To Howl or not to Howl? The Nature of Synchrony in Wolf Chorus Howling

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Abstract

As apex predators, grey wolves (*Canis lupus*) are in direct conflict with humans and have been historically persecuted due to livestock depredation. Their numbers fell dramatically in the 20th century but following reintroduction and the establishment of a protected status, wolves are recolonizing many areas of their historical range. Effective wild management schemes are necessary to minimise further human-wolf conflict. To develop effective management schemes, a more complete understanding of wolf vocal behaviour is needed. The recording of wolf howls could provide a non-invasive method to monitor population sizes and territories in the wild. Wolf howls are used for long range communication and play a role in territory maintenance and pack assembly. Chorus howls are thought to have similar functions. In this study we recorded howls of captive wolves to investigate the structure of choruses and elucidate whether this is related to individual wolf characteristics such as howl frequency, age, sex or dominance rank. We found that while some individuals led more chorus howls than others, the initiation success of each wolf was equal. Chorus leadership does not appear to be dominated by specific individuals or determined by specific howl characteristics. Instead, the leading of choruses appears to be a by-product of increased howling rate and duration. These results improve our understanding of wolf chorus howls and could prove useful in the development of monitoring schemes and long-range pack identification in the wild.

Introduction

Acoustic signals play an important role in animal communication by allowing messages to be conveyed rapidly over long distances without the need for physical or visual contact (Hopp et al. 1998). Animals communicate in a variety of contexts ranging from competitive to cooperative. One of the benefits of acoustic signalling is the high degree of variation possible within each sound type which enables animals to express variation in meaning (Harrington & Asa 2010).

The wolf, *Canis lupus*, is a markedly social species (Passilongo et al. 2010) and much of their social behaviour is accompanied by vocalisations (Harrington & Asa 2010). The basic social unit of wolves is the pack. Wild wolf packs are typically made up of a dominant breeding pair and their offspring. The dominant male and female lead many aspects of pack life. The dominant male initiates foraging, food provisioning and travelling whereas the female initiates pup care and defence (Mech 2000). Observations of wolves in Yellowstone National Park found that newly initiated behaviours were prompted almost three times more often by dominant breeders than by subordinate members of the pack (Peterson et al. 2002). Social interactions within the pack and agonistic interactions between packs require a sophisticated vocal repertoire with both short-range and long-range vocal types (Holt 1998). The vocal repertoire has been found to contain moans, whines, squeaks, yelps, squeals, screams, barks, growls, yawns and howls (Harrington & Asa 2010 after Coscia 1991, 1995). To humans, the howl is arguably the most conspicuous and recognisable wolf vocalisation and acts as the principal long-distance vocalisation in the wolf, as its acoustic properties facilitate low attenuation. Features of the environment such as landscape structures and vegetation affect the evolution of acoustic signals. For example, higher frequency sounds are often more strongly attenuated than lower frequency sounds (Wiley & Richards 1978). To maximise the distance travelled and minimise distortion and loss, sounds should be of a lower frequency and harmonically simple (Harrington & Asa 2010). The howl satisfies these criteria. Howling fundamental frequencies range from 150 to 780Hz (Theberge & Falls 1967) and it is estimated that howls can carry at least 9km in wooded areas (Harrington & Mech 1979) and 16km in open tundra (Henshaw & Stephenson 1974).

Grey wolves are apex predators and are frequently in conflict with humans because of livestock depredation (Sillero-Subiri & Laurenson 2001). They have been directly persecuted as a result. Loss of top predators from an ecosystem as a result of persecution could have major influences on prey populations (Elmhagen & Rushton 2007). In southern California, it has been suggested that removal of the coyote (apex predator) caused an increase in the population of the red fox (mesopredator) and this led to a higher extinction rate of small prey including scrub-breeding birds (Sæther 1999).

Furthermore, reintroduction of wolves or expansion of wolf populations, as is occurring in Scandinavia, could have similar trophic cascades (Sæther 1999). Effective management of wild grey wolf populations is essential to minimise human-wolf conflict and monitor the effects of wolf populations on the rest of the ecosystem. One way to develop more effective monitoring schemes would be by gaining a more complete understanding of wolf vocal behaviour including the role of chorus howling in pack activities. For example, if it were known that wolf packs have a signature chorus howl structure, this may assist in the long-range identification of packs, important for non-invasive management of species.

Previous studies have laid the foundations for understanding wolf howling. Howling is thought to play a role in pack cohesion (Theberge & Falls 1967; Tooze et al. 1990), territory maintenance (Harrington & Mech 1979), mate attraction and possibly in the formation of new packs (Zimen 1981). Howls are also thought to carry information on individual identity (Tooze et al. 1990; Palacios et al. 2015). Recent software advances have allowed solo and chorus howls to be assigned to individual wolves in captivity with a high level of accuracy (Root-Gutteridge et al. 2014b).

To a wolf, the howling of another wolf is a powerful stimulus to follow suit. Zimen (1981) observed that an initial howl by one animal will often quickly lead to a chorus howl by the whole pack. The length of a bout of chorus howling may (Klinghammer & Laidlaw 1979) or may not (Harrington 1989) be related to pack size. The fundamental frequencies of wolves joining the chorus usually differ by at least 15Hz (Filibeck et al. 1982) and howls typically become more variable further into the chorus (Harrington 1989). This complicates the counting of the number of individuals taking part. Howling is not the only vocalisation heard during choruses. Holt (1998) described squeaks, barks and growls as well as howls although these other vocalisations are only heard over close range and thus, are likely to be more important for within-pack communication.

The motivation for and functions of chorus howling are thought to be similar to those of individual howling. Chorus howling may advertise or exaggerate group size (Harrington 1989) which may play an important role in territory maintenance. Zimen (1981) observed that chorus howling often preceded an evening or morning phase of activity and suggested that this may serve the purpose of synchronising and coordinating the activity that follows. The dominant wolves in the pack are often the most responsive to howling stimuli (Harrington & Mech 1979) and play an key role in chorus howl initiation (Klinghammer & Laidlaw 1979; Zimen 1981). Given the observed importance of the dominant male in pack activities, it follows that the dominant wolves may try to elicit chorus howling

more and may be more successful at initiating chorus howling if the howling plays a role in the control of the same activities.

We aimed to build on previous research in chorus howling by investigating the synchrony in timing between individual howls in a chorus and the features of individual howls that make them more likely to be followed. We predicted that wolves that lead chorus howls would have particular characteristics, be that in their leading howl characteristics or their age, sex or dominance rank. We attempted to answer the following questions: (1) Are there individuals that lead more chorus howls than others? (2) Are there individuals that solo howl more than others and thus, have the potential to start a chorus? (3) Are there individuals that have greater success starting choruses? (4) Do the characteristics of solo howls determine whether they are followed or not? (5) Do wolves vary their howl characteristics depending on whether they are solo howling or chorus howling?

Improving our understanding of wolf long-range vocal-communication and how this relates to wolf pack dynamics and pack movements would be useful when devising management schemes. Identification of the order in which wolves howl in the chorus or specific characteristics of howls that vary within a chorus could improve individual identification within a chorus howl recording. Furthermore, determining chorus howl features could allow long-distance identification on a pack level (rather than individuals from solo howls as has been possible previously). This study provides a platform for future research into the role and structure of chorus howling in wolves.

Methods

Location

We obtained howl recordings at the UK Wolf Conservation Trust (UKWCT) located in, Berkshire, UK, RG7 5NT. The UKWCT is home to ten grey wolves living in four small packs. All the wolves are housed outside throughout the year with each group kept in a separate enclosure as shown in **Figure 1**. The UKWCT was chosen primarily based on facility layout which was able to provide good visual access to all the wolves. Nine of the wolves are socialised to humans and all receive close physical contact with humans on a daily basis. Recording in a captive environment overcomes several limitations of wild studies primarily the ability to maintain close observation throughout the study period and individually identify wolves at all times. Furthermore, socialised wolves are less inhibited by human presence. The details of each wolf at the Trust are detailed in **Table 1** and the relatedness is shown in **Figure 2**. From this tree it can be seen that the wolves are generally grouped according to relatedness. The only exceptions are Mosi and Mai which had to be separated due to problems of aggression.

Figure 1 - *Organisation of the enclosures. The enclosure boundaries are indicated by the blue lines. Within each enclosure there is a treed area, a mound and wooden platforms. The dimensions of each enclosure are under the name. Packs contain three individuals as detailed in Table 1. (Map sourced from Google Maps ©)*

Table 1 - *Details of the wolves at UKWCT. The asterisks (*) indicate the dominant individuals in the packs. The pack/packmate indicates the enclosure groupings.*

| Wolf	Sex	DOB	Subspecies	Pack/Packmate
Mosi	F	27/4/06	*Canis lupus occidentalis,* Northwestern wolf	Torak
Torak	M	Late4/06	*C. l. lupus x C. l. occidentalis,* European / Northwestern wolf cross	Mosi
Sikko	F*	8/3/11	*Canis lupus arctos,* Arctic wolf	Arctics
Massak	M*	8/3/11	*Canis lupus arctos,* Arctic wolf	Arctics
Pukak	M	8/3/11	*Canis lupus arctos,* Arctic wolf	Arctics
Mai	F	27/4/06	*Canis lupus occidentalis,* Northwestern wolf	Motomo
Motomo	M	19/5/08	*Canis lupus occidentalis,* Northwestern wolf	Mai
Nuka	M*	3/5/11	*Canis lupus occidentalis,* Northwestern wolf	Beenhams
Tundra	F*	3/5/11	*Canis lupus occidentalis,* Northwestern wolf	Beenhams
Tala	F	3/5/11	*Canis lupus occidentalis,* Northwestern wolf	Beenhams
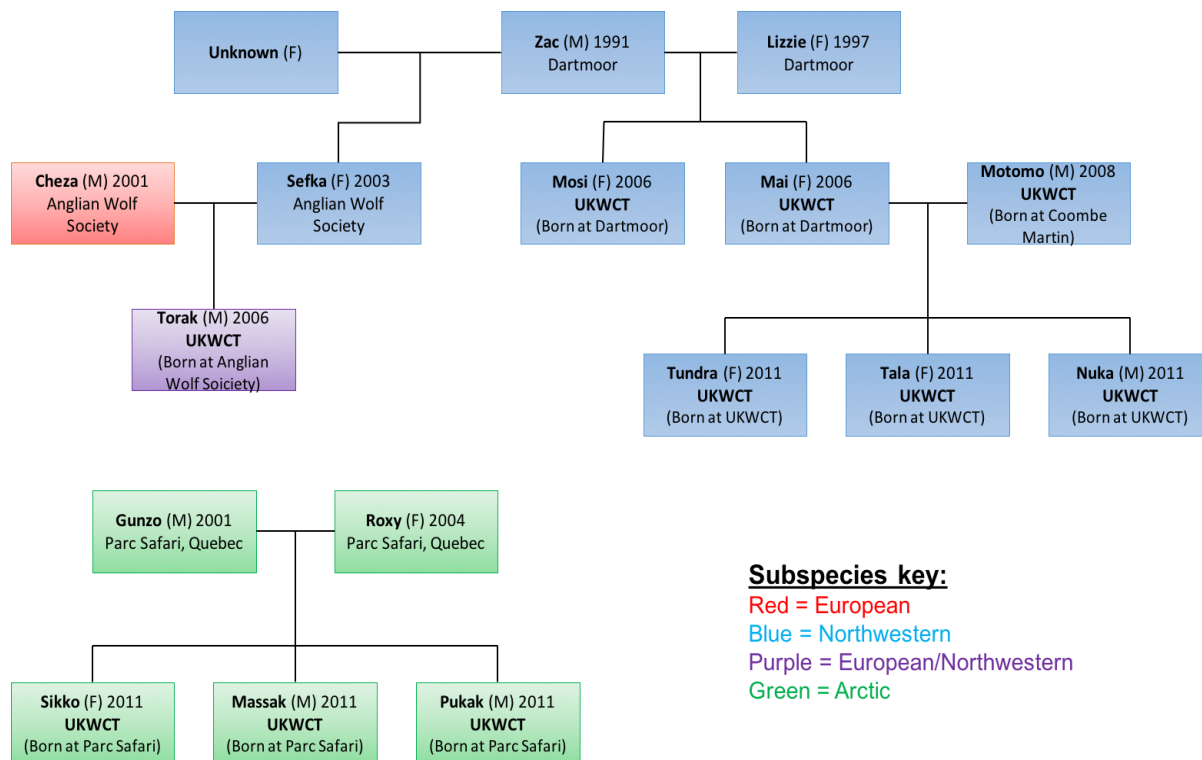
Figure 2 - *Family tree of the wolves at the Trust. The colour code represents the different species as shown in the key. The format for each box is: Name (sex) Year of birth, Current Location, (place of birth for the wolves currently at UKWCT).*

Dates and times of recording

Following a preliminary check of the suitability of the UKWCT, we visited on a further two occasions during January and February 2016 to obtain all of our recordings. Recordings were made at this time of the year to coincide with the breeding season when solo and chorus howling in wild and captive wolves has been shown to peak (Harrington & Mech 1979; Klinghammer & Laidlaw 1979). Wolves tend to howl more at dawn and dusk and during the night (Klinghammer & Laidlaw 1979; Nowak et al. 2007). However, due to the need to obtain video footage as well as audio recordings, we were limited to recording during daylight hours. Recordings were made between 12pm and 5pm.

Obtaining the recordings

We had to elicit howling because "spontaneous" howling is not very common during the day. We tried to elicit howling with playbacks of wolf howls and sirens. The keepers advised us that the wolves will generally ignore this and are most responsive to human howling. The wolves are involved in howling sessions with the public on a regular basis and so are used to responding to humans. Live human imitation of wolf howls, especially that of the keepers, were usually successful in eliciting howling. The use of human imitation of howls is a common way to elicit howling (Harrington & Mech 1979; Palacios

et al. 2007; Passilongo et al. 2015) and is often more successful than recorded alternatives (Joslin 1967; Theberge & Falls 1967).

Howling sessions were recorded using a Canon PowerShot SX50 HS, Canon S3is, Nikon Coolpix P500, Nikon Coolpix P510, Nikon Coolpix L23, Apple IPhone 6s, Samsung S4-mini, and a Bushnell HD Trailcam.

We used four cameras to film the different enclosures simultaneously from the most convenient viewing places. We began filming when the human started to howl. We were only interested in occasions when the wolves responded to the human stimulus and recorded the subsequent howls that occurred between the wolves. We discarded the occasions when human howls were ignored.

Sound analysis

We had four simultaneous recordings on different devices (saved as a ".wav" file). These files had to be synchronised so that we could work out the order in which wolves howled across the four enclosures. We visualised the spectrograms of each sound file using Raven Pro 1.4 (Bioacoustics Research Program 2011) and identified points of synchronisation manually by looking for obvious identical features in the spectrograms of overlapping files and noting the time in the file at which they occurred (**Figure 3**). In some recordings we blew a whistle that could be heard on all four devices to aid the synchronisation process; however, this noise disturbed the wolves and distracted them from howling so we stopped using the whistle after very few trials. We used Matlab® version 9.0 (The Mathworks, Inc. 2016) to combine the spectrograms into one four-track ".wav" file with the appropriate spacing.

Once the sound files were synchronised, they were viewed again in Raven and each individual howl was identified and attributed to a wolf using the video recordings. For the purposes of this study we concentrated on howls (long-range communication) and ignored other vocalisations that occurred during the chorus such as barks and growls described in Holt (1998). Also, we concentrated on only the fundamental frequency (F_0) of each wolf's howl which generally had the greatest intensity. To be able to identify the wolves from video footage, we learned to recognise the wolves individually based on morphological characteristics (e.g. size, face shape) and the presence of distinctive marks (e.g. dark patches on the flank). Once a howl was identified in the video we looked for the corresponding line
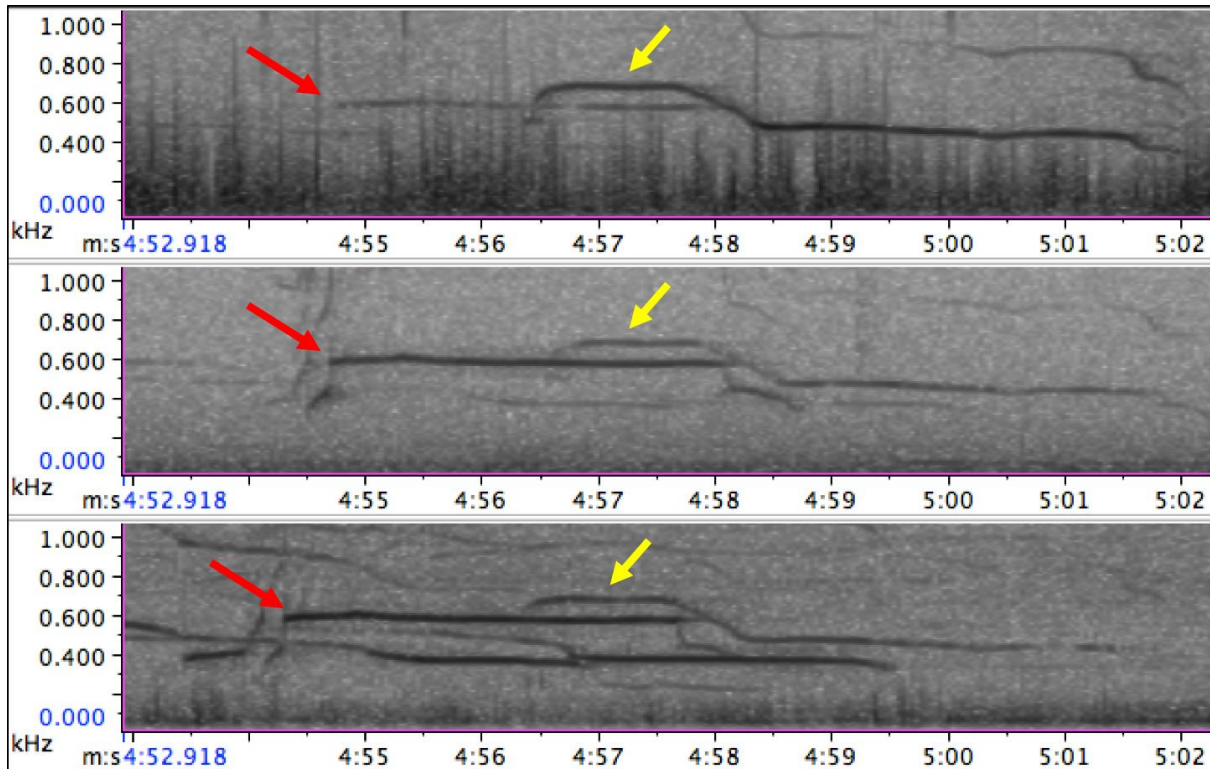
Figure 3 - *Example of a synchronised spectrogram for three files. The red arrows indicate an easily recognisable flat howl from an arctic wolf that is visible on all three spectrograms. The yellow arrows indicate a second howl visible on all three spectrograms that confirms the synchronisation point.*

on the spectrogram. Using the Raven selection table function we labelled each howl with the name of the wolf and also recorded the maximum frequency (Hz), minimum frequency (Hz), start time and end time of each howl. From this data we calculated the mean frequency (Hz), the frequency range (Hz) and the howl length (s).

We identified a total of 731 individual howls across all the spectrograms. Any recordings in which howls could not be accurately attributed to an individual wolf due to suboptimal video footage were discarded. This left 594 useable howls across both days.

Using the start times and end times in the selection table output we grouped the howls into bouts. A bout is defined as a continuous session of howling involving at least one wolf. A howl that followed any pause or period of silence was classed as a new bout. A bout may consist of a solo or chorus howl. A solo howl is defined as a howl that starts when no other wolves are howling and ends before another howl begins. A chorus howl is defined in the literature (Holt 1998) as a form of howl, whereby two or more pack members vocalize together. Each chorus has a leader. The leading howl is defined as a howl which starts when no other wolves are howling and is overlapped during the time course of that howl.

All subsequent howls in the chorus bout are classed as following howls. In some instances, we grouped leading and solo howls together as 'lone' howls because leading and solo howls both begin when no other wolves are howling and in both cases the wolf does not know whether it will be joined. Lone howling gives the wolf the potential to lead a chorus.

We viewed humans as a howling stimulus rather than a member of the pack with whom a wolf could chorus howl. Therefore, if a wolf howled in response to a human, we would say that that wolf was solo howling or leading a chorus, not chorus howling with humans. Humans howled 57 times and these howls were removed before carrying out any analyses. With humans removed, we identified a total of 129 bouts. 65 were solo howls and 64 were chorus howls. Within the choruses we have a total of 64 leading howls and 408 following howls.

Statistical analyses

A mixture of chi-squared, t-tests and analysis of variance (ANOVA) were used to test the hypotheses. Statistical analyses were carried out in R version 3.2.4 (R Core Team 2016) and Microsoft Excel version 15.15.

To see whether howl characteristics varied between individual wolves we ran an ANOVA for each characteristic with the wolf name as the independent variable and the howl characteristic as the dependent variable. We visualised the raw data in a histogram and used a Shapiro-test to see if the raw data were normally distributed. Upon seeing that frequency range and length were not normally distributed, we square-root transformed these data so as not to contravene the assumptions of the ANOVA. In conjunction with the ANOVA, we performed a TukeyHSD (honest significance difference) test for each characteristic to compile the wolves into groups that are significantly similar to one another. The TukeyHSD test compared the means of each wolf to every other wolf and identified any difference between two means that was greater than the expected standard error.

We used a chi-squared test (CHITEST function in Excel) to test how likely an individual was to be the leader of a bout of chorus howling. A chi-squared test is used to determine whether there is a significant difference between the expected values for a variable and those actually observed. When the expected values were less than 5, they were removed before calculating the p value to conform to the assumptions of the chi-squared. The formula to calculate the chi-squared test statistic is $X^2 = \sum[(\text{observed-expected})^2 / \text{expected}]$. The p-value is calculated using the test statistic and the degrees of freedom. The expected values for the chi-squared tests were calculated using the null hypothesis.

For the hypothesis that some wolves lead more howls than others, the null hypothesis predicts an equal number of chorus howls were led by each wolf. Therefore, the expected value is the overall number of chorus howls divided by the number of wolves.

Chi-squared tests were also used to test the hypotheses that some individuals lone howl more than others and that some individuals are more likely to be followed than others. The former is a measure of an individual's potential to start a chorus and the latter is a measure of an individual's success at starting a chorus. To calculate the expected number of lone howls for each wolf, we multiplied the overall proportion of lone howls by the total number of each individual's howls. The null hypothesis predicts the proportion of lone howls for each individual does not differ from the overall proportion of lone howls for all the wolves. To calculate the expected number of each wolf's lone howls that were followed, we multiplied the overall proportion of lone howls that successfully started choruses by the number of lone howls for each individual. The null hypothesis predicts that all individuals are followed the same proportion of the time.

T-tests were used to investigate whether the characteristics of solo and leading howls are different. This was done to determine whether there are characteristics of a lone howl that make it more or less likely to be followed. A t-test is used to determine whether two sets of data are significantly different from one another. Prior to performing the t-test, an f-test was performed on the data sets to check for equal variances. As a result of the f-test, t-tests assuming equal or unequal variances were used accordingly. For data that was not normally distributed and a significant difference was found with a t-test, we ran a non-parametric test as well to check whether the result was still significant.

We used a two-way ANOVA to investigate whether wolf howls changed depending on whether they were lone howling or chorus howling. Each howl was classified as being either a lone howl (including solo and leading howls) or a chorus howl (including all following howls). The wolf name and howl-type (lone or chorus) were factors in the ANOVA and the howl characteristic (maximum frequency, minimum frequency, mean frequency, frequency range or length) was the response. The model initially included an interaction term between name and howl type but this was removed as it was found to have no significant effect on the model.

An alpha level of 0.05 was used for all statistical tests. All statistical values are reported to two or three significant figures where appropriate.

Results

Number of howls

We identified 537 howls which could be accurately assigned to a wolf. The breakdown of the number of howls by each wolf and the number of howls of each type (solo, leading and chorus) is shown in **Figure 4**. There is a significant difference between the number of howls by each wolf (X^2= 71.6, df = 9, p<0.0001). Mai and Mosi howl significantly more than expected and Motomo, Tundra and Tala howl significantly less than expected.
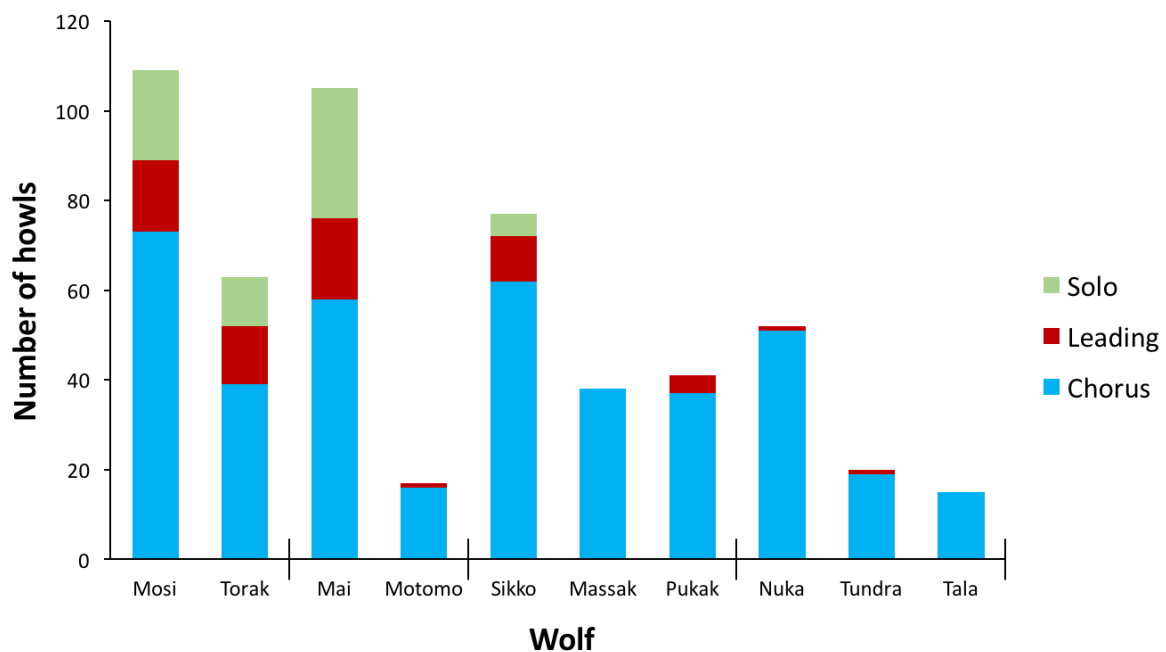

Figure 4 - Graph showing the number of howls of different types by each wolf. Blue represents howls that occurred within a chorus (not including the leading howl). Red and green indicate leading and solo howls respectively. The divisions on the x-axis separate the wolves into packs.

Individual howl characteristics

An ANOVA showed there are significant differences among the wolves' howls for maximum frequency (**Figure 5a**), minimum frequency (**Figure 5b**), mean frequency (**Figure 5c**), frequency range (**Figure 5d**) and howl length (**Figure 5e**). The TukeyHSD test grouped the wolves such that those sharing the same letter are not significantly different. This analysis shows that there are fundamental differences in the howl characteristics for each wolf.

Torak consistently had the lowest frequency howl having been assigned his own letter for minimum, maximum and mean frequency. Mai's howl is significantly shorter than all other wolves.
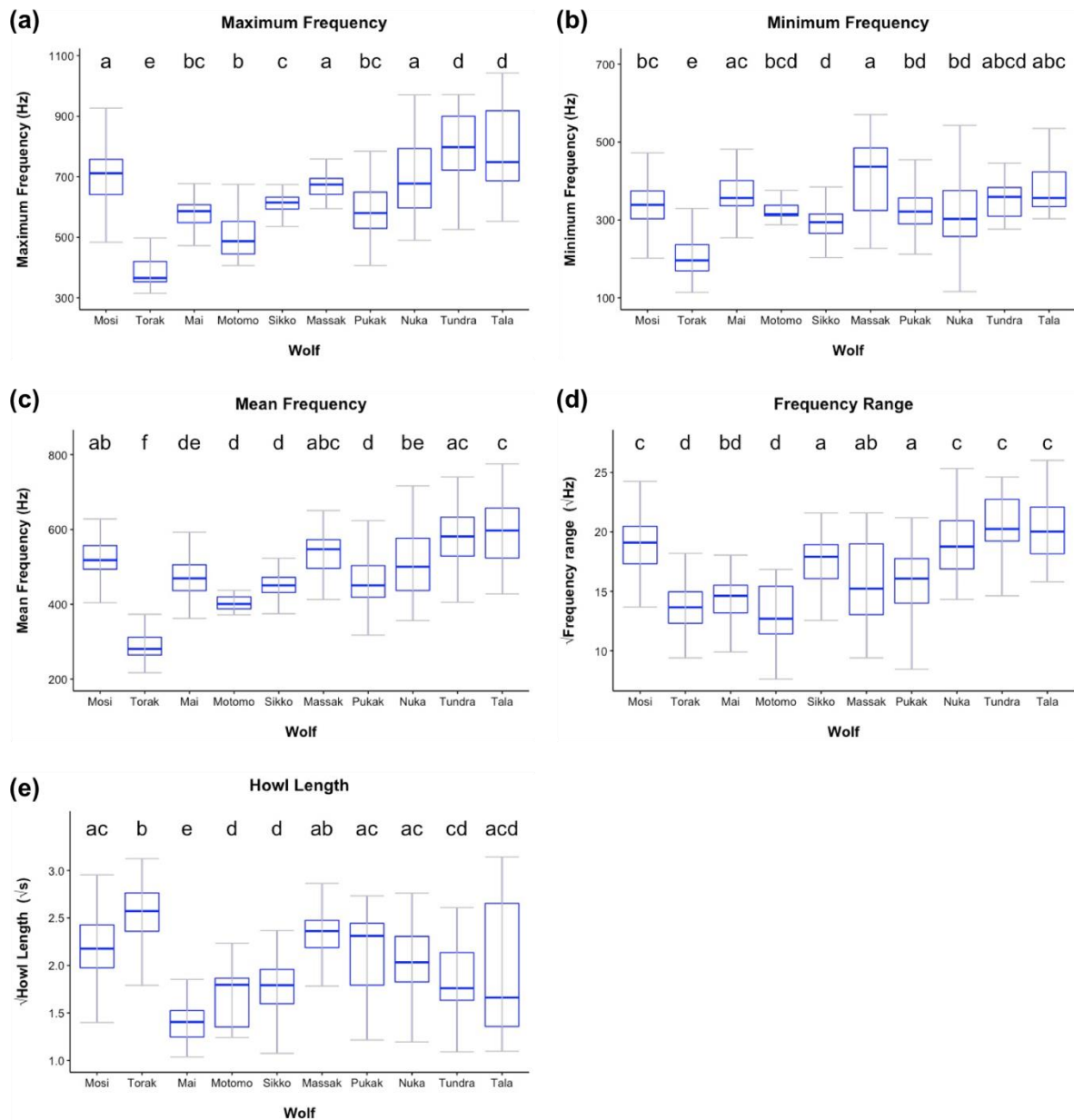
Figure 5 - Graphs showing the individual howl characteristics for each wolf. The middle blue line represents the mean; the blue box shows the interquartile range, and the grey whiskers show the standard error about the mean. Significant variation was found for each characteristic: **5(a)** – Maximum frequency (F = 64.67, df = 9, p<0.0001); **5(b)** – Minimum frequency (F = 26.66, df = 9, p<0.0001); **5(c)** – Mean frequency (F = 54.2, df = 9, p<0.0001); **5(d)** – Frequency range (F = 40.54, df = 9, p<0.0001); **5(e)** – Howl length (F = 54.2, df = 9, p<0.0001). Note that the raw data for frequency range and howl length was square-root transformed before running the ANOVA.

It is interesting to observe that for frequency range, the wolves that are related and share an enclosure have been assigned the same grouping. Nuka, Tala and Tundra are all group C which represents the greatest range of individual howling frequencies while Massak, Sikko and Pukak are all group A which

represents a smaller range of frequencies. However, no statistical analyses have been performed on this and the sample size is too small to provide meaningful results.

Chorus Howling

There is a significant difference between the number of leading howls by each individual (X^2 = 71.6, df = 9, p<0.0001). The number of leading howls by each wolf and the expected value assuming an equal proportion of leading howls from each individual is shown in **Figure 6**. While the chi-squared can only reveal a significant variation from the expected values across all the wolves, we can infer from the data that Mosi, Torak, Mai and Sikko led the overwhelming majority of chorus howls whereas the rest of the wolves led very few. Overall we can say that some wolves led significantly more chorus howls than others.
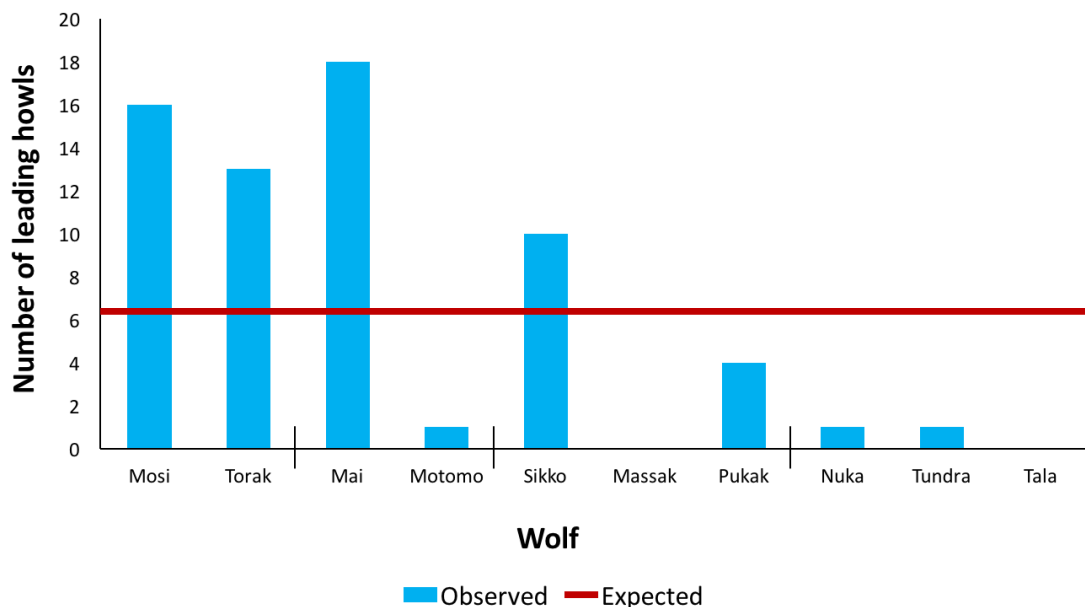

Figure 6 - *Number of leading howls by each individual wolf. The divisions on the x-axis separate the wolves into packs. The red line shows the expected value used to calculate the chi-squared. The expected value is the total number of leading howls/ the total number of wolves.*

Some wolves howl significantly more than others while there are no other wolves howling (X^2 = 51.5, df = 6, p<0.0001). This gives those wolves a greater potential to start a chorus. The observed and expected number of lone howls by each wolf are shown in **Figure 7**.

When a wolf solo howls it can either be followed or ignored. We found that there were no wolves that were followed significantly more than lone howls were followed overall (*X^2 = 2.39*, df = 3, p = 0.50 [NS]). The observed values did not differ significantly from the expected values (**Figure 8**).
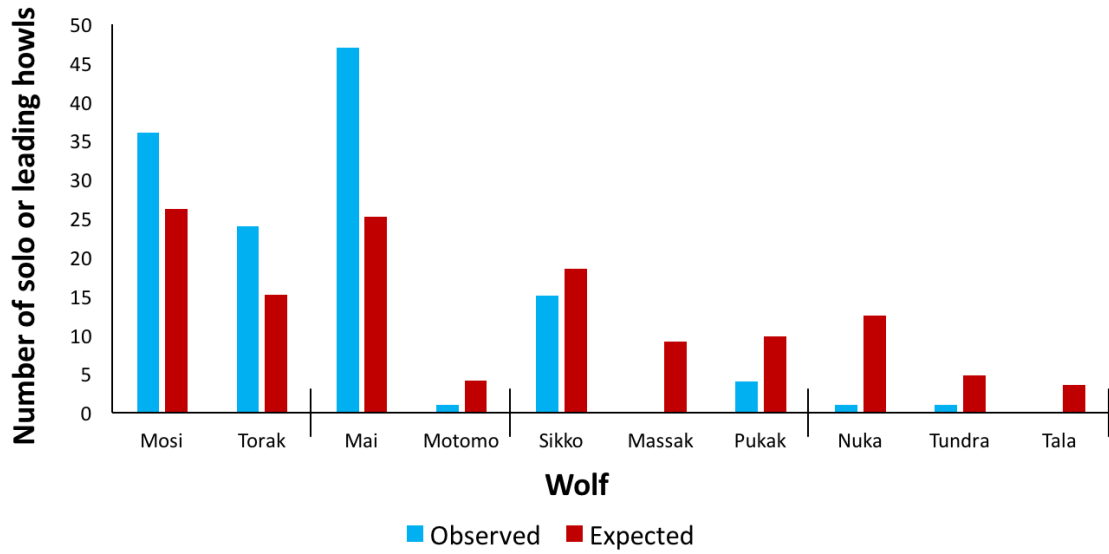
Figure 8 - Number of solo or leading howls by each individual wolf. The divisions on the x-axis separate the wolves into packs. The red bars give the expected values. The expected values were calculated for each wolf by multiplying the overall proportion of total howls that were solo or leading by the total number of howls by each wolf. We expect the proportion of howls by each individual that are leading or solo to be the same. Expected values less than 5 were removed before performing the chi-squared test.
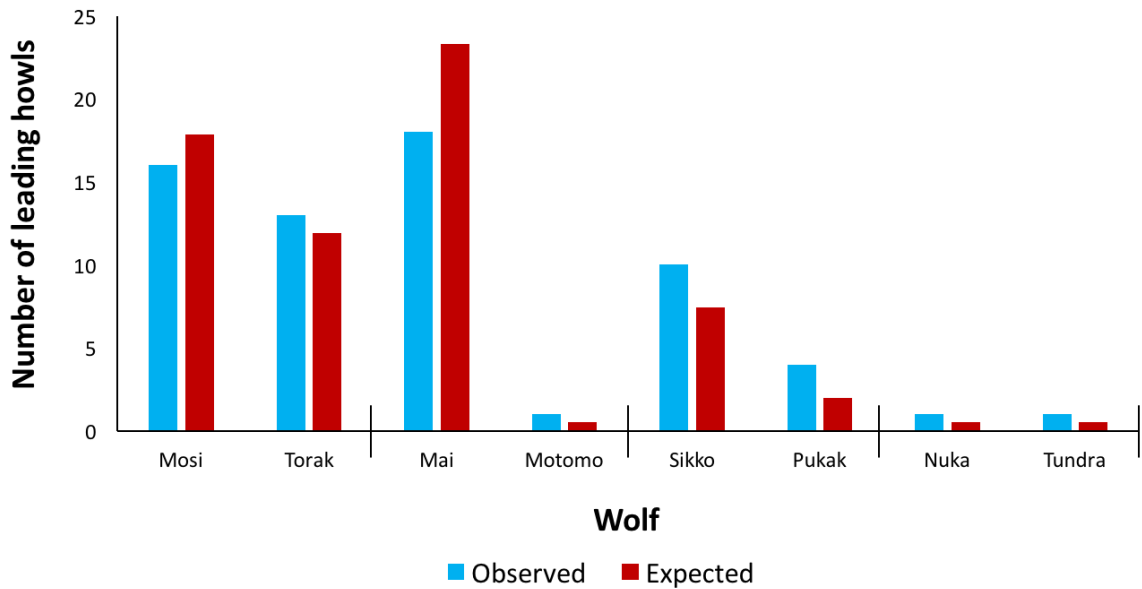

Figure 8 - Successful leading howls by each wolf. The divisions on the x-axis separate the wolves into packs. The expected values are calculated by multiplying the overall proportion of lone howls that were leading howls by the number of lone howls for each wolf. Expected values less than 5 were removed before performing the chi-squared test.

Approximately half of all lone howls are followed; out of 129 bouts, 64 (49.6%) were leading howls and 65 (50.4%) were solo howls. The wolves which never lone howled are not included in this analysis – if they never lone-howled they could not lead a chorus.

Solo – leader howl comparison

There is no significant difference in maximum frequency (t-Stat = 1.33, df = 127, p=0.18), minimum frequency (t-Stat = 0.016, df = 127, p=0.99), mean frequency (t-Stat = 0.91, df = 127, p=0.36) or frequency range (t-Stat = 1.74, df = 127, p=0.08) between solo and leading howls (**Figure 9**). There is a significant difference between the lengths of the solo and leading howls (t-Stat = 2.40, df = 127, p=0.018). Longer howls are more likely to start a chorus howl. When tested with a non-parametric test (Mann-Whitney U test), the result was still significant (Z-score = -2.19, df = 127, p=0.029).
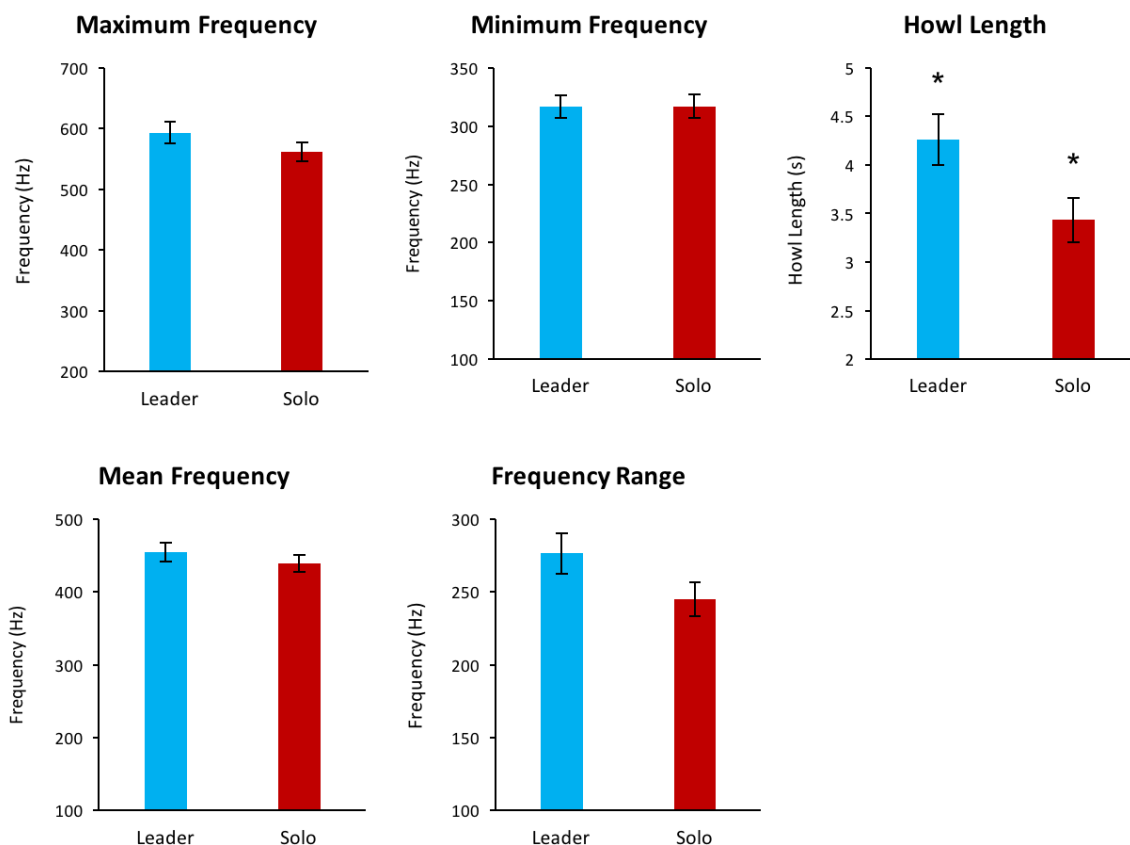

Figure 9 *- Comparison of the characteristics of solo and leading howls. Error bars show the standard error. The asterisks (*) indicate a significant difference between the means.*

<u>Lone – chorus howl comparison</u>

A simple t-test revealed a significant difference between lone and chorus howls for all the howl characteristics ($p<0.05$) except length. However, we cannot disentangle whether this difference is because there are more wolves which chorus howl (which we have already shown to have different howl characteristics) or whether each individual is changing their howl when part of a chorus. We performed a two-way ANOVA to look for the effect of howl type (lone or chorus) on howl characteristics for each wolf. The two-way ANOVA showed no significant effect of howl type on maximum frequency ($F = 2.52$, df = 1, $p = 0.11$), minimum frequency ($F = 2.35$, df = 1, $p = 0.13$), frequency range ($F = 1.54$, df = 1, $p=0.70$), mean frequency ($F = 3.41$, df = 1, $p=0.06$), or howl length ($F = 0.035$, df = 1, $p=0.85$). There are no significant differences between lone and chorus howls for each wolf.

Discussion

In this study we analysed 537 solo and chorus howls from ten captive grey wolves. We found that the total number of howls by each wolf was highly variable. Some wolves lone howl more than others. Chorus leadership also varied among the wolves with some initiating more choruses than others. When wolves lone howled, we found they had approximately a 50% chance of being followed. There were no wolves which were preferentially followed more than others when a chorus was initiated. We found no differences in frequency characteristics between leading and solo howls and thus, there appears to be no specific frequency characteristic which causes howls to be followed. Also, there were no differences between the characteristics of lone and chorus howls; wolves do not appear to be changing their howl according to whether they are leading or following.

The wolves that led more choruses are also the wolves that howled the most. Mosi, Mai, Torak and Sikko account for the majority of total howls (65.9%) and lone howls (94.6%) and lead the overwhelming majority of choruses (89.1%). It is likely that the larger number of leading howls was a by-product of the increased howling rate. When a wolf solo howls, it has the potential to be joined and start a chorus. Therefore, a solo howling wolf could be seen as attempting to start a chorus. Solo howls were interpreted in this way by Zimen (1971) [cited in Klinghammer & Laidlaw 1979]. Zimen described solo howls as "Vorheulen" which is best translated as "attempt to elicit howling in others". He regards solo howling as attempting to stimulate others to join in a chorus. We found that some wolves do indeed have greater potential to start a chorus than others by lone howling more. Furthermore, the howling of one wolf is a powerful stimulus for other wolves to howl (Zimen 1981). Therefore, by solo howling more frequently, it is likely that at least some of the time that wolf will be followed. Extending the duration of the solo howl further increases the chance that it will be overlapped by another wolf.

The seemingly random nature of chorus leadership is surprising. Chorus howling is such an iconic part of a wolf's behaviour that we might expect it to be controlled by the dominant members of the wolf pack like other group activities such as hunting and travelling (Mech 2000). The dominant male has been found to be the most responsive to human howling in the wild (Harrington & Mech 1979). We did not specifically investigate the responsiveness of the wolves to humans. We used humans as a howling stimulus assuming that wolves treated humans in the same way as an intruding wolf pack or coyote in the wild; both elicit howling (Joslin 1967; Klinghammer & Laidlaw 1979). Harrington & Mech (1979) revealed that human imitations of howling are regarded by adult wolves as an intruder's call and are responded to accordingly in an "agonistic, interpack context".

The wolves at the Wolf Conservation Trust are socialised to humans. Socialised wolves are extremely gregarious and exhibit friendly behaviour towards humans (Woolpy & Ginsburg 1967). The process of socialisation changes wolf behaviour from an unsocial state of avoidance to a social state of approach. The wolves in Minnesota studied by Harrington & Mech (1979) were wild wolves and therefore unsocialised. We cannot say with certainty that the captive wolves at the UKWCT treat humans in the same agonistic manner as seen in wild wolves but we worked on this assumption as has been done in other studies (Klinghammer & Laidlaw 1979; Nowak et al. 2007), although several studies make no reference to this assumption when using human imitation (Theberge & Falls 1967; Palacios et al. 2007). It is likely that the socialisation of the animals makes them more responsive to humans. Motomo, the only unsocialised wolf, howled very little (3.2% of total howls), which could be due to his alternative relationship with humans. Relationship with humans could be influencing howling rate instead of any intrinsic trait of the wolf.

Previous studies have found that the dominant male controlled the howling response of the pack (Harrington & Mech 1979) and that dominant individuals do more solo howling and are joined in choruses more frequently than lower-ranking individuals (Klinghammer & Laidlaw 1979). They did not perform a compensation for the rate of solo howling and therefore, the greater following success of the alpha male could be a by-product of that wolf's increased rate of solo howling, as we found in this study. We did not find that being joined in a chorus was related to dominance or age. However, the increased howling rate which leads to more chorus opportunities could be due to dominance or age. If we group the animals into age categories of 'older animals' (8-10yrs, 4 individuals) and 'younger animals' (5yrs, 6 individuals), older animals account for 92% of the solo howls and 75% of the leading howls which suggests that they are indeed solo howling more and therefore joined in choruses more. These groupings are rather arbitrary though, as both age groups are considered adults.

In the Beenham pack, the more vocal wolf was the dominant male, Nuka, as we would expect (Harrington & Mech 1979) but the same was not seen in the Arctic pack. The dominant Arctic male, Massak, never solo howled or led a chorus and actually howled the least out of all the members of that pack. The female, Sikko, howled the most out of the Arctics, and as the only female in that pack, she would be the equivalent of the dominant female. Based on this, we cannot say with any certainty that dominance was impacting the howling rate but it may be having some effect.

Our study found no evidence of acoustic characteristics differentiating leading howls from solo howls; both were variable. Harrington (1989) suggested that variation found in initial howls of a chorus could come from two major sources: (1) variation among the howls of an individual wolf, which initiated all the choruses; or (2) variation among the howls produced by several individual wolves, each of which initiated a proportion of the choruses. To test this, Harrington looked for the presence of a distinctive 'signature' howl for the initiating wolf but found no evidence for this. This suggests that one wolf may initiate all replies during a session but does so using quite variable howls, or several different adults initiated choruses during a session. We have shown that several different wolves start choruses and that there is no 'signature' initiating howl. Variation in the initial howls is likely due to multiple wolves initiating a proportion of the choruses. Eight different individuals successfully started at least one chorus during our study and their individual howling characteristics show significant variation. By varying the initiating howl, a wild wolf pack makes it difficult for rival packs to associate individual howls or voices with a specific pack (Harrington 1989). This may be important in avoiding conflicts between packs.

In contrast, Zaccaroni et al. (2012) found that chorus howling in wolf packs has a group-specific vocal signature with significant differences between packs. This may facilitate group recognition. Other species with complex social structures like the wolf, such as chimpanzees (Crockford et al. 2004) and killer whales (Ford 1991) have been shown to have group-specific vocal signatures. Within species, these signatures may serve to avoid conflicts between nearby groups by minimising the uncertainty of an approaching individual's identity (Crockford et al. 2004), and coordinate activities of group members (Tyack 2000).

We found that the wolves did not significantly change their howl characteristics when howling as part of a chorus compared to when howling alone. Harrington (1989) found that choruses started with simply-structured howls and became, shorter, higher and more frequency modulated as the chorus continues. Howls at the end of the chorus were the most frequency modulated. An overall comparison between lone and chorus howls at the Trust revealed the maximum frequency of chorus howls to be significantly higher. We could not disentangle whether this difference was due to individual wolves changing their howls within the chorus or whether it was the result of more individuals taking part in the chorus. A comparison for each wolf revealed that it was not due to wolves changing their characteristics. We did not look into frequency modulation during the time course of the howl due to software and time limitations. As Harrington reported, this may well have been the variable that changed (if any). Furthermore, we did not determine the order of each howl within the chorus, and

so we were unable to observe whether howls became more variable as time went on. This was partly due the high variability in the number of howls in each chorus. Some chorus bouts included only two howls from two wolves whereas other bouts included nearly 50 howls.

Our research was limited, at this time, to the study of only ten wolves in one location. This prevents us generalising our findings beyond the location we studied. Despite the small sample size, we believe we have still found interesting results that will provide a basis for future work. We must also be cautious when commenting on the howling rate of individual wolves. We targeted the human howling towards wolves known to be the most responsive to be more certain of getting a response. Therefore, any measures of responsiveness would be biased towards certain individuals. Further studies in different locations are needed to test whether the chorus structure and characteristics observed at the UKWCT are mirrored in other captive wolf packs. We are planning to extend this research to include more packs in different captive locations.

Furthermore, the organisation of the packs was quite unique and does not resemble the wild situation. There are four small packs located in very close proximity separated by only a fence. They are not one combined pack but neither can they be thought of as fully separate because each wolf can see and hear its neighbours at all times. As a result, we were unable to fully assess the effect of dominance ranking on howling rate for all the wolves there. We cannot say that the wolves in one enclosure are dominant to another enclosure. In packs containing two wolves (one of each sex) we said they are equally dominant as they are the equivalent of a breeding pair in the wild. In packs containing three individuals, the dominant individuals were identified by the keepers' long term observations.

It is important that we understand the social structure of wolf packs to be able to interpret the interactions that we observe occurring between individuals in the wild. The prevailing view of wolf packs is a group of individuals competing for dominance and held in check by the alpha male. Mech (1999) argues that this view does not apply to wild wolves. Random assemblages of unrelated individuals are very uncommon in the wild and as such, there is little competition for the dominant role. Instead, dominance is a reflection of the age, sex and reproductive status of the wolves. This is in contrast to captive wolves where a random assemblage of unrelated individuals is more common (Mech 1999). Dominance interactions do still occur in the wild and submission behaviours help promote friendly relations between pack members. Subordinate members including the breeding female will submit posturally to the breeding male and all young members and post-reproductive females will submit to the breeding pair. Previous studies have focused on social interactions such as

posture changes and food exchange. It is possible that chorus howling also plays a role in determining the social structure.

Our research may help to determine whether chorus howling does play a role in enforcing wolf social structure. Previous studies found that the alpha male howled the most frequently (Harrington & Mech 1979; Klinghammer & Laidlaw 1979). This may be a way of asserting dominance within the pack and the high howling rate may subsequently lead to increased chorus howling initiated by that individual. Chorus howling is a way to synchronise group activities (Zimen 1981). It makes sense that the leading or dominant individuals in the pack would instigate chorus howling as a means of coordinating such activities. While our research did not find such strict leadership it will be interesting to see if the same is seen in other packs. Having found no specific leading individuals as we might have expected calls into question our current understanding of wolf social structure. It is possible that pack structure may be less despotic and more consensual or cooperative than currently thought.

Cooperation between the pack members may in fact be responsible for the initial evolution of group howling, with all individuals gaining a selective advantage from participation. For example, chorus howling is important for territory maintenance and defence. Harrington (1989) found that howling by larger packs was a more successful deterrent to rival packs and therefore pack size is often the factor that determines the outcome of interpack conflicts. As well as avoiding energetically-costly conflicts, the maintenance of a territory may provide additional benefits to the pack, especially if the territory is large. The possession of a large territory increases the amount of prey available to a pack and therefore increases the likelihood of a successful hunt. Hunting is not an easy task for wolves, with only 1 in 10 hunts being successful (BBC 2002) but success rate may be increased with a larger group size and synchronisation (MacNulty et al. 2014) that may stem from chorus howling. On the contrary, chorus howling could be costly if performed at inappropriate times and a trade-off between whether to howl or not to howl may arise. The dominant individual in the pack may initiate the chorus having surveyed the relative risk of howling and then deemed it beneficial to respond to another pack at that time. It has been observed that wolves choose not to respond to the howls of another pack when they are in a vulnerable situation or when the energetic costs of howling outweigh the benefits of defending their current site. For example, when a pack has nearly exhausted the food supply from a kill they respond less or retreat upon hearing another pack howl as it is not worth responding for such a small reward (Harrington & Mech 1979) whereas, when the kill is fresh they chorus howl to alert the other pack of their presence.

This research may also assist with long-range identification of wolf packs in the wild. It has been shown that individual wolves (Theberge & Falls 1967; Tooze et al. 1990) and other canids (Frommolt et al. 2003) can be identified from their vocalisations using fundamental frequency and duration. Recent research suggests that howl amplitude may also improve the identification (Root-Gutteridge et al. 2014b). Individual identification is useful in monitoring programmes and conservation schemes in birds and could be applied to mammals (for review, see Terry et al. 2005). If individuals can be identified using frequency and temporal parameters, it follows that groups could be identified in a similar way. However, it must be stressed that the individual identification in these studies is the result of statistical analysis of large quantities of howls. When such large amounts of data are not available, group signatures could speed up identification and may be of use for long-range identification of distinct animal groups in monitoring schemes. In this study, individual howls were isolated spectrographically and, using the parameters previously mentioned, the howls could have been assigned to specific wolves. It may be possible to use individual howl characteristics from multiple individuals or an overall group signature to identify a specific pack in the wild. Coupling this with Zaccaroni et al. (2012)'s research that packs have stable vocal signatures over time, it could improve our ability to monitor wolf pack movements and count wolf pack numbers non-invasively. By looking for differences between howls in the chorus we could discriminate between individuals without needing to observe them (but see Harrington 1989 for difficulties in counting individuals joining a chorus though this could possibly be overcome with more modern spectrographic software). In a recent study, Passilongo et al. (2015) were able to recognise chorus size from bioacoustic analysis with very high accuracy. With spectrographic evidence, they could distinguish seven concurrent vocalisations in a chorus of nine wolves. Any additional knowledge of the structure or motivations of chorus howling, which this study provides, may help improve the accuracy of such population measures. Monitoring population sizes can help inform when population control measures are needed and to what extent they should be employed.

A key aim for the management of wild wolves is minimising their negative interaction with humans and livestock because conflicts with human populations are still the major threat to carnivore populations (Chapron et al. 2003). Listening to chorus howling could help in monitoring pack movements and alerting people when wolves are ranging close to livestock. The use of stationary recording devices to triangulate the location of wolves is currently being tested in Yellowstone National Park with high success (Kershenbaum, ongoing research). Much of the time, these recording devices will be picking up chorus howling. Understanding the dynamics of chorus howls for specific packs could allow us to identify the specific pack which is howling, as well as the location they are at.

Overall, this study provides a base point for future research into the structure and synchrony of chorus howling. We have shown that studies of this type with precise individual identification from simultaneous direct observations are possible. Understanding the dynamics of chorus howls in captive wolves may help to improve methods to monitor wolf numbers and movements non-invasively in the wild and provide a greater insight into their complex social structure.

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