amount of data, with only 5 usable recordings between the two islands. Sangihe did not yield duet phrase values, as we were unable to

discern a difference between possible phrases and the song in its entirety. Thus the only

measured values for deepwater duets can be found in Siau Island, but cannot be

extrapolated to represent both of the islands.

For non–duet male and female notes, we still had a small sample size, with only

n=25 for females and n=50 for males. Deepwater island males showed high

differentiation from the rest of the populations, with males differing from shallow water

island males in almost every category (p = 0 in: note type, low frequency, high frequency,

Q1, 90% bandwidth, center frequency, change in frequency, duration, 95% frequency,

and gap frequency), except for 3rd quartile, 5% frequency, and gap time (p = 0.261, 0.588,

and 0.406, respectively). Males on the deepwater islands also varied greatly from

mainland islands, with significant differences in 1st and 3rd quartile frequencies, 5%

frequency, rate of change, and gap time (p = 0.907, 0.235, 0.311, 0.422, and 0.074).

For female non–duet notes, low frequency was significantly different in all

categories (p = 0), while high frequency differentiated deepwater islands from both

mainland (p = 0.001) and shallow water islands (p = 0.041). 1st and 3rd quartile

frequencies also were significantly different between deepwater island females and all

others (p = 0), while 90% bandwidth showed significant differences for both mainland

and shallow water islands (p = 0.001, 0.014). In female notes, center frequency, IQR, 5%

frequency, and rate of change were significantly different across every island category,

while duration, maximum frequency, and 95% frequency all differed between "deep"

islands and the other two categories (p = 0).

Shallow Water Islands

Our analysis of shallow water islands includes 21 sites distributed across Bunaken, Mantehage, Manado Tua, Lembeh, Talisei, and Bangka. Duet sample size was n=30, encompassing n=461 female duet notes, n=180 female non–duet notes, and n=360 male notes.

Shallow water island males differed significantly from mainland island males in note type ($p = 0.170$), low frequency ($p = 0.353$), duration ($p = 0.348$), IQR ($p = 0.554$), gap frequency ($p = 0.801$), and gap time ($p = 0.200$).

Shallow water island female non–duet notes did not differ significantly from mainland notes when it came to low frequency ($p = 0.071$), 1st and 3rd quartiles ($p = 0.198$ and 0.289, respectively), or 90% bandwidth ($p = 0.289$). Center frequency, IQR, 5% frequency, and rate of change were statistically significant, as mentioned above. Duration, maximum frequency, and 95% frequency, however, did not exhibit a statistical difference.

Mainland of Sulawesi

Our two mainland locations, Klabat and Tangkoko, each yielded 4 recording sites, which together resulted in a sample size of n=16 duets, n=233 female duet notes, n=90 non–duet female notes, and n=180 male notes.

Male notes recorded at mainland sites were significantly different from shallow water islands when high frequency, 1st and 3rd quartiles, maximum frequency, 5% frequency, and rate of change were assessed. For female non–duet notes, low frequency,

center frequency, change in frequency, IQR, 5% frequency, and rate of change all

showed statistical differences ($p = 0$, 0.011, 0, 0.044, 0.044, and 0.001, respectively).

DISCUSSION

We found duetting behavior at every location in which tarsiers were present (though distinct duet phrases were not observed on Sangihe). Males and females coordinate sex–specific, stereotyped, repetitive phrases in which they adjust their timing and acoustic patterns to one another. Duets found on the mainland and shallow water islands all conformed to McKinnon & McKinnon's "Manado Form," with only small adjustments differentiating the locations (McKinnon & McKinnon 1980). Duets on Sangihe had no discernable "duet phrase," in which the notes changed during divocalism, indicating either a secondary loss of the mechanism, or dispersal before its development. Siau, the only other inhabited "deep" island, had a visible duet phrase in which both males and females adjusted their acoustic patterns in a simultaneous call; however, the Siau "duet phrases" proved variable and highly modulated, with little to no visible patterns or stereotyped notes.

Duets

In primates, duetting is found in few species, and is thought to be an example of functional convergence in stable, monogamous, territorial species. In birds, duetting correlates loosely with tropical breeding, sexual monochromatism, and social monogamy—all traits shared by the mammalian Sulawesi tarsiers (Haimoff, 1986, Hall, 2004). It is thought that the main function of duetting functions jointly in resource and territory defense, since duet calls are typically loud and the callers easy to locate. In addition, duetting provides reinforcement, maintenance, and cohesion of the pair bond

formation while maintaining spatial organization among neighboring family groups

(Burton & Nietsch, 2010; Haimoff, 1986; Molles & Waas, 2006). Theoretically, based on

the Sulawesi tarsiers' social structure and territoriality, we would expect to find duetting

behavior, which we did at almost every location. The differences in note ritualization

between the deepwater locations and remaining sites may be due in part to differences in

social structure (which has not been assessed on either island), to genetic drift, or

environmental factors.

Timing

All recordings were carried out around dawn, when tarsiers return to their

sleeping trees; first calls were emitted between 4:58 am and 7:54 am. Temperature

gradients cause refraction into cooler mediums due to lower air density (Naguib et al.,

2009). At dawn, temperatures are such that refraction occurs downwards, keeping the

signal within hearing of the tarsiers' intended receivers. Reduced wind and turbulence at

daybreak are likewise advantageous to long distance signal transmission, as these

conditions contribute to signal degradation (Haimoff, 1986; Henwood & Fabrick, 1979;

Naguib et al., 2009). While the acoustic benefits of dawn communication are well

documented across taxa, with signal broadcast up to 20 times more effective than midday

(Henwood & Fabrick, 1979), it is interesting to note that the timing of the tarsiers' duet

call is important to their social behavior as well, as it marks a circadian shift between

nocturnal foraging and the selection of the daily sleeping site.

While signal efficiency is optimal in dawn conditions, there is a trade–off:

signalers from across the animal kingdom flock to take advantage of it. With each new

voice in the dawn chorus, the signal/noise ratio decreases, masking tones and lowering

efficiency. Frequency partitioning is often seen in insects, birds, and amphibians, where

multiple signals sharing communication channels create masking interference (Goodwin

& Podos, 2013; Naguib et al., 2009; Schmidt et al., 2013). We found most birdsong, and

some insects, fell below the tarsiers' typical bandwidth, though all locations featured

insects occupying a constant frequency and spanning a band 400 Hz–1,200 Hz in height,

somewhere between 3,000 Hz and 6,500 Hz, well within the tarsiers' bandwidth (see

Figure14). Sunbirds were recorded on Lembeh, Talisei, and Siau, where their broadband,

repetitive chirps reached just above 8,000 Hz. Spectrograms of sunbird notes resemble

low–pitched, type 1 male tarsier notes. It is interesting that on Lembeh recordings, males

have the highest frequencies of any of our locations; unlike on Talisei or Siau, the latter

of which conversely has the lowest average male frequency we recorded.

Figure 15. Spectrogram of dawn tarsier duetting on Klabat with an insect frequency band at around 7kHz, and a bird calling from 2–3kHz
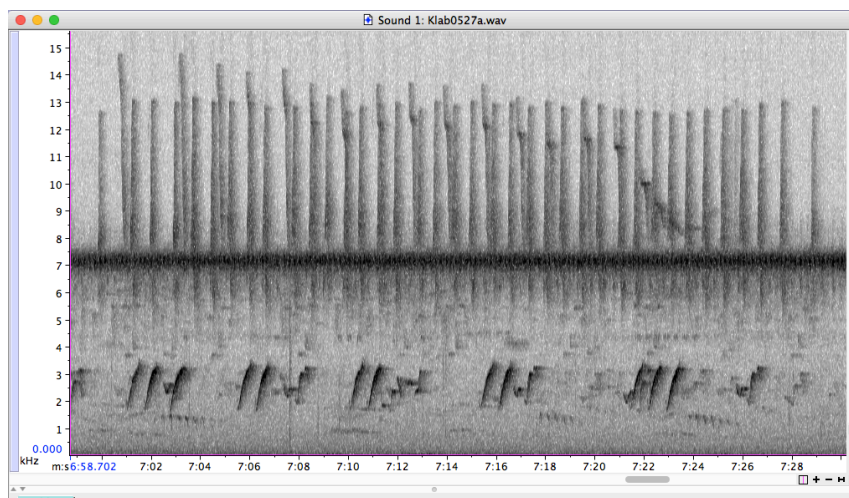
All of our recorded tarsier calls measuring between 1,553.5 Hz and 15,713 Hz,

high above the 300–2,000Hz typical of other primates' duet calls (Haimoff, 1986).

Recent studies by Gursky (2015) also have reported tarsiers in Tangkoko communicating in the ultrasonic frequencies, showing that frequency filtering from predators has been successful at least for anthropogenic threats. Like most mammals, tarsiers have adaptations of the middle and inner ear allowing them to hear frequencies well above 10 kHz, which reptiles and birds cannot, thus affording them a private communication channel off limits to their predators (Fitch, 2006; Gursky, 2005; Sachs et al., 1978).

While frequency filtering and partitioning may account in part for the tarsiers' high pitched signal, they are likely most constrained by their body size. Frequency is modulated by vocal fold vibration, and the longer the vocal fold, the slower it is able to vibrate, thus the lower the frequency of the sound produced (Fitch, 2006). The tarsiers are consequently constrained by their small larynges, which impose physical limitations on how low a frequency they can achieve. We found both male and female notes on the deep water islands displayed consistently low frequencies compared to the other locations. On Sangihe, males' highest frequencies ($\bar{x} = 9{,}677$Hz $\pm\ 608.7$) were significantly lower than all but Siau's ($\bar{x} = 9{,}886$Hz $\pm\ 725.46$). For non–duet female notes, low frequencies averaged lower for both Sangihe and Siau than for any other location ($\bar{x} = 2{,}871$Hz $\pm\ 324.5$, and $3{,}804$Hz $\pm\ 776.4$ respectively), as did center frequency, 5% and 95% frequency, and 1st and 3rd quartiles—all significantly different, save for center frequency, which was not significantly different from Bangka. Data on body mass for the different North Sulawesi populations are not available, but it is interesting to note that six animals measured by Shekelle & Salim on Sangihe in 2009 weighed between 120 and 157g, making them slightly larger on average than the 100–140g otherwise cited for *Tarsius* species (Driller et al., 2009; Shekelle & Salim, 2009).

Small body size may impose limits on signal design, as do the ecological constraints of the animal's habitat. These constraints give us a framework within which selection acts on the signal to increase effectiveness (Davies & Krebs, 2012). The acoustic adaptation hypothesis proposes that animals will adjust their song usage and structure to adapt to the sound transmission characteristics of the environment (Davies & Krebs, 2012; Endler, 1992; Ey & Fischer, 2009; Morton, 1975). Tarsiers have spent roughly the last 50 million years adapting to the tropical jungles of SE Asia; we expect their acoustics should be well adapted to their habitat.

All the tarsiers recorded were observed in moderate to thick foliage characteristic of secondary vegetation, whether deep in the jungle or in a garden. Our examination of whether sleeping site vegetation was correlated with acoustic measurements yielded no results, likely because the ancestral environments of all the observed populations were very similar. Tarsiers evolved their sensory systems and signals in tandem with the hot and humid forests throughout SE Asia, developing those signals least affected by attenuation and degradation (Endler, 1992; Morton, 1975). Tropical forest vegetation presents a large number of surfaces to reflect and reverberate signals, bouncing and scattering sound waves that result in signal degradation. Since selection favors signals that are least affected by transmission degradation, we expect to see pure notes, narrow frequency bands, slow modulation, and repetition, in Tropical forest environments (Davies et al., 2012; Endler, 1992; Ey & Fisher, 2009). We find all of these characteristics in female notes, while male notes exhibit repetition and little modulation.

High frequency sounds are, however, absorbed more quickly by humidity, and they attenuate quickly in forest environments, creating "sound windows" that amplify

mid–range signals, with frequencies of about 2 kHz transmitting optimally (Davies et al., 2012; Morton, 1975; Waser & Brown, 1984). If low frequencies travel farther, while high frequencies are absorbed and attenuated at much higher rates, why do tarsiers consistently call at such high frequencies? Tarsiers have relatively small territories to demarcate, so frequencies with less interference and private communication channels may have been selected for over long–distance reach.

During spectrogram analysis, we found a visual pattern of high energy frequency tics in many of the male notes. We were unable to capture this in statistical analysis, as peak frequency did not reliably reflect their presence (see Figure 16). These tics typically had a frequency band of 200–350 Hz, and were found beginning around 3,000 Hz (on Bangka) all the way to 10,500 Hz (on Mantehage).

Figure 16. Spectrogram of a Mantehage duet call showcasing the male "tic," seen here at around 10.5kHz.
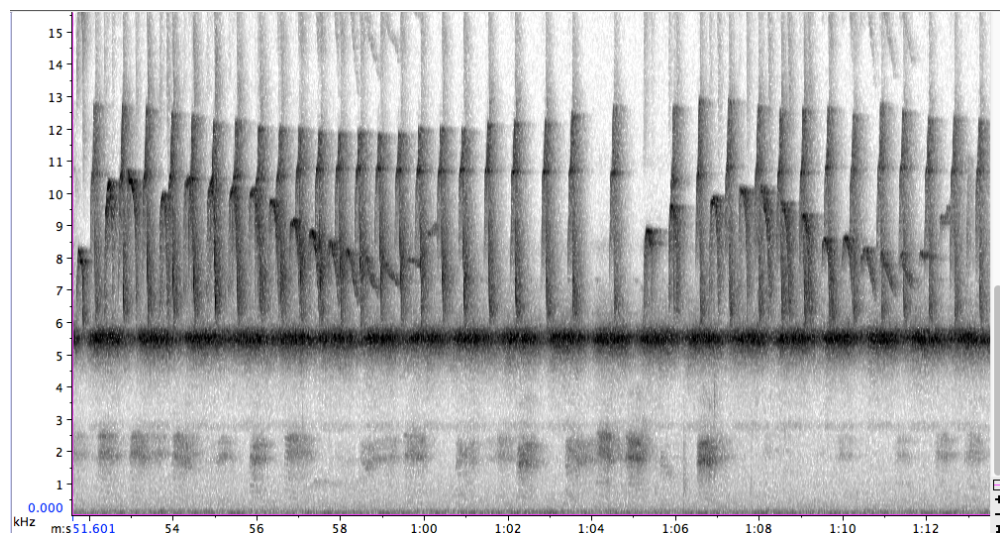
Not all duet phrases contained these "tics", even within a single recording. All tics within a recording did tend to stay at the same frequency, which was often visible as a straight or slightly oscillating line of higher power. The most interesting example we found of these male tics was on Manado Tua, where all of our sites featured a dynamic male tic, in which the males coordinated these vocalizations with the female's duet phrases—not only temporally, but spectrally as well (see Figure 18).

Figure 17. Male note taken from the Mantehage duet in Figure 16, with observable tic between 10.5 kHz and 13kHz.
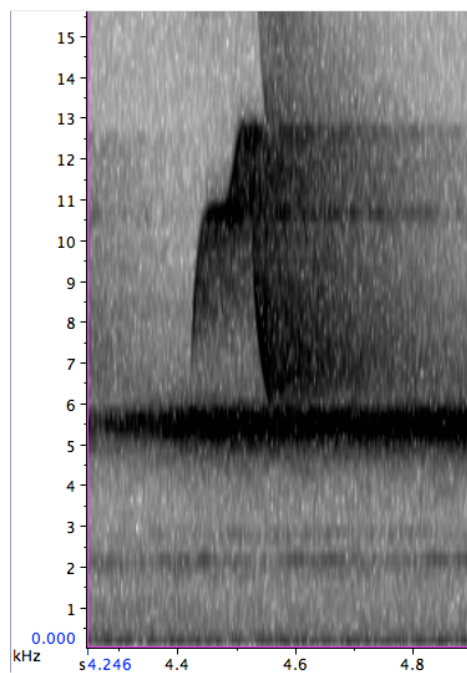
Our first hypothesis was that we would see greater correlations between the mainland locations than were apparent between mainland and shallow water islands, which in turn would be more strongly correlated among each other and the mainland than with the deep water islands. For male and non–duet female notes, we found a higher degree of similarity between the mainland locations and the shallow water islands than

we did between the deep water islands and either of the other two categories. We did not, however, find any more conformity between the two mainland locations than among the shallow water islands, or even between different recording sites at any given location.

Figure 18. Manado Tua duet call showcasing the dynamic male "tic," as it begins around 5kHz and rises to meet the female's swooping duet phrase.
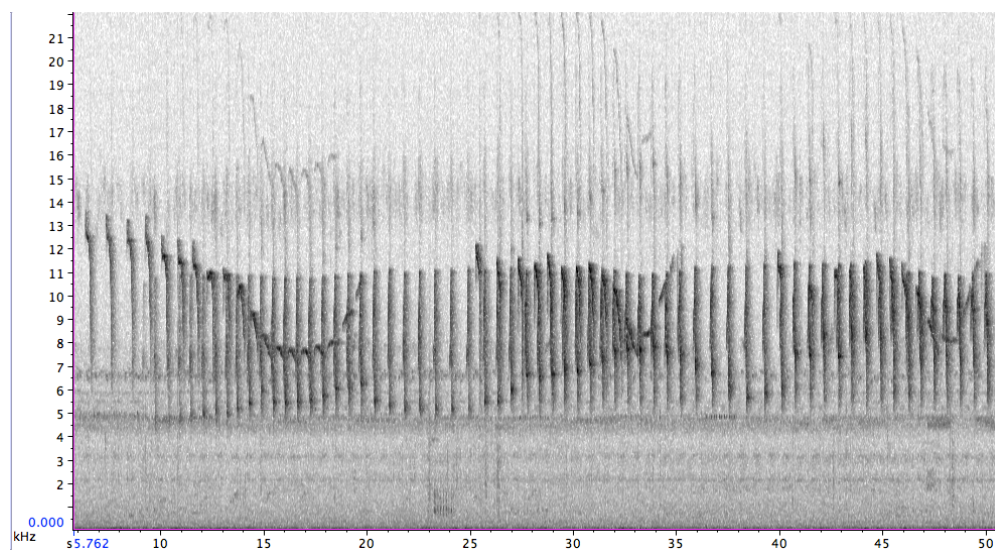
Our second hypothesis was that a stepping stone pattern of colonization would be evidenced in the acoustic structure of tarsiers from the Sangihe Arc, with each island showing vocalizations more similar to its immediate neighbors than to other islands. Since tarsiers were not found to be present on Tagulandang or Biaro, we were unable to trace the entire arc as planned. We did find significant differences between some of the islands, though they did not follow expected patterns for a stepping stone colonization event. Sangihe, the largest island and the farthest north, was the most acoustically unique, as expected. While male notes there were not statistically different from male notes on Siau, female notes were significantly different. Siau female's non–duetting notes are

visually intermediate to Tangkoko form and Sangihe form, but have more in common, statiscally, to Bangka (frequency range and center frequency). Talisei had more in common with Bunaken and Manado Tua than with Bangka, and Bangka was more similar to Bunaken, Klabat, Lembeh, and Manado Tua, than to Talisei.

It is likely that the absence of tarsiers on Tagulandang and Biaro is due to extinction. Islands in the Sangihe Arc get successively larger as their distance from the mainland increases. If we consider mainland Sulawesi to be the source of colonists in MacArthur & Wilson's (1967) theory of island biogeography, then immigration and emigration to the Sangihe Arc should be affected by distance to the mainland, while size of the island affects extinction rates. (MacArthur & Wilson, 1967; Shekelle & Salim, 2009). Both Tagulandang and Biaro are relatively small and isolated, and have little habitat available for biological colonists to exploit, and little chance of a rescue effect from the mainland. It should be added that one local on Tagulandang had spoken of seeing tarsiers there, though he was not corroborated by anyone else on the island.

We found that the "Manado form" duet as described by MacKinnon and MacKinnon (1980) was conserved across all of the mainland and shallow locations. Sangihe duets featured divocalism with temporal and spectral adjustments by both sexes, but did not exhibit a clear "duet phrase," as did the mainland and shallow water island tarsiers. Siau duets did have a definitive duet phrase, however, it was much more modulated and dynamic, and less stereotyped, than the Manado form.

Tarsiers likely colonized the Sangihe Arc via rafting, millions of years before the recurrent glaciations of the Pleistocene made terrestrial travel to the islands on Sulawesi's continental shelf possible. Tagulandang and Biaro have likely experienced extinction events, as their status as small, distant islands makes them more vulnerable to extinction than other islands in the study.

Both genetic drift and environmental factors pay a role in evolving animal communication, but we hypothesize that it is more likely the former at work in this case, as the habitats are similar, and we found no strong evidence of short term habitat adaptations or frequency partitioning. The spectral and temporal structure of the duet calls on the mainland and shallow water islands showed no clear geographical bias or patterns, suggesting that panmixia and hybridization during recurring glaciations may function in preventing subdividions among the populations.

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