COMMUNICATION AND COOPERATION *IN SILICO* AND NATURE

By

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ABSTRACT

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Sociality involves repeated interactions among individual members of an animal group. Because sociality represents one of the major transitions in evolution, it remains a significant focus for behavioral ecologists. Two associated phenomena, communication and cooperation, are closely tied to sociality, and all three appear to be interdependent. Much research effort has been devoted to investigating sociality, communication, and cooperation, but how the three interact, how interdependent they are, and which, if any, precedes the others, remain open questions My dissertation focuses on the intersections among communication, sociality, and cooperation. Within the subsequent chapters of this dissertation, I investigate signaling systems and the interactions that may drive the evolution of signaling systems. I begin by investigating the evolution of a mimicking signal from an aposematic cue precursor and test the conditions that maintain the resulting dishonest signal. I then 1) quantify the repertoire of a socially complex and cooperative species, the spotted hyena, 2) test hypothesis about the information and transmission of information in one of their vocalizations, and 3) investigate a risky type of social behavior that likely played a role in shaping the communication system in this species.

For The Mara Hyena Project and all its participants and contributors

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CHAPTER 1

GENERAL INTRODUCTION

Sociality involves repeated interactions among individual members of an animal group (Ward and Webster 2016). Because sociality represents one of the major transitions in evolution (Maynard Smith and Szathmary 1997), it remains a significant focus for behavioral ecologists. Two associated phenomena, communication and cooperation, are closely tied to sociality, and all three appear to be interdependent. Modeling suggests that communication and cooperation can be stabilized by social interactions (Silk et al. 2000; Sachs et al. 2004; Pfeiffer et al. 2005). At the same time, we know that communication reduces the potential costs of intergroup competition (Cheney et al. 1995; Preuschoft and van Schaik 2000), and cooperation represents one way of realizing the benefits of sociality (Ward and Webster 2016). Further, a number of evolutionary hypotheses predict that complexity in one of these phenomena leads to, or requires, complexity in the other two (Kershenbaum and Blumstein 2017).

Much research effort has been devoted to investigating sociality, communication, and cooperation, but how the three interact, how interdependent they are, and which, if any, precedes the others, remain open questions (Freeberg and Krams 2015). These are the questions that drive my research. For example, we know communication can promote social bonding (Fedurek et al. 2013) and reduce aggression (Cheney et al. 1995), but it is unclear whether repeated social interaction leads to the evolution of communication signals, or whether communication signals must preexist to allow for repeated social interactions. Can social relationships be stably maintained without communication to facilitate them (Preuschoft and van Schaik 2000)? Communication also facilitates cooperation (Balliet 2010; Rawal et al. 2010; Santos et al. 2011)--often by coordinating movement (Gersick et al. 2015)--and cooperation is common within many animal groups. Does cooperation require social relationships (Freeberg and Krams 2015), communication, neither, or both? Some models suggest that cooperation can take place without social relationships or communication, instead relying on by-product mutualism or stigmergy, which means cooperation based on simple rules that are followed by independently-acting group-mates with no reliance on communication signals (Theraulaz and Bonabeau 1999; Noë 2006). This assumes that all individuals act according to these simple rules; but in nature, there is often a great deal of variation in individual behavior (Sih et al. 2004). This variation in behavior leads to uncertainty in an individual's ability to predict conspecific behavior during collective actions. Such uncertainty can be reduced by 1) repeated social interactions that provide information necessary to predict conspecific behavior, or 2) communication signals contemporaneous with cooperation that provide information about the conspecific's intentions or future behavior. Such contemporaneous signals may require repeated interactions to maintain honesty (Pfeiffer et al. 2005).

Territorial animals living in complex social environments face a number of social challenges, including how to recognize group mates, recruit help, coordinate movements, build and maintain relationships, and partition resources (Ward and Webster 2016). These challenges can all come into play during coordination of complex cooperation in the face of great danger. For example, groups of spotted hyenas (Crocuta crocuta) regularly cooperate to drive lions from a kill, despite facing both danger from lions and intragroup competition for those same resources (Kruuk 1972; Périquet et al. 2015).

Spotted hyenas live in fission-fusion societies, called 'clans,' where sub-groups of individuals come together and move apart many times throughout the day (Smith et al. 2008). Food competition within a clan is fierce, and priority of access to food is determined by each individual's position in a linear, despotic, dominance hierarchy (Kruuk 1972; Frank 1986). Nonetheless, all clan-mates defend carcasses of their prey as well as a common territory from neighboring hyena clans and lions, which are their direct interspecific competitors. Interestingly, mean relatedness within clans is no greater than relatedness between neighboring clans (Van Horn et al. 2004). How then, do clans composed of both related and unrelated animals navigate the perpetual changes in sub-group membership and partition resources, all while maintaining the social bonds required to cooperatively fight off dangerous competitors?

My dissertation focuses on the intersections among communication, sociality, and cooperation. Within the subsequent chapters of this dissertation, I investigate signaling systems and the interactions that may drive the evolution of signaling systems. I begin by investigating the evolution of a mimicking signal from an aposematic cue precursor and test the conditions that maintain the resulting dishonest signal. I then 1) quantify the repertoire of a socially complex and cooperative species, the spotted hyena, 2) test hypothesis about the information and transmission of information in one of their vocalizations, and 3) investigate a risky type of social behavior that likely played a role in shaping the communication system in this species.

In chapter 2, I use digital evolution to test how easily an interspecific signaling system can evolve from a cue, using a classic predator-aposematic prey-mimicking prey paradigm. A signal is a trait that has evolved for the purpose of transmitting information from a sender to a receiver, in an attempt to alter the receiver's behavior in a way that benefits the signaler. In contrast, a cue is a trait or occurrence that contains some information but did not evolve to transmit that information. I demonstrate that predators quickly evolve to recognize and avoid a cue informing them of their prey's unpalatability. I also show that another species in the system evolves a signal to mimic the cue of the unpalpable prey, thereby protecting itself from predation. This dishonest mimicry remains stable under all conditions that support cue recognition in the predator.

In chapter 3, I quantify the vocal repertoire of the spotted hyena, a highly social carnivore whose complex vocal signals have previously only been described verbally. I use a large dataset of recorded vocal units and a fuzzy clustering algorithm to classify and quantitatively describe seven distinct core call types as well as many hybrid calls where one core vocalization grades into another. The fuzzy clustering paradigm allows me to quantify gradation within the repertoire. I find that the hyena repertoire contains a high level of gradation among these seven core vocal types, and discuss what this may tell us about the evolution of this vocal repertoire.

In chapter 4, I use machine learning to determine whether the hyena's long-distance vocalization contains a group signature, individual signature, or both. Contrary to my prediction, my analysis of this vocalization reveals that it does not contain a group signature but does contain an individual signature. I also find that the prominent call features that distinguish individual vocalizations are likely to transmit over long distances and demonstrate how the repetition of the vocalization within a bout increases receivers' likelihood of identifying the caller.

In chapter 5, I describe the conditions that lead to risky cooperation in spotted hyenas and whether that risky cooperation results in group benefits. Lions and hyenas are direct competitors throughout most of their range. As such, they often interact; although lions are larger and more dangerous than hyenas, hyenas live in much larger social groups than do lions. Hyenas often recruit groupmates and participate in a risk cooperative behavior called mobbing, where they approach one or more lions as a group to drive them away so their food can be usurped. I show that larger groups of hyenas are more likely to mob lions than smaller groups, and that mobbing is more likely to result in spotted hyenas feeding at a contested carcass than when no mobbing occurs. This kind of risky but rewarding cooperation is the kind of social behavior that likely requires a complex signaling system to maintain.

This dissertation is the product of multiple productive collaborations. Chapter 2 is the product of collaboration with computer and biological scientists in BEACON, an NSF Funded Center for the Study of Evolution in Action. The remaining chapters would not have been possible without the many research assistants, field staff, graduate students, and faculty that have been involved in the Mara Hyena Project. All writing was extensively reviewed by collaborators and advisors. Therefore, I will use the first-person plural instead of first-person singular throughout the remainder of this dissertation.

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CHAPTER 2

FROM CUES TO SIGNALS: EVOLUTION OF INTERSPECIFIC COMMUNICATION VIA APOSEMATISM AND MIMICRY IN A PREDATOR-PREY SYSTEM

Lehmann, K. D. S., B. W. Goldman, I. Dworkin, D. M. Bryson and A. P. Wagner. 2014. From Cues to Signals: Evolution of Interspecific Communication via Aposematism and Mimicry in a Predator-Prey System. PloS one 9:e91783.

ABSTRACT

Current theory suggests that many signaling systems evolved from preexisting cues. In aposematic systems, prey warning signals benefit both predator and prey. When the signal is highly beneficial, a third species often evolves to mimic the toxic species, exploiting the signaling system for its own protection. We investigated the evolutionary dynamics of predator cue utilization and prey signaling in a digital predator-prey system in which prey could evolve to alter their appearance to mimic poison-free or poisonous prey. In predators, we observed rapid evolution of cue recognition (i.e. active behavioral responses) when presented with sufficiently poisonous prey. In addition, active signaling (i.e. mimicry) evolved in prey under all conditions that led to cue utilization. Thus, we show that despite imperfect and dishonest signaling, given a high cost of consuming poisonous prey, complex systems of interspecific communication can evolve via predator cue recognition and prey signal manipulation. This provides evidence supporting hypotheses that cues may serve as stepping-stones in the evolution of more advanced communication and signaling systems that incorporate information about the environment.

INTRODUCTION

Signaling systems represent a basic form for inter- and intraspecific communication. Signals are an evolved means of actively conveying information and influencing the behavior of receivers. In contrast, cues are passive, non-evolving biological and environmental traits that inherently provide the observer with information (Bradbury and Vehrencamp 1998; Smith and David 2003). Organisms have evolved to use both signals and cues to inform their behavior. One of the most pervasive examples of signaling systems in the animal world is aposematism: the warning coloration of poisonous and distasteful species. Aposematism occurs in a wide variety of taxa (Wickler 1968; Randall 2005; Lev-Yadun 2009) and represents a striking example of evolution (Fisher 1930). The characteristics of these aposematic signaling systems are highly variable. Chief among these, the toxicity (ranging from merely distasteful to poisonous or venomous) of the aposematic prey (i.e. the model) and the occurrence, palatability, and accuracy of mimics differ between systems. Studies of a variety of aposematic systems have provided insight into a vast number of evolutionary concepts including character displacement (Pfennig and Kikuchi 2012), frequency dependence (Ries and Mullen 2008; Iserbyt et al. 2011), species diversity (Joron and Mallet 1998; Przeczek et al. 2008), gene flow (Harper and Pfennig 2008), co-evolution and co-evolutionary arms races (Dawkins and Krebs 1979; Gavrilets and Hastings 1998), and adaptive landscapes (Turner et al. 1984). Despite these studies, and the development of theoretical models on aposematic signaling and mimicry(Smith 1975; Hasson 1991; Lindstrom et al. 1997a; Edmunds 2000; Sherratt 2002; Sherratt and Beatty 2003; Holen and Johnstone 2004; Franks et al. 2009), we do not fully understand the conditions necessary for their evolution because no studies have been able to investigate their evolution from a primordial system. To complicate matters, predator learning is not fully understood and its importance for the evolution of aposematism and mimicry likely varies between species (Servedio 2000).

Current theory suggests that evolution of aposematic signaling in toxic species is adaptive (Hasson 1991). Prey benefit when predators learn to avoid them, and predators benefit by avoiding harmful prey. Such signaling systems often include one or more additional species that mimic the aposematic signal, reducing their predation risk. In Müllerian mimicry, a number of species share a common warning signal to advertise toxicity. The species within such mimicry rings share the costs associated with educating a common predator (Müller 1879). In contrast, Batesian mimics advertise a warning signal while remaining palatable (Bates 1862). This dishonest signal benefits only the toxic signaler and the receiving predator because it degrades the information quality of the aposematic signal (Rowland et al. 2010).

In terms of accuracy of mimic signals, for both Müllerian and Batesian mimicry, predation on imperfect mimics is expected to select for perfect mimicry, or automimicry (e.g., Mappes and Alatalo 1997). For automimicry systems, quantitative models suggest that levels of protection enjoyed by the mimic scale with the toxicity of the model prey (Brower et al. 1970; Pough et al. 1973). In addition, a number of experimental studies have shown that accurate mimicry evolves when the model is scarce relative to the abundance of mimics (Iserbyt et al. 2011).

Although selection often favors automimicry, stable systems of imperfect mimicry are prevalent in nature and occur under many conditions (Edmunds 2000; Sherratt 2002; Holen and Johnstone 2004). In order to describe the processes by which they can evolve, systems of imperfect mimicry have been widely studied from both the experimental (Brower 1960; Lindstrom et al. 1997b; Mappes and Alatalo 1997; Pfennig et al. 2006; Barnett et al. 2007; Aronsson and Gamberale-Stille 2008; Kikuchi and

Pfennig 2010a; Wilson et al. 2013) and theoretical perspectives (Emlen 1968; Pough et al. 1973; Estabrook and Jespersen 1974; Sherratt 2002; Holen and Johnstone 2004; Balogh and Leimar 2005; Franks and Sherratt 2007; Gamberale-Stille et al. 2012). From these, two primary effects seem to support the evolution of imperfect mimics. First, selective pressure for perfect mimicry relaxes when imperfect mimics are rare relative to the model or when imperfect mimics are unprofitable due to other factors (e.g., size or agility) (Sherratt 2002; Penney et al. 2012). Second, predators exert less selective pressure on imperfect mimics when they generalize the poisonous prey's characteristics, leading to behavioral avoidance of any species that exhibit these generalized traits (Sherratt 2002; Caley and Schluter 2003; Kikuchi and Pfennig 2010b).

In order to determine the evolutionary trajectories, beginning from a naïve predator-prey system through to a fully functional aposematic signaling system, quantitative models have outlined the theoretical conditions under which aposematic signaling systems stabilize (e.g., Huheey 1964, 1976; Matessi and Cori 1972; Pough et al. 1973; Kannan 1983; Turner et al. 1984; Avery 1985; Holmgren and Enquist 1999; Lindström et al. 2004; Tsoularis and Wallace 2005; Honma et al. 2008). However, such models require substantial simplification of the signaling system, assumptions of unnatural conditions, or reliance on extant signaling systems. Additionally, no studies have been able to experimentally examine the conditions necessary for a predator-prey signaling system to evolve from scratch. This is a difficult challenge, given that, in order to fully evaluate the conditions necessary for the evolution of aposematic signaling, one must observe its evolution in a naïve system where signaling has not yet evolved. However, all natural predators available for experiments have preexisting and established signal recognition systems. To resolve these issues, we used the digital evolution research platform Avida (Ofria et al. 2009) to test the conditions leading to the evolution of a mimicry signal from an aposematic cue in a coevolutionary predator-prey system. We tested for levels of toxicity necessary for the evolutionary emergence of (1) recognition of signaling cues by predators and (2) dishonest signaling by prey mimics. Highly toxic model species are predicted to support more numerous and less accurate mimics (Edmunds 2000; Sherratt 2002; Holen and Johnstone 2004; Kikuchi and Pfennig 2010b; Penney et al. 2012). Further, the maintenance of a dishonest signal, as in Batesian mimicry, is expected to require accurate mimicry when an abundant signal accompanies low toxicity (Brower et al. 1970; Pough et al. 1973). Accordingly, we also tested the level of mimic accuracy required to support a successful Batesian mimic population while varying the levels of model toxicity. We hypothesized that these two conditions (high toxicity or accurate mimicry) provide the necessary selective pressures for dishonest signaling to arise from an existing cue.

MATERIALS AND METHODS

We used the digital evolution software Avida to assess the conditions facilitating the evolution of predators that utilize cues to inform their behavior and prey that actively signal via mimicry, thereby influencing the feeding habits of cue-receptive and cue-sensitive predators. Avida organisms have a sequence of program instructions that controls their behavior and serves as genetic information inherited by their offspring. Instructions executed on the genome dictate the actions taken by an organism, allowing it to sense and interact with the environment (e.g., obstacles, food, other organisms), process information, or reproduce (Ofria et al. 2009). The genome replication process is imperfect, allowing for the introduction of mutations into offspring genomes. Differential fitness in the populations occurs as a consequence of mutations producing novel combinations of operations. Combined, these properties of Avida provide the conditions necessary for adaptive evolution via natural selection: replication, inheritance, variation, and differential fitness (Lewontin 1970). Over the course of evolution, digital organisms in Avida often exhibit behaviors similar to biological organisms observed in natural systems (Fortuna et al. 2013).

We configured Avida to enable predator-prey interactions (Fortuna et al. 2013) using a modified form of Avida's Heads-EX hardware (Bryson and Ofria 2013b). To allow for the evolution of predators, we included an attack instruction that, when mutated into the genome, enabled organisms to consume other organisms. In our digital ecosystems, prey species consumed spatially distributed, limited resources across a 2,516,251 grid-cell environment. Once resources in a cell were consumed, the environmental resource was replenished at a rate of 0.01 resource units per cell per update, to a maximum per-cell level of 1 full unit (1 unit = minimum level consumable by prey). Prey organisms could utilize sensory information and movement instructions to locate and reach edible resources. Predators (once evolved) had the same set of potential genetic instructions as prey. Thus, predators could evolve to use the same instructions to locate and consume prey, through which they gained 25% of their captured prey's previously collected resources. For comparison, we conducted additional experiments using conversion efficiencies of 10% and 50% (Figure A1). While a conversion efficiency of 10% would have more closely reflected natural efficiencies (Lindeman 1942; Wagner et al. 2013), at this level, most populations did not evolve stable predator subpopulations in the allotted time, particularly at high poison levels (see below). Ultimately, varying the conversion efficiency appeared to impact only threshold poison levels for the evolution of predator cue recognition, not overall evolutionary or behavioral patterns (see Figure A1).

All organisms were required to consume a total of 10 units of resource before they could reproduce, either directly from the environment (prey) or from consuming prey that had consumed resources (predators). Reproduction was thus limited by resource consumption: the faster an organism gathered food, the sooner it could reproduce. Accordingly, for predators, advantageous mutations were those that allowed for more rapid targeting and capturing of prey. Likewise, any prey mutations that conferred greater foraging efficiency or predator avoidance skills would give prey a selective advantage.

Prey organisms were divided into three classes of morphs: non-poisonous or ‘safe’, poisonous (toxic), and mimic. To control for subpopulation size effects, and because predators could not act as a top-down control on the poison prey class, classes were assigned at birth such that 50% were poisonous, 25% were safe, and the remaining 25% were potential mimics. The designated class was a part of the prey’s phenotype visible to other organisms. Each morph class foraged for separate environmental resources.

‘Non-poisonous’ prey organisms directly transferred 25% of their gathered resources to predators when consumed. The ‘poison’ prey organisms, upon consumption, reduced a predator’s gathered resources by a factor (i.e. ‘poison level’, see below) of what that prey had previously gathered. The ‘mimic’ prey provided the same resource benefit as ‘non-poisonous’ prey when consumed by a predator. However, mimic prey were unique in that, if appropriate mutations had occurred, they could execute an instruction that allowed them to change their visible phenotype to that of a different prey class. This instruction had no effect in organisms not in the mimic class, even if the relevant mutations occurred. Displayed and visible classes provided a cue for predators, and served as an evolutionary opportunity for them to evolve abilities for recognizing and avoiding poisonous prey. If these behaviors evolved, mimics would then be able to further evolve to manipulate that cue, avoiding predation by providing the predators with a false signal.

Organisms were classified as predators after they made their first kill. The prey class preference of each predator was determined by a specific instruction sequence defining the 'attack organism operation' (see Table 2.1). The default sequence, constituting a single attack instruction, performed a 'generalist' attack, targeting any prey organism in the cell in front of the predator, regardless of the prey's displayed signal. Three additional attack options (first requiring the acquisition of appropriate mutations) consisted of an attack instruction followed by one of eight modifying instructions that specified the target prey morph type. If the victim's displayed class did not match the specified attack type (i.e. predator preference), the attack would fail. As a result, a predator's prey preference was explicitly heritable, though multiple preferences could be expressed if multiple attack instruction sequences existed within the predator's genome. Under most treatments, for a successful kill of a mimic, the predator's expressed prey preference had to match the mimic's displayed class (i.e. what it mimicked), not its true class. However, select treatments, as noted in the results, further altered the fidelity of the apparent prey phenotype such that predators perceived the true class, instead of the displayed class, with the specified probability (i.e. imperfect mimicry).

All experiments were started with the introduction of one prey organism from each class. Each organism's genome was 100 instructions long, and each of these ancestors moved randomly through the Avida landscape, attempting only to consume resources and reproduce. Genetic mutation rates applied to offspring genomes were a 0.25 probability of a single instruction substitution, and a 0.05 probability each that a single instruction would be inserted or deleted (after Bryson and Ofria 2013a). Assignment of offspring to prey classes ensured that half of all prey born were safe for predators to eat, and that the cue from the poisonous prey outnumbered any signaling by the mimics. Importantly, this does not mean heeding the cue was always advantageous: the reward for eating a signaling mimic may have outweighed the penalty for eating a poisonous prey. Furthermore, as we controlled only birth ratios, it was possible for the number of mimics in a given population to outnumber the poisonous prey. In such cases, if enough mimics successfully signaled that they were poisonous, it could be advantageous for a predator to ignore the signal and feed on the excess mimics. Assigning classes in this way also helped stabilize the system, preventing the extinction of any one prey class.

Table 2.1: Attack instruction sequence targeting.

Instruction sequence	Non-poisonous	Mimic	Poisonous
attack	•	•	•
attack + nop-A	•		
attack + nop-B		•	
attack + nop-C	•	•	•
attack + nop-D			•
attack + nop-E	•		
attack + nop-F		•	
attack + nop-G	•	•	•
attack + nop-H			•

The prey type targeted by each of nine possible attack instruction sequences are shown above, indicated by the • symbol.

Because predators could not act as a top-down control on the poisonous prey class, we limited the number of prey in each class to 1,000 organisms and imposed the following method of class-specific population size limitations. Whenever an organism was born, it was assigned to a prey class. If the inclusion of the newly born organism would increase the number of prey in that class beyond the prey type cap, a random existing individual from that class was removed from the population. This method follows the same logic used in Avida by default in which population sizes are limited by physical space constraints (Ofria et al. 2009), except that we applied independent limits to each class, instead of to the population as a whole. We set resource inflow levels sufficiently high to ensure that they did not directly limit prey population levels. Consequently, cases in which a prey class population was substantially less than 1,000 specifically indicated that the class was top-down limited by predators. Unlike the three prey classes, predators were not limited to a maximum class size: predator resources (i.e. prey) were always finite and in a negative frequency relationship with the predator population size. To prevent population extinctions and standardize prey population sizes, the total minimum prey levels across all morph classes, below which predator attacks would be blocked (until another birth occurred), was set to 900 (after Wagner et al. 2013 and Fish et al. n.d.).

All populations were evolved for 500,000 updates (an update is an arbitrary time unit in Avida, roughly equivalent to the time required for each organism in the population to execute 30 genomic instructions), or approximately 4,200 generations. We utilized identical configuration parameters for all treatments, only varying the efficacy of the poison by manipulating the poison level associated with the poison prey (levels used: 0.01, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.75, and 1.0), as specified in the results. We used a logistic regression model to determine the relationship between the toxicity (poison level treatment) of the poisonous prey and the probability that the predators would evolve to avoid eating poisonous prey. We conducted 30 replicate runs for each treatment.

We used Avida version 2.14.1 for all experiments. Data were post-processed using Python 2.7.2. Statistical analyses and plotting were conducted in R (Team 2013) version 2.15.2 using the libraries ggplot2 (Wickham 2009) version 0.9.3.1, gridExtra (Auguie 2012) version 0.9.1, and boot (Canty and Ripley 2013) version 1.3–5.

RESULTS

We tested for levels of toxicity necessary for the evolutionary emergence of signal recognition from a cue by predators and dishonest signaling by prey mimics. We also tested the level of mimic accuracy required to support a successful mimic population.

Predator Recognition of a Cue

We assessed the conditions under which predators evolved to preferentially avoid consuming poisonous prey using a logistic regression model that related predation levels to the eight levels of toxicity of the poisonous prey (0.01, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.75, 1.0). In this model, we classified each of the 30 poison prey sub-populations as being under predation pressure if their realized abundance fell below 800 individuals (80% of the maximum) at the end of the experimental trial. This threshold was chosen arbitrarily during preliminary data analysis in an effort to reduce noise as experimental abundances were either significantly above or significantly below this value, but the individual abundance values were highly stochastic. Based on the proportion of evolved populations in which poison prey were not under predation pressure, the model predicts the probability that predators will evolve to preferentially avoid consuming poisonous prey at a given a poison efficacy level (Figure 2.1). At poison levels below 0.2, the selective pressure to avoid such prey is weak, resulting in a low probability that predators evolved selective predation habits. However, when the efficacy of the poisonous prey is above 0.2, predator populations nearly universally evolve to avoid predating poisonous prey. To illustrate the potential realized cost of consuming a poisonous prey, a poison level of 0.1 would cause a 10% reduction in the resources available for an attacking predator to satisfy the threshold for reproduction (10 units). As such, in order to compensate for 10% 'energetic' loss, if a predator had previously stored 9 units, it would now have to consume eight young prey that each had consumed 1 unit of environmental resource, or one better-fed prey that had consumed 8 units. On the other hand, had that same predator killed a non-poisonous species, it could reproduce immediately.

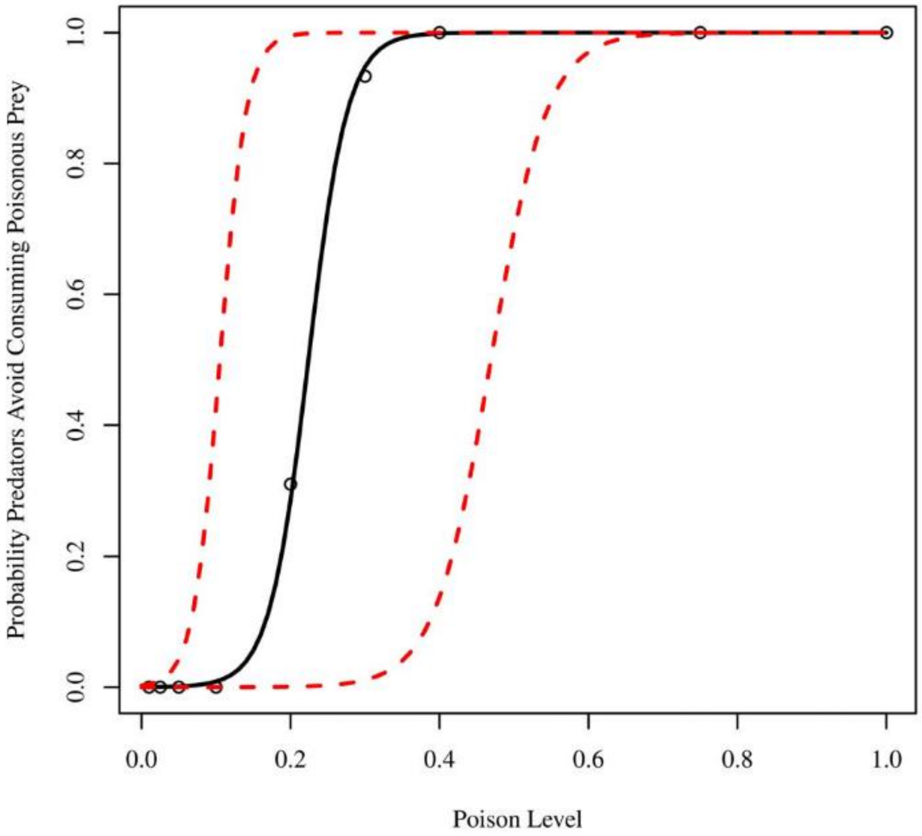

Figure 2.1: Predators evolve to recognize and avoid consuming poisonous prey and poison-signaling mimics, even at relatively low poison efficacy levels. Data shown represent a fit from a logistic regression model relating poison level to the probability that predators will evolve to avoid consuming poisonous prey (based on proportion of evolved populations in which poison prey were no longer under predation pressure). Solid black line indicates the predicted probability. The red dashed lines represent the 95% bootstrap confidence intervals of the model. Circles indicate the observed values in our experiments.

Selective Targeting of Prey Types

For all tested poison levels, the three prey classes rapidly grew toward the population cap until predators began exerting top-down controlling pressure (Figure 2.2). At low poison levels (Figures 2.2a and 2.2b), mimic and safe prey subpopulation sizes remained relatively comparable and constant throughout evolution, suggesting that predators were consuming non-poisonous prey in proportion to their availability, predators were not distinguishing between poisonous and non-poisonous phenotypes, and the cue was not affording protection to the mimic class. However, at higher poison levels (Figures 2.2c and 2.2d), the poisonous prey subpopulations converged to the maximum sub-population size, indicating that they were no longer under top-down predation control and that predators had evolved selective targeting of non-poisonous prey. At the same time, realized predator population sizes were higher in high poison efficacy trials. This indicates that predators could have benefited from evolving skills for discriminating prey at low poison levels. However, at those levels, the selection pressures were not (apparently) strong enough for predator populations to realize this potential in the allotted evolutionary time.

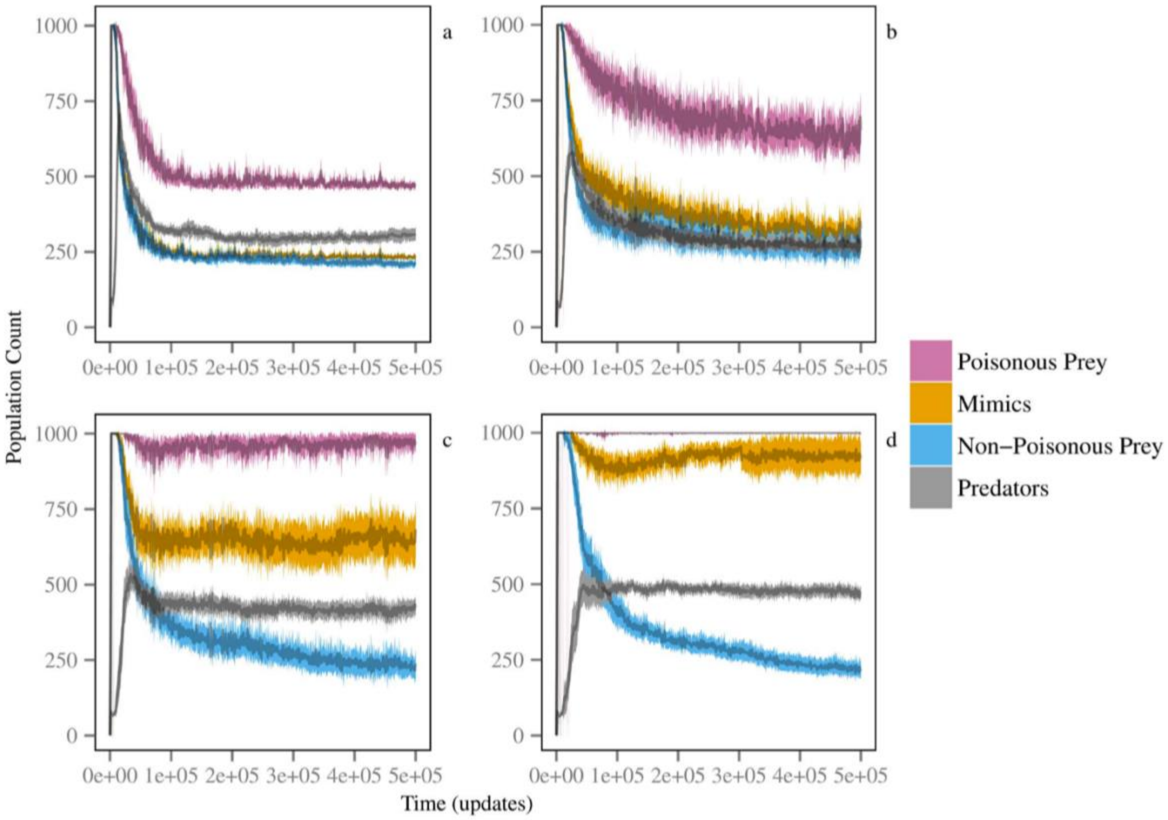

Figure 2.2: Predators benefit from evolving to distinguish and preferentially avoid poisonous prey at higher poison efficacy levels. Shown are mean population sizes for the three prey classes; non-poisonous (blue), poisonous (pink), and mimics (orange), as well as predator abundance (grey) at four of the tested poison levels, 0.1 (a), 0.2 (b), 0.3 (c), and 0.4 (d). Shaded regions show the 95% bootstrap confidence intervals calculated from 10,000 iterations.

Evolved Manipulation of a Communicative Signal

In all populations, prey evolved to alter their apparent phenotypic signal (i.e. appearance) when in the mimic class (Figure 2.3). However, at low poison levels (0.1 and 0.2, Figure 2.3a and 3b, respectively), mimic class prey did not demonstrate a clear preference for mimicking poisonous prey. In contrast, at high poison levels (0.3 and 0.4, Figure 2.3c and 3d, respectively), mimic prey showed a clear preference toward mimicking poisonous prey. Thus, it was only when poison efficacy was high and predators evolved to selectively avoid poisonous prey (Figure 2.1) that prey evolved to selectively mimic that prey type.

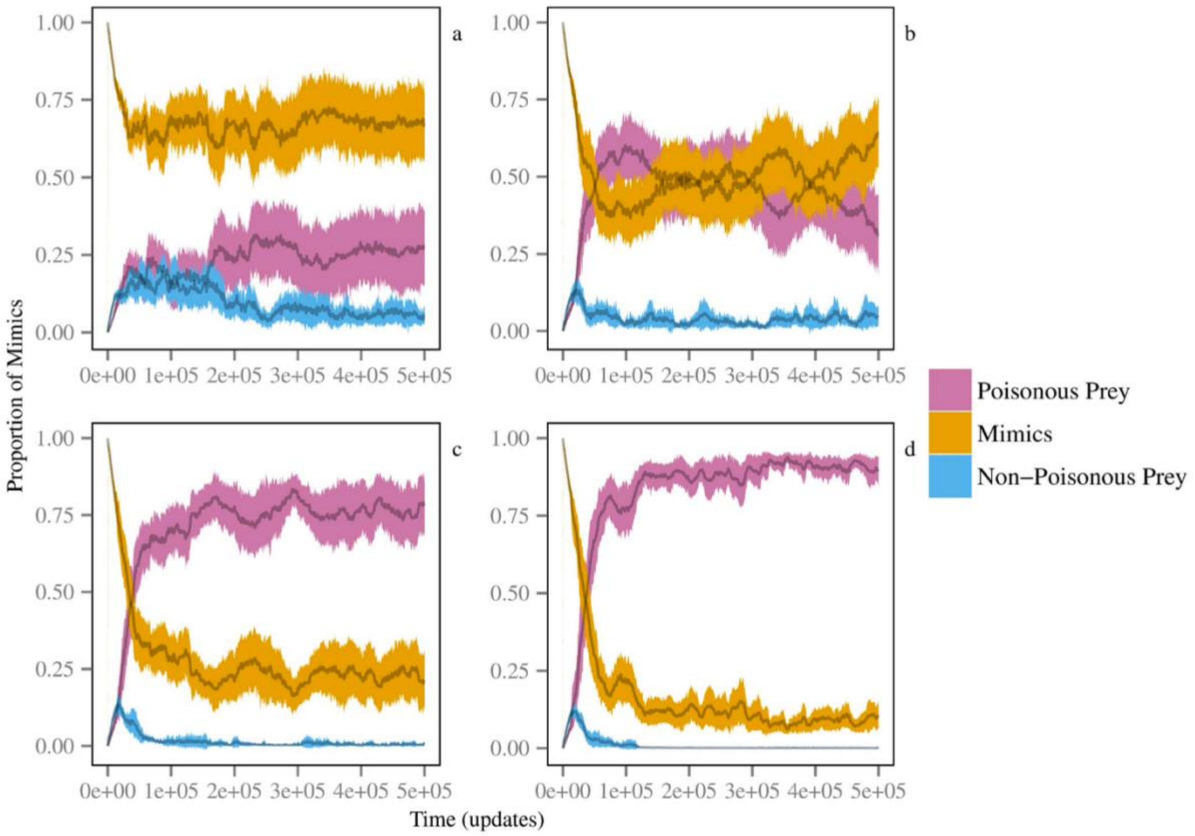

Figure 2.3: Prey preferentially mimic poisonous prey under conditions of high poison efficacy and associated evolved predator selectivity. Shown are proportion of mimic class organisms presenting phenotypes representing each available prey class: mimic (orange), non-poisonous (blue), and poisonous (pink) at four tested poison levels, 0.1 (a), 0.2 (b), 0.3 (c), and 0.4 (d). Shaded regions show the 95% bootstrap confidence intervals calculated from 10,000 iterations.

Signal Noise and the Effect of Information Loss

In the treatments considered above, the phenotypic appearance of mimics was perceived with perfect fidelity by the predators (i.e. predators always saw what the mimics intended them to see). In order to test the robustness of strategies for mimicking poisonous prey, we further evaluated mimicry choices by evolving populations under 'imperfect' mimicry conditions. Under imperfect mimicry, predators perceived the intended mimic signal 10% of the time, with the true (mimic) phenotype apparent to the predator the rest of the time. From the final populations, we calculated the ratio of the mean proportion of organisms of each population that were mimicking poisonous prey under the low accuracy mimicry conditions to the mean proportion mimicking poisonous prey when mimicry was perfect for each of the five poison levels (0.10, 0.20, 0.30, 0.40, 0.75, 1.0; Figure 2.4). By this measure, ratios under 1.0 would indicate a greater proportion of organisms were attempting to mimic poisonous prey when mimicry was perfect than when it was imperfect. We calculated 95% bootstrap confidence intervals (Davison and Hinkley 1997) for these measures by repeatedly calculating the ratio from sampled subsets of the source populations (i.e. 10% and 100% accuracy populations) over 1000 iterations. At the two lowest poison levels, 0.10 and 0.20, the ratios (95% CIs) were 0.58 (0.50–0.64) and 0.73 (0.65–0.80), respectively. The higher poison levels, 0.3, 0.4, 0.75, and 1.00 had realized ratios of 0.37 (0.34–0.39), 0.41 (0.39–0.43), 0.29 (0.27– 0.31), and 0.33 (0.32–0.35), respectively. Overall, these data indicate that relatively low proportions of organisms in the mimic class chose to mimic poisonous prey when mimicry was imperfect. However, moderate and high poison efficacy (.= 0.30) provided enough protection to poison prey phenotypes that a higher proportion of prey in the mimicry class had evolved to appear poisonous, even though mimicry was highly imperfect.

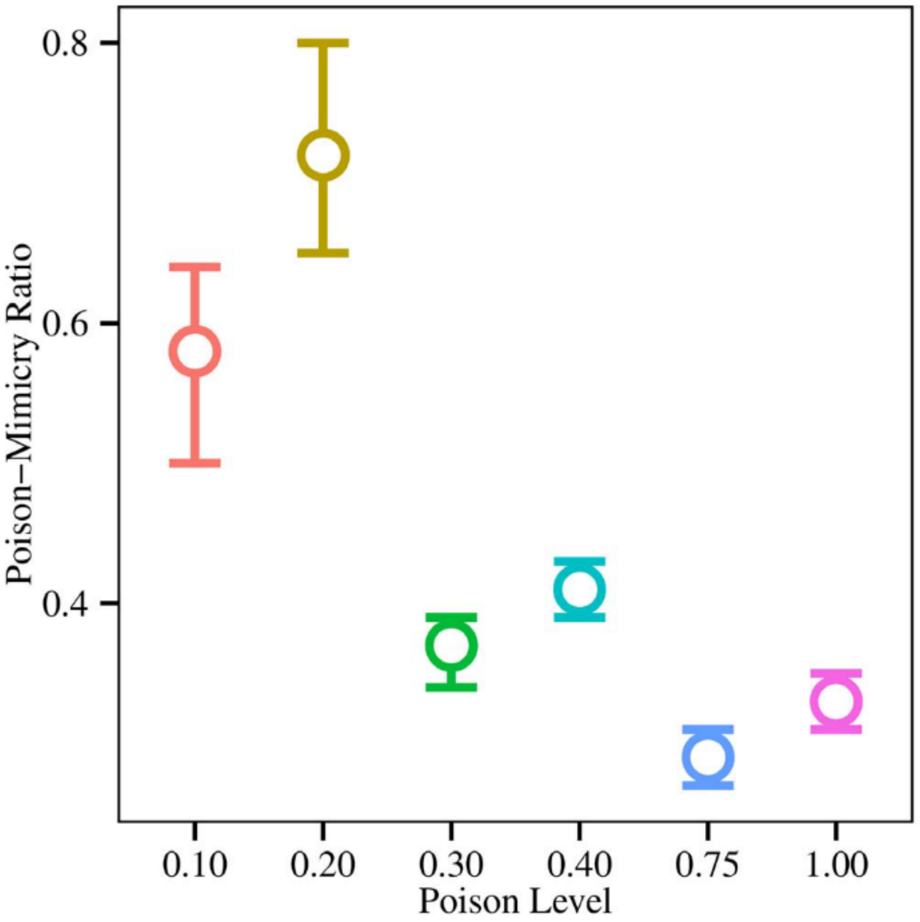

Figure 2.4: Moderate and high poison efficacy levels promote the evolution of mimicry, even when mimicry is highly imperfect. Shown are mean ratios of the mean proportion of organisms in each population that were mimicking poisonous prey under low accuracy mimicry conditions (10%) to the mean proportion mimicking poisonous prey when mimicry was perfect (100% accuracy). At low poison levels, a lower proportion of organisms in the mimic class mimic poison prey when mimicry is imperfect. CI's given are 95% bootstrapped confidence intervals. Bootstrapping was performed by repeatedly calculating the ratio from sampled subsets of the source populations (i.e. 10% and 100% accuracy populations) at 500,000 updates.

DISCUSSION

We have demonstrated that adequate toxicity is required for aposematic cue recognition to evolve and inform predatory behavior. At poison levels below 0.2, predators never distinguished between prey types. However, at higher poison efficacy levels, predator recognition and selection of prey types increased, with the behavior fixing in all trial populations for poison levels at and above 0.4. This agrees with previous findings that predator learning is enhanced by highly toxic prey (Lindstrom et al. 1997b). At the same time, selective pressures on prey were strong enough to promote the evolution of dishonest signaling through mimicry of the aposematic signal (Figure 2.3). Mimics and dishonest signaling did not cause predators to ignore the aposematic cue (Figures 2.1–2). Instead, while the presence of mimics increased the poison level triggering evolution of predator cue and signal recognition (Figure A2), at the given prey class 'immigration' rates used here, the evolution of deceptive signaling by mimics did not destabilize predator cue recognition (Figure 2.3). Additionally, once predators began to cue in on and respond to prey signals, higher proportions of mimics signaled that they were poisonous, leading to an increase in mimic survival relative to safe prey (Figure 2.2).

Despite being capable of mimicking poisonous species, mimics did not universally evolve to employ this strategy. Instead, as has often been observed in nature and is predicted in mathematical models (Huheey 1988; Holen and Johnstone 2004; Speed and Ruxton 2010), the ratio of expressed mimic signals appears to reach equilibrium. This occurred at all poison levels, suggesting that the toxicity of the model can only provide protection to a certain number of mimics and that this level of protection is governed by negative frequency dependence. Any non-poisonous species that becomes highly abundant will experience increased selection pressure, potentially driving it into rarity while other prey species populations increase, causing an increase in predation on that species and ultimately leading to stable signaling ratios, as are apparent in Figure 2.3.

Brower's model (Brower et al. 1970) demonstrated that highly toxic prey can support an abundance of perfect mimics. Similarly, we found that the proportion of mimics signaling toxicity increased as the toxicity of the model increased (Figure 2.3). Under conditions of imperfect mimicry (Figure 2.4), predators increasingly generalize the signals of toxic prey as the prey's toxicity increases, because the cost of failing to identify a model as a mimic is too high (Ihalainen et al. 2007; Kikuchi and Pfennig 2010b), particularly in the presence of alternative prey (Lindström et al. 2004). However, our experiments also show that in environments with coevolving naïve predators, imperfect mimicry is supported without requiring high levels of toxicity. This finding lends support to Fisher's theory (Fisher 1930) of gradual evolution of mimicry. Our results suggest that other dishonest signals may have evolved gradually in situations where the cost of incorrectly distinguishing a dishonest from honest signal is high. Similarly, in the coral snake mimicry complex, the most perfect mimics appear at the edge of the model range (Harper and Pfennig 2007) and high model abundance at the center supports imprecise mimicry because of predatory generalization (Kikuchi and Pfennig 2010b). At the same time, our findings of predator generalization and the evolution of imperfect mimicry contrast with the assertion that mimicry must evolve in a two-step process that starts with feature saltation (Franks and Sherratt 2007; Balogh et al. 2010). Such feature saltation would allow a species to jump the adaptive valley between crypsis and mimicry, and then gradually evolve toward the adaptive peak defined by the model's appearance (Balogh et al. 2010; Gamberale-Stille et al. 2012). Our results suggest that this two-step process is not a necessary mechanism for the evolution of mimicry systems.

Overall, we have demonstrated that mechanisms of communication based on cue recognition readily evolve when it provides adequate benefits to both parties. Further, we have shown that these systems are robust to high levels of noise: cues, once recognized, can support the evolution of dishonest mimicry signaling, even when the mimicry signal is highly imperfect, without disrupting the communication system. Understanding how basic signaling systems evolve can help us understand the selective pressures leading to more complex communication and language systems. Our findings suggest that communicative signaling systems can evolve readily and gradually, without feature saltation, and can confer adaptive advantages allowing populations to cross adaptive valleys toward increasingly sophisticated signal-receiver communications systems.

ACKNOWLEDGMENTS

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APPENDIX

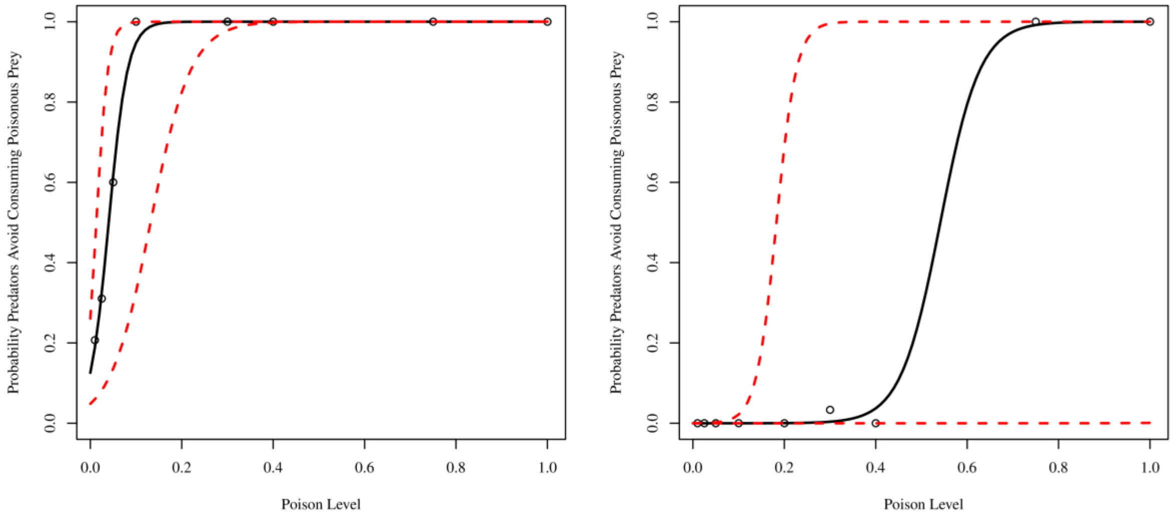

Figure A1: Prey to predator conversion efficiency shifts critical poison level thresholds, without altering overall patterns. Data shown represent fits from logistic regression models relating poison level to the probability that predator species will evolve to avoid consuming poisonous prey (based on proportion of evolved populations in which poison prey were no longer under predation pressure) when predators receive 10% (left) and 50% (right) of the value of their preys' consumed resources. Solid black line indicates the predicted probability. Red dashed lines represent the 95% bootstrap confidence intervals of the model. Circles indicate the observed values in our experiments. Due to low prey densities, in many populations, predators did not evolve into the systems when conversion efficiency was low and poison levels were high (only populations with at least 100 predators were considered here, n=92 out of 270 for 10% and 270 out of 270 for 50%).

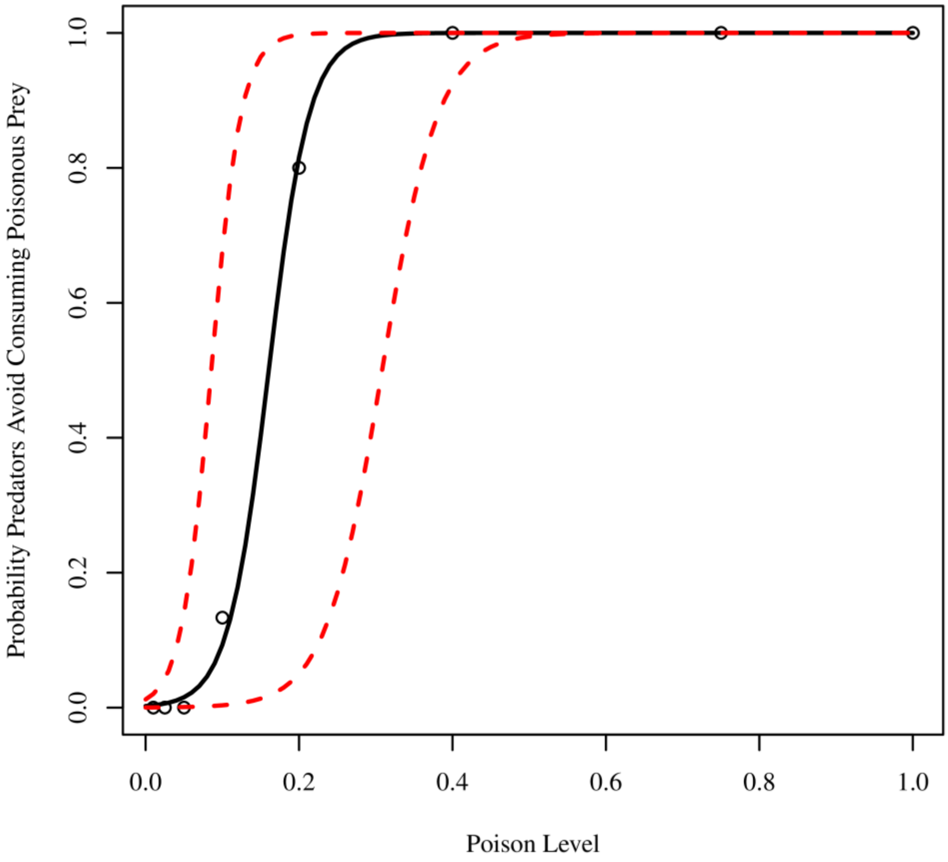

Figure A2: Exclusion of mimicry behaviors reduces poison levels needed to trigger the evolution of cue recognition. Data shown represent fits from logistic regression models relating poison level to the probability that predator species will evolve to avoid consuming poisonous prey (based on proportion of evolved populations in which poison prey were no longer under predation pressure) when mimic morphs were prevented from mimicking (compare to Figure A1). Solid black line indicates the predicted probability. Red dashed lines represent the 95% bootstrap confidence intervals of the model. Circles indicate the observed values in our experiments.

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CHAPTER 3

THE VOCAL REPERTOIRE OF THE SPOTTED HYENA

Kenna D. S. Lehmann and Kay E. Holekamp

INTRODUCTION

For productive study of animal behavior, we must first describe and quantify of specific behaviors (DeWaal 1988). Behavioral definitions serve to 1) standardize the terms and definitions used among researchers studying a species, 2) provide a solid foundation to determine the function of, and selection pressures shaping, behaviors within a species, and 3) facilitate comparative studies aimed at determining how behaviors have evolved among species and clades. Defining and quantifying vocal signaling behavior is especially productive, as such signaling behavior can be used to identify cryptic species and subspecies (Henry 1994; Henry and Wells 2010; Sinsch et al. 2012; Passilongo et al. 2013), identify trait variation and the function of that variation among populations (Sewall 2009), test a species' cognitive abilities(Cheney et al. 1986; Benson-Amram et al. 2011; Benítez et al. 2017), and investigate the causes and consequences of sociality. Ultimately, such work allows us to gain insight about what animals perceive, communicate to others, and ultimately act upon (Seyfarth et al. 2005).

There is a huge amount of variation in animal vocal signals, both within and between species. The diverse set of vocal signals emitted by members of a given species is referred to as its vocal repertoire. To appropriately compare repertoires among populations and species, we must also quantify the variation within, and the level of gradation between, the signals in a repertoire. Such quantification then allows us to develop definitions that can unify and standardize future research. Determining whether signals in a repertoire are graded, discrete, or some combination of these is also intrinsically valuable because there is a predicted tradeoff between flexibility and reliability along the continuum between discrete and graded signals (Marler 1975). Graded signals and signaling systems are thought to be more flexible and able to transmit more kinds of information, whereas discrete signals are thought to transmit information more reliably because they are easily distinguished (Marler 1975; Cheney and Seyfarth 1990). Quantifying the level of gradation among the signals in a vocal repertoire allows us to investigate this predicted tradeoff and determine how it has shaped signal evolution.

Here, we study and define the vocal repertoire of the spotted hyena (*Crocuta crocuta*), a gregarious carnivore that has been studied intensively in multiple long-term research projects in eastern and southern Africa (Kruuk 1972; Mills 1990; Green et al. 2019; Vullioud et al. 2019). Spotted hyenas live in social groups, called 'clans,' composed of up to 126 individuals (Holekamp and Dloniak 2010; Green et al. 2018), and they have a complex vocal repertoire that enables communication over both short and long distances. Hyena society is characterized by fission-fusion dynamics (Aureli et al. 2008; Couzin and Laidre 2009), such that individuals and sub-groups break apart and come together many times throughout a day (Smith et al. 2008). Their complex communication via multiple modalities (olfactory (Theis et al. 2012, 2013), vocal, visual and tactile (Kruuk 1972; Mills 1990) appears necessary for clan-mates negotiating group fission and fusion, as well as for communication between spatially distant clan members.

Spotted hyena socioecology also closely parallels that of cercopithecine primates, making spotted hyenas a powerful system in which to test predictions of hypotheses about the evolution of intelligence(Holekamp et al. 2007a). Previous research has shown that hyena social intelligence is consistent with predictions of the social intelligence hypothesis (Byrne and Whiten 1988; Holekamp et al. 2007b). With a deeper understanding of the spotted hyena vocal repertoire, additional predictions of this hypothesis, and many others, could be tested (Johnson-Ulrich et al. 2018). Furthermore, hyenas could be used as a model organism to test the social complexity hypothesis of communicative complexity, which suggests that social complexity drives the evolution of communicative complexity (Freeberg et al. 2012). Thus, a quantitative description of the vocal repertoire of the spotted hyena is long overdue.

The complex vocal repertoire of spotted hyenas has previously been described in detail but has never before been quantified. Table 3.1 presents comprehensive descriptions of the hyena's vocal repertoire published by Hans Kruuk and Gus Mills, based on their studies of spotted hyenas in the Serengeti in East Africa (Kruuk 1972) and the Kalahari in Southern Africa (Mills 1990). Subsequent studies of the vocal repertoire of spotted hyenas have thus far focused on two call types, the whoop and the giggle. Previous studies of the whoop, the hyenas' long-distance call, described variation in these calls both within and among individuals (East and Hofer 1991a; b; Theis et al. 2007), and determined that mothers respond most strongly to the whoops of their own cubs (Holekamp et al. 1999). Earlier work has also shown that whoops are individually recognizable and that whoops emitted in quick succession, or fast whoops, function to recruit groupmates (Benson-Amram et al. 2011; Gersick et al. 2015). Hyenas are also well known for their giggle vocalization, earning them the moniker "laughing hyenas," but research on the giggle is limited to a single study of variation within the calls of captive hyenas (Mathevon et al. 2010). Here we build upon this previous research by providing a quantitative description of the spotted hyena's vocal repertoire. We 1) establish an objective categorization of call types and quantify variation within these categories, 2) determine the amount of gradation between call types, and 3) reconcile our results with earlier call classification schemes.

Table 3.1: Comparison of call descriptions.

Mills Term*	Kruuk Term**	Mills Description	Kruuk Description	Mills Situation	Kruuk Situation
Whoop	**Whoop**	Starts off as a deep lowing sound (phase 1), followed by a slow rise in pitch (phase 2), rising to a high (phase3). This is sometimes followed by a descending low (phase 4) which may then occasionally rise again (phase 5).	Series of up to 15 (usually 6-9) calls, each lasting 2-3 sec, spaced 2-10 sec. apart; -oo-tone, each call beginning low sending high, though calls in beginning of series may be low-high-low. Last calls of series shorter, reduced to only the low-pitched part. Very loud, may be heard more than 5 km away.	Most (60%) whooping was spontaneous. Also occurred in response to others whooping, prior to leaving, attempting to locate clan members, after unsuccessful hunt, immigrant male approaching another immigrant male [paraphrased]	Both sexes use it, usually when walking alone, sometimes in company. Rarely "answer" each other's calls; usually appears to be spontaneous, without external cause.
Fast whoop	**Fast Whoop**	variation of the whoop…higher pitched with the calls and intervals between them shorter than in the whoop	As above, higher pitched, calls and intervals shorter, not "petering out."	During meeting ceremonies, encounters with lions and conspecifics. [paraphrased]	With many other hyenas present, often in confrontation over kill with lion or other clan; often just before a group of hyenas attacks together.
Rumble	**soft grunt-laugh**	series of soft, rapid staccato grunts	Rapid succession of low-pitched, soft staccato grunts, series lasting several seconds.	Alarm call, typically when several are feeding on a carcass. All scatter.	When surprised by and fleeing from lion or man when on the den or on a kill; when attacking a large prey (infrequent).

Table 3.1 (cont'd)

Soft growl	**Grunt**	low pitched, soft, deep-throated growl	Soft, very low pitched growling sound, several seconds	By females to cubs, also in agonistic situations such as two strangers meeting.	On approach of another hyena (e.g., by female with cubs when approached by male, or by either sex when approached by member of another clan) often followed by chasing.
low	**Groan**	-	As grunt, more -ooo-sounding, pitch higher but variable, lasting intermittently for up to 15 sec.	By dominant during the meeting ceremony and during aggressive intra and interspecific inter-actions. Also heard around dens usually by females with cubs.	Just before and during meeting ceremony.
	low	Ooo-sound of variable pitch and loudness	"-ooo- sound with pitch varying during call, usually low, several seconds."		As fast whoop, less likely to lead to immediate attack.
Snarl	**growl**	high frequency, guttural sound	Loud, with varying but low pitch, up to several seconds, with -aa- and -oh- tones, often with a rattling quality in it.	In defensive posture, when being threatened or approached by dominant, in both intra- and inter- specific interactions	When attacked and bitten, often when about to bite back, loudest when actually returning a bite.
Roar-growl	**-**	loud, explosive growl	-	When being bitten	-

Table 3.1 (cont’d)

hoot-laugh	**loud grunt-laugh**	soft sound, consisting of several low, but variable pitched, tonal-staccato elements	Louder than soft grunt-laugh, but still not a very loud call. Series often lasting more than 5 sec.	Accompanied by tendency to attack posture. …often uttered by several animals simultaneously	In encounters between clans or between hyenas and lions over kills, especially during mass approach or mass withdrawal
Laugh-grunt	**-**	short, medium amplitude sound of variable pitch	-	During play-wrestling and low intensity agonistic situations	-
giggle	**giggle**	loud, high pitched staccato sound	Loud, very high-pitched and rapid series of hee-hee-hee, total usually shorter than 5 sec. reminding one of human "mad laughter."	By submissive animal when being attacked or chased by a con-specific, particularly at food.	When attacked or chase, often over a kill.
squeal	**-**	high pitched, tonal sound of medium to high amplitude		By submissive animals when being attacked	-
yell	**yell**	loud of varying pitch, starting low and working into a crescendo	Loud, with varying but very high pitch, up to several seconds, like a human yell.	By an animal being bitten	As giggle, usually when actually being bitten; strong tendency to flee than giggle.
harsh whine	**whine**	loud, high pitched staccato squeal ee-ee-ee-ee	Loud, high-pitched, long-drawn-out squeals of -eeee- sounds, often with a staccato element (-ee-ee-ee-ee-), very rapid. May continue for minutes with short breaks	With appeasement posture; beg call of cubs wanting to suckle	By cubs when following a female before suckling, of when thwarted in attempts to get food from kill.

Table 3.1 (cont'd)

whine	**soft squeal**	softer, less staccato squeal	As whine, but soft, no staccato, several seconds.	With appeasement posture by cubs and sometimes submissive adults during the meeting ceremony.	By cubs, but also adults; when meeting a well-known individual after long separation.

*Kruuk 1972

**Mills 1990

METHODS

Study animals, behavioral observations, and call recordings

Every hyena clan is structured by a strict linear dominance hierarchy (Kruuk 1972), and an individual's priority of access to resources is determined by its social rank. Each clan contains multiple matrilines of females and their offspring as well as one or more immigrant males that sire most young born. Female hyenas are philopatric, but most males disperse from their natal clan, and join a new clan, at 2 to 6 years of age (Kruuk 1972; Frank 1986; Mills 1990; Smale et al. 1997).Females usually give birth to 1 or 2 offspring in an isolated natal den and move those offspring to a communal den at around one month of age. This communal den protects the clan cubs until they are around nine months of age, when they begin to venture out into the territory (Boydston et al. 2005). The den also serves as a social hub for the clan.

The Mara Hyena Project monitors the behavior of spotted hyenas in multiple clans in the Maasai Mara National Reserve, Kenya. Since 1988, researchers have conducted daily observations around dawn and dusk, weather permitting. We routinely identify all members of each clan by their unique spot patterns, assign birthdates (to ± 7 days) to natal animals based on cub appearance when first seen (Holekamp et al. 1996), and assign a sex to each individual based on the shape of its erect phallus (Frank et al. 1990). We defined adult hyenas as those older than 24 months, and younger hyenas were called juveniles.

From April 2010 to January 2011 and July 2014 to April 2016, recordings of hyenas in four clans were obtained opportunistically from the window of 4WD vehicles, which were used as mobile blinds. Observers deployed recording equipment once the vehicle was turned off and silent, and aimed the handheld directional microphone (ME-

66/K6 and ME-67/K6, sensitivity: 50 mV/Pa, frequency range: 40 Hz–20 kHz62.5 dB; Sennheiser Electronic Corporation, Old Lyme) toward the vocalizing animals within 50 meters of our vehicle. For these recordings, observers used a Marantz PMD661 handheld solid-state recorder (Marantz America, Inc., Mahwah, NJ) at sampling rates of 44.1, 48, or 96 kHz and 16 or 24-bit sampling depths. These recordings were then downsampled to 44.1 kHz and 16-bit sampling depth for consistency and analysis.

We next isolated vocalizations from recordings, and documented the hyena's identity, date, time, context, and call type based on the ethograms published by Kruuk (1972) and Mills (1990) (see Table 3.1). Some of these call types occur naturally in bouts (giggle, groans, whoops). In an effort to quantify vocalization types within bouts each call was segmented into its smallest component, or unit (Figure 3.1a). A unit was defined as a continuous sound, as with syllables in bird song (Brenowitz et al. 1997). Thus, giggle and whoop bouts were reduced to isolated giggles and whoops for analysis.

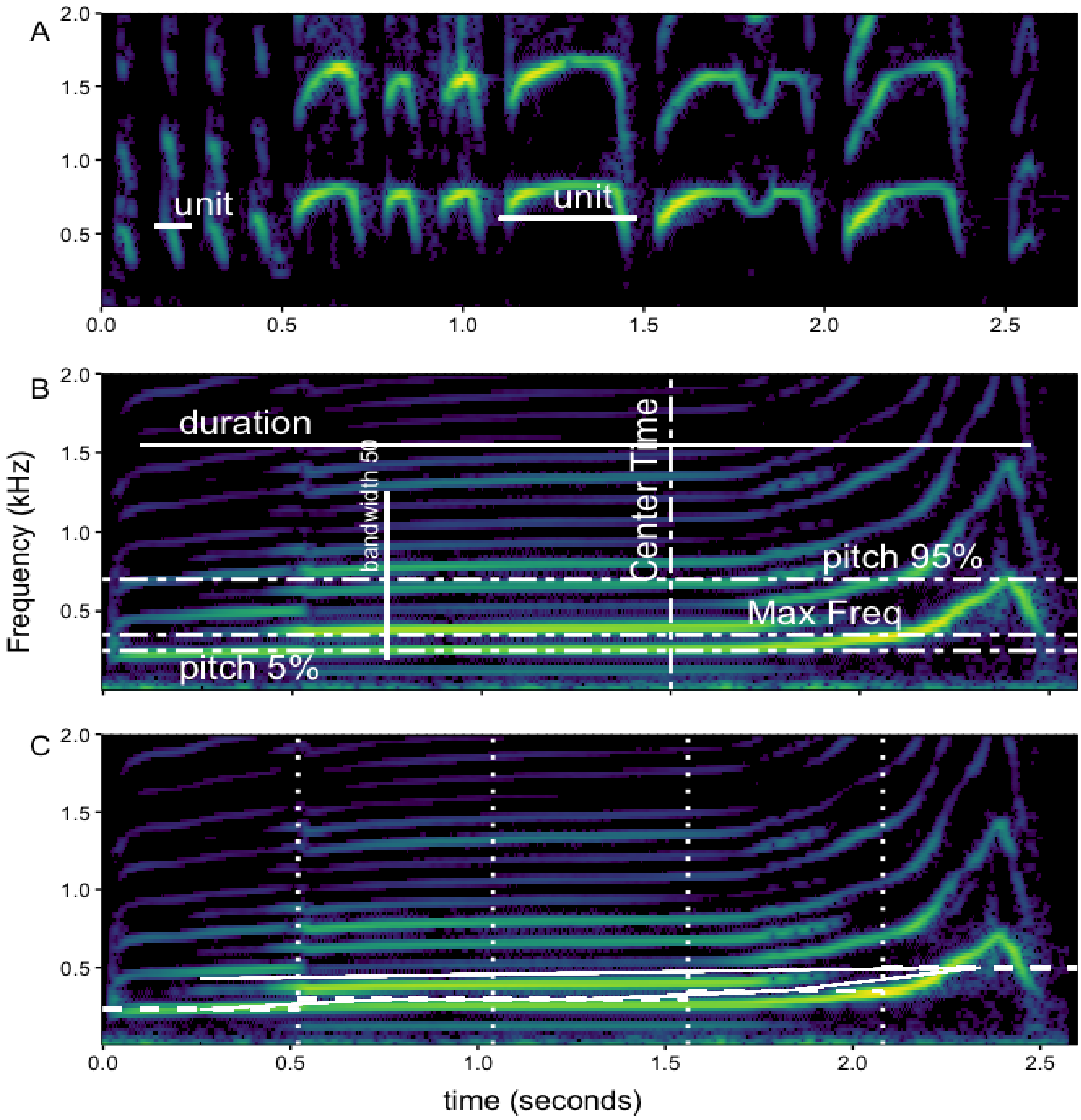

Figure 3.1: Spectrogram of a vocalization, demonstrating the units within the vocalization (a). Spectrogram of a single whoop, indicating the call features measured for each vocal unit in the dataset (b). Spectrogram of a single whoop, demonstrating how some measures were taken on 5 segments of the call. Dotted lines delineate the segments. Dashed lines indicate the median frequency of the segment and solid lines indicate the slopes between each segment and over the entirety of the call.

We created spectrograms (FFT size of 8291, block size .04 ms, block overlap 0.035 ms) of the vocalizations emitted from known adult hyenas. We visually inspected all spectrograms to ensure the signal to noise ratio was high and eliminated any recordings with interfering background noise (e.g. wind, birds, cow bells, wildebeest). This resulted in a dataset of 1307 hyena vocalization units emitted by 109 (33 males and 76 females). We extracted 41 sound features from these spectrograms using Raven Pro 1.5 (Conservation Bioacoustics 2014) and seewave in R (Sueur et al. 2020). Most of these measures were obtained by taking the mean across the entire length of the call. We supplemented these overall measurements with measures of variation during the call. To do this, each call was segmented into five equal portions and a subset of measures was obtained from each portion. Five was the most we could segment many of the very short calls and still obtain frequency measurements. Using five segments for all calls allowed us to compare the change over time among calls of varied lengths. These measures were then standardized by subtracting the overall call mean, such that each segment measure represents a negative or positive difference from the mean. These measured aimed to capture the variation in these measurement over the entire duration of the call. For example, the median frequency of a call measures the central tendency of the call frequency whereby 50% of the call power is above and 50% is below. A measure of "medianFreq_1" is the median frequency measure of the first segment of the call divided by the median frequency of the entire call. A positive median frequency of the first segment indicates that the first fifth of the call has a higher median frequency than the entire call. A number of call features were also calculated from a trace of the call's fundamental frequency. This pitch trace was obtained by employing seewave's integration of multiple pitch tracking methods (Sueur et al. 2020). See Figure 3.1b&c for examples of call measures and Table 3.2 for a full descriptions of call measures.

One of our goals was to quantify the hyena repertoire based purely on vocal characteristics, rather than based on prior human descriptions of a call type. To do so, we used prcomp in R to reduce the 41 sound features to 18 principal components, which together explained >85% of the variation in the dataset, and included all components that explained >1% of the variation (Kaiser-Guttman test, Table 3.3). These principal components were then used to establish fuzzy clusters (Wadewitz et al. 2015), using the Gustafson, Kessel and Babuska algorithm with noise cluster variation of fuzzy clustering in the *fclust* R package (Giordani et al. 2019). This variation allows for non-spherical clusters (Gustafson and Kessel 1978) and improved covariance estimation (Babuska et al. 2002). In addition, this algorithm assigns outlying data points to a noise cluster to prevent outliers from heavily influencing the formation of meaningful clusters (Dave 1991).

In contrast to hard clustering, where each data point is assigned to a single cluster, fuzzy clustering identifies clusters and assigns each data point to each of these clusters with a "membership degree" between 0 and 1, with 0 representing "sharing no similarity with the cluster" and 1 representing "complete similarity with the cluster." Values between 0 and 1 indicate some, but not complete similarity with a cluster. Thus, data points can belong to more than one cluster. The fuzziness of the clustering is adjusted via the fuzziness parameter (m>1). When m=1, fuzzy clustering assigns each data point to a single cluster and the algorithm performs hard clustering. As the fuzziness parameter increases from 1, data points can be assigned membership degrees that are increasingly distributed more evenly among the clusters. At a sufficiently high fuzziness parameter (dependent on the dataset, but commonly m>3 (Kroll 2011)), data points are assigned to each cluster with an equal membership degree, resulting in a single indistinct group. As the fuzziness parameter is decreased from m>3, fuzzy clustering assigns the data points to increasingly unique and less pooled clusters. Thus, the fuzziness parameter is a somewhat arbitrary value that must be determined along with the appropriate number of clusters in a dataset.

To determine the ideal fuzziness parameter and number of clusters in our dataset, we ran the fuzzy clustering algorithm with multiple fuzziness parameters, first increasing from m=1.1 to m=2.5 by 0.05 and second increasing from m=1.3 to m=1.75 by 0.005. We calculated the fuzzy silhouette index (SIL.F) for each clustering solution resulting in 2 to 30 clusters, as previous research suggests hyenas have less than 15 (Kruuk 1972; Mills 1990). The cluster solution that yielded the highest SIL.F value at each fuzziness parameter was retained. From these solutions, we then 1) selected the most stable clustering solution above 5 (i.e. the number of clusters that yields the highest SIL.F value for the largest range of fuzziness parameters) and 2) used the lowest (e.g. most sharp or 'clear') fuzziness parameter yielding that number of clusters.

Fuzzy clustering allows us to identify both calls that fall definitively within a cluster (membership degrees close to 1) and calls that may lie between two or more clusters (2 membership degrees close to 0.5). We calculated a typicality measure for each call by subtracting its second highest membership degree from its first highest membership degree. Fuzzy clustering also allowed us to calculate the average membership degree of a cluster, which indicates whether a cluster is composed of very similar (high average membership degrees) or highly variable (low average membership degrees) calls.

All analyses were conducted in R (R Core Team 2019), figures were created with ggplot2 (Wickham et al. 2020), cowplot (Wilke 2019) and scatterplot3d (Ligges et al. 2018) and tables were created with flextable (Gohel 2020).

Table 3.2: Table of all call features measured.

Abbreviation	Parameter	Units	Description	Program
Center.TimeR	Relative center time	percent of call	Time point in the call where power on either side is equal, divided by the length of the call	Raven
Dur.90	conservative measure of duration	seconds	length of the call with the center 90% of the power	Raven
Max.Freq	Maximum or peak frequency	Hz	frequency at which the maximum power occurs	Raven
pitch_mean	mean pitch of the call	Hz	mean of pitch trace	seewave
pitch_5per	conservative pitch minimum	Hz	Fifth quantile of the pitch trace values	seewave
pitch_95per	conservative pitch maximum	Hz	95th quantile of the pitch trace values	seewave
pitch_sd	standard deviation of the pitch	Hz		seewave
medianFreq_mean	mean center frequency	Hz	mean of the center frequencies of all time slices	seewave
medianFreq_sd	standard deviation of the center frequency	Hz	standard deviation of the center frequencies of all time slices	seewave
f1Freq_mean	mean frequency of the first formant	Hz		seewave
f1Width_mean	mean width of the first formant	Hz		seewave
f2Freq_mean	mean frequency of the second formant	Hz		seewave
f2Width_mean	mean width of the second formant	Hz		seewave

Table 3.2 (cont'd)

harmonics_mean	mean power in harmonics	dB	the amount of energy in upper harmonics, namely the ratio of total spectral power above 1.25 x F0 to the total spectral power below 1.25 x F0 (dB)	seewave
harmonics_sd	standard deviation of power in harmonics	dB		seewave
HNR_mean	harmonics-to-noise ratio	dB	a measure of harmonicity. If HNR = 0 dB, there is as much energy in the harmonics as in noise.	seewave
entropy	mean Weiner entropy of all frames	bits	Close to 0: pure tone or tonal sound with nearly all energy in harmonics; close to 1: white noise	seewave
entropy_sd	standard deviation of Weiner entropy of all frames	bits	variation in entropy over the call	seewave
ampl_sd	standard deviation of amplitude	dB	variation in amplitude over the call	seewave
bandwidth50_mean	mean distance between the 25th and 75th quantile	Hz		seewave
entropy_1-5	entropy of the 1st-5th segments	bits	standardized by subtracting the mean to capture variation over the call	seewave
medianFreq_1-5	center frequency of the 1st-5th segments	Hz	standardized by subtracting the mean to capture variation over the call	seewave

Table 3.2 (cont'd)

voiced_90percent	proportion of call that is voiced	percent of call	number of voiced time frames divided by the total number of time slices within the 90% duration	Raven
voiced_1-5	voiced proportion of 1st-5th segment	percent of call	standardized by subtracting the percentage voiced of the call	Raven
slope_call	slope of entire call	Hz/seconds	center frequency of the 5th segment - center frequency of the 1st segment divided by the length of the call	Raven
slope_1-4	slope between 1st-5th segments	Hz/seconds	difference between two segments, divided by the length of the segment	Raven

RESULTS

Objective call types

We quantified the spotted hyena repertoire using 1307 vocalizations from 109 adult hyenas. The principal component analysis reduced the 41 call features to 18 principal components. PC1 explains 16.2% of the variation within the call dataset and is largely composed of frequency measures and harmonic versus non-linearity measures (Table 3.3. PC2 contains measures aimed at capturing the change in frequency over the course of the call (median frequency standard deviation and the median frequencies of all five call segments). PC3 is composed of measures that capture the relative harmonicity of the call, including the standard deviation of the harmonic measure (or how much power is in the call's harmonics above the fundamental frequency) and the proportion of the call that is voiced (e.g. has a detectable fundamental frequency).

Table 3.3: Factor loadings of the first seven principal components from standardized call measures. These components explain >50% of the variation within the call dataset.

call measures	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Center.TimeR	0.042	-0.001	0.138	-0.088	**0.254**
Dur.90	**0.214**	0.109	0.135	**0.168**	0.009
Max.Freq	**-0.268**	-0.067	0.048	0.058	0.043
pitch_mean	**-0.303**	**-0.171**	-0.079	-0.053	0.101
pitch_5per	**-0.280**	**-0.190**	-0.155	-0.150	0.042
pitch_95per	**-0.261**	-0.071	0.041	0.069	0.155
pitch_sd	-0.045	0.105	**0.175**	**0.252**	**0.160**
medianFreq_mean	**-0.331**	0.150	0.000	0.016	0.044
medianFreq_sd	-0.140	**0.336**	-0.019	-0.068	0.003
f1Freq_mean	-0.043	0.082	**-0.325**	**0.276**	**0.180**
f1Width_mean	0.115	**0.201**	**-0.198**	**0.339**	0.081
f2Freq_mean	0.134	0.121	**-0.362**	0.154	0.096
f2Width_mean	**0.273**	0.137	-0.143	0.039	-0.020
harmonics_mean	**-0.210**	0.148	0.122	**0.300**	0.016
harmonics_sd	-0.011	0.038	**0.340**	**0.166**	-0.002
HNR_mean	-0.033	**-0.213**	0.009	**-0.222**	0.031
entropy	**-0.262**	0.124	-0.145	**0.182**	0.044
entropy_sd	**-0.205**	**0.250**	-0.027	0.071	-0.077
ampl_sd	-0.096	-0.002	**0.173**	0.105	-0.043
bandwidth50_mean	**-0.308**	0.076	-0.082	0.147	0.076
entropy_1	-0.057	**0.165**	0.010	**-0.263**	**0.222**
entropy_2	0.095	0.008	0.078	0.018	**0.387**
entropy_3	0.149	-0.081	0.116	**0.165**	0.025
entropy_4	-0.042	-0.085	-0.029	0.131	**-0.369**
entropy_5	-0.154	0.152	-0.059	-0.086	**-0.315**
medianFreq_1	-0.007	**0.227**	0.019	**-0.299**	**0.182**
medianFreq_2	0.017	**-0.296**	-0.012	0.102	**0.167**
medianFreq_3	-0.004	**-0.373**	-0.036	**0.184**	0.006
medianFreq_4	0.006	**-0.323**	0.002	**0.216**	-0.115
medianFreq_5	-0.013	**0.198**	0.018	-0.070	**-0.240**
voiced_1	-0.065	0.031	**0.187**	0.124	**-0.280**
voiced_2	0.014	-0.015	**-0.212**	-0.086	**-0.177**
voiced_3	0.065	-0.031	**-0.332**	-0.091	0.007
voiced_4	0.034	-0.009	**-0.162**	-0.135	0.134
voiced_5	-0.005	-0.001	**0.246**	0.063	**0.303**

Table 3.3 (cont'd)

voiced_90percent	-0.146	-0.029	**0.254**	**-0.208**	-0.088
slope_call	0.009	0.063	0.031	-0.001	0.012
slope_1	**-0.167**	-0.109	-0.130	-0.036	0.080
slope_2	-0.047	-0.060	-0.068	0.061	-0.055
slope_3	0.089	0.106	0.098	-0.022	-0.032
slope_4	0.117	0.120	0.115	0.008	-0.001
Proportion of Variance	0.162	0.126	0.084	0.067	0.062
Cumulative Proportion	0.162	0.287	0.371	0.438	0.500

Bolded loading values exceed the square root of one divided by the number of measures.

Using these principal components, we then determined the ideal cluster size at a range of fuzziness parameters, first from *m*=1.1 to *m*=2.5 by every 0.05 and then *m*=1.3 to *m*=1.75 by every 0.005 (Figure 3.2). The most stable number of clusters above five is seven because seven clusters yield the highest SIL.F value over the widest range of *m* (*m*=1.53-1.57).

We used the *m*=1.53 to classify all calls and call units into seven clusters (Figures 3.3 to 3.6 and Table 3.4, SIL.F = 0.174). These clusters fall into two main types 1) short calls with medium to high-pitched notes (clusters 2, 3, 5, and 6; Figure 3.7) and 2) long, low pitched calls (clusters 1, 4, and 7; Figure 3.8). Nearly a quarter (23.3%) of the calls were categorized as noise because their highest cluster membership degree was <0.5. Some clusters were characterized by common changes in call features over the length of the call (Figure 3.6).

The seven clusters detected at m=1.53 appear to correspond to the descriptions of call types by Kruuk (1972) and Mills (1999). Furthermore, there was considerable gradation between clusters (Figures 3.9 to 3.13).

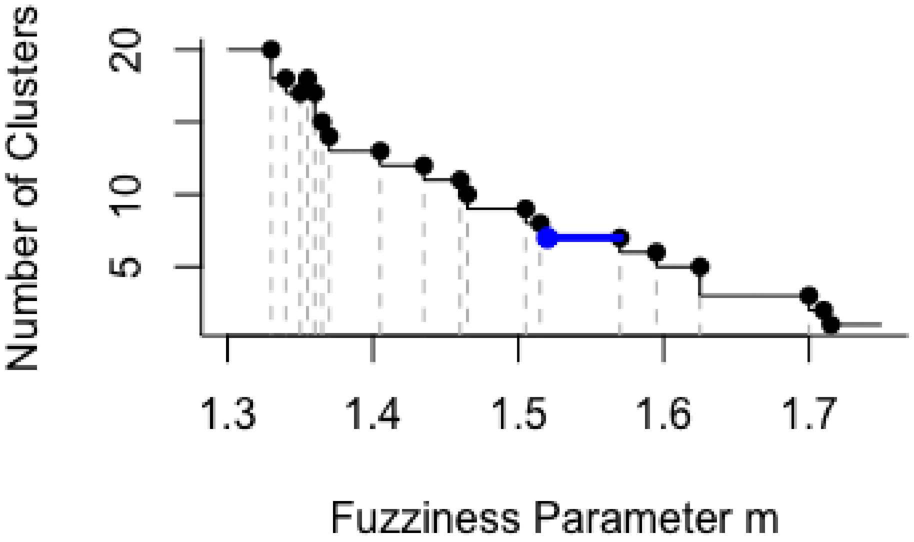

Figure 3.2: The number of clusters with the highest SIL.F value for fuzziness parameters between 1.3 and 1.75 in steps of 0.005. The most stable number of clusters (i.e. the number of clusters that yields the highest SIL.F value for the largest range of fuzziness parameters) above five is highlighted in blue along with the ideal fuzziness parameter.

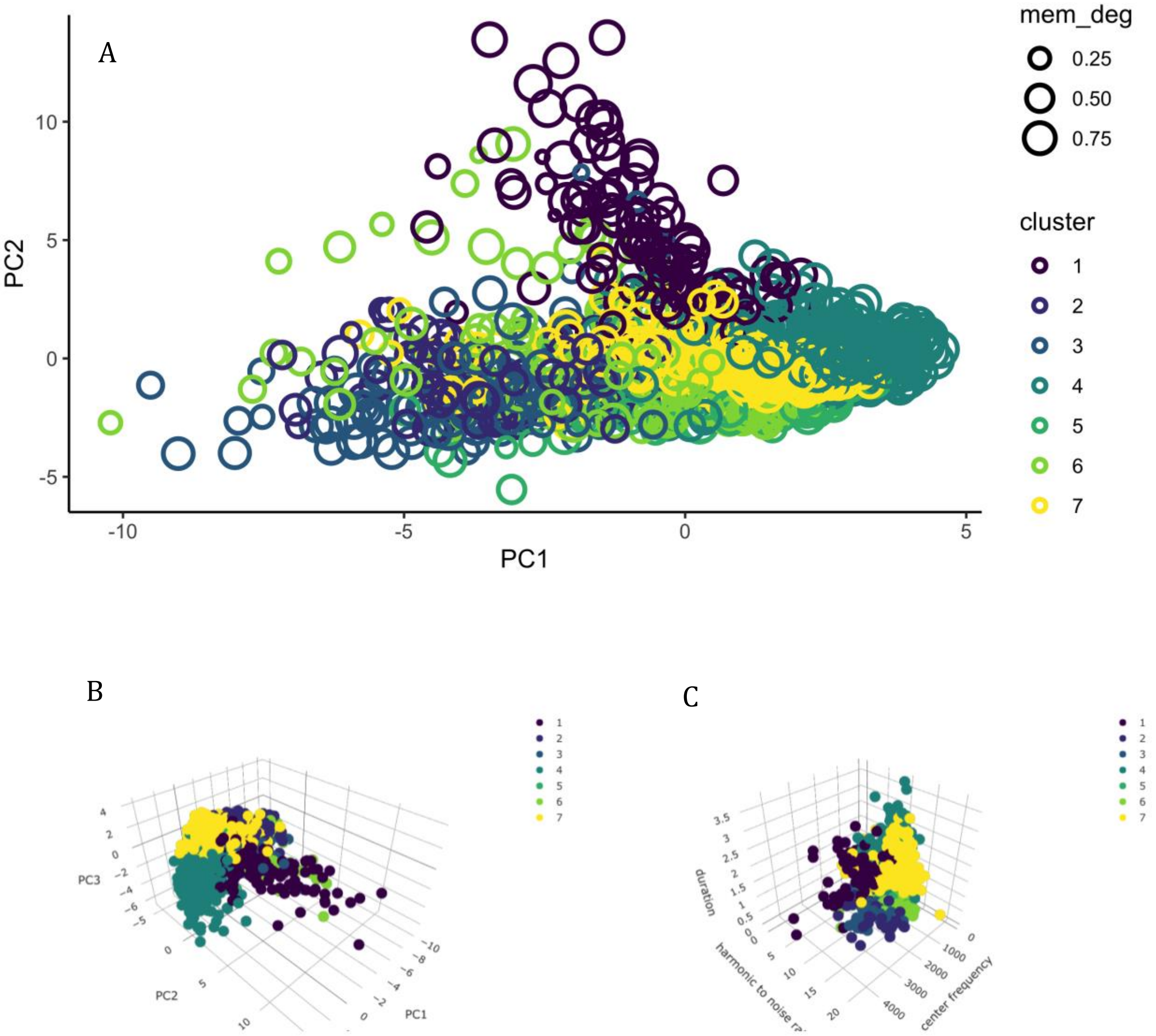

Figure 3.3: All calls plotted by PC1 and PC2 (a). Size of the points indicate the membership degree of the primary cluster. All calls plotted by PC1-PC3(b) and duration, harmonic to noise ratio, and center frequency (c).

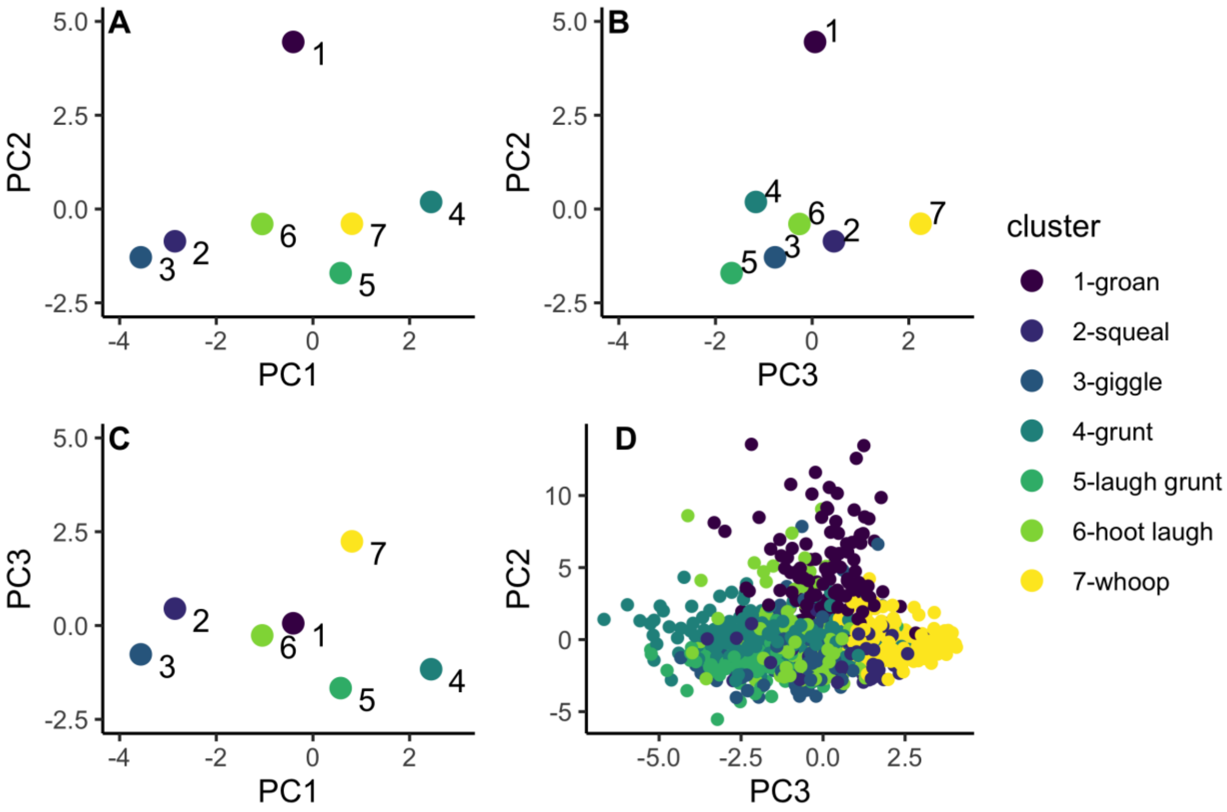

Figure 3.4: Cluster centers plotted by combinations of the first three principal components (a-c). All calls with a primary membership degree > 0.6 with point color representing the cluster that holds their primary membership degree (d).

Table 3.4: Cluster descriptions and characteristics and a comparison of call features within call type clusters. Boxplots include calls that are assigned to their main cluster with a membership degree (MD) > 0.6.

name	**description**	**#calls**	**average membership degree (MD)**	**# MD > 0.6**
groan	long, low pitch with frequency modulation and power in the harmonics	121	0.664	76
squeal	medium length, harmonic and high pitch with variable frequency modulation	98	0.512	31
giggle	short, high pitched note, that quickly rises and falls with significant energy in the harmonics	126	0.596	67
grunt	long, gravelly, low frequency, with a gradual, small rise and fall in frequency	346	0.773	282
laugh grunt	short medium frequency note with little energy in the harmonics	114	0.697	75
hoot laugh	short medium frequency note with some energy in the harmonics	163	0.567	74
whoop	whoops, as described by Mills	339	0.730	245

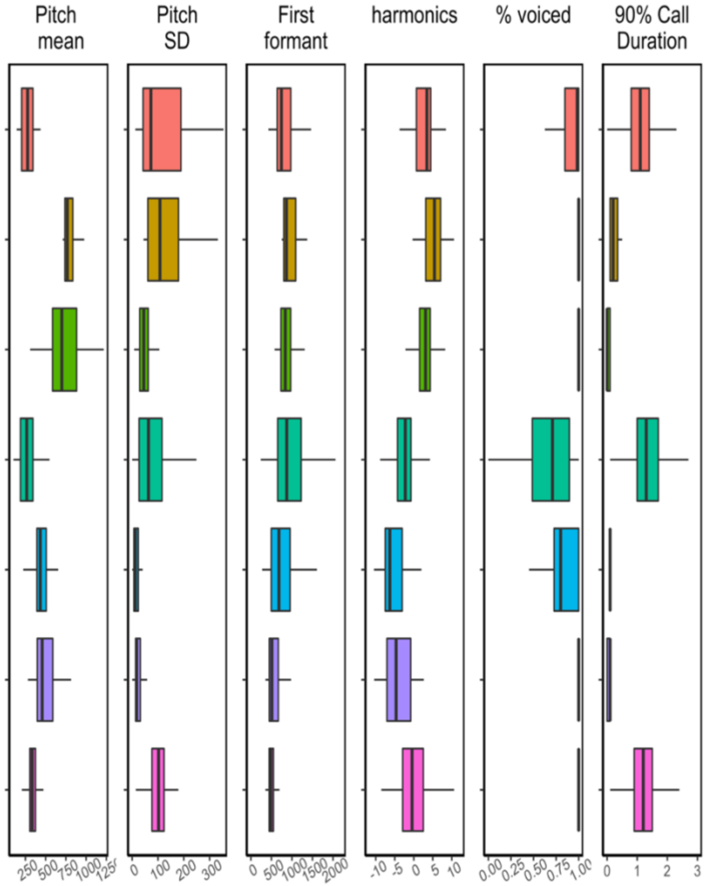

Figure 3.5: Cluster descriptions and characteristics and a comparison of call features within call type clusters. Boxplots include calls that are assigned to their main cluster with a membership degree (MD) > 0.6.

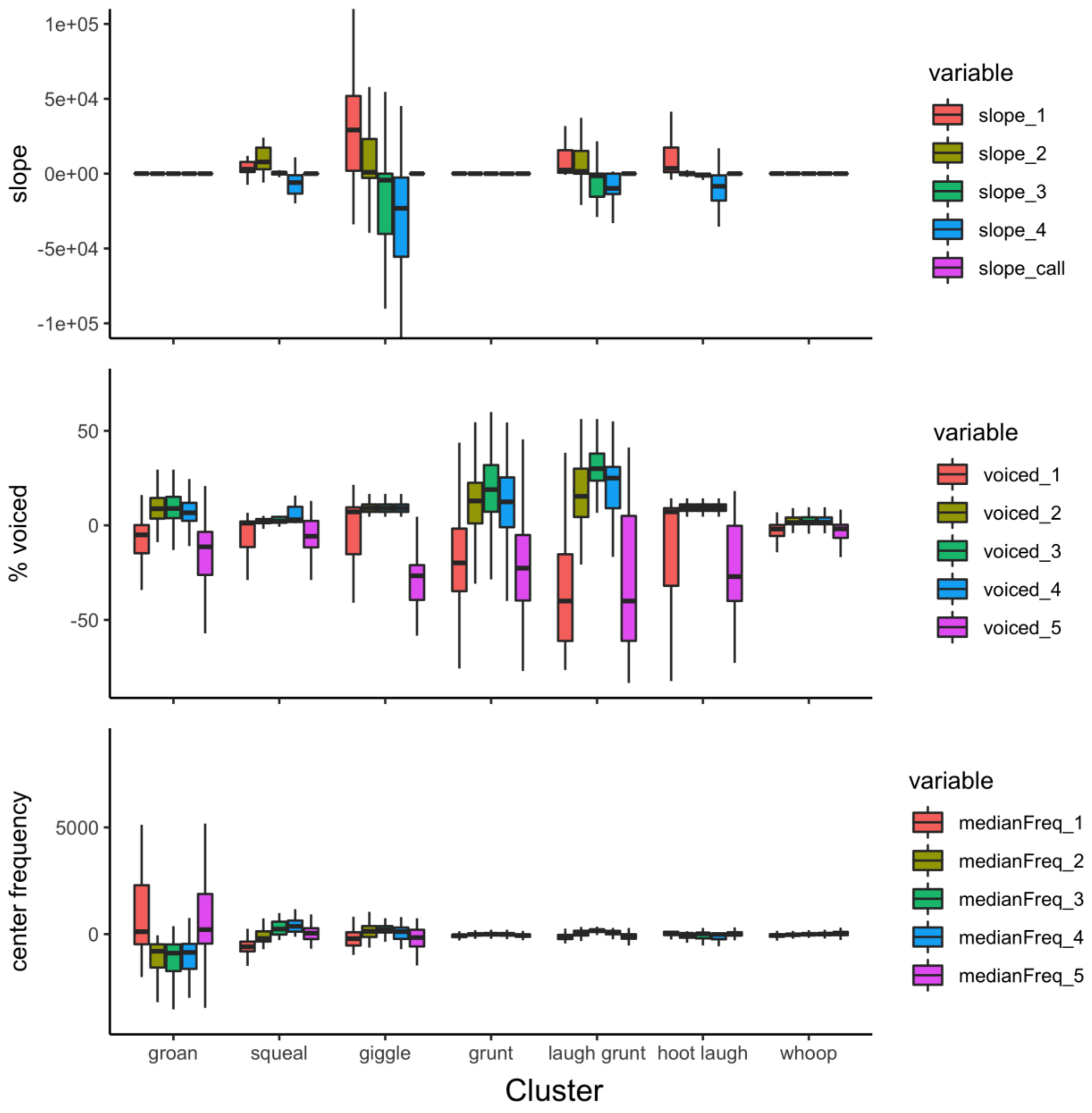

Figure 3.6: Comparison of call segment features. For these features, each call was separated into five equal segments. We then subtracted the mean feature of the entire call from each segment to standardize each segment to the overall feature mean. For example, the overall slope of the call was calculated, along with the slope between each of the five call segments, resulting in slope measures 1-4. The overall slope of the call was then subtracted from each slope segment.

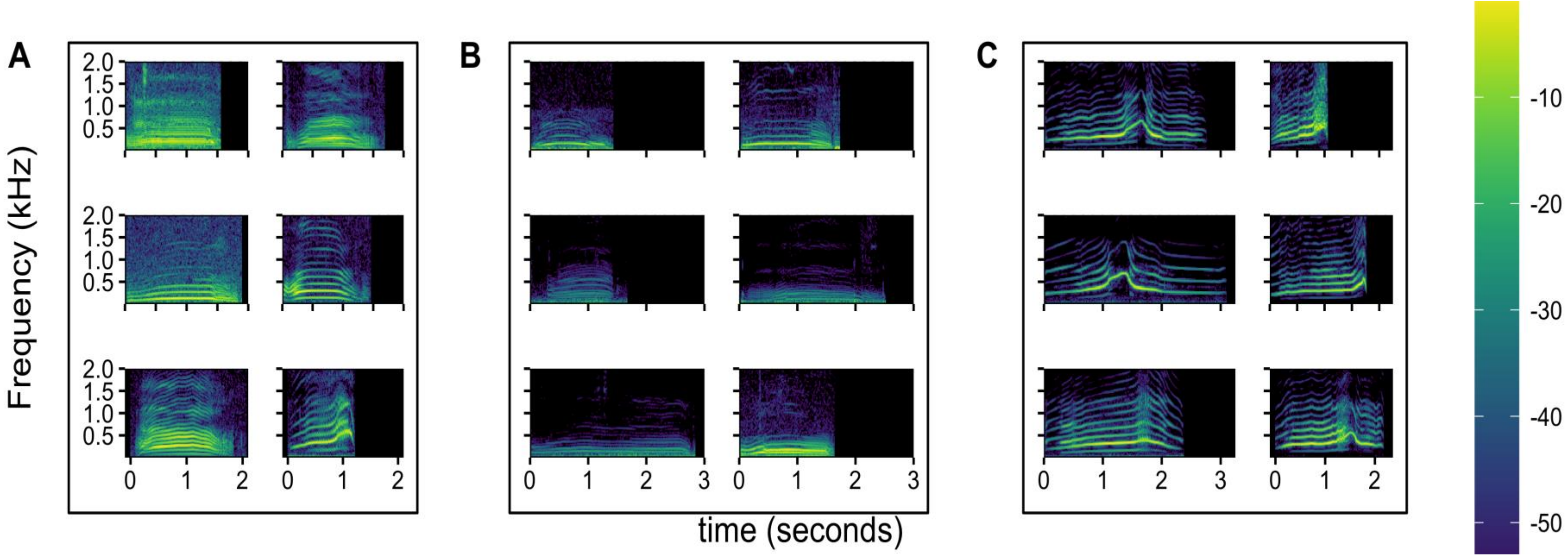

Figure 3.7: Spectrograms of calls representative of groan (a), grunt (b), and whoop (c) clusters.

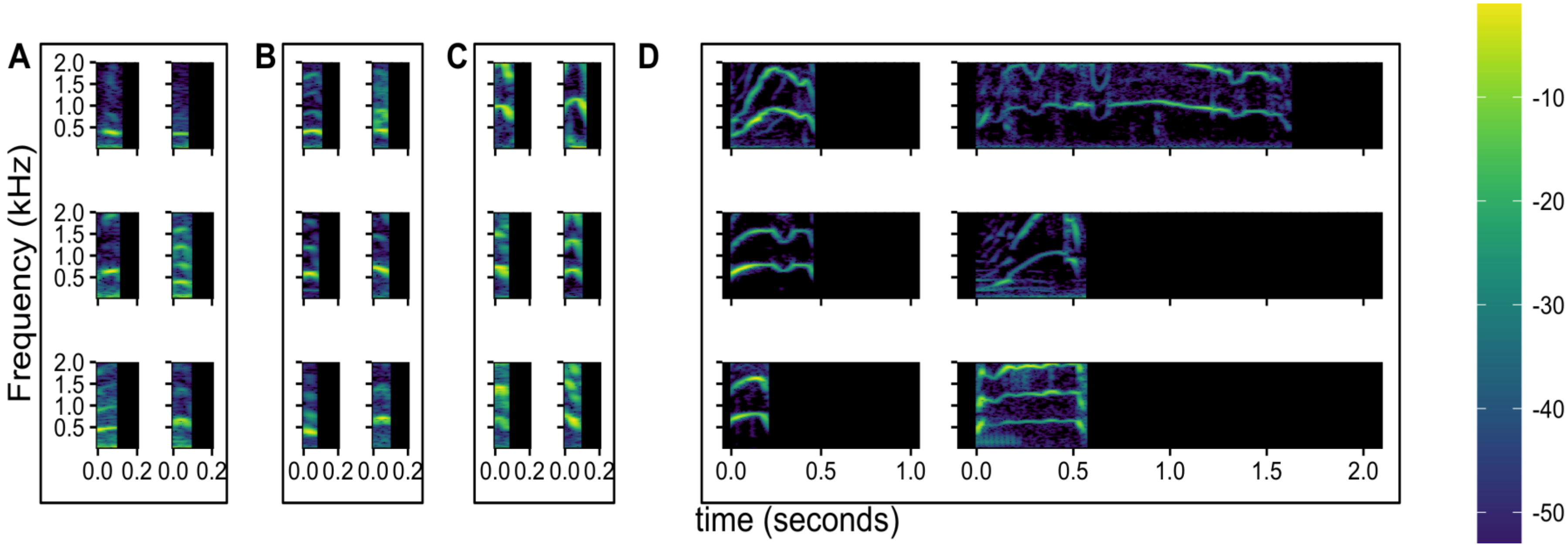

Figure 3.8: Spectrograms of call units representative of the laugh (a), grunt (b), hoot laugh (c), giggle (d), and squeal clusters (e).

Gradation between call types

One advantage of the fuzzy clustering method is the ability to quantify gradation between clusters. The gradation between clusters can be described in two ways: The highest membership degree and the typicality measure. A call's highest membership degree indicates which cluster it most resembles. Clusters with a larger proportion of calls with high membership degrees have calls that are more similar than clusters with low membership degrees (Figure 3.9). The typicality measure describes how typical the call itself is. A call with a high typicality measure is similar to a single cluster, while a call with a low typicality measure likely lies between two or more clusters (Figure 3.10. Thus, grunts and whoops belong to more solid, or less fuzzy clusters than do the remaining call types.

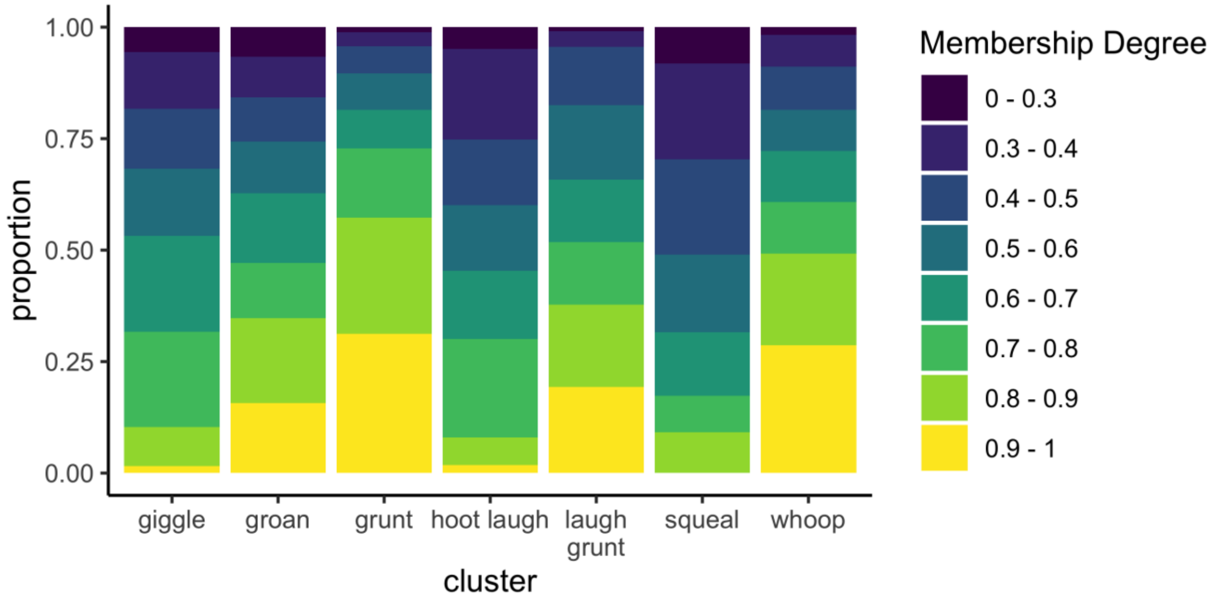

Figure 3.9: Membership degrees of all calls assigned to each cluster, including calls assigned to the noise category. Each call is assigned a membership degree for each cluster. Thus, a call that belonged equally to all clusters would be assigned a membership degree of 0.143 for each of the seven clusters. The higher the membership degree for a cluster, the higher the likelihood the call belongs in that cluster.

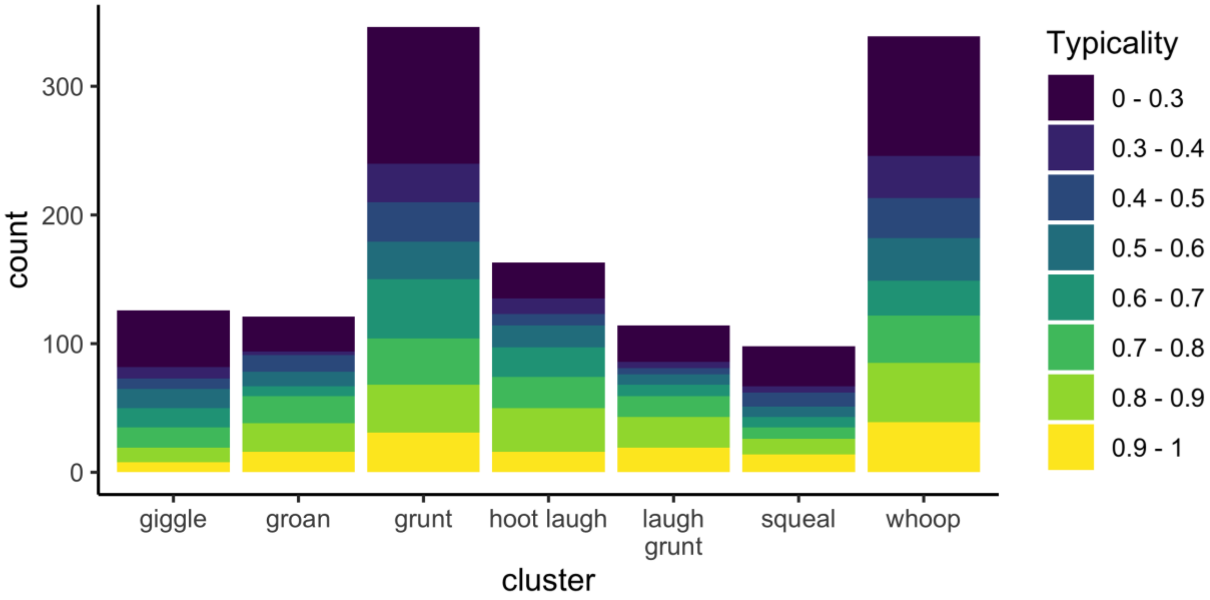

Figure 3.10: Typicality measure for calls in each cluster. Typicality is calculated by subtracting the second highest membership degree from the highest membership degree. Thus, calls with a low typicality measure were assigned high membership degrees to two or more clusters. Clusters with calls of higher typicality are more stereotyped and have many similar calls within their main cluster.

Figure 3.11 demonstrates which clusters grade into each other by showing how many calls within a cluster have a high membership degree in a second cluster. The color of the stacked bars indicates the secondary membership of each call in that cluster. For example, the giggle cluster contains calls with a high secondary membership degree in the hoot laugh, laugh grunt, and squeal clusters, while groans and grunts have a large proportion of calls that lie between them.

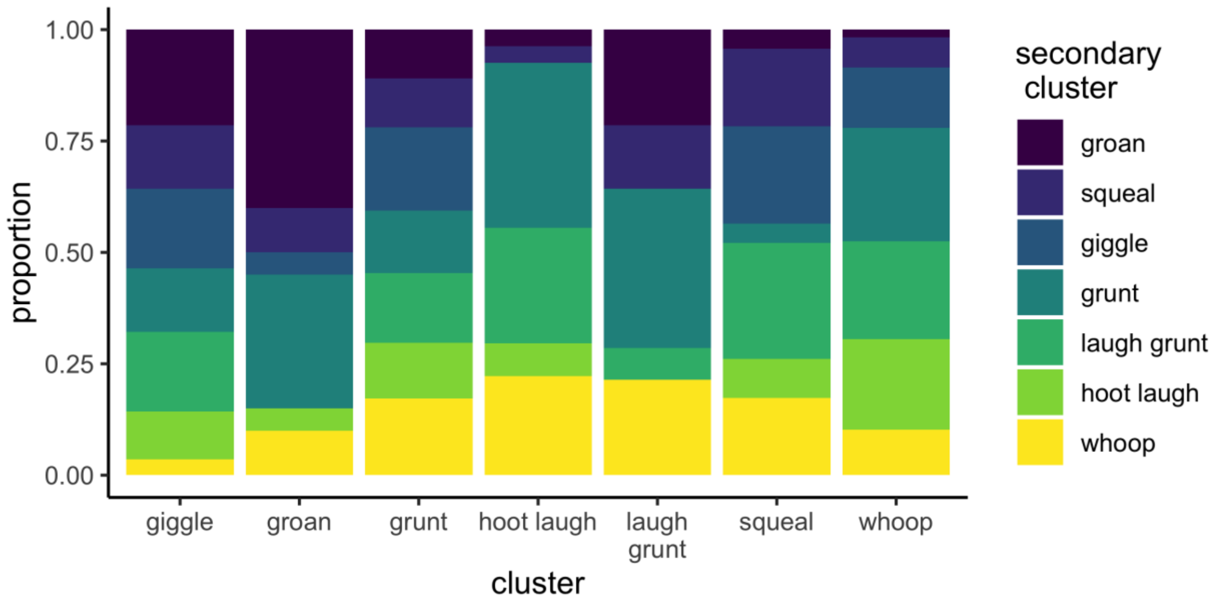

Figure 3.11: The number of calls with a secondary cluster membership greater than 0.25. These calls were assigned high membership degrees to two clusters and represent graded calls between those clusters. This plot shows the number of calls in each cluster that had a high secondary membership. Bars represent the primary cluster membership and color represents the secondary cluster membership. Only calls that had high membership in two clusters are represented here.

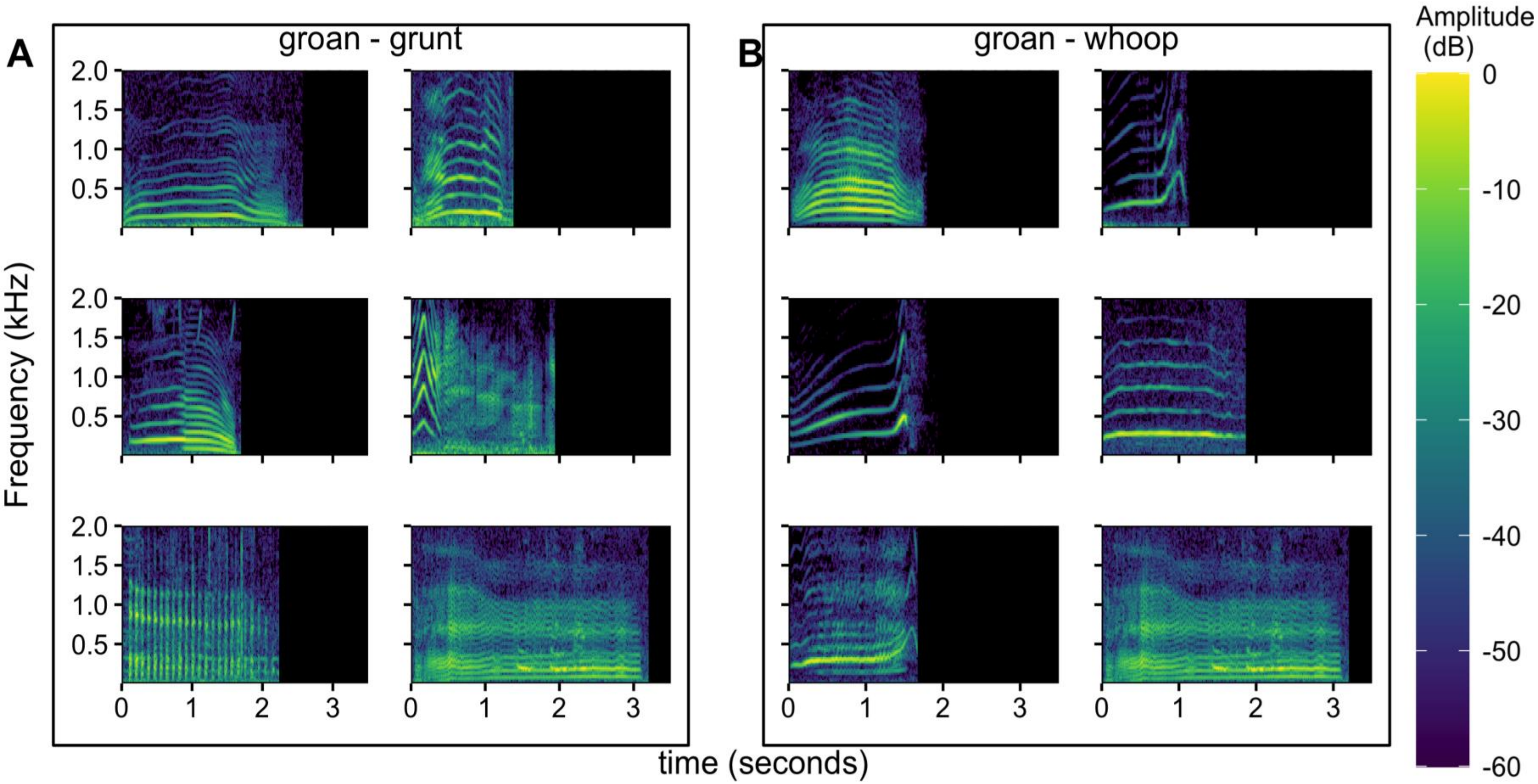

Figure 3.12: Spectrograms of calls with high membership degrees for both grunts and whoops (a) and squeal and whoop (b) clusters.

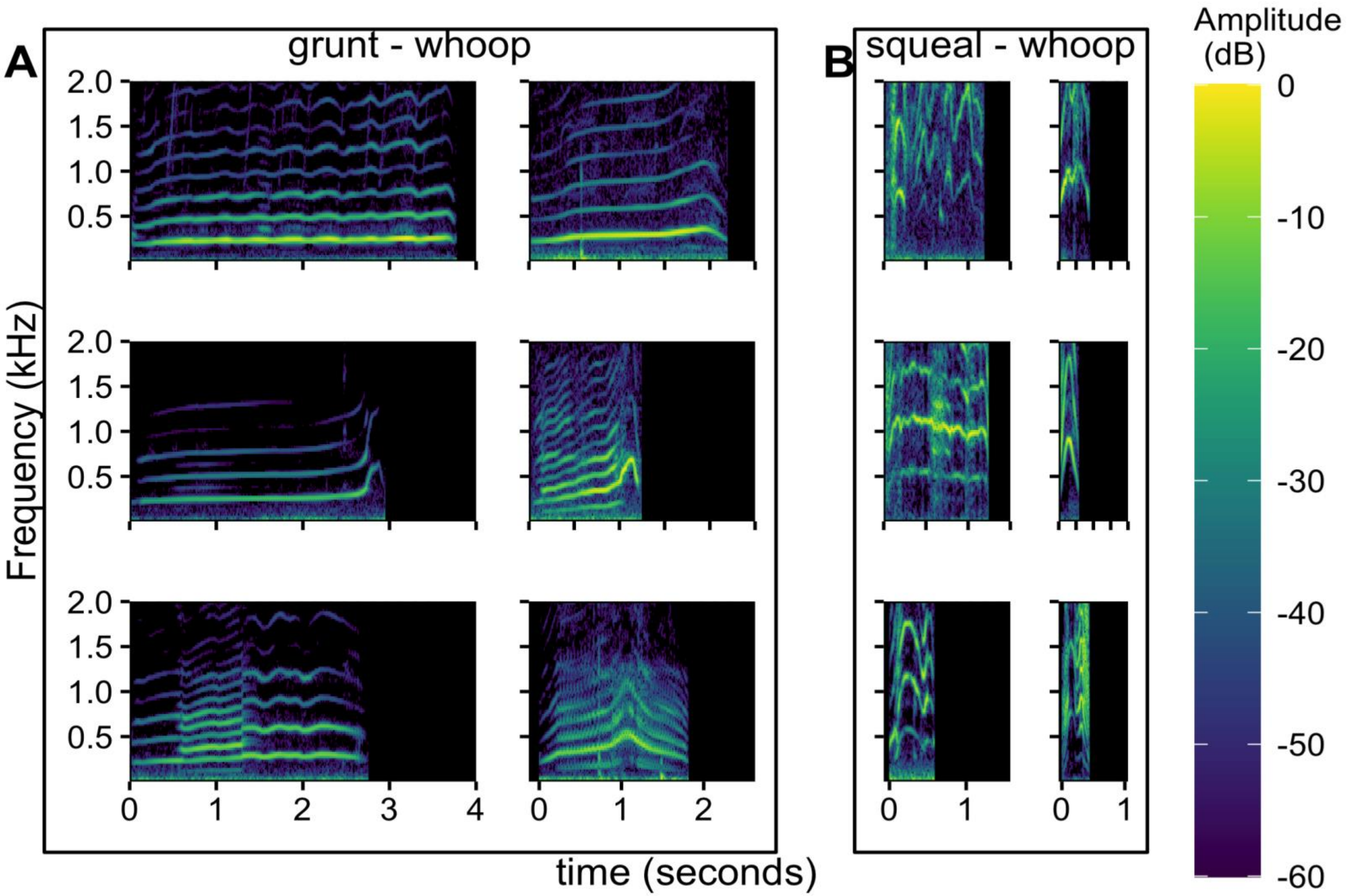

Figure 3.13: Spectrograms of calls with high membership degrees for both grunt and whoop (a) and squeal and whoop (b) clusters.

DISCUSSION

Here we provide the first quantitative description of the vocal repertoire of spotted hyenas. Using 41 measures grouped into 18 principal components, we identified seven call types that agree with previously described categories of calls. Four of these clusters are short vocalizations with medium to high frequencies that correspond to the giggles, squeal, laugh grunt, and hoot laugh, respectively. These were largely recorded in bouts of vocalization while hyenas interacted with one another during agnostic encounters. Two other clusters correspond to the groan and grunt, vocalizations that seem to be directed toward social partners during affiliative interactions. The final cluster corresponds to the whoop, the spotted hyenas long-distance and most well-studied vocalization. Therefore, the unsupervised learning approach we employed here quantified biologically meaningful clusters of vocalizations.

Gradation between call types

This analysis uncovered significant gradation within the spotted hyena's vocal repertoire, in full agreement with Mills' observation that "Most vocalizations of hyaenas grade into related vocalizations: few are discrete" and "..often comprising several sounds in combination with each other, making them particularly difficult to unravel" (1990, pg. 179-182). Discrete signals are predicted to transmit information more reliably as they are easier to distinguish whereas graded calls allow for greater flexibility and perhaps more subtle communication (Marler 1975; Mills 1990). Thus, we expect graded signals when other signaling modalities are available to provide redundancy via multimodal signaling (Marler 1973). Many of hyenas' vocalizations are emitted during close contact when visual, olfactory, and tactile signals can also be transmitted. In fact, hyenas have an impressive postural repertoire, and we know there is a great deal of information available in their olfactory signals (Theis et al. 2012, 2013). Adding to this modal redundancy, many of these signals are repeated in bouts, potentially providing redundancy through repetition. However, it is not clear whether such modal and repetitive redundancy simply allows for gradation as a byproduct or instead that this gradation was shaped by natural selection by a need to communicate subtle messages to social partners.

Future work will need to answer these questions and many others with carefully designed playback experiments. Importantly, this call type categorization must be validated by the hyenas themselves. Graded signals are perceived as categorical signals in a variety of species and signal modalities (Green et al. 2020), and a species' ability to discriminate calls is tuned to their species-specific repertoire (Zoloth et al. 1979). Computers - although less biased than humans - may tune into specific aspects of the calls that are not relevant to the hyenas themselves, and vice versa. Future work should also build on our classification of call units to determine whether there is information stored in their combination, order, or inter-unit-intervals as we know occurs in whoop bouts (Gersick et al. 2015).

Reconciling classification

Beyond describing the vocal repertoire of the spotted hyena, this work highlights the difficulties inherent in quantitatively describing a graded repertoire. It should be noted that a number of clustering techniques were employed on this set of vocalizations with little success. The fuzzy clustering with noise category method ultimately used here, allowed classification of common vocal units and quantification of the natural gradation between those units (Wadewitz et al. 2015) without requiring *a priori* determination of which recordings constituted call types or variant calls (Phillips and Stirling 2001).

It should be noted there are trade-offs involved with employing this “unbiased” categorization of calls. This analysis does not rely on pre-held conceptions of call types, which are often based on the situation under which the call was emitted. This categorization of hyena call types does not incorporate the contextual information that we see while observing the hyenas. Given the similarities between human and animal perception and information integration (Diehl et al. 2004), it is likely that the animals themselves are using similar intuition that is missing from this analysis. Such pre-held conceptions can lead to two types of error. First, researchers run the risk of assigning two (or more) categories of signal to a single call type, thereby missing potential variation that animals may be perceiving and responding to (Fischer 1998). Second, at the other extreme, it is possible researchers may categorize two call types to a signal that is emitted in two different contexts but ultimately has the same signal structure. This mis-categorization runs the risk of missing the reality that an animal is integrating information from both the signal and context to inform their behavior. Nonetheless, we believe that the work described represents a starting point.

Notably, this method will facilitate the study of unit sequencing in spotted hyena vocalizations (Kershenbaum et al. 2014). Many of these the call types identified in our analysis are emitted in bouts. These range from the long, loud, whoop bouts to the short units of a giggle bout, for which the spotted hyena is often called the “laughing hyena.” By focusing on the short units within these vocalizations we were able to separate out four clusters that grade together. From visual inspection of spectrograms, these call types are interspersed within the same bout. Future work should investigate the order of units within these bouts and whether that order encodes information that hyenas use or serve another function.

There are a number of remaining calls that we may not have captured in this study and our fuzzy clustering paradigm. Two are vocalizations that are almost exclusively emitted by cubs (harsh whine and whine) and so were not included in our analysis of adult calls (but see Figure 3.14). We also did not find clusters corresponding to Kruuk's low, snarl or growl, or corresponding to Mills' roar-growl, and the yell. There appears to be some confusion between Kruuk and Mills' categorization of the 'low' vocalization. Kruuk describes a groan and a yell as two separate vocalizations while Mills combines these in his table and labels them 'low.' Meanwhile, cluster one of our analysis corresponds to Kruuk's groan and Mill's low, in both their verbalized and situational descriptions. Perhaps this discrepancy is due to regional dialects and Mill's population did not emit lows as described by Kruuk. Alternatively, groans and lows lie at either end of a gradation that Kruuk split and Mills lumped. The remaining vocalizations described by Kruuk and Mills and missing from our classification system may be variants of our defined clusters, fall between clusters, were not recorded at high enough quality (i.e. vocalizations from other animals often overlap these), or are not present in our study population.

Finally, even though our dataset included rumbles (Kruuk: soft grunt-laugh)–which Mills describes as a discrete call–this method did not assign them to their own cluster. This is likely because rumbles are the most quiet and low frequency of hyena calls leading to very few high-quality recordings from known individuals and this small number (12) went undetected by the fuzzy clustering algorithm (but see Figure 3.15).

In the end, it is these undetected vocalizations that most epitomize the need for accessible, quantitative description of a species' vocalizations. Without spectrograms or recordings to directly compare to, it is impossible to appropriately integrate new analyses with previous work. Call for sharing repertoires, not just as publication and verbal description with a few spectrograms (unless repertoire is truly discrete). Sounds files should also be made readily available (this has been especially helpful in the study of birds and should become the standard for mammals as well). It is our hope that this quantitative description of the spotted hyena's vocal repertoire will serve as a starting point for future investigation of the function of spotted hyena calls and potential variation among populations, and ultimately, be used to further our understanding of the function of gradation within signaling systems and the development and evolution of the diversity of signals we observe in nature.

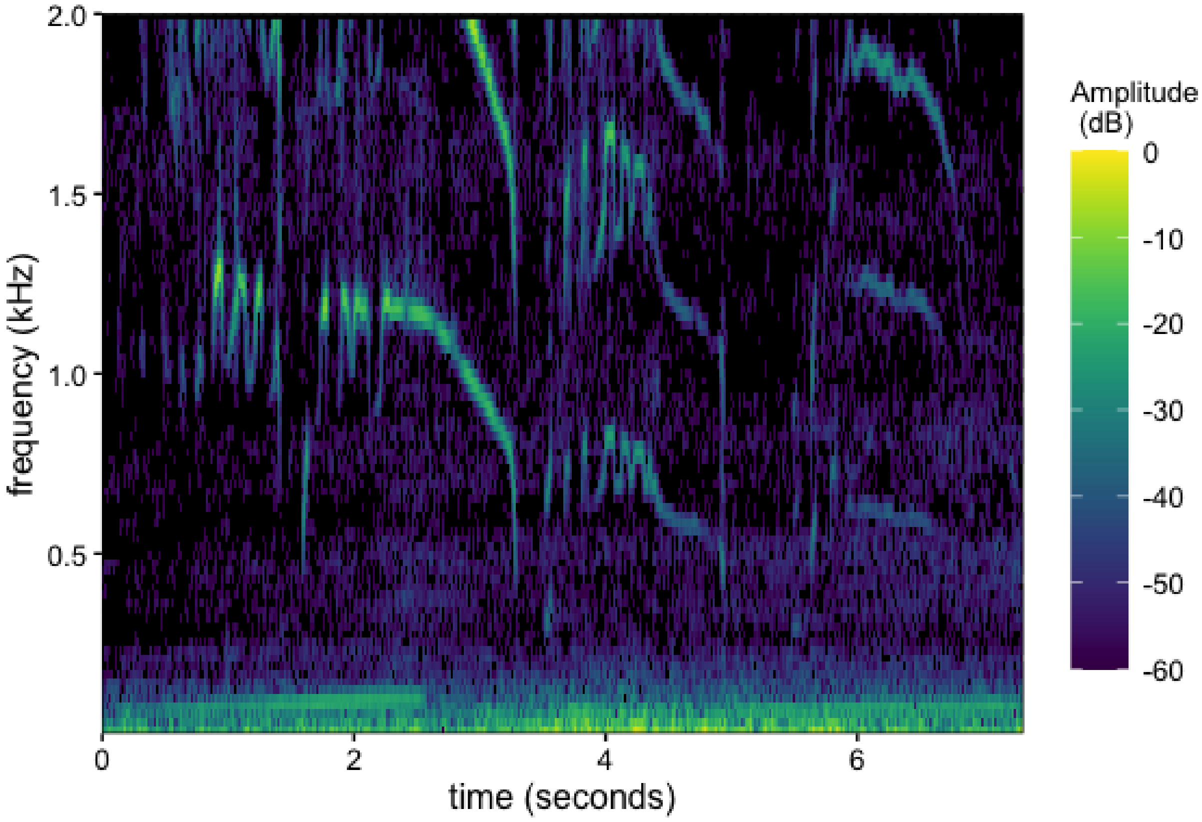

Figure 3.14: Spectrogram of the whine or harsh whine cub vocalization. Most often given during weaning disputes.

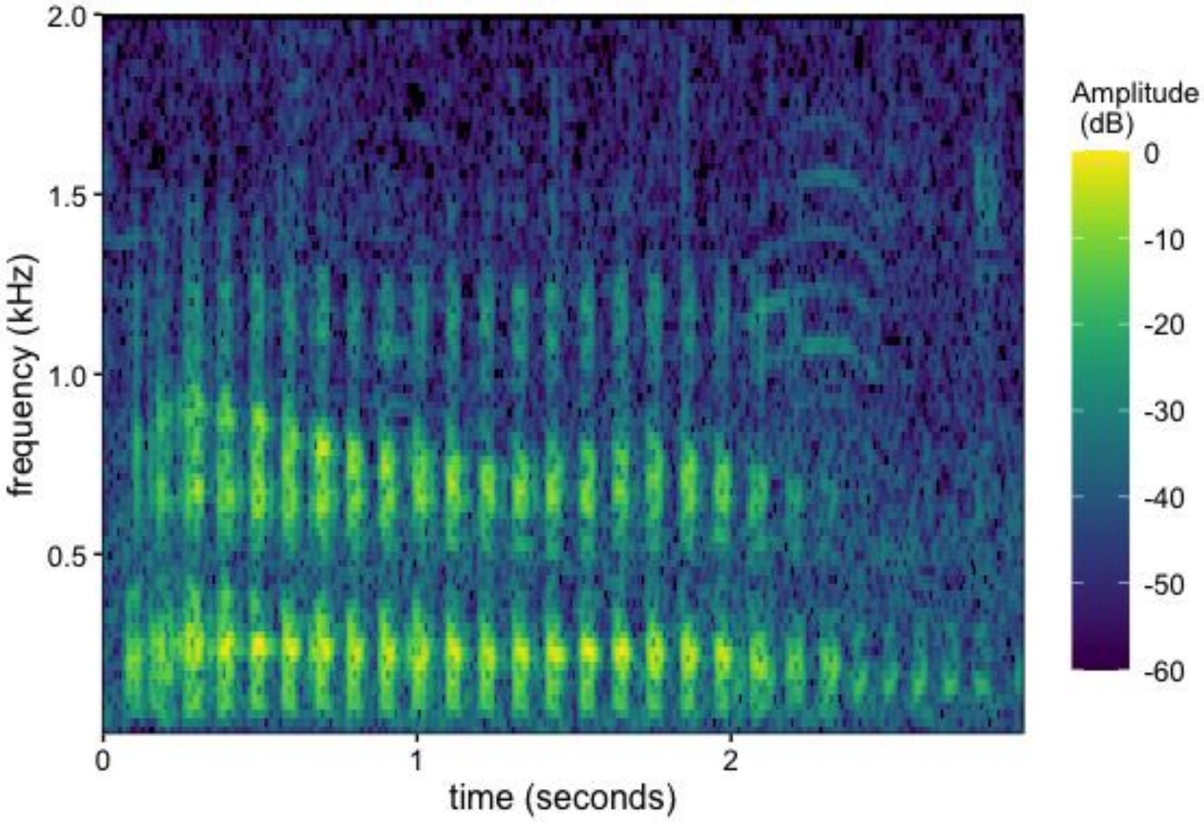

Figure 3.15: Spectrogram of an exemplar rumble.

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CHAPTER 4

THE SPOTTED HYENA'S LONG-DISTANCE VOCALIZATION CONTAINS INDIVIDUAL, BUT NOT GROUP, SIGNATURES

Kenna D. S. Lehmann, Frants Jensen, Andrew Gersick, Ari Strandburg-Peshkin, and Kay E. Holekamp

INTRODUCTION

Signal detection theory (SDT) is a powerful framework within which to investigate the information content and adaptive structure of animal signals. SDT describes how noise is omnipresent in signaling systems and how noise shapes signal evolution, resulting in a suboptimal equilibrium for both signalers and receivers (Green and Swets 1996). In complex animal societies, receivers face a series of categorization tasks after detecting a species-specific signal embedded in noise; in order to respond adaptively to it, they must be able to categorize the signal as relevant or irrelevant to their own interests, determine whether to respond, and decide which responses are appropriate for the given signal (Wiley 2006). These tasks become more difficult as social interactions increase in complexity, the social group grows, or unpredictable variation in environmental noise increases. Signalers have a number of ways to enhance detection in the presence of increased noise (reviewed in Wiley 2006), but signalers are not always able to detect or predict the amount of noise present between themselves and their intended receivers. For example, an individual in a dispersed society, where groupmates are not always in close contact, may not know how far away many of its groupmates are located. How then do signals evolve to be easily detected and distinguishable enough to transfer information among many individuals in a complex, dispersed social group?

SDT can be used to infer how the structure of a signal facilitates the transfer of information and determines the signaler's subsequent categorization tasks, especially under challenging conditions. SDT predicts that signals should 1) encode information with the fewest number of categories necessary for proper function, 2) encode information in features that are robust to the channel's noise, and 3) be structured to maximize detection.

Distinguishing groupmates from non-groupmates is a common social problem often solved by vocal signals. Because fewer categories are easier to detect and categorize, SDT predicts that signals that evolved to distinguish groupmates from non-groupmates should include as few categories as necessary. In many species, this results in one or more signals that contain a group signature. In chimpanzees (*Pan troglodytes*), the long-distance calls of three adjacent groups were more easily distinguished from one another than from a fourth, distant group, suggesting that this call is used to identify nearby callers as groupmates or non-groupmates (Crockford et al. 2004). Green wood hoopoes (*Phoeniculus purpureus*) also have group signatures in their group choruses (Radford 2005), while the entire vocal repertoire of orca whales (*Orcinus orca*) are group-specific (Ford 1991; Yurk et al. 2002) with consistent, but small, individual differences (Nousek et al. 2006). Interestingly, the production of group signatures may be learned and thus require vocal flexibility in production; in many species, individuals must learn new signatures during the course of their lives, as in mated pairs of red crossbills (*Loxia curvirostra* (Sewall 2009)), flocks of chickadees (*Parus atricapillus* (Nowicki 1989)) and foraging groups of spear-nosed bats (*Phyllostomus hastatus* (Boughman 1998)). Thus, group signatures are hypothesized to be a selective force for flexible vocal production (Sewall et al. 2016).

However, the binary categorization of a caller to “group-mate or not” may not always be sufficient, especially when relationships vary among groupmates or change quickly over time. These varied relationships may require the receiver to identify signalers individually in order to respond adaptively to their signals. Where relationships vary among individuals in the group, and where identifying signalers without additional cues is advantageous, SDT suggests that signals should contain an individual signature. Dolphin signature whistles are known to identify individuals (Caldwell et al. 1990), and are used to maintain group cohesion (Janik and Slater 1998). Dingoes (*Canis lupus dingo*) and coyotes (*Canis latrans*) have individually distinct features in their howls (Hallberg 2007; Deaux et al. 2016) whereas wolf (*Canis lupus*) howls include both group (Zaccaroni et al. 2012) and individual signatures (Watson et al. 2018) in their howls. Signals in a set, such as individual signals within a group, become harder to detect as the size of the set increases (Nolte and Jaarsma 1967; Cary and Reder 2003); as a social group grows, the amount of individual categorization required is compounded. Therefore, individual signatures are only expected to evolve if receivers have the ability to remember which individuals are groupmates and if distinguishing among groupmates is necessary to maximize fitness.

Vocal signals that contain individual or group signatures are often long-distance calls. According to SDT, important information is predicted to be encoded in call features that avoid attenuation. Therefore, long-distance acoustic signals should be tonal because pure tones travel better than broadband noise, which is susceptible to scattering (Wiley and Richards 1978). These calls are also expected to be relatively low-frequency and frequency-modulated because high frequencies and amplitude modulations–where the sound level varies over the course of the call or call bout– within a call and attenuate more quickly due to refraction, scattering, and heat loss, although the lowest frequencies may be susceptible to ground attenuation (Marten and Marler 1977; Wiley and Richards 1978). Low frequencies are also easier to locate than high frequencies (Wiley and Richards 1978) and are expected to be used in long-distance calls that advertise the caller's location. Overall, long-distance vocalizations are most easily detected and distinguished if they 1) are tonal, with frequency modulation over a range of frequencies, 2) have redundant amplitude modulations to compensate for attenuation or 3) incorporate both 1 and 2 (Wiley and Richards 1978).

To maximize detection, signalers can increase amplitude, avoid noise either in time or signal space, or increase redundancy in a signal (summarized in Wiley (2006)). Here, we focus on redundancy via repetition, an often-neglected signaling strategy. A common way to increase redundancy is by employing multiple modes to transmit the same or similar information in multiple sensory channels simultaneously (Higham and Hebets 2013). However, long-distance communication is often most effective in the auditory channel, and this often precludes the use of multimodal signaling. Thus, we expect long-distance vocalizations to be literally redundant via repetition.

Here, we investigate the information encoded in the long-distance vocalizations of spotted hyenas (*Crocuta crocuta*), and we inquire how the structure of these signals facilitates transmission of information in a noisy, unpredictable channel. Spotted hyenas are large carnivores that live in social groups, called 'clans,' composed of 3-126 individuals (Holekamp and Dloniak 2010; Green et al. 2018) that often cooperate to defend a large common territory (13-1095 km2, Holekamp and Dloniak (2010)). Female hyenas are philopatric, but most males disperse from their natal clan to join a new clan at 2 to 6 years of age (Kruuk 1972; Frank 1986; Mills 1990; Smale et al. 1997). Each clan contains multiple matrilines of females and their offspring as well as one or more immigrant males that sire most young born (Engh et al. 2002). Hyena society is characterized by fission-fusion dynamics (Aureli et al. 2008; Couzin and Laidre 2009) such that individuals and sub-groups break apart and come together many times throughout a day (Smith et al 2008). Each hyena clan is structured by a strict linear dominance hierarchy (Kruuk 1972), and an individual's priority of access to resources is determined by its social rank. Relationships among clan-mates thus vary based on rank, sex, age, and kinship. Spotted hyenas also have an elaborate vocal repertoire; the complexity of their social environment suggests that SDT predicts that their vocal signals might contain both individual and group signatures.

Spotted hyenas' long-distance call, the whoop vocalization, has multiple hypothesized functions (Kruuk 1972), including coordination of movements by clan-mates within their territory (Gersick et al. 2015), sexual advertisement (East and Hofer 1991a), finding specific groupmates (Holekamp et al. 1999), and territory maintenance (Mills 1990). The whoop vocalization is most often emitted in bouts that range from 2 to 34 whoops (East and Hofer 1991b). These calls are loud and can be heard up to 5 km away (Kruuk 1972; East and Hofer 1991b). Each whoop is a harmonic, frequency modulated, tonal call (Figure 4.1a). The fundamental frequency of a whoop provides reliable information about the caller's general age and, for adult callers, information about sex as well (Theis et al. 2007). Previous research indicates that mothers recognize and respond strongly to the whoops of their young offspring (Holekamp et al. 1999), suggesting that whoops may contain an individual signature and that mothers can identify the voices of their cubs. This individual distinctiveness also appears to extend into adulthood (East and Hofer 1991a). Playback experiments have revealed that hyenas can distinguish among whoops emitted by multiple unfamiliar callers, and that they respond to unfamiliar calls played from inside their clan's territory as they would to a territorial incursion (Benson-Amram et al. 2011). The inter-whoop interval, or the period of silence between whoops within a whoop bout, is shortest in the contexts of social excitement and interspecific competitors, and recordings manipulated to shorten the inter-whoop interval recruit individuals to the caller's location (Theis et al. 2007; Gersick et al. 2015). Thus, whoops appear to encode information about the caller's age, sex, location, affective state, individual identity, and perhaps also group membership.

Here, we use supervised machine learning via random forests to test whether spotted hyenas' long-distance vocalizations contain group or individual signatures, and to identify which call features are important for this discrimination. We use these results to quantify the increase in classification accuracy that results from the repetitive nature of the whoop bout. We then discuss the implications of these findings for signal evolution and acoustic communication in socially complex species.

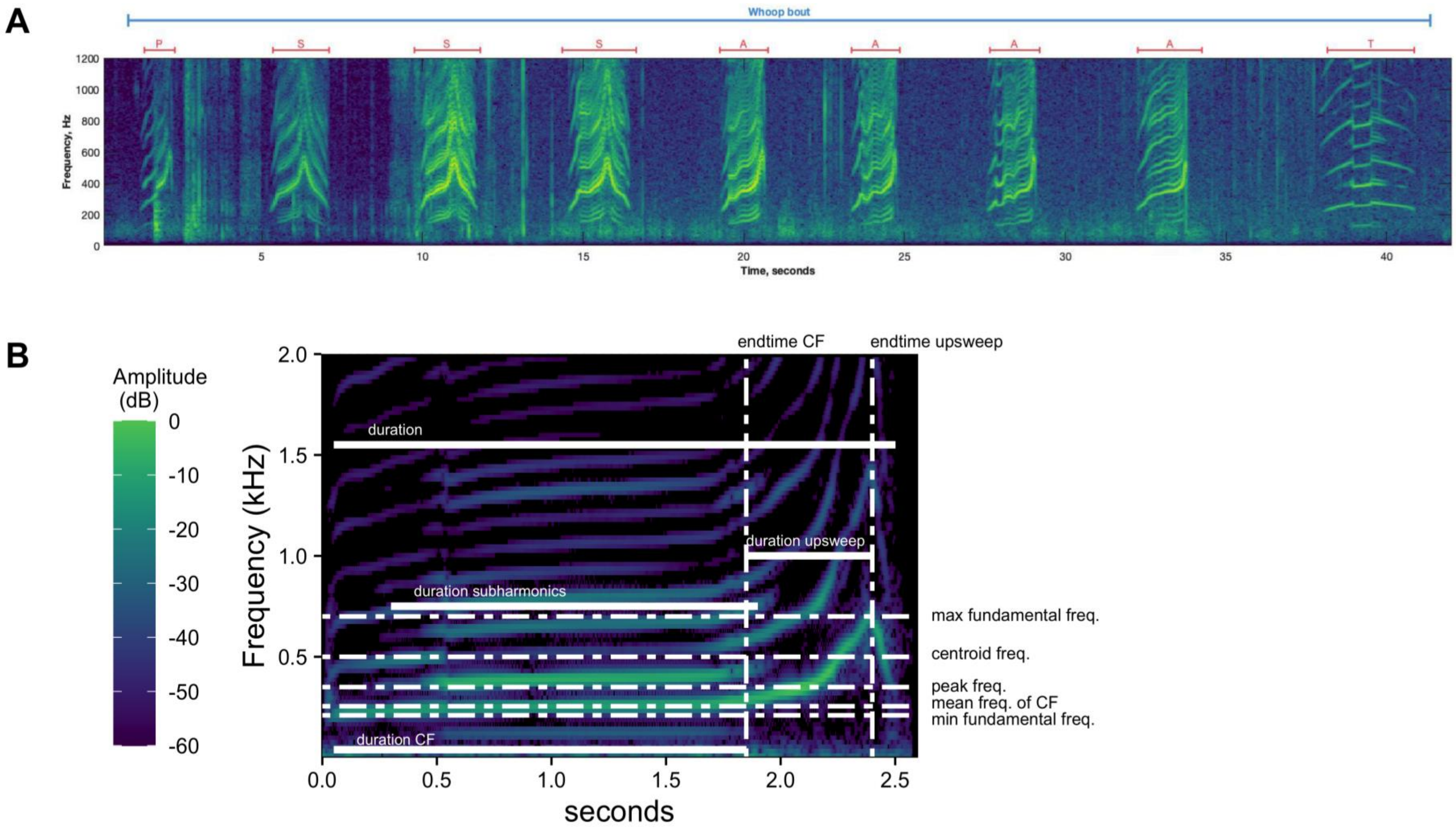


Figure 4.1: Spectrogram of exemplar whoop bout with all four whoop types labelled (a). Call features extracted from each whoop, superimposed on an exemplar A type whoop.

METHODS

Study animals and call recordings

We obtained recordings of whoops emitted by spotted hyenas from four clans monitored by the Mara Hyena Project in the Maasai Mara National Reserve, Kenya. The Talek West (TW) territory lies on the eastern side of the Reserve, approximately 25 km from the remaining three clans which lie adjacent to one another on the western side of the Reserve: Happy Zebra (HZ), Serena South (SS), and Serena North (SN). We identified all members of each clan by their unique spot patterns, assigned birthdates (± 7 days) to natal animals based on cub appearance when first seen (Holekamp et al. 1996), and assigned a sex to each individual based on the shape of its erect phallus (Frank et al. 1990).

We obtained recordings of whoops in two ways. First, from April 2010 to January 2011 and from July 2014 to April 2016, recordings were obtained opportunistically from the window of off-roading vehicles, which are used as mobile blinds. Observers deployed recording equipment once the vehicle was turned off and silent, and aimed the handheld directional microphone (ME-66/K6 and ME-67/K6, sensitivity: 50 mV/Pa, frequency range: 40 Hz–20 kHz62.5 dB; Sennheiser Electronic Corporation, Old Lyme) toward the vocalizing animals within 50 meters. For these recordings, observers used a Marantz PMD661 handheld solid-state recorder (Marantz America, Inc., Mahwah, NJ; Figure 4.2a) at sampling rates of 44.1, 48, or 96 kHz and 16 or 24-bit sampling depths. These recordings were then downsampled to 44.1 kHz and 16-bit sampling depth for consistency. Second, custom-made sound-, movement-, and position-recording Tellus radio collars were deployed on five TW adult females from January 2017 to March 2017 (Figure 4.2b). Each collar consisted of a base Followit Wildlife (Followit AB, Lindesberg, Sweden) Medium Iridium collar with a round, reinforced belting, integrated VHF antenna and a GPS and iridium module for telemetering location and battery state. Each collar was wired to a secondary sound and movement module consisting of a modified digital acoustic tag (DTAG: Johnson and Tyack, 2003, Johnson et al. 2009) connected using a serial cable to a high sample rate Gipsy-5 GPS module (Technosmart Europe, Rome, Italy. This module was placed on the top of the collar and thus located on the back of the neck with the microphone facing forward and protected by an oleophobic acoustic vent (GAW325, 3.2mm ID, W. L. Gore and Associates, Elkton, MD, USA). Collars digitized sound using a sigma-delta ADC with an oversampling rate of x6, for a final 32 kHz sampling rate and 16-bit depth.

Figure 4.2: Recording devices used: shotgun microphone handheld in the off-road vehicles we use as mobile blinds (a). and collars fitted with recording device, VHF, GPS, and accelerometers (b).

We isolated whoop bouts from both types of digital field recordings, noted the time, date, and identity of the calling hyena and matched this information with the age, sex, and clan membership of the caller. We then cut the whoop bouts into single whoops for analysis, using only whoops from adult hyenas that were at least 24 months of age at the time of recording. This ensured that recordings were obtained from hyenas that had completed their physical development (Smith and Holekamp 2019) and eliminated the possibility that young hyenas might not yet have learned a group signature if they have one.

We also assigned each whoop to a whoop type, from the classification scheme provided by East and Hofer (East and Hofer 1991b), but modified to include a fourth category, the preliminary whoop or P type (Figure 4.1a). Preliminary whoops are often emitted at the beginning of the whoop bout and are typically very short relative to the other whoops in the bout. Symmetric (S type) whoops resemble a flattened bell curve, with the peak frequency near the center of the call. In contrast, asymmetric (A type) whoops have a long constant frequency portion that rises to peak frequency toward the end of the call. Terminal (T type) whoops are often the last whoop in a bout. They maintain a relatively constant, low frequency, and are often of lower amplitude than the other whoops in the bout (East and Hofer 1991a).

Sound Analysis

We used our field recordings to create spectrograms (high-pass filter 100 Hz, low-pass filter 10,000 Hz, FFT size of 8291, block size .04 ms, block overlap 0.035 ms) and extracted 6 call features from each whoop using custom functions in MATLAB (MATLAB 2019). In addition, we extracted 11 call features from a trace of the fundamental frequency acquired using a supervised tracing algorithm in MATLAB. Thus, we extracted 17 measurements from each whoop. These whoop features are described in Table 4.1.

Table 4.1: Spectral and temporal features extracted from each whoop.

Abbreviation	Measurement	Units
ID	identity of individual	(categorical)
bout	bout code for each whoop	(categorical)
dur	Duration of call (99% energy criterion)	[seconds]
dur.cf	Duration of constant-frequency (CF) component	[seconds]
dur.upsweep	Duration of upsweep (until max frequency)	[seconds]
dur.subharm	Duration of call with dominant subharmonics (Energy > harmonics)	[seconds]
endtime.cf	End time of CF component relative to call	[fraction of call]
endtime.upsweep	End time of upsweep (max frequency) relative to call	[fraction of call]
freq.centroid	Centroid frequency	[kHz]
freq.peak	Peak frequency	[kHz]
freq.min	Min fundamental frequency	[kHz]
freq.max	Max fundamental frequency	[kHz]
freq.mean.cf	Mean fundamental frequency within CF component	[kHz]
harmonic.ratio.total	Ratio of harmonic to subharmonic energy within entire call	[dB]
harmoic.ratio.fc	Ratio of harmonic to subharmonic energy within CF component	[dB]
mean.entropy	Mean spectral entropy within 99% energy duration	[0(pure tone) to 1(white noise)]
centertime.wobble	Center time of wobble relative to call	[fraction of call]
cpp.mean	Mean of cepstral peak prominence of call	
cpp.sd	Standard deviation of cepstral peak prominence of call	

Random Forest

Random forest classification is a type of supervised machine learning algorithm that uses a set of decision trees (i.e. a 'forest') to classify objects that are represented by measured features of the objects. Each tree in a forest attempts to parsimoniously split the training objects into the correct categories based on the objects' features. The ensemble of trees in a forest is much more accurate than any single tree and once a forest is grown, its accuracy can be calculated with a novel set of pre-labeled objects, or 'test data'. This entire process is then bootstrapped by randomly selecting test and training data for each forest.

To test the clan signature hypothesis without pseudoreplication of individual data, we used all available whoop bouts (n=525; Table B1) and reserved all whoops from a single hyena from each clan for testing data. We then trained the random forest of 500 trees on the remaining data. This resulted in a single hyena's whoops being in either the test or training dataset, but not both (Figure 4.3a). We repeated this 1000 times, with a random selection of the test data each time. Because each random selection of test data creates a different expected proportion correct, we calculated a weighted expectation (WE), which is the expected proportion correct due to chance alone. Because most male hyenas disperse from their natal clan (Smale et al. 1997; Höner et al. 2007), male hyenas may retain their natal group signature instead of learning signature of the clan we recorded them in. We tested this possibility by rerunning the analysis with males only and also with females only. This subsetting of the dataset did not affect the outcome of the analysis and is not discussed further (Figure B1).

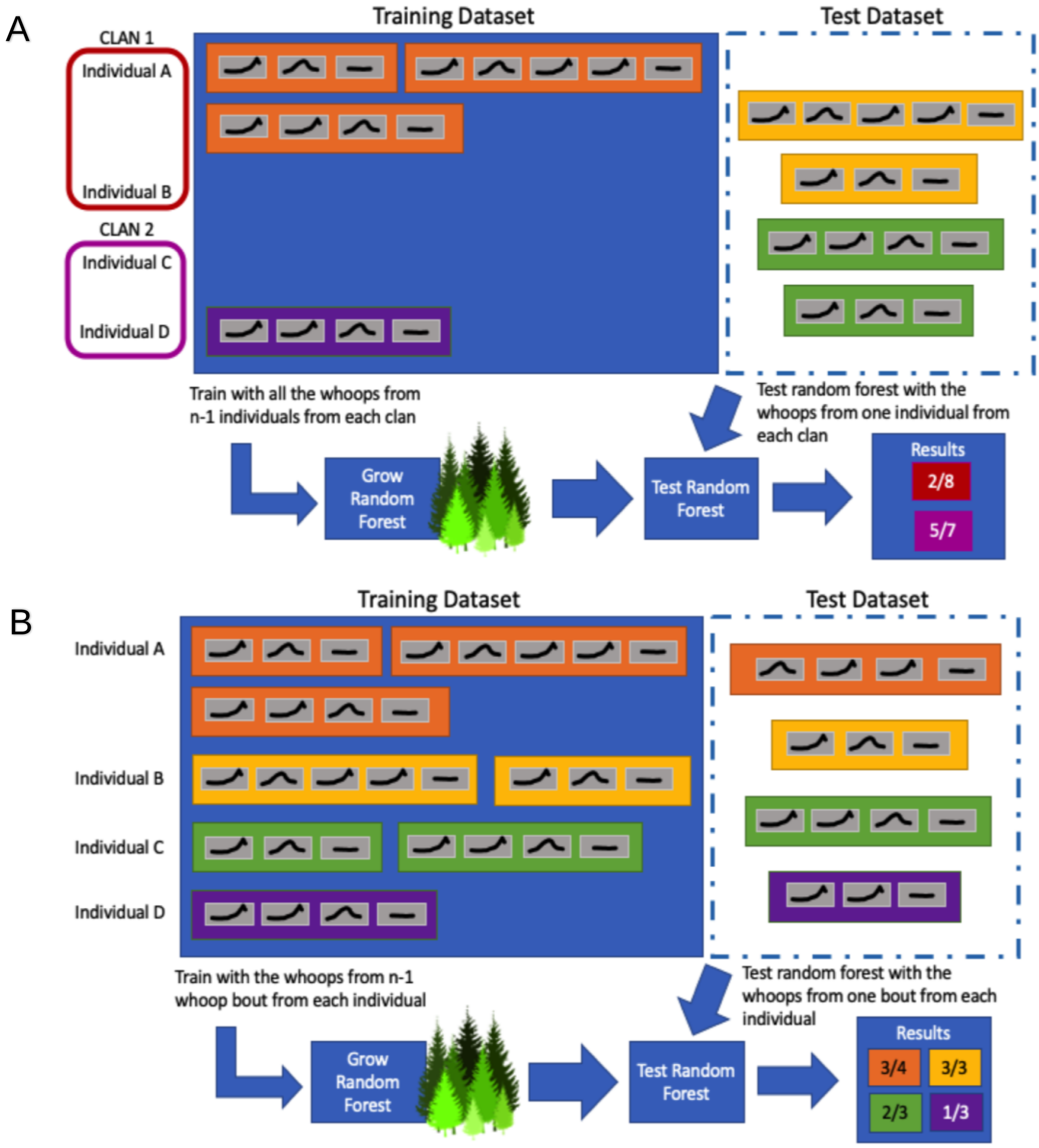


Figure 4.3: Random forest testing and training dataset for clan signature analysis (a) and individual signatures analysis (b). Colored rectangles represent whoop bouts, while the color of the boxes indicate the individual. Clan one is composed of individuals A and B while clan two is composed of individuals C and D. To prevent pseudo-replication of individual in the test for clan signature, we removed all of one individual's whoops from each clan and retained them in the testing dataset. The random forest was then trained with the remaining whoops from each clan. The random forest was trained on single whoops and was blind to the individual and bout. The random forest was then used to predict the clan membership of each whoop and the proportion of correct guesses was recorded. To prevent pseudo-replication of bouts and eliminate the possibility of within-bout similarities affecting the analysis of individual signatures, we removed a single bout from each individual and retained it for testing. The random forest was then trained on the remaining whoops and we used the random forest to predict the caller of each whoop in the test dataset.

To test the individual signature hypothesis, we reduced the dataset to all hyenas that had two or more whoop bouts (and excluded one additional female because we had few recorded whoops from her, Table B1). To prevent the random forest from assigning individual identity based on variation present within a whoop bout instead of common variation among an individual's whoop bouts, we saved one bout from each individual for the test dataset and used the remaining whoops to grow a random forest with 500 trees (Figure 4.3b). We repeated this 1000 times, with a different random selection of test and training data each time. To test the possibility that the random forest's individual accuracy was influenced by the two recording methods, we reran this analysis twice, once on microphone recordings only and a second time on collar recordings only. We also reran the random forest analysis with males and females separated to determine if one sex has more individually distinctive whoops than the other. Neither subsetting of the data affected the outcome of our analysis so they are not discussed further (Figure B2).

For each test, we calculated the proportion of correct guesses by each random forest for the entire dataset and each clan or individual separately, resulting in 1000 estimates of random forest accuracy per test. Next we plotted these estimates to compare their accuracy distributions to the weighted expected proportion correct distributions.

We constructed confusion matrices for the analyses of clan and individual whoops (Figures B3 to B4). For the random forest analyses that achieved accuracies above those expected by chance, we plotted the accuracy of assignment by whoop type. We also reran the random forest analysis, dropping one call feature (Table 4.1) at a time to determine the importance of each feature in classifying whoops to the correct category.

All analyses and figures here were generated in RStudio with R version 3.6.2 (R Core Team 2019) and Bookdown (Xie 2020). We analyzed data using tidyr (Wickham and Henry 2020) and randomForest (Breiman et al. 2018), and created figures using gplots (Warnes et al. 2020), ggplot2 (Wickham et al. 2020), and cowplot (Wilke 2019). Diagrams were created in PowerPoint and colors were generated from viridis (Garnier 2018).

Sequence analysis for repeated whoops within a bout

To investigate how multiple whoops in a bout might reduce a receiver's uncertainty about the identity of the caller, we simulated a receiver's likelihood of assigning a whoop bout to the correct caller, given 1, 2, 3, etc. whoops in the bout. We first determined the proportion of trees in each random forest that voted for each individual for each whoop from each bout and then calculated the mean proportion of correct trees. Thus, for each whoop, we had a distribution of the proportion of trees that assigned each whoop to each individual, with this distribution summing to a total of one. We saved each distribution for use in calculating the expected accuracy with each additional whoop in each bout. We then assumed an uninformed prior with a 1 in 14 possibility that the whoop bout in question belongs to any of the 14 individuals in this analysis. It is important to note that the prior probability that a bout belongs to any individual will be much different for hyenas in the wild as they are likely incorporating information from cues and other sources. Although most spotted hyenas must discriminate between many more than the 14 individuals we distinguish here, they may also likely have prior information regarding which individuals are nearby or in a particular direction. The uninformed prior was next multiplied by the distribution of the first call, first and second calls, first through third calls, etc. With each addition of the distribution belonging to the next whoop in the bout, the resulting new distribution was normalized, such that the proportions totaled to one. For these simulations, the number of whoops and their order in the bout was maintained. We then plotted the expected proportion of correct guesses for each whoop bout with as many whoops as the bout contained.

RESULTS

The random forest for assigning clan membership was not more accurate than expected by chance (Figure 4.4, mean: 0.36, sd: 0.172, chance: 0.26). In contrast, the random forest for assigning individual identity was much more accurate than expected by chance (Figure 4.5, mean: 0.44, sd: 0.054, chance: 0.07), although the accuracy of assignment to individual varied among the individual callers (Figure 4.5). The accuracy of individual assignment also varied with whoop type (A, S, T or P), but whoop type variation is likely explained by the frequency of whoop types in the dataset (Figure 4.6).

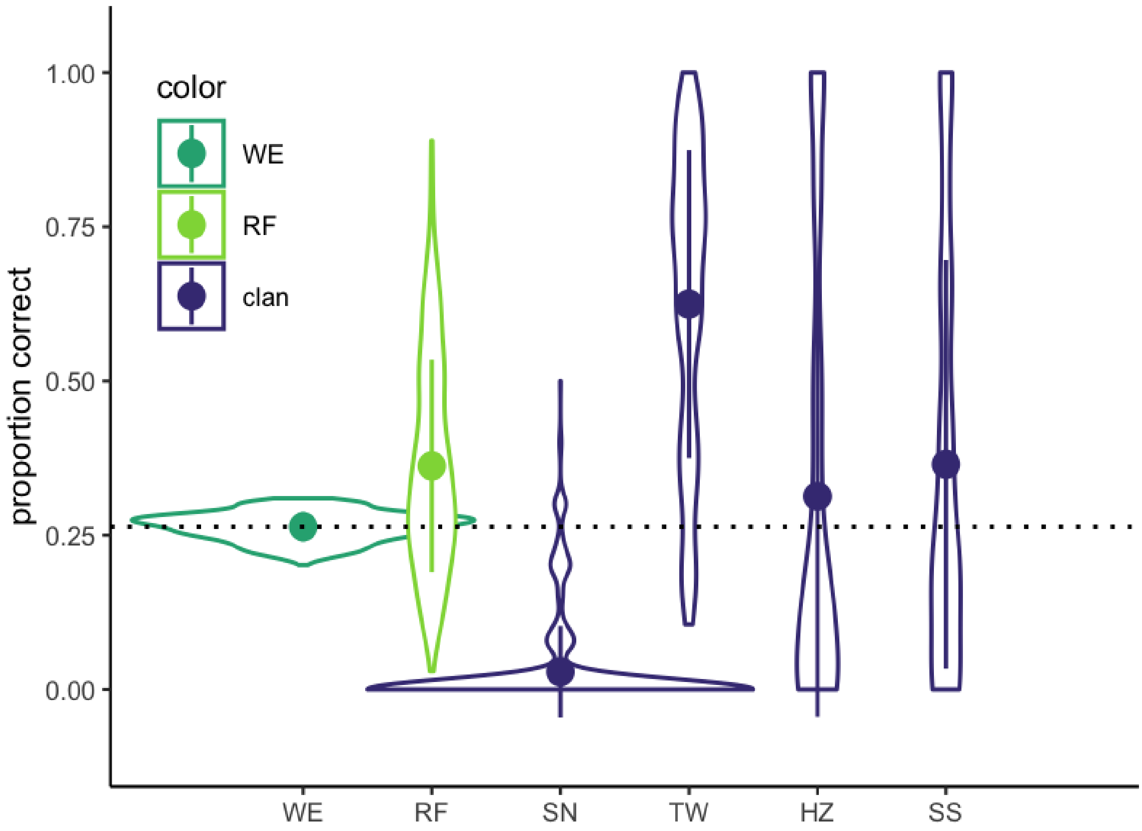


Figure 4.4: Violin plots of proportion of test data correctly assigned to clan for random weighted expectation (WE), performance of the random forest overall (RF), and random forest accuracy for each hyena clan. Each violin plot represents the results from the same 1000 random forests, thus each random forest is represented six times, once in each violin. Points and bars represent means and standard deviations of random forest accuracy. Dotted line indicates mean random weighted expectation.

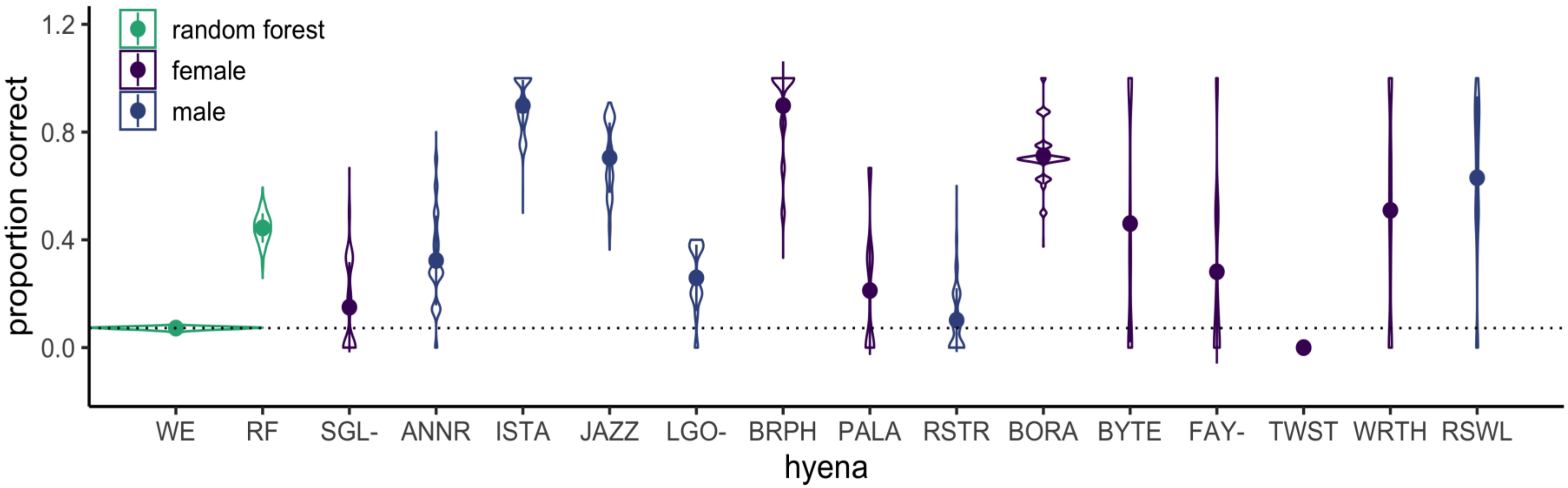


Figure 4.5: Violin plots showing the spread of proportion of correct guesses from 1000 random forests. Each random forest is represented in each violin, showing the distributions of the proportion correct from the weighted expectation of random guessing (WE), overall random forest (RF), and random forest accuracy for individual hyenas. Points and bars represent means and standard deviations of random forest accuracy. Dotted line indicates mean random weighted expectation.

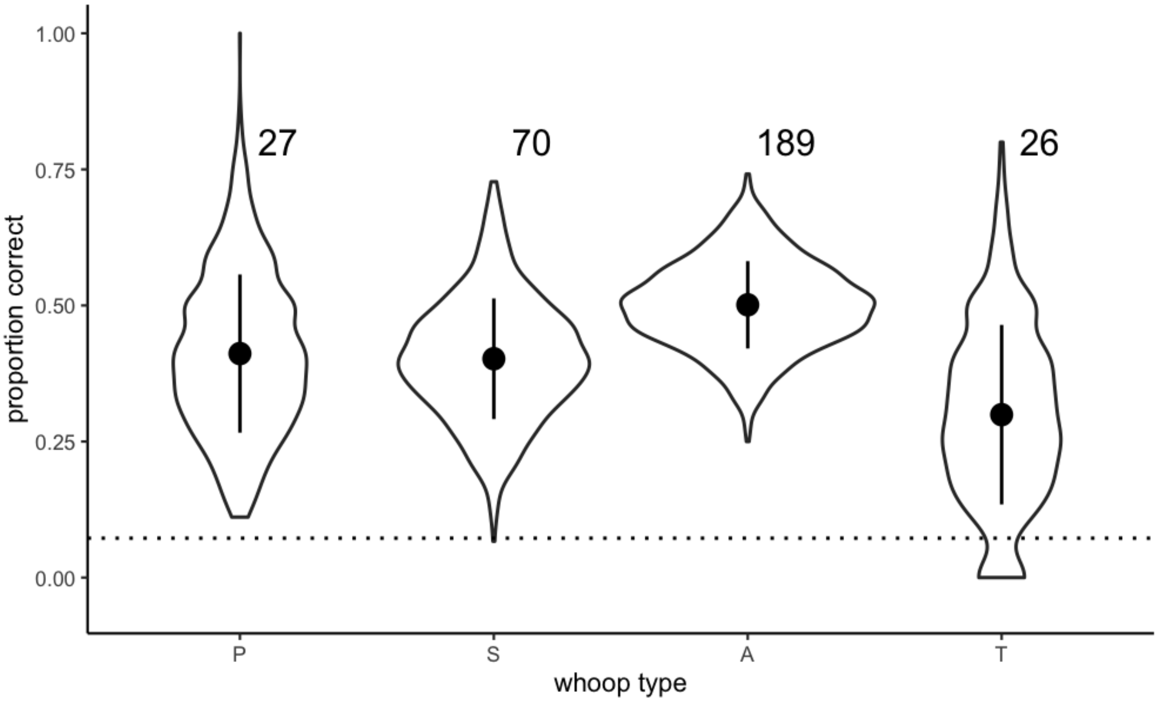


Figure 4.6: The proportion of correct guesses from 1000 random forests predicting individual, tallied by whoop type.

Some call features were more important than others for predicting the correct individual (Figure 4.7). The features included frequency measures of multiple portions of the whoop, with the most important being the frequency of the flat, initial portion of the whoop. The constant frequency portion is protracted in A type whoops. Unfortunately, we cannot disentangle whether this is a result of A whoops being over-represented in the dataset or whether A whoops are more common because their constant frequency portion is a better indicator of individual identity. The importance of the entropy and CPP measures suggest hyenas could also attend to the disorder or dysphonia within an individual's vocal signature. While these measures are unlikely to transmit over long distances, they are a common identifier in the voices of a number of species (Shapiro 2010).

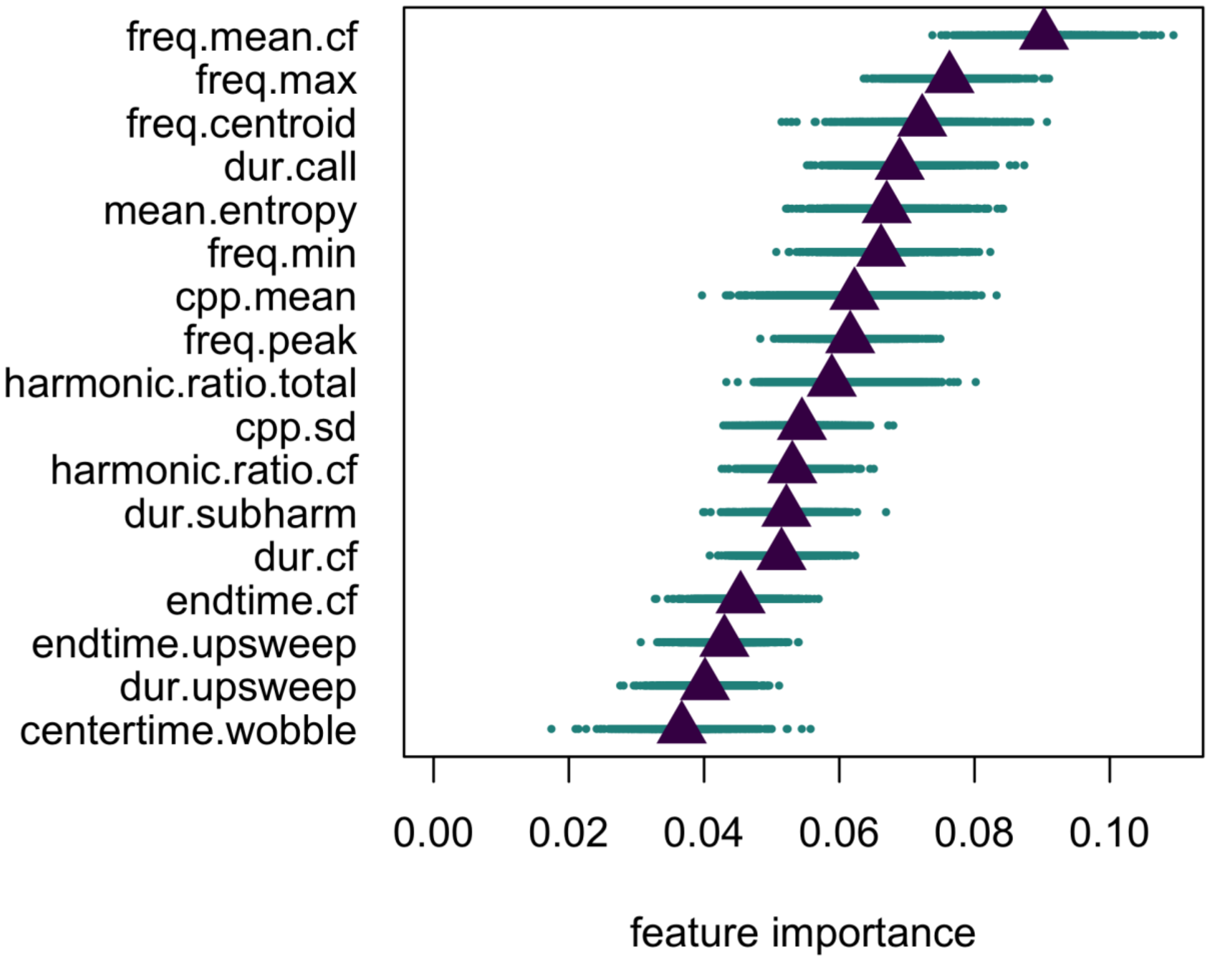


Figure 4.7: The importance of each whoop feature. Feature importance is measured as the decrease in normalized mean proportion of accuracy decreases after the feature is removed from the random forest analysis. See Table 4.1 for full feature names and descriptions.

Our analysis of whoop repetition within a whoop bout supported the hypothesis that the repetitive nature of the whoop bout increases receiver certainty about the identity of the caller. With more whoops in a bout, the proportion of correct guesses approaches 1 for most whoop bouts (Figure 4.8). In some cases, as few as two whoops are needed to achieve greater than eighty percent accuracy and five whoops are sufficient for 66.7 percent of the whoop bouts to achieve an assignment accuracy greater than ninety percent. In contrast, some bouts do dip in accuracy toward the end of the bout. This may be explained by the presence of T whoops at the end of most bouts, and raises the question: What is the function of the terminal whoop, or does it even have a specific function?

The variation in individual identification accuracy (Figure 4.5) is also apparent in in the whoop bouts that reach zero percent accuracy. This may be a product of our recordings dataset, or it could reflect real challenges that hyenas face in the wild. Some individuals may benefit from being more difficult to identify, or the acoustic space may not be large enough to accommodate a large number of distinct signatures. Further, hyenas likely need to distinguish themselves from their clan mates and individuals in neighboring clans, but they do not need to be distinguishable from individuals many miles away (as, for example, SS, SN, and HZ are from TW).

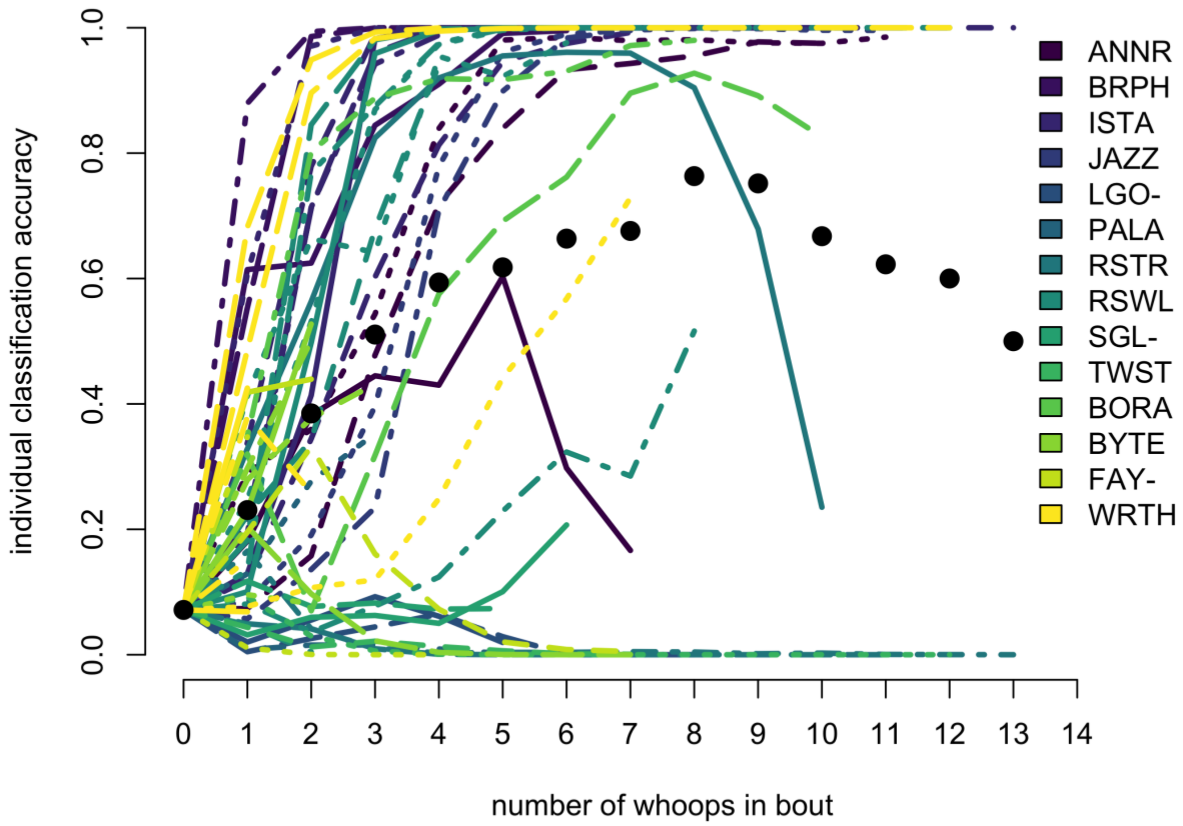


Figure 4.8: The expected proportion of correct guesses of caller identity improves with number of whoops examined in the bout. Each line represents a single whoop bout from a particular individual. Individual is indicated by line color. Different whoop bouts are indicated by line type. Whoop order was maintained in the bout. Proportions correct were calculated from an aggregation of all votes from all 1000 random forests.

DISCUSSION

Animals are expected to use cues and signals to gain information and reduce their uncertainty about the surrounding environment and social interactions (Dall et al. 2005). For social species with fission-fusion dynamics that range over large areas, such as lions, spotted hyenas, capuchin monkeys and chimpanzees, long-distance vocalizations represent an important way to maintain contact and coordinate movements (Spehar and Di Fiore 2013; Gersick et al. 2015). Here we used the principles of signal detection theory to investigate the whoop, a long-distance vocalization in the complex fission-fusion society of the spotted hyena. We found evidence that whoops contain an individual signature, but not a group signature, suggesting the identity of the whooping hyena is salient information for receivers. Frequency, duration, and level of dysphonia are the most important call features for distinguishing the caller's identity and likely facilitate the transfer of information over long distances. These call features are repeated in the whoop bout and increase the accuracy of identifying the caller. Together, these findings contribute to our understanding of the evolution of vocal flexibility and communication signals in general.

Group signatures

SDT predicts that species to evolve signals that meet their minimum needs (Wiley 2013) while using as few categories of signals as possible to maximize detection and discrimination (Wiley 2006). If whoops only functioned to coordinate and recruit at the group level, hyenas would only need to distinguish the group membership of the caller and it would be surprising to find an individual signature in such calls. Instead, we found that the hyena's long-distance whoops contain significant acoustic variation among individuals, but not among clans. The lack of a group signatures in spotted hyena whoops suggests that a simple 'group-mate or not' classification is insufficient for spotted hyenas to respond adaptively to whoop vocalizations. Instead, we suspect that the complex and varied nature of social relationships in this species requires individual identification of callers, be they allies, kin, or otherwise. These individual signatures then provide the requisite group membership information, thus obviating the need for a group signature in spotted hyenas.

This lack of a group signature also has implications for the evolution of vocal plasticity and learning in this species. The "signaling group membership hypothesis" posits that selection pressures favoring the evolution of a group signature also favored the evolution of learned control of flexible vocal production (Sewall et al. 2016), a relatively rare trait in animals (Seyfarth and Cheney 2010). Without this, an individual would be unable to learn and produce a new group signature after changing groups, as occurs in bats (Boughman 1998). Female hyenas are philopatric, but the majority of males emigrate after reaching sexual maturity (Smale et al. 1997; Höner et al. 2007). Males would thus need to learn how to produce the group signature of their new clan. Instead, it appears that male hyenas must learn an entire new suite of voices after dispersal. Overall, our work fails to support the "signaling group membership hypothesis" of vocal learning (see also (Smith-Vidaurre et al. 2020)).

Individual signatures

Although our results suggest hyenas do not need flexible vocal production to produce a group signature, the individual signatures we detected suggest these animals are capable of associative learning and flexible vocal comprehension (Seyfarth and Cheney 2010). We do expect individual recognition in social species (Dale et al. 2001), especially in large groups with both differentiated relationships within groups and competition among groups (Tibbetts and Dale 2007). In such groups, individual recognition represents the basis of social cognition (Seyfarth and Cheney 2015) because it is necessary for receivers to tailor their response to the current situation (Reeve 1989) and to the signaling individual (Johnstone 1997; Tibbetts and Dale 2007; Wiley 2013). This may mean only responding to the calls of offspring (Aubin et al. 2000; Charrier et al. 2002; Bohn et al. 2007) or alarm calls of reliable signalers (Cheney and Seyfarth 1988; Blumstein et al. 2004).

Individual signatures represent a significant cognitive load, as these signals require receivers to develop and maintain a template for each of their groupmates. Nevertheless, some species are clearly capable of recognizing individual callers and even associating them with traits lying on multiple axes (such as rank and kinship

(Bergman et al. 2003; Schino et al. 2006)). Our data suggest that spotted hyenas have distinctive signatures that can be used to recognize individuals, and past work indicates that spotted hyenas have the cognitive capacity to recognize and remember individuals even in their large (100+) social groups. Although it remains possible that these animals do not actually use the identity information we detected here, as also occurs in meerkats (Schibler and Manser 2007), this seems unlikely given previous studies indicating that hyenas are able to discriminate the number and identity of callers (East and Hofer 1991a; Holekamp et al. 1999; Benson-Amram et al. 2011; Gersick et al. 2015). However, playbacks are necessary to determine what portions of the whoops hyenas use to distinguish callers, and whether they associate rank, kinship, or other traits with that individual and their template.

Call features

The call features that were most important for discrimination of individuals included a number of frequency measures (mean frequency of the constant frequency portion of the whoop, maximum and minimum frequency of the fundamental, and centroid frequency), call duration, and measures of noisiness (entropy) and dysphonia (CPP mean). Frequency measures are common identifiers in other species with individual vocal recognition (Shapiro 2010). The importance of the mean frequency in the constant-frequency portion of the whoop may be attributed to the fact that higher frequencies attenuate more and are more difficult to locate than low frequencies due to scattering in open habitats (Wiley and Richards 1978). In addition, the frequency modulation captured by the multiple frequency measures may increase detectability due to the energy concentrated at a narrow bandwidth that changes over the duration of the call (Ryan 1985; Bosch and De La Riva 2004). Long-distance vocal signals often contain frequency modulation, amplitude modulation, or both (Alexander 1967; Marler

1969, 1972, 1973; Waser and Waser 1977), as seen here in hyena whoop bouts. Each whoop contains modulation over a wide range of frequencies while the repetition of the whoops and inter-whoop intervals together represent an amplitude modulation that is distinct enough to travel over long distances.

These features of the hyena whoop bout may serve a second function, which is to facilitate locating the caller. Complex, repeated calls with a wide frequency range are expected to facilitate location of the sound source (Marler 1955). Indeed, East and Hofer (1991a) noted this as a possible function of the structure of hyena whoop bouts. The high frequency portions, which degrade more quickly, may allow a receiver to ascertain the distance of the caller from it while the low frequency portions of the call ensure it reaches as many receivers as possible. The repetition of whoops within the bout also provides multiple opportunities for receivers to localize the caller (Tenaza and Tilson 1977) while coding information within the tonic features of the bout, specifically the inter-whoop-interval (Gersick et al. 2015). The information stored in the amplitude modulation of the bout requires considerable redundancy as amplitude modulations are prone to degradation over long distances (Wiley and Richards 1978). Given the heterogeneity of hyena social relationships, it makes sense that calls used to located individuals would also encode information about the identity of the caller.

Call redundancy

This redundancy through repetition within a bout likely increases the probability of detection and the receiver’s ability to identify the caller. This notion was supported by our calculations of increasing classification accuracies over the course of most whoop bouts. In a perfect system, this redundancy would be unnecessary, but the unavoidable noise in animal communication systems requires some level of redundancy. In systems where signalers are unable to predict the amount of noise between themselves and their receivers - in this case, due to incomplete knowledge of receiver locations - additional repetition ensures the message is received. Such redundancy also allows for subsequent divergence between repeated elements and may allow the evolution of a derived element for a new purpose. For example, whoop bouts often start with a truncated whoop, a simple tonal call that may serve as an alerting component (Richards 1981). Thus, it is possible that each whoop type within a bout conveys a different kind of information. Unfortunately, our sample size was not large enough to directly test that here.

Whoop bouts themselves may also be repeated at intervals to reinforce signal memory and facilitate future individual recognition. A large proportion (47.1% (Theis et al. 2007) to 60% (Mills 1990)) of whoop bouts are "spontaneous" or "slow" and do not appear to recruit individuals (Gersick et al. 2015), suggesting they serve an additional function. We concur with East and Hofer's (1991a) hypothesis that spontaneous whoops display the identity and location of the caller, and suggest one further function: these bouts may reinforce the templates, or mental representations, of receivers within hearing distance (Guilford and Dawkins 1991; Wiley 2006). Such memory reinforcement should improve future detection and discrimination as it does in humans (Wiley 2006). This function of spontaneous whoops may be especially important given that receivers must discriminate among many groupmates.

While there have been a number of studies on increased redundancy in calls due to increased noise in the environment, to our knowledge no studies have previously attempted to quantify the increase in accuracy of information transfer as the redundancy of the signal increases. There is an important push in animal behavior to investigate signal redundancy in multimodal signals (Higham and Hebets 2013; Partan 2013), especially when studying the interaction between social and communicative complexity (Peckre et al. 2019). We suggest this should also extend to redundancy over time because animals are constantly integrating signals and new information into their decisions.

CONCLUSION

Overall, our study emphasizes how social complexity via heterogeneous social relationships ("relational complexity"; (Lukas and Clutton-Brock 2018)) relates to evolution of vocal flexibility and the structure and function of acoustic calls. Signaling group membership in the whoop would require learned control of flexible vocal production. Either this control is beyond the hyena vocal apparatus or, more likely, a group membership is extraneous information. Instead, hyena whoops encode caller identity, providing receivers with both group membership and the opportunity to adjust their behavior based on their social relationship with the caller. Spotted hyenas show social preferences for certain groupmates based on kinship and dominance (Holekamp et al. 1997; Smith et al. 2006), and social alliances can restructure the social hierarchy (Vullioud et al. 2019) to influence rank and fitness (Strauss and Holekamp 2019). However, in order to socialize with a preferred groupmate or benefit from social alliances, a hyena must first find these social partners, despite large-scale dispersion in space and time. Therefore, these long-distance calls encoding individual identity may be crucial to the functioning of hyena societies, allowing group members to manage numerous social relationships occurring over large spatial scales. Matching predictions of SDT, this process is facilitated by acoustic structures that improve the efficacy of signal transmission over long distances and are encoded redundantly to increase the accuracy of detection and discrimination. Our results are also consistent with the hypothesis (Beecher 1989) that individual signatures in vocal calls are tied to the evolution of differentiated social relationships in complex societies.

APPENDIX

Table B1: Summary of datasets used in random forest analyses. All whoops were used in the analysis of clan signatures and individuals with two or more whoop bouts were used in the analysis of individual signatures. Bolded individuals' recordings were obtained from recording collars

hyena	**clan**	**status & sex**	**bout #**	**whoops per bout**
JLYR	HZ	natal female	1	8
PIKE	HZ	natal female	1	6
SGL-	HZ	natal female	2	5,6
SILK	HZ	natal female	1	1
SNAP	HZ	natal female	1	8
ANNR	HZ	imm. male	3	7,10,11
ISTA	HZ	imm. male	6	7,6,4,10,13,9
JAZZ	HZ	imm. male	2	11,7
PGLG	HZ	imm. male	1	5
TEMP	HZ	imm. male	1	7
ANGI	SN	natal female	1	8
JONI	SN	natal female	1	4
LOGC	SN	natal female	1	9
RBC-	SN	natal female	1	12
RMON	SN	natal female	1	6
SHRM	SN	natal female	1	1
LGO-	SN	imm. male	2	8,5
LPR-	SN	imm. male	1	1
RALI	SN	imm. male	1	10
SST-	SN	natal male	1	2
BADG	SS	natal female	1	7
BBW-	SS	natal female	1	6
BRPH	SS	natal female	3	6,8,8
GRIM	SS	natal female	1	6
JAVA	SS	natal female	1	10
KOMO	SS	natal female	1	7
KS--	SS	natal female	1	5
MTN-	SS	natal female	1	8
PALA	SS	natal female	2	6,3
TAJ-	SS	natal female	1	6
DEE-	SS	imm. male	1	6
ONEK	SS	imm. male	1	5

Table B1 (cont'd)

RSTR	SS	imm. male	3	11,13,10
BORA	**TW**	**natal female**	**2**	**8,10**
BYTE	**TW**	**natal female**	**5**	**2,7,2,3,2**
FAY-	**TW**	**natal female**	**4**	**2,7,4,1**
HRPY	TW	natal female	1	5
MGTA	**TW**	**natal female**	**2**	**4,2**
ROOS	TW	natal female	1	12
TWST	TW	natal female	2	7,12
WRTH	**TW**	**natal female**	**7**	**1,12,1,7,4,1,2**
DDMA	TW	imm. male	1	8
RSWL	TW	imm. male	8	6,8,2,8,12,9,7,4
ZITI	TW	natal male	1	5

Bolded lines are data obtained from recording collars.

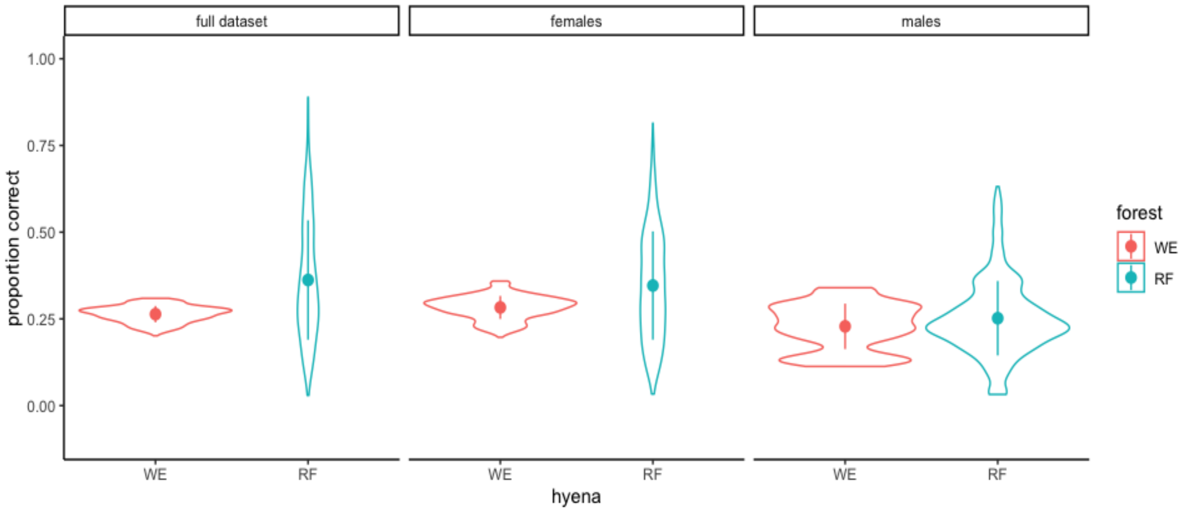


Figure B1: Violin plot of the accuracies of 1000 random forests identifying clan compared to the weighted accuracy expected by random guess for the full dataset, female vocalizations only, and male vocalizations only.

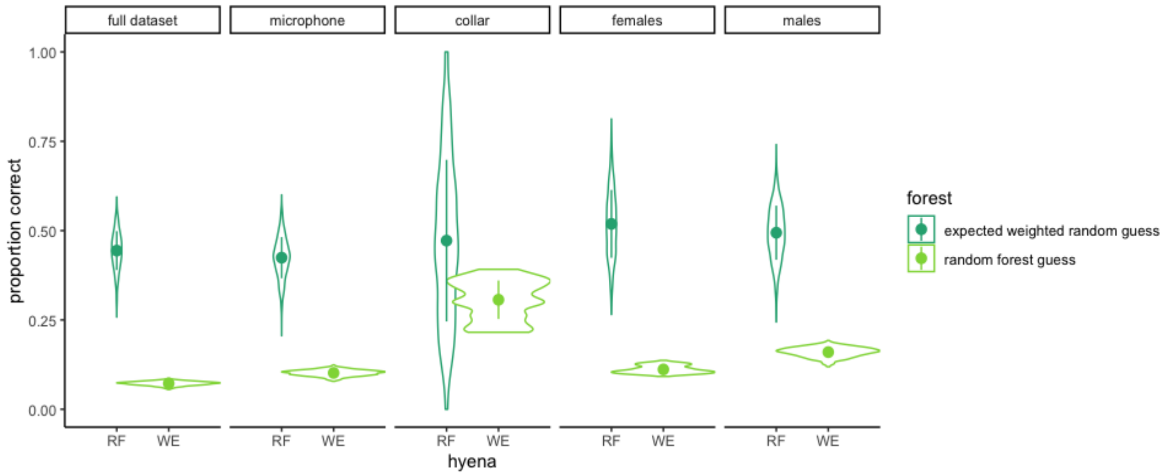
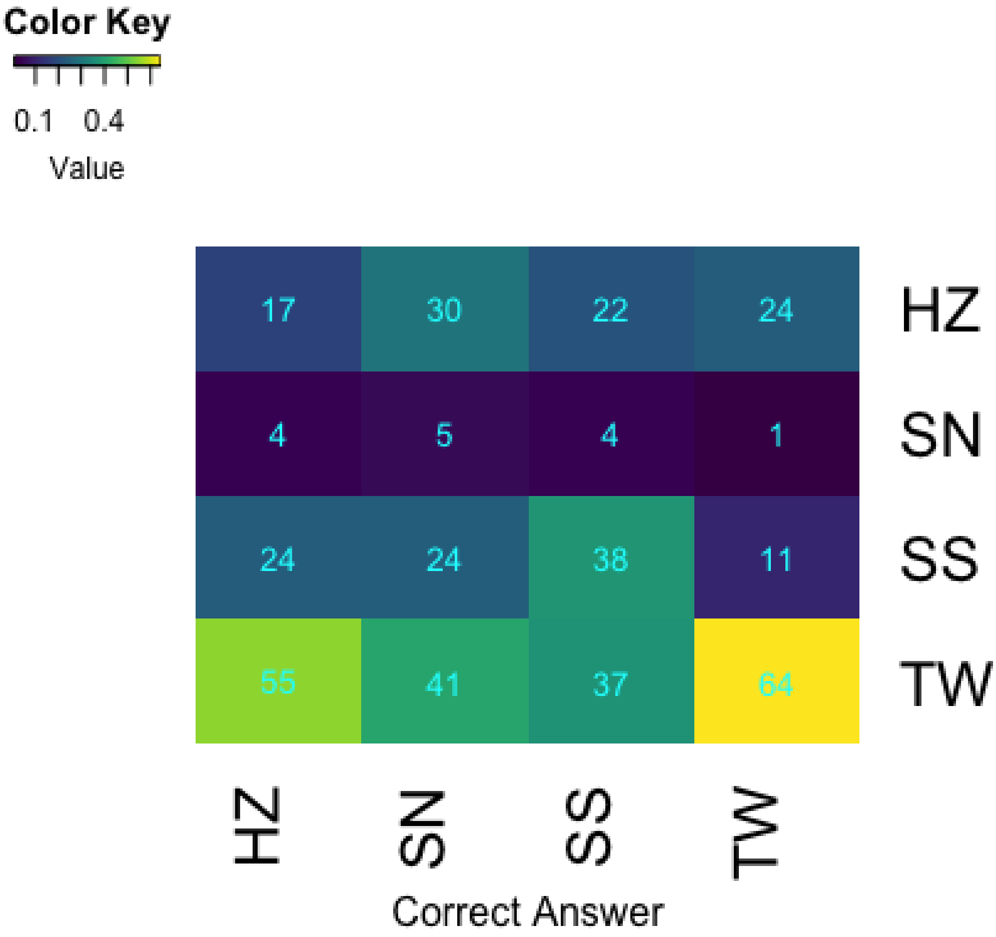
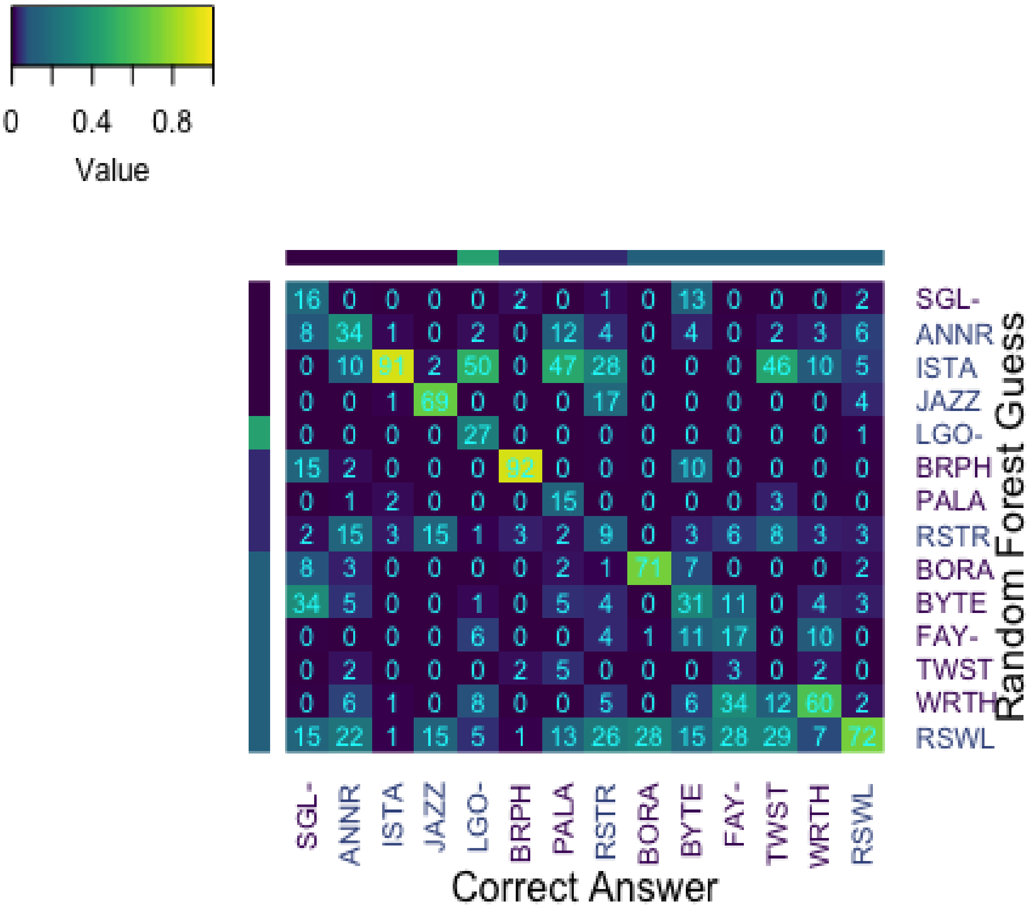


Figure B2: Violin plot of the accuracies of 1000 random forests identifying individual compared to the weighted accuracy expected by random guess for the full dataset, shotgun microphone recordings only, collar recordings only, female vocalizations only, and male vocalizations only.



	HZ	SN	SS	TW
HZ	17	30	22	24
SN	4	5	4	1
SS	24	24	38	11
TW	55	41	37	64

Figure B3: Confusion matrix for clan signature. Columns represent true categories while rows represent the random forest assignments. Each column shows how the random forest classified calls for that particular clan. Each cell represents the percentage of calls that were assigned to the row category from the true or column category. Guesses that lie along the diagonal are correct assignments while guesses on either side of the diagonal are incorrect assignments. Numbers in cyan show the percentage of calls assigned to category y (row y) when it came from category x (row x).



	SGL-	ANNR	ISTA	JAZZ	LGO-	BRPH	PALA	RSTR	BORA	BYTE	FAY-	TWST	WRTH	RSWL
SGL-	16	0	0	0	0	2	0	1	0	13	0	0	0	2
ANNR	8	34	1	0	2	0	12	4	0	4	0	2	3	6
ISTA	0	10	91	2	50	0	47	28	0	0	0	46	10	5
JAZZ	0	0	1	69	0	0	0	17	0	0	0	0	0	4
LGO-	0	0	0	0	27	0	0	0	0	0	0	0	0	1
BRPH	15	2	0	0	0	92	0	0	0	10	0	0	0	0
PALA	0	1	2	0	0	0	15	0	0	0	0	3	0	0
RSTR	2	15	3	15	1	3	2	9	0	3	6	8	3	3
BORA	8	3	0	0	0	0	2	1	71	7	0	0	0	2
BYTE	34	5	0	0	1	0	5	4	0	31	11	0	4	3
FAY-	0	0	0	0	6	0	0	4	1	11	17	0	10	0
TWST	0	2	0	0	0	2	5	0	0	0	3	0	2	0
WRTH	0	6	1	0	8	0	0	5	0	6	34	12	60	2
RSWL	15	22	1	15	5	1	13	26	28	15	28	29	7	72

Figure B4: Confusion matrix for individual signature.

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CHAPTER 5

LIONS, HYENAS AND MOBS (OH MY!)

Lehmann, K. D. S., Tracy M. Montgomery, Sarah M. MacLachlan, Jenna M. Parker, Olivia S. Spagnuolo, Kelsey J. Vandewetering, Patrick S. Bills, and Kay E. Holekamp. 2017. Lions, hyenas and mobs (oh my!). Current Zoology 63:313–322.

ABSTRACT

Understanding the factors that facilitate the emergence of cooperation among organisms is central to the study of social evolution. Spotted hyenas (*Crocuta crocuta)* frequently cooperate to mob lions (*Panthera leo)*, approaching the lions as a tightknit group while vocalizing loudly in an attempt to overwhelm them and drive them away. Whereas cooperative mobbing behavior has been well documented in birds and some mammals, to our knowledge it has never been described during interactions between 2 apex predators. Using a 27-year dataset, we characterize lion–hyena encounters, assess rates of mobbing behavior observed during these interactions, and inquire whether mobbing results in successful acquisition of food. Lions and hyenas interacted most often at fresh kills, especially as prey size and the number of hyenas present increased. Possession of food at the beginning of an interaction positively affected retention of that food by each predator species. The presence of male lions increased the probability of an interspecific interaction but decreased the likelihood of hyenas obtaining or retaining possession of the food. Hyena mobbing rates were highest at fresh kills, but lower when adult male lions were present. The occurrence of mobbing was predicted by an increase in the number of hyenas present. Whether or not mobbing resulted in acquisition of food from lions was predicted by an increase in the number of mobs formed by the hyenas present, suggesting that cooperation among hyenas enhances their fitness.

Key words: competition, cooperation, hyena, lion, mobbing, sociality.

INTRODUCTION

A central focus in the study of social evolution is the emergence of cooperation among organisms, including the factors that facilitate or impede cooperation. We define cooperation as collective action among individuals for mutual benefit (Dugatkin et al. 1992a). In many animal species, cooperation involves coordinated action by multiple individuals (Stephens and Anderson 1996). Cooperative actions must, on average, increase the fitness of the individuals involved, even though the direct or indirect benefits accruing to any specific individual may not be obvious (Noe 2006; Brosnan and Bshary 2010). Cooperative behaviors function importantly in animal societies to allow groups of individuals to accomplish objectives that would not be achievable by any individual acting alone (Hammerstein 2003).

After cooperative breeding (e.g., Moehlman and Hofer 1997), most research on cooperation among mammalian carnivores has focused on group hunting, which occurs in many gregarious species (Bailey et al. 2013). However, mobbing is another important form of cooperative behavior, which occurs when 2 or more individuals in a high state of arousal synchronously approach or attack a threatening stimulus in the environment. Mobbing behavior is commonly observed in many groups of animals, including birds (Altmann 1956; Sandoval and Wilson 2012), ground squirrels (Owings and Coss 1977), primates (Gursky-Doyen and Nekaris 2007), and cetaceans (De Stephanis et al. 2015). In these species, mobbing functions to deter or harass potential predators. Among mammalian carnivores, mobbing behavior has been observed in gregarious mongooses (Rood 1975) coatis (*Nasua narica*; Janzen 1970), meerkats (*Suricata suricatta*; Graw and Manser 2007), and spotted hyenas (*Crocuta crocuta*; Kruuk 1972; Mills 1990).

Mobbing by spotted hyenas is observed most frequently during their interactions with lions (*Panthera leo*; Kruuk 1972).

Lions and spotted hyenas are the dominant large carnivores in most African ecosystems, and these 2 species are one another's main competitors for resources (Periquet et al. 2015). When interacting with lions, hyenas frequently mob them by making synchronous coalitionary attacks on the lions. Hyenas live in fission–fusion societies (Smith et al. 2008) and often recruit groupmates to locations where they encounter lions by emitting long-distance vocalizations (Gersick et al. 2015). Once multiple hyenas are present, individuals may cooperate to mob the lions, approaching them as a cohesive group and vocalizing loudly together, a behavior that can enable the hyenas to overwhelm the lions and drive them away (Kruuk 1972; Mills 1990). However, there are considerable fitness costs associated with this form of cooperation. Lions are significantly larger and stronger than hyenas, and they represent the leading cause of mortality in many hyena populations (Cooper 1991; Hofer and East 1995; Trinkel and Kastberger 2005). An attack from a lion can result in serious injury or death for a mobbing hyena (Kruuk 1972).

Here, we focus on interspecific interactions between lions and spotted hyenas, and the intraspecific cooperative mobbing behavior that occurs among hyenas during many of these encounters. Because lion–hyena interactions are complex and highly variable (Kruuk 1972; Mills 1990), their analysis requires a large sample size, which can only be obtained from detailed long-term observational data. We utilize a dataset spanning 27 years to characterize the lion–hyena interactions taking place within the territories of 7 hyena clans at 2 study sites in Kenya. Our first goal is simply to describe lion–hyena encounters, asking where they occur and under what circumstances, and when they result in interspecific encounters. We next focus on mobbing behavior, inquiring about specific variables recorded during lion–hyena encounters that predict whether or not this cooperative behavior will occur. Finally, we test a hypothesis suggesting that mobbing behavior increases the probability that hyenas will obtain food from lion-controlled kills or carcasses, thus likely enhancing the fitness of the hyenas participating in those mobs.

MATERIALS AND METHODS

Study animals

Spotted hyenas are gregarious, long-lived predators that live in social groups called clans. Clans in east Africa are comprised of multiple matrilines of adult females and their offspring, and several adult immigrant males (Kruuk 1972; Frank 1986). Clans can contain up to 130 individuals (Holekamp et al. 2015), and all female clan-mates concurrently nursing young cubs rear them together at a communal den (Kruuk 1972). Each clan is structured by a linear dominance hierarchy that determines the priority of access to resources by individual group members (Frank 1986). Spotted hyenas cooperate in coalitionary aggression against clan-mates, a behavior that serves to enforce rank-relationships and defend resources within their societies (Engh et al. 2000; Smith et al. 2010). Although mean relatedness among clan-mates is very low (Van Horn et al. 2004), clan-mates also frequently join forces to defend a common territory against conspecifics, to guard their kills, and to secure resources from sympatric carnivores (Cooper 1991; Henschel and Skinner 1991). Here all hyenas were identified individually by their unique spot patterns and sexed based on the morphology of the erect phallus (Frank et al. 1990).

Study populations

Between 1988 and 2014, we monitored 7 clans of free-living spotted hyenas in 2 different national parks in Kenya, 5 in the Masai Mara National Reserve and 2 in

Amboseli National Park (Table C1). Both of these national parks are areas of open tropical grassland that support large herds of resident and seasonally migrant herbivores, which serve as prey for the resident carnivores (Kolowski et al. 2007; Watts and Holekamp 2008). Prey availability was recorded during biweekly surveys by counting all herbivores within 100 m of 2–4 line transects of 1–5.4km in each territory (Holekamp et al. 1999; Watts and Holekamp 2008; Green 2015; Table C1). We calculated the prey density within the territories of each of our study clans on a monthly basis, and used the monthly number of standard deviations above or below the yearly mean to determine prey availability for each clan during each month of study.

Observation sessions

All methods of data collection were identical among populations. We monitored clans daily during 2 observation periods, in the morning from 6 to 10 AM and in the evening from 4 to 8PM. When we encountered a subgroup of one or more hyenas, we initiated an observation session, and all hyenas within 200 m of that subgroup were considered to be present in that session. Observation sessions (hereafter, "sessions") lasted from 5min to several hours and ended when interactions ceased, and observers left that individual or group. Session length was recorded as the duration of each session in minutes. "Den" sessions occurred within 200 m of an active hyena den, "kill" sessions occurred within 200 m of a fresh kill (made less than 24h previously), and "carcass" sessions occurred within 200 m of a kill older than 24h (Boydston et al. 2003). Carcass age was determined by observers based on its odor, appearance, and the presence or absence of fresh blood. Locations of all remaining sessions were categorized as "other" sessions, which usually involved animals resting or travelling far from a den, kill, or carcass.

We defined a lion–hyena “encounter” as occurring whenever we found members of the 2 species within 200 m of one another. We identified an interspecific “interaction” as occurring whenever hyenas and lions approached within 10 m of one another. At each encounter, we recorded the total number and identities of all hyenas present, as well as the total number, age class, and sex of all lions present (Whitman and Packer 2006). All encounters occurred within or on the boundaries of the territory of a study clan.

Feeding behavior

Many lion–hyena interactions involve contests over food resources. Here “food” included both fresh kills and older carcasses. In each kill or carcass session, we recorded all observed feeding behavior by any lions or hyenas using one-zero sampling (Altmann 1974). A predator species was considered to be feeding if at least 1 member of that species obtained at least 4 kg of food, which represents the spotted hyena’s typical daily food intake (Green et al. 1984; Henschel and Skinner 1990). Food mass consumed was estimated from archived field notes in which our observations of lion–hyena encounters were recorded. These notes contained detailed descriptions of the kill or carcass, how this changed over the course of the session, which individuals fed, and which specific body parts were consumed. Whenever possible, we also recorded which prey species had been killed. Kill size was categorized by prey species weight (Sachs 1967) as small (<100 kg; e.g., gazelles, impala), medium (100–300 kg; e.g., zebra, wildebeest, topi), or large (>300 kg; e.g., eland, buffalo, giraffe).

Interspecific competition between lions and hyenas

Both the prevention of kleptoparasitism of food by lions and the usurpation of food resources from lions are achieved primarily through synchronous mobbing behavior by hyenas (Trinkel and Kastberger 2005). Throughout each session involving both lions and hyenas, we recorded all mobbing events using all-occurrence sampling (Altmann

1974). We defined “mobbing” as a group of 2 or more hyenas, usually side-by-side and within 1 m of one another, with tails bristled over their backs, approaching within 10 m of at least 1 lion. In association with each mobbing event, we recorded the identities of all participating hyenas and the age/sex classes of the lions being approached. We counted all mobs that occurred during each session and calculated an hourly mobbing rate for each session as the total number of observed mobbing events divided by the total number of minutes in that session, multiplied by 60.

At sessions where kills or carcasses were present, we recorded which species controlled the food throughout the session, including which predator species was in control when observers arrived on the scene and which controlled the food, if any remained, when observers left. When observers arrived after a kill had been made, we determined which predator species had made the kill based on 2 criteria. A predator species was assigned credit for killing the prey animal if members of that species were considerably bloodier than members of the competing species when the session began (Cooper 1991), or if no members of the competing predator species were present when observers arrived on the scene of a very fresh kill (Watts and Holekamp 2008). If both predators were present when observers arrived, but neither species was obviously bloodier than the other, the predator species making the kill was assigned as unknown.

Statistical analyses

Four trained research assistants (S.M.M., J.M.P., O.S.S., and K.J.V) extracted data on lion–hyena encounters from detailed written field notes describing these interactions. To ensure consistency, all work was overseen by T.M.M. and K.D.S.L. 13% of all sessions were randomly reviewed by T.M.M. and K.D.S.L., and this subset did not differ from the remaining sessions with respect to hyena count, lion count, male lion count, or number of mobs (Wilcoxon–Mann– Whitney P>0.05). Mean inter-observer reliability was calculated by determining the research assistants' agreement with a "correct dataset" generated by T.M.M. and K.D.S.L. on 12 complex lion– hyena interaction sessions. The agreement score between the 4 individuals extracting data from field notes averaged 83% for all 7 variables of interest: hyena count, lion count, male lion count, number of mobs, who controlled the food at the start and end of the session, and whether hyenas fed.

All datasets and their criteria are described in Table 5.1, as well as below. We used nonparametric statistical tests for between-group comparisons, as all datasets failed to meet the assumption of normality for parametric tests.

Table 5.1: The criteria for inclusion in datasets analyzed in Results

Dataset	**Criteria**	**Number of Sessions**	**Complete Cases Modeled**
Lion–Hyena Sessions	Sessions in which a known number of lions and hyenas occurred within 200 m of each other ("encounters")	935	903
Food Sessions	Lion–Hyena Sessions in which a kill or carcass was present with known food possession	394	–
Known Killer Sessions	Food Sessions in which the species that made the kill was known	221	216
Potential Mobbing Sessions	Lion–Hyena Sessions in which more than 1 hyena was present	761	736
Potential Feeding Sessions	Potential Mobbing Sessions in which the session began with lions in control of the food	235	227
Talek Kill Sessions	Talek clan sessions in which a fresh, identifiable kill was present	2,558	2,239

All datasets include both "encounters" (within 200 m) and "interactions" (within 10 m) between lions and hyenas. All sample sizes represent the number of observation sessions meeting inclusion criteria. Nonparametric statistical tests and other calculations were conducted using the relevant full "number of sessions" dataset. Any sessions for which prey availability could not be calculated were excluded from modeling analyses, which used the reduced "complete cases modeled" dataset

Generalized linear mixed models (GLMMs) were built to include all biologically relevant predictors as well as our predictors of interest. Sessions for which prey availability could not be calculated were excluded from modeling analyses; thus, modeling was conducted on datasets comprised exclusively of "complete cases." We included year nested within clan as random effects in all models using data from more than 1 clan, and we included year as a random effect in all models using data from a single clan. This allowed us to determine whether our predictors of interest were significant while controlling for variation in territory size, territory quality, and predator and prey density. Session length in minutes was included in all models to control for variation in observation time. Prey availability was included in all models to control for likely variation in hunger levels and motivation in both predator species due to seasonal fluctuations in prey abundance. All numeric model predictors were z-score standardized using the scale function in R to simplify comparing coefficients. Coefficients were estimated via maximum likelihood and Laplace approximation. All model predictors were tested for multi-collinearity that would justify their elimination from the model and none were found to be collinear. All model residuals were visually inspected to confirm assumptions of homoscedasticity and comparable variance between groups. All groups and observations were inspected for disproportionate influence on the models and none warranted exclusion. All responses were modeled via logistic regression and the logit link function.

All analyses were conducted using RStudio Version 0.99.489 and R Version 3.2.3 utilizing the lme4 package for all GLMMs, the car package to test for multicollinearity in model predictors, and the popbio package to create Figures 5.3 and 5.4.

Characterizing lion–hyena interactions

We created dataset "Lion–Hyena Sessions" using all sessions where known numbers of lions and hyenas occurred within 200 m of each other (n=935). Using the subset of "Lion–Hyena Sessions" where the 2 species approached within 10 m of one another (n=410), we calculated the median and mean numbers of hyenas and lions present at sessions where the 2 predators actually interacted. We used "Lion–Hyena Sessions" with known prey availability to build a GLMM of the log odds of lions and hyenas interacting (n=903 complete cases), with presence or absence of an interspecies interaction as the binary response variable and session location, hyena count, lion count, presence of male lions, the interaction between session location and session length, the interaction between lion count and hyena count, and the interaction between hyena count and presence of male lions as fixed effects.

To determine how often hyenas encountered lions at a food source, we used all fresh kill sessions with an identifiable prey species in the Talek clan territory (n=2,558; hereafter, "Talek Kill Sessions") to calculate the percentage of kills at which lions were present. We restricted this analysis to the Talek clan because killed prey species' identities were reliably recorded there. We used "Talek Kill Sessions" with known prey availability to build a GLMM to inquire whether lions were present more or less often at kills of prey of different sizes (n=2,239 complete cases). This GLMM modeled lion presence or absence as the binary response variable, with prey size and the interaction of prey size and session length as fixed effects.

We created dataset "Food Sessions" using "Lion–Hyena Sessions" with a kill or carcass present in which control of the carcass was unambiguous at both the beginning and end of the session (n=394). These sessions were used to calculate the percentage of fresh kills made by hyenas versus lions, as well as the number and percentage of sessions in which each species started and ended with food. We used "Food Sessions" where lions had possession of the food at the start of the session (n=255) to calculate the mean hyena:lion ratio in sessions where hyenas either won or lost contests over kills and carcasses against groups of lions with and without adult male lions present.

Using the subset of "Food Sessions" where the predator species making the kill was known (n=221; hereafter "Known Killer Sessions"), we built a GLMM to determine whether the predator species that killed the prey animal, or controlled its carcass at the beginning of the session, affected that species' retention of the food until the end of the session (n=216 complete cases). We modeled control of the food at the end of the session as the binary response variable (hyenas=1, lions=0), and included the following variables as fixed effects: which species started with food, which species killed the prey animal, hyena count, lion count, presence of male lions, number of mobs formed, the interaction between session length and which species started with food, the interaction between hyena count and lion count, the interaction between hyena count and presence of male lions, and the interaction between session length and number of mobs formed.

Variables predicting whether or not mobbing occurs

We used "Lion–Hyena Sessions" with multiple hyenas present (n=761; hereafter "Potential Mobbing Sessions") to calculate the percentage of all lion–hyena encounters in which hyenas mobbed the lions. Using the subset of "Potential Mobbing Sessions" containing mobs (n=157), we calculated the total number of mobs observed, the median and mean number of hyenas per mob, and the median and mean number of mobs per session.

Using "Potential Mobbing Sessions," we compared mobbing rates between session locations using Kruskal–Wallis and Wilcoxon–Mann–Whitney tests with Bonferroni corrections. We also counted the number of sessions in which hyenas mobbed lions when no discernable resource was present. Using the subset of "Food Sessions" where multiple hyenas were present (n=353), we calculated the percentage of encounters involving mobbing when male lions were either present or absent. We also used Wilcoxon– Mann–Whitney tests on this subset to compare mobbing rates between kills with and without male lions present. Using "Talek Kill Sessions" at which both lions and multiple hyenas were present (n=209) and a Kruskal–Wallis test, we compared mobbing rates among kills of known sizes.

We built a GLMM using "Potential Mobbing Sessions" to identify variables that increased the probability of hyenas forming mobs against lions (n=736 complete cases). Here our response variable was binary, whether or not at least 1 mobbing event occurred during the session. Potential predictors included as fixed effects were session location, hyena count, lion count, presence of male lions, the interaction between session length and session location, the interaction between hyena count and lion count, and the interaction between hyena count and presence of male lions.

Does mobbing affect the probability that hyenas will feed?

Using a subset of "Potential Mobbing Sessions" in which the session began with lions in control of the food (n=235; hereafter "Potential Feeding Sessions"), we built a GLMM to identify variables that increased the probability of hyenas feeding (n=227 complete cases). This allowed us to test the hypothesis that cooperation, in the form of mobbing, increased the probability of hyenas obtaining food. Here our response variable was binary, whether or not any hyena obtained at least 4 kg of food during the session. Potential predictors included as fixed effects were hyena count, lion count, presence of male lions, number of mobs formed, the interaction between hyena count and lion count, the interaction between hyena count and presence of male lions, and the interaction between session length and the number of mobs formed.

RESULTS

In our 7 study clans, lions and hyenas were observed within 200 m of one another in 1,038 different observation sessions. Of these, observers could accurately determine the number of lions and hyenas present in 935 sessions that lasted on average 37min (median 25 range 5–240). Data from these "Lion–Hyena Sessions" were used to characterize lion–hyena encounters and interactions, and the mobbing behavior occurring therein.

Characterizing lion–hyena encounters

Lions and hyenas interacted in 43.9% (n=410) of the 935 interspecific encounters. In these 410 interaction sessions, the median number of hyenas present was 14 (mean 14.9, range 1–48), and the median number of lions present was 3 (mean 3.6, range 1–

20). In sessions where lions and hyenas co-occurred without interacting, the 2 species were either resting or traveling in proximity to one another, but the only relevant behavior we observed was watchfulness.

In our model of the likelihood of lions and hyenas interacting using complete cases (n=903) from "Lion–Hyena Sessions," the 2 species were more likely to interact at kill sessions than at any other session type (P=0.02; Table 5.2). There were also 2 significant interactions between session location and session length (P<0.02; Table 5.2; Figure C1). In shorter sessions, den and kill locations were more likely to have interactions than carcass or "other" locations. A larger number of hyenas (P<0.0001) and the presence of male lions (P<0.04) increased the log odds of lions and hyenas interacting, although the number of lions present did not (P>0.7; Table 5.2; Figure 5.1). In addition, a significant interaction between hyena count and lion count indicated that, as the number of hyenas present increased, so did the positive effect of the number of lions present on the probability that the 2 species would interact (P<0.02; Table 5.2).

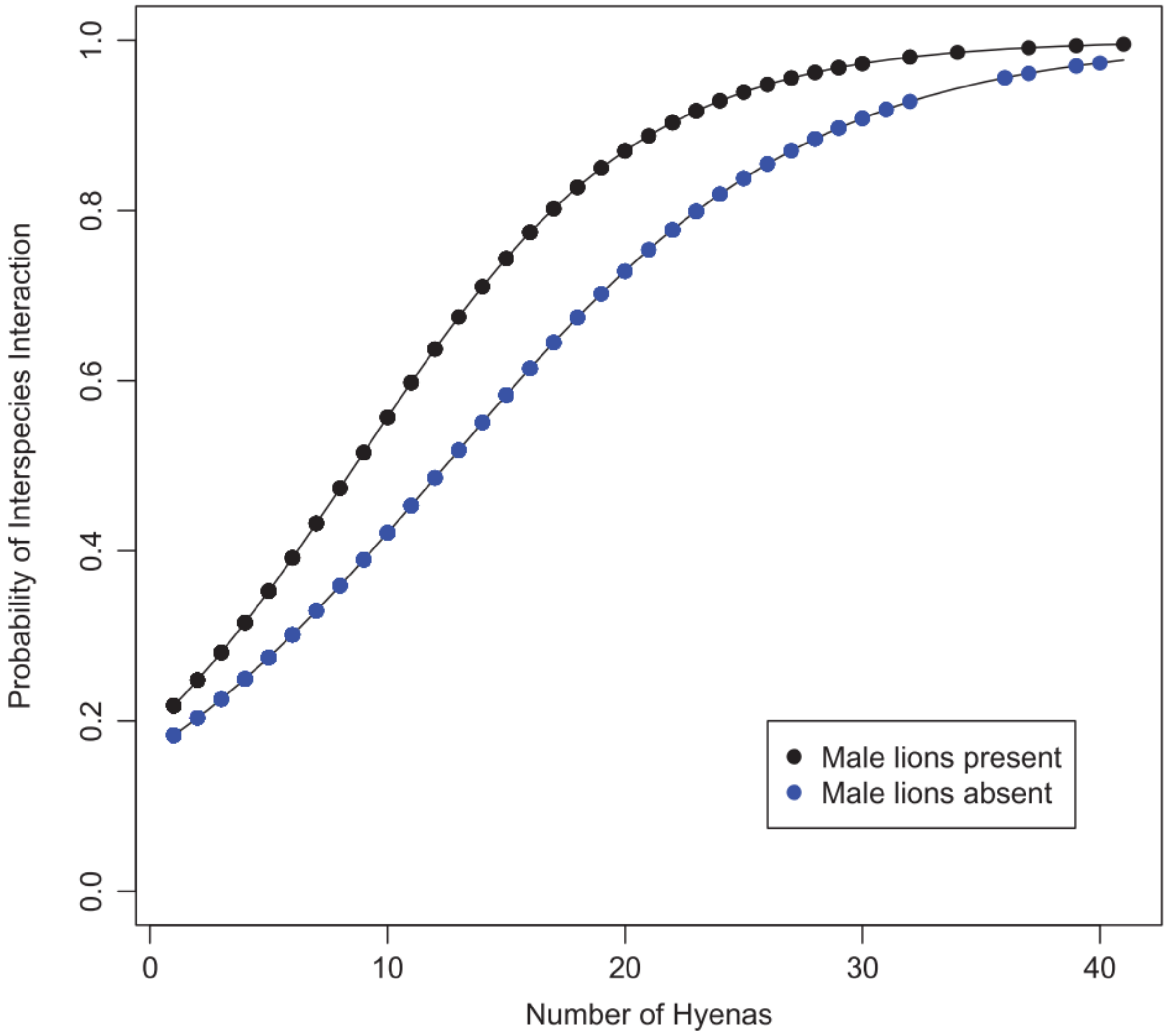

Figure 5.1: The probability of lions and hyenas interacting based on number of hyenas present and the presence or absence of adult male lions using complete cases in dataset "Lion–Hyena Sessions." Each line plots the modeled log odds that lions and hyenas would interact in sessions in which they were within 200 m of one another. Data points are true hyena counts plotted on the curves predicted by the model. Hyenas and lions were more likely to interact when male lions and more hyenas were present (Table 5.2).

Table 5.2: GLMM of the log odds of lions and hyenas interacting using complete cases in dataset "Lion–Hyena Sessions"

Modeling log odds of lions and hyenas interacting (*n*=903)	**B**	**SE**	***z***	**P**
Full Model				
Intercept	-0.46	0.18	-2.58	0.0100
Session length	1.14	0.24	4.83	< 0.0001
Prey availability	0.16	0.09	1.72	0.0847
Location – carcass	0.03	0.35	0.09	0.9263
Location – den	-0.07	0.33	-0.20	0.8437
Location – kill	0.48	0.21	2.33	0.0200
Hyena count	0.76	0.14	5.45	< 0.0001
Lion count	-0.03	0.09	-0.31	0.7543
Male lions present	0.42	0.20	2.11	0.0346
Session length x Location – carcass	-0.10	0.52	-0.20	0.8424
Session length x Location – den	-1.14	0.38	-3.01	0.0026
Session length x Location – kill	-0.63	0.26	-2.38	0.0173
Hyena count x Lion count	0.32	0.13	2.53	0.0115
Hyena count x Male lions present	0.49	0.25	1.95	0.0507

The response is binary: lions and hyenas interact (1) or not (0). Year is nested within clan, with both included as random effects. All numeric predictors are standardized. SE: standard error. Bolded rows indicate P-value<0.05.

Lion–hyena encounters occurred at 10.3% (n=263) of all 2,558 "Talek Kill Sessions." While controlling for session length, the size of the prey animal had a significant effect on the log odds of a lion–hyena encounter at a kill (Table 5.3; Figure C2). Small prey reduced the probability of lions being present (P<0.0001), whereas large prey increased the probability of lion presence (P<0.04; Table 5.3).

Table 5.3: GLMM of the log odds of lions encountering hyenas at a kill session using complete cases in dataset "Talek Kill Sessions"

Modeling log odds of lion presence with Talek hyenas at a kill (*n*=2,239)	**B**	**SE**	***z***	**P**
Full Model				
Intercept	-2.02	0.11	-18.62	< 0.0001
Session length	0.62	0.09	6.70	<0 .0001
Prey availability	-0.11	0.07	-1.60	0.1092
Prey size – small	-0.71	0.17	-4.17	< 0.0001
Prey size – large	0.69	0.33	2.07	0.0387
Session length ? Prey size – small	-0.03	0.19	-0.18	0.8604
Session length ? Prey size – large	-0.06	0.27	-0.23	0.8175

The response is binary: lions are present (1) or not (0). Year is included as a random effect. All numeric predictors are standardized. Bolded rows indicate P-value<0.05.

In analyzing “Food Sessions” (n=394), we found that hyenas made the majority of kills to which a predator species could be assigned (Table C2); this was unsurprising given that hyenas are our primary study animals. In our model of food possession at lion–hyena sessions using complete cases from “Known Killer Sessions” (n=216), the species in possession of the food when the session began positively affected retention of the food by both hyenas and lions regardless of session length (P<0.003; Table 5.4; Supplementary Figure S3). However, which species actually made the kill in the first place had no effect on retention of the food by either species (P>0.1; Table 5.4). Neither hyena count (P>0.7) nor lion count (P>0.1) affected the log odds of hyenas controlling food at the end of a session (Table 5.4), although hyena:lion ratios were higher at sessions in which hyenas successfully usurped food than when lions retained control of it throughout (n=255, W=4,525, P<0.0001; Table C3). Unsurprisingly, the presence of male lions significantly decreased the log odds of hyenas possessing the food at the end of a session (P=0.0002; Table 5.4; Figure 5.2), and the hyena: lion ratio required to usurp the food was higher when male lions were present than absent, although this trend was not significant (n=98, W=1,042.5, P>0.3; Table C3). Somewhat surprisingly, the number of mobs did not significantly increase the log odds of hyenas controlling the food at the end of the session (P>0.1).

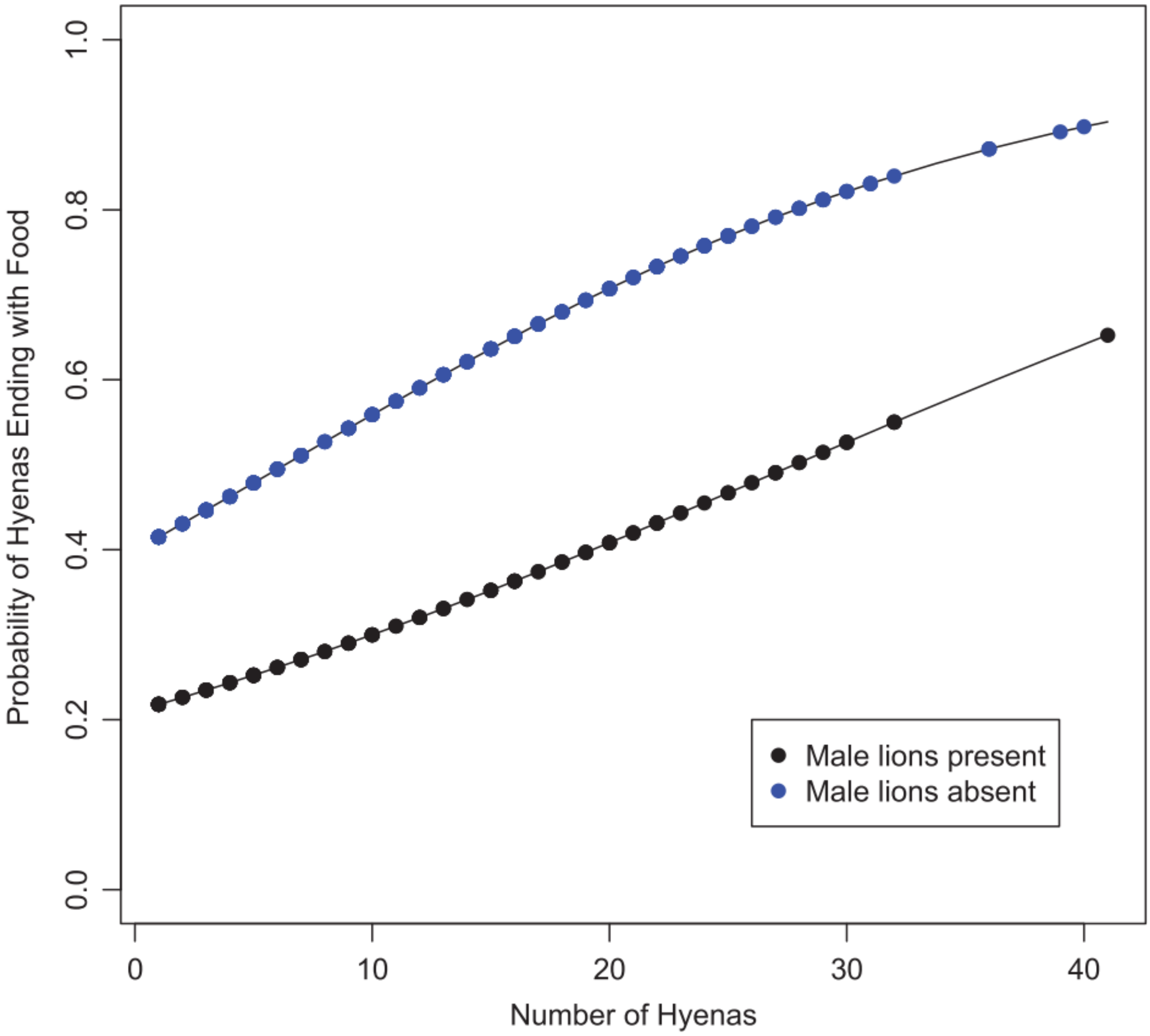

Figure 5.2: The probability of hyenas possessing the carcass at the end of the session based on number of hyenas present and the presence or absence of adult male lions using complete cases in dataset "Known Killer Sessions." Each line plots the modeled log odds that hyenas would have possession of the carcass at the end of the session. Data points represent true hyena counts plotted on curves predicted by the model. Hyenas were more likely to end the session with food when male lions were absent, and more hyenas were present (Table 5.4).

Table 5.4: GLMM of the log odds of lions or hyenas controlling the carcass or kill at the end of a session using complete cases in data- set "known killer sessions"

Modeling log odds of lions (0) vs. hyenas (1) possessing the food at the end of a session (*n*=216)	**B**	**SE**	***z***	**P**
Full Model				
Intercept	1.06	0.43	3.76	0.0002
Session length	0.25	0.36	0.70	0.4834
Prey availability	-0.04	0.18	-0.20	0.8403
Lions start with food	-1.22	0.40	-3.08	0.0021
Lions killed food	-0.81	0.52	-1.57	0.1173
Hyena count	0.08	0.28	0.27	0.7851
Lion count	-0.32	0.23	-1.35	0.1763
Male lions present	-1.57	0.42	-3.71	0.0002
Number of mobs	0.40	0.26	1.54	0.1240
Session length ? Lions start with food	0.21	0.40	0.53	0.5986
Hyena count ? Lion count	0.26	0.25	1.07	0.2827
Hyena count ? Male lions present	-0.43	0.42	-1.01	0.3118
Session length ? Number of mobs	-0.10	0.14	-0.70	0.4860

The response variable is binary: lions (0) or hyenas (1) end the session in possession of the carcass. Year is nested within clan, with both included as random effects. All numeric predictors are standardized. Bolded rows indicate P- value<0.05.

Variables predicting whether or not mobbing will occur

Hyenas formed 1 to 9 mobs (median 2, mean 2.5) against lions in 20.6% (n=157) of 761 "Potential Mobbing Sessions" for a total of 394 mobs observed. The median size of these hyena mobs was 4 individuals (mean 6.0, range 2–28). Both adult male and female hyenas participated in mobbing, as did both high and low-ranking individuals. Mobbing hyenas often approached within 1 or 2 m of the lions, which put them within the lions' reach and thus at considerable risk.

In analyzing "Potential Mobbing Sessions," we found that hyena mobbing rates were highest at fresh kills, followed by carcass, den and then "other" locations, but only kill sessions and "other" sessions differed significantly from one another (Bonferroni corrected critical value for multiple pairwise comparisons=0.0083; U=40,567, P<0.0001). Nonetheless, hyenas did mob lions in 29 "other" sessions where neither food nor den-dependent cubs were present. In sessions with multiple hyenas, mobbing occurred in 20.7% (n=25) of 121 "Food Sessions" with male lions present, and in 31.9% (n=74) of 232 "Food Sessions" at which male lions were absent. In these same sessions, mobbing rates were significantly higher when male lions were absent than when they were present ("Food Sessions" with multiple hyenas: n=353, W=12,312, P<0.02). At kills, mobbing rates did not differ based on variation in prey size ("Talek Kill Sessions" with lions and multiple hyenas: n=209, U=1.846, df=2, P>0.3).

Our model of mobbing probability using complete cases from "Potential Mobbing Sessions" (n=736) determined that hyenas were more likely to mob when larger numbers of hyenas were present (P<0.0001; Table 5.5; Figure 5.3). Location (P>0.3), lion count (P>0.2), and presence of male lions (P>0.1) were not significant predictors of mobbing (Table 5.5).

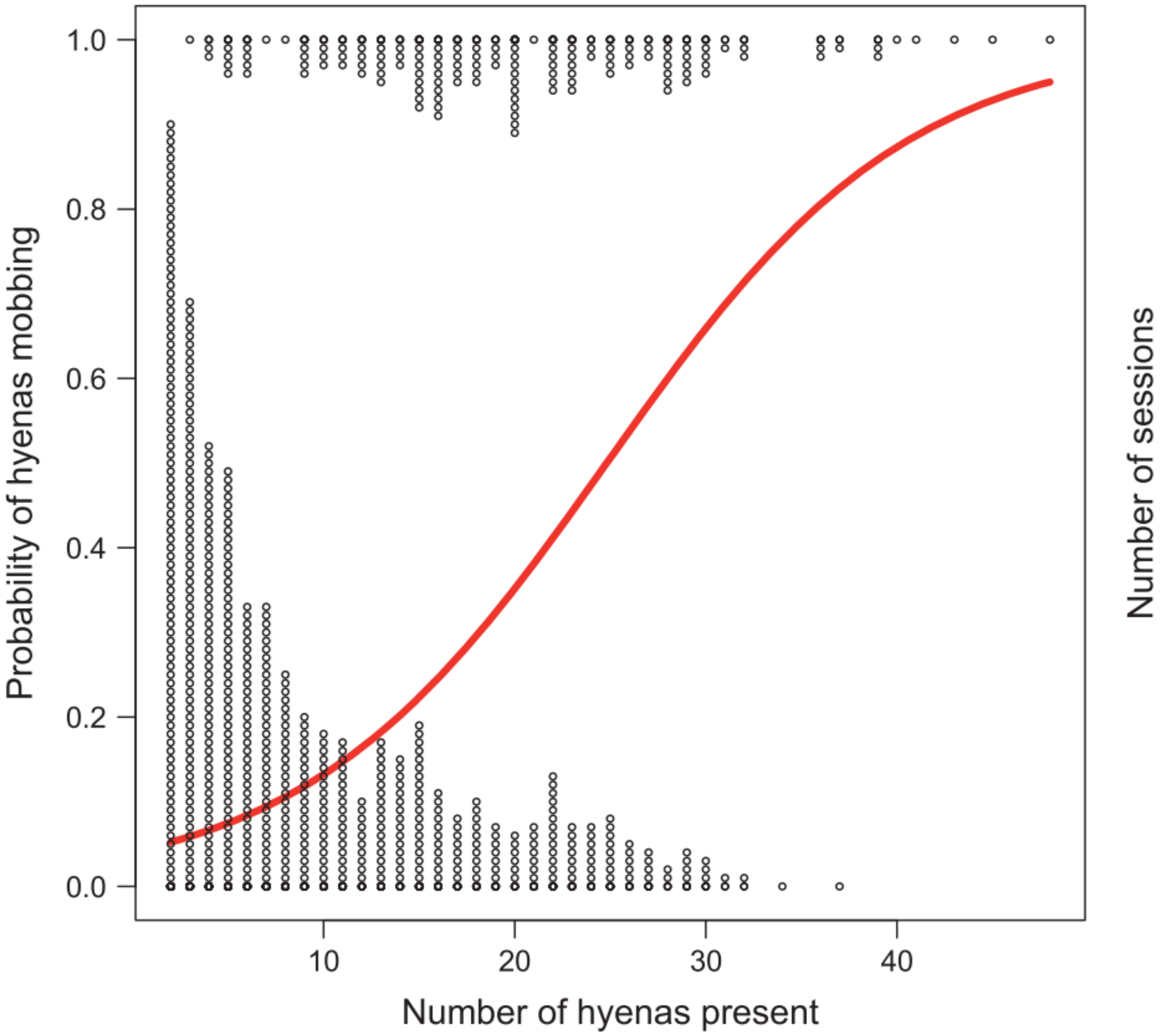

Figure 5.3: Probability of hyenas mobbing. The probability of mobbing function (red) plotted against the number of hyenas present in each session using complete cases in dataset "Potential Mobbing Sessions." The open circles represent the number of sessions in which mobbing does (top) or does not (bottom) occur, with sessions binned by the number of hyenas present. As the number of hyenas present increased, the log odds of mobbing also increased (Table 5.5).

Table 5.5: GLMM of the log odds of hyenas mobbing lions using complete cases in dataset "potential mobbing sessions"

Modeling log odds of hyenas mobbing lions (*n*=736)	**B**	**SE**	***z***	**P**
Full Model				
Intercept	21.80	0.25	27.27	<0.0001
Session length	0.66	0.25	2.58	0.0098
Prey availability	0.19	0.11	1.78	0.0756
Location – carcass	20.25	0.54	20.47	0.6353
Location – den	0.30	0.45	0.66	0.5070
Location – kill	0.28	0.28	1.02	0.3096
Hyena count	0.92	0.15	6.14	<0.0001
Lion count	20.18	0.14	21.27	0.2056
Male lions present	20.45	0.30	21.52	0.1293
Session length ? Location – carcass	0.83	0.62	1.34	0.1807
Session length ? Location – den	20.83	0.47	21.77	0.0775
Session length ? Location – kill	20.43	0.27	21.60	0.1097
Hyena count ? Lion count	0.11	0.12	0.90	0.3660
Hyena count ? Male lions present	0.27	0.26	1.05	0.2940

The response variable is binary: hyenas mob lions (1) or not (0). Year is nested within clan, with both included as random effects. All numeric predictors are standardized. Bolded rows indicate P-value<0.05.

Does mobbing affect the probability that hyenas will feed?

Our model of the probability of hyenas feeding using complete cases from "Potential Feeding Sessions" (n=227) found that hyenas were more likely to feed when the number of mobs in the session was higher (P=0.0003). A significant interaction between session length and number of mobs indicated that the shorter the session, the more influential the number of mobs were on feeding occurrence (P<0.002; Table 5.6; Figure 5.4). Hyena count (P>0.6), lion count (P>0.1), and presence of male lions (P>0.1) were not significant predictors of feeding (Table 5.6). These data support the hypothesis that mobbing behavior increases the likelihood that hyenas will obtain food from lion-controlled kills or carcasses.

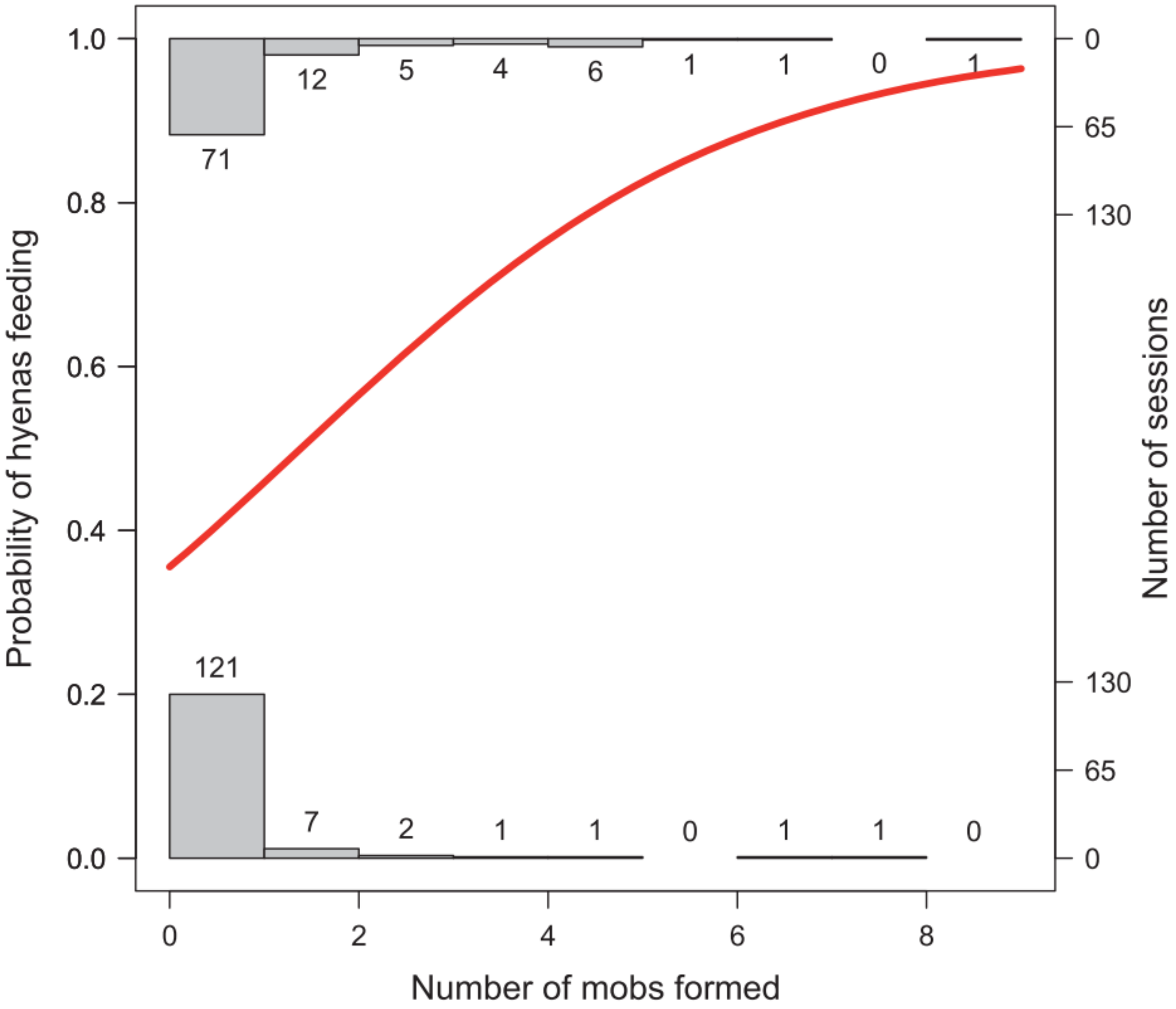

Figure 5.4: Probability of hyenas feeding. The probability of hyenas feeding function (red) plotted against the number of mobs formed during each session using complete cases in dataset "Potential Feeding Sessions." Histogram bars and sample sizes indicate the number of sessions in which mobbing occurred when hyenas fed (top) or did not feed (bottom). As the number of mobs increased, the log odds of hyenas feeding also increased (Table 5.6).

Table 5.6: GLMM of the log odds of hyenas feeding using complete cases in dataset "potential feeding sessions"

Modeling log odds of hyenas feeding (*n*=227)	**B**	**SE**	***z***	**P**
Full Model				
Intercept	-0.33	0.24	-1.36	0.1741
Session length	0.58	0.21	2.78	0.0055
Prey availability	0.17	0.17	1.03	0.3014
Hyena count	-0.11	0.23	-0.47	0.6369
Lion count	-0.29	0.19	-1.55	0.1212
Male lions present	0.58	0.37	1.58	0.1140
Number of mobs	1.15	0.32	3.63	0.0003
Hyena count ? Lion count	0.27	0.18	1.56	0.1191
Hyena count ? Male lions present	0.51	0.39	1.30	0.1946
Session length ? Number of mobs	-0.50	0.16	-3.23	0.0012

The response variable is binary: hyenas feed (1) or not (0). Year is nested within clan, with both included as random effects. All numeric predictors are standardized. Bolded rows indicate *P-value*<0.05.

DISCUSSION

Interactions between spotted hyenas and lions offer a particularly interesting case study compared with other species considered in the literature on risk-taking behavior in shifting cost/benefit landscapes. Although hyenas are top predators themselves, they are frequently killed or wounded by lions (Periquet et al. 2015). Hyenas and lions have a high degree of dietary overlap and often compete directly for the same food resources (Kruuk and Turner 1967; Hayward 2006). Lions are larger and more powerful than hyenas, which puts the hyenas at risk of injury or death during competitive interactions over food (Kruuk 1972; Schaller 1972; Hofer and East 1995). However, the possible benefits of acquiring food may outweigh the risk of injury from lions (Watts and Holekamp 2008). Here we document some important situational variables, such as the relative numbers of lions and hyenas present, the size of the contested carcass, the presence of male lions, and whether or not hyenas form mobs against the lions, that affect the probability that the hyenas will benefit from their risky interactions with lions. Our data enhance the understanding of the role of cooperation in mediating interspecific competition between these 2 competitors.

Characterizing lion–hyena interactions

Where the 2 species co-occurred, lions and hyenas were most likely to interact over fresh kills (Table 5.2), which is unsurprising for 2 predators whose diets overlap by more than 68% (Periquet et al. 2015). Fresh kills are extremely rich but highly ephemeral resources in the Mara ecosystem (Jones et al. 2015) and are thus sites of intense interspecific feeding competition. Lions and hyenas also had a high probability of interacting at the communal dens of hyenas (Figure C1). The communal den is a valuable resource for hyenas to guard, as it contains the young offspring of all females in the clan (Kruuk 1972). Furthermore, lions are known to kill den-dwelling cubs when the opportunity arises (Mills 1990; Hofer and East 1995; Watts and Holekamp 2009), so hyena fitness should be enhanced by driving lions away from dens.

Lions and hyenas were more likely to interact as the number of hyenas increased (Table 5.2; Figure 5.1), which is not surprising given that the risks involved in interacting with lions can be more widely distributed when more hyenas are present. The probability of successfully defending or obtaining food from lions also likely increases with the number of allies present (Table C3).

Lions and hyenas encountered each other more often as prey size increased (Table 5.3; Figure C2). This is consistent with the notion that acceptable fight costs should increase with resource value (Enquist and Leimar 1987). Furthermore, hyenas can completely consume smaller kills in a few minutes (Kruuk 1972), leaving little or nothing for lions to kleptoparasitize. A large group of hyenas can ingest even medium-sized prey, such as an entire fresh topi or wildebeest, in as little as 13min (Holekamp and Smale 1998). Local prey availability, however, did not significantly affect the probability of lions and hyenas interacting (Table 5.2), indicating that the intensity of interspecific competition is more situational (i.e. , a fresh kill within hearing distance of both species) than dependent on larger scale environmental factors such as low prey abundance.

When lions and hyenas did clash over food, the species with control of the carcass when observers arrived was most likely to maintain possession of the food until the end of the session (Table 5.4). This is consistent with the hypothesis that the resource holder enjoys an inherent advantage over potential usurpers (Parker 1974). Interestingly, when a session began with hyenas in possession of the food, session length did not appear to affect their likelihood of retaining the food. In contrast, the longer the session, the more likely lions were to lose control of the food to hyenas (Figure C3). This may indicate the hyena's persistence in remaining near the food (Kruuk 1972), the lions' willingness to abandon food once satiated (Kissui and Packer 2004), or the hyenas' ability to recruit more allies, given enough time (Gersick et al. 2015).

Presence of male lions

Several past studies of lion–hyena interactions found that the presence of male lions either partially (Mills 1990) or completely (Elliott and Cowan 1978; Cooper 1991; Honer et al. 2002) prevented hyenas from obtaining food from lion-controlled carcasses. Here, although the presence of male lions increased the probability that lions and hyenas would interact (Table 5.2; Figure 5.1), it decreased the probability that hyenas would possess the food at the end of the session (Table 5.4; Figure 5.2). Male lions may increase the probability of interspecies interaction by instigating the interactions themselves, as male lions are more likely to approach feeding hyenas on a kill than are females (Elliott and Cowan 1978). Furthermore, males are known to stalk hyenas and even detour in attempts to kill them (Frank et al. 1995).

Once in possession of the carcass, male lions, due to their larger size, are far more effective than female lions at keeping hyenas at bay and preventing hyenas from obtaining food (Cooper 1991; Kissui and Packer 2004). Accordingly, our descriptive data revealed that hyenas were only able to obtain food from lion groups containing adult males at sessions with high hyena:lion ratios (Table C3). Surprisingly, hyenas were nonetheless able to secure food in the presence of male lions on 26 of 93 occasions (Table C3). We believe that our large sample of observations enabled us to document this otherwise rare behavior. We also documented mobbing of male lions in 25 of 121 sessions involving a kill or carcass, although mobbing rates were much lower when male lions were present than when they were absent. This suggests that, although hyenas view male lions as a source of added danger, they are nonetheless willing to risk approaching male lions when the potential benefits warrant it.

Variables predicting whether or not mobbing occurs

The number of hyenas present, rather than the presence of food, was the key variable predicting the occurrence of mobbing (Table 5.5; Figure 5.3). Mobbing in large groups may reduce the risk of injury, either by distributing it among participants or by making it more difficult for lions to select an individual to pursue (Miller 1922). This supports the notion that availability of potential support from conspecifics has a stronger effect on mobbing behavior than do either lions or the presence of food.

Nevertheless, mobbing rates were highest around food resources, especially fresh kills. However, mobbing rates did not vary based on the size of the prey animal killed, indicating that mobbing depends on more than just the resource value. Mobbing rates were next highest at hyena communal dens, where hyenas presumably mobbed to protect their young. Additionally, hyenas mobbed lions in 29 sessions without any discernable resource present to fight over, which is surprising due to the danger inherent in mobbing behavior. One possible explanation for this is that hyenas use mobbing to gather information about potential threats, as also occurs in meerkats (Graw and Manser 2007). Lions, like hyenas, are territorial, so mobbing hyenas can expect to re-encounter the same individual lions within their lifetimes (Packer et al. 1990). Hyena mobbing may thus have a broader function beyond deterrence of lions, in that it may facilitate situational risk assessment on which subsequent decisions by group-members are based.

Mobbing affects the probability that hyenas will feed

Cooperative mobbing significantly increased the hyenas' ability to feed from lion-controlled carcasses, as the number of mobbing events observed in a session was a significant predictor of whether or not hyenas fed under these circumstances (Table 5.6; Figure 5.4). Thus, cooperation appears to enhance fitness in spotted hyenas by increasing their probability of feeding when competing with lions for control of a food resource. The ephemeral nature of kills and carcasses (Jones et al. 2015), and the lions' ability to steal a significant proportion of hyena kills (Periquet et al. 2015), should theoretically have imposed strong selection pressure in the past, promoting the evolution of cooperative mobbing in this species.

Future directions

Given that cooperative mobbing does indeed increase access to food resources by hyenas, several further investigations now seem warranted. First, we will ask whether all group members who participate in mobbing events realize comparable direct benefits, and whether cheating occurs with individuals who fail to participate in mobs yet feed when food is usurped from lions. Our future work will also inquire about the individuals who participate in mobs, their age, sex, and rank, and the relationships among members of individual mobs. In intragroup aggression, adult females provide coalitionary support most frequently to their close kin regardless of intensity of aggression or risk of counterattack (Smith et al. 2010). However, evidence from previous work suggests that relatedness within a clan is not high enough to select for cooperation without additional direct benefits (Van Horn et al. 2004). Unless hyena mobs are composed of related individuals, a mechanism other than kin selection must maintain cooperative mobbing behavior. For example, ritualized greeting ceremonies reinforce social bonds in hyenas (Smith et al. 2011), and can provide a mechanism to assess and strengthen bonds with potential mobbing allies. Cooperative mobbing against lions might also serve a social bonding function through quality advertisement to potential mates and social allies (Dugatkin and Godin 1992b).

We are also interested in the mechanisms mediating collective movement during mobbing in spotted hyenas. Social facilitation is a predominant feature of hyena behavior (Glickman et al. 1997), and promotes coalition formation among captive juveniles (Zabel et al. 1992). Social facilitation could function to synchronize movement in mobbing behavior (Zabel et al. 1992) and has been hypothesized to play a role in the development of cooperative behavior (Glickman et al. 1997; Drea and Carter 2009). Furthermore, in contrast with intraspecific coalitionary aggression, lion–hyena interactions are accompanied by raucous signaling. It is unclear whether these vocalizations function to facilitate cooperation or to intimidate the mobbed lions. One of these, the lowing vocalization, is usually emitted during mobbing and may help individuals synchronize their movements (Kruuk 1972).

Given that risk-taking behavior has been correlated with hormone concentrations in a variety of species (e.g., Martins et al. 2007; Stanton et al. 2011), future work should focus on the hormones modulating behavior in participating hyenas before and during these cooperative encounters. Understanding the mechanisms maintaining cooperation, including the relevant communication signals and endocrine mediation of mobbing, will undoubtedly enhance our understanding of the relationships among communication, cognition, and cooperation.

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APPENDIX

Table C1: Comparison of the seven study clans. a) Ecological conditions in the two study areas in Kenya. Data from Watts & Holekamp (2008). b) Sociological variables for each clan of spotted hyenas. Data for Masai Mara from Green (2015) and data for Amboseli from Watts & Holekamp (2008). Although the Peekaboo clan was not one of our study clans, we passed through the Peekaboo territory on our way to the Ol Tukai territory, and have included data from 7 lion-hyena encounters we witnessed there.

Populations (a)	**Masai Mara National Reserve**	**Amboseli National Park**
Mean prey density (animals/km$_2$)	234.0 +/- 32.1	90.5 +/- 13.7
Mean prey biomass (kg/km$_2$)	33461 +/- 14712	15977 +/- 2592
Lion density (adults) (lions/km$_2$)	0.439	0.079 - 0.135
Hyena density (adults) (hyenas/km$_2$)	0.95	1.65

Clans (b)	**Talek**	**Fig Tree**	**Serena North**	**Serena South**	**Happy Zebra**	**Air strip**	**Ol Tukai**	**Peeka boo**
Territory size (km$_2$)	77.0	71.1	42.7	28.3	41.3	28.0	26.4	unk
Mean clan size	73	49	49	41	32	51	39	unk
Years monitored	1988-2013	2007-2014	2008-2011			2003-2005		-
Encounters between hyenas and lions (number of sessions)	775	24	23	22	17	10	57	7
Total encounter observation time (hrs)	469.7	12.4	19.2	13.7	13.4	10.7	37.4	2.7
Number of Prey Transects	3	3	2	2	2	3	4	-
Total Length of Prey Transects (km)	12	12	6.8	4.9	7.7	6	10	-

Table C2: Observation sessions involving kills or carcasses using dataset "Food Sessions." Sample sizes represent number of observation sessions.

	Sessions with fresh kills (n=394)			
	Killed by	**End with food**	**Start with food**	**End with food**
Hyenas	44.6% (n=176)	Hyenas: 58.0% (n=102) Lions: 42.0% (n=74)	139	Hyenas: 75.5% (n=105) Lions: 24.5% (n=34)
Lions	12.1% (n=45)	Hyenas: 37.8% (n=17) Lions: 62.2% (n=28)	255	Hyenas: 38.4% (n=98) Lions: 61.6% (n=157)
Unknown	43.3% (n=173)	Hyenas: 48.6% (n=84) Lions: 51.4% (n=89)	-	-

Table C3: Comparison of hyena:lion ratios in sessions where hyenas either won or lost contests over kills and carcasses against groups of lions with and without adult male lions present. Data includes the subset of "Food Sessions" where lions had possession of the food at the start of the session. Numbers shown in each cell represent the mean number of hyenas present per lion. Sample sizes in parentheses represent the number of observation sessions.

	Do hyenas win control of carcass? (n=255)	
	Succeed	**Fail**
Male Lions Absent	5.8 +/- 0.6 (n=72)	3.5 +/- 0.5 (n=90)
Male Lions Present	7.5 +/- 1.4 (n=26)	3.3 +/- 0.5 (n=67)

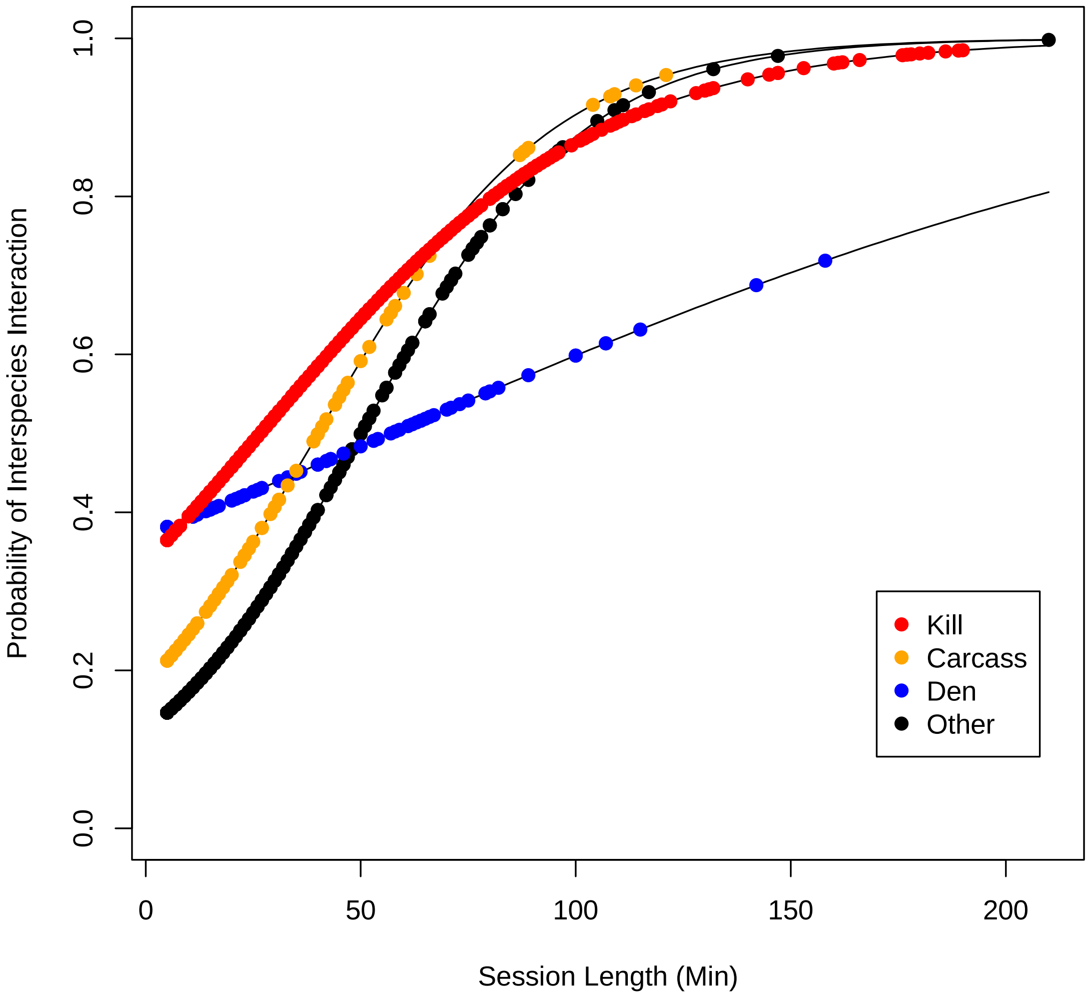

Figure C1: The probability of lions and hyenas interacting based on session length and session location. Each line plots the modeled log odds that lions and hyenas interact using complete cases in dataset "Lion-Hyena Sessions." Data points are true session lengths plotted on the curves predicted by the model. Sessions with fresh kills were most likely to include interactions between lions and hyenas. There was a significant interaction between session length and session location when the location was either at a den or a kill site (Table 2).

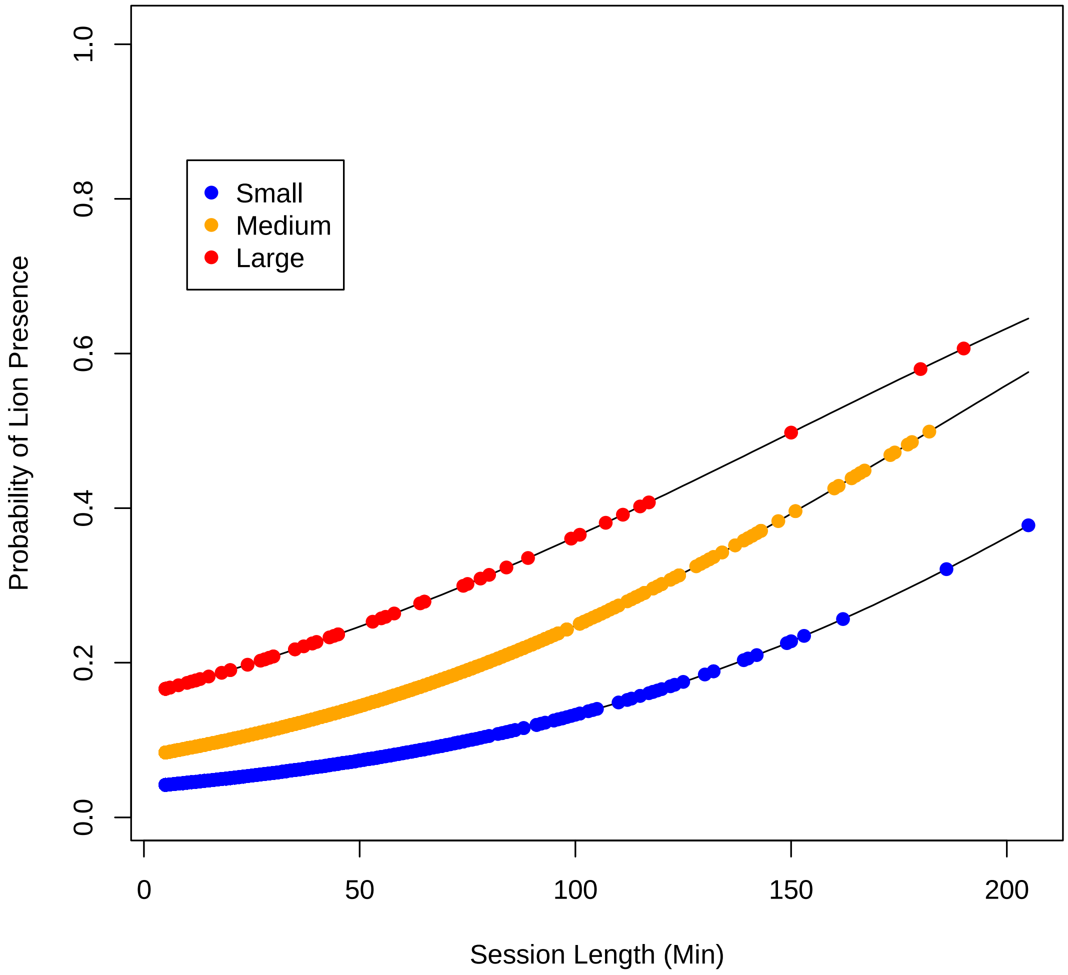

Figure C2: The probability of lion presence with Talek hyenas at a kill based on session length and carcass size. Each line plots the modeled log odds that lions would be present with hyenas at a kill using complete cases in dataset "Talek Kill Sessions." Data points represent true session lengths plotted on the curves predicted by the model. Lions were more likely to be present at larger carcasses (Table C3).

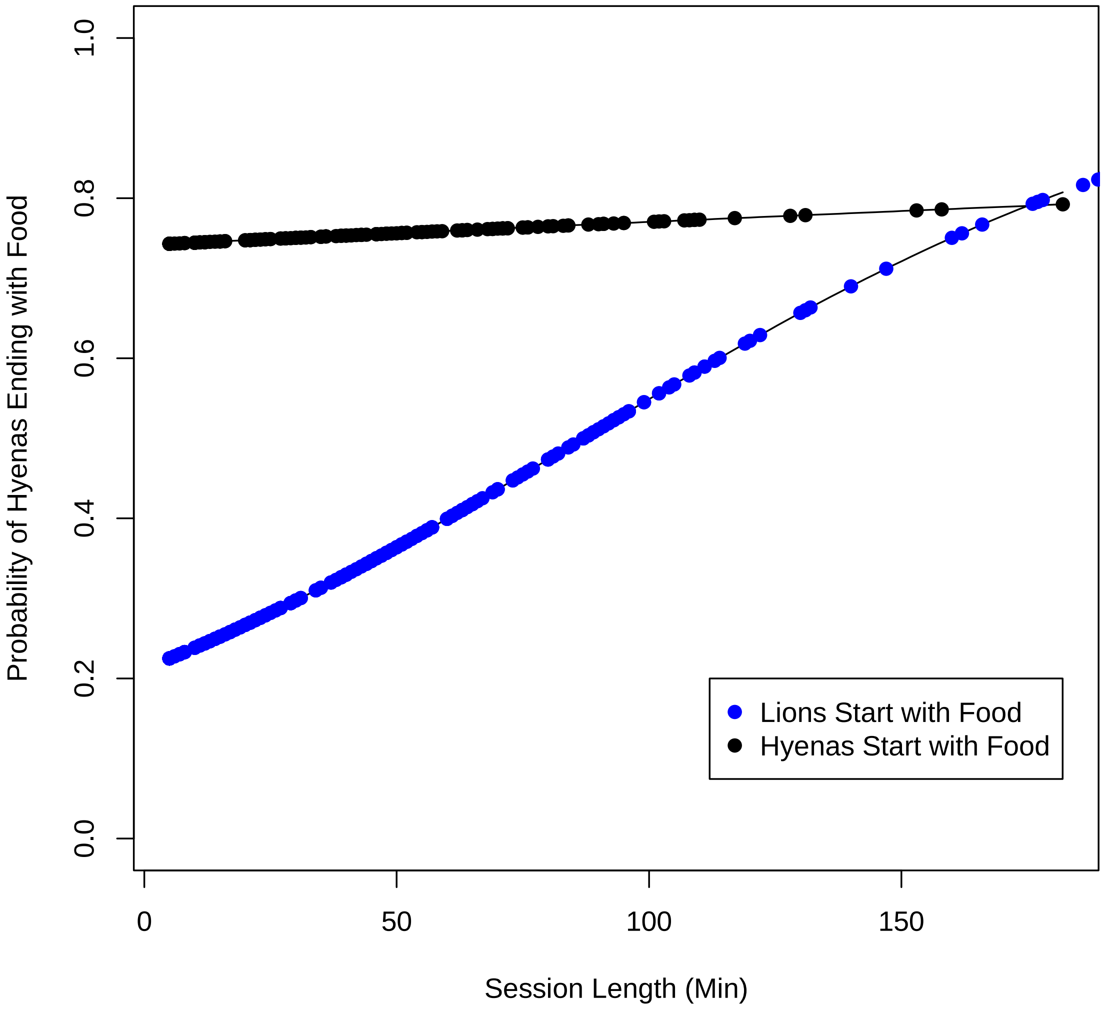

Figure C3: The probability of hyenas possessing the carcass at the end of the session based on session length and on which species had possession of the carcass at the start of the session using complete cases in dataset "Known Killer Sessions." Each line plots the modeled log odds that hyenas would have possession of the carcass at the end of the session. Data points represent true session lengths plotted on curves predicted by the model. When lions started the session in possession of the carcass, hyenas were more likely to possess the food at the end of the session as session length increased. When hyenas started the session in possession of the carcass, session length did not affect their retention of the carcass (Table 4).

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