ORIGINAL ARTICLE

Social modulation of spatial dynamics in treefrog choruses

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Abstract

In anuran amphibians, as well as many other animals, it is common for males to form breeding aggregations where they advertise to females of the same species. For female gray treefrogs (*Hyla versicolor*), the characteristics of male calls are integral to their preferences while the distance to the males represents a key feature of the required investment. Therefore, the spatial arrangement of males and the quality of their advertisement calls can influence sexual selection processes. We recorded the calls of male gray treefrogs, as well as the spatial position of individuals in the chorus, for aggregations in northern Michigan over three breeding seasons. Males were not randomly distributed across the chorus but showed both clustered patterns and dispersed patterns, depending on the scale of analysis. On active chorus nights, we identified clusters of males and individual males closest to the centers of those clusters ("medoids") using a novel analytical approach. Medoids displayed some call characteristics that are preferred by females significantly more often than the other members of their clusters and thus may represent "hotshot" males. Non-medoid males in the clusters displayed less preferred call features. Irrespective of overall cluster membership, the size of the chorus (number of males calling per night) and nearest neighbor distances were also correlated with specific call features. We suggest that the smaller clusters within choruses that we identified may represent the spatial scale over which female gray treefrogs sample and choose male mates.

Significance statement

Aggregations of animals for the sole purpose of mating are common and conspicuous occurrences. However, such aggregations provide both costs and benefits to participants. For males, the quality of their displays and spatial location in the aggregation can determine whether they mate at all. Females also face constraints as their preferred mates may represent costly choices. Using an objective point pattern analysis technique, we demonstrated that the male advertisement choruses of the gray treefrog can be subdivided into smaller local groups that contain higher quality males at the center. These local groups display some features of classic "hotshot" style leks and may represent the units females consider for choice of male mates.

Introduction

Aggregation of animals for breeding purposes is widespread, found in hundreds of vertebrate and invertebrate species (Alexander 1974; Beehler and Foster 1988; Mackenzie et al. 1995; Jones et al. 1998; Perez and Backwell 2019). The contribution of biotic and abiotic factors to the formation and function of the aggregations, however, may well be unique to each such group. For example, aggregations may be spatially constrained by the presence of critical environmental features related to food, nesting sites, or predator avoidance (Boyko et al. 2004; Evans et al. 2016; Brockmann et al. 2018; Macedo et al. 2018; Bowyer et al. 2020). In other cases, apparently identical habitat may sometimes

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host breeding aggregations and sometimes not (Whitney and Krebs 1975; Nekola and Kraft 2002; Johnson and Semlitsch 2003; Rudolf and Rodel 2005; Glos et al. 2008). Discovering the underlying factors responsible for the non-random spatial distributions of breeding aggregations can help us better understand the costs and benefits of aggregation membership.

The spatial scales over which males and females search for, compare, and choose mates may profoundly influence sexual selection processes (Jennions and Petrie 1997). In the common case, males form aggregations and then females choose mates from the assembled group. Female mate choice may then depend upon the particular location of males, with females displaying preferences for males in the center of the groups or closest to the edges, for example (Beehler and Foster 1988; Westcott 1994; Meuche et al. 2013). Females may adjust searching behaviors based on the perceived costs of travel (Jennions and Petrie 1997; Bonachea and Ryan 2011). Some females may choose only the closest male or evaluate a smaller group of local males, rather than choosing from among all males in the aggregation (Schwartz and Gerhardt 1989; Gibson 1996; Meuche et al. 2013; Stratman et al. 2021). On the other hand, females may sample from multiple distinct aggregations located large distances apart (Duraes et al. 2009). The specific spatial location of males in aggregations may thus result in an individual male not acquiring a mate at all.

Elements of male quality also usually interact with location to influence female preferences and choices of mates (Jennions and Petrie 1997). As an example, leks represent a special subset of breeding aggregations (Hoglund and Alatalo 1995; Jiguet et al. 2000). Classic leks contain no resources of direct value to females and males aggregate to perform courtship displays. Females then visit leks solely to mate. One perspective on lek formation therefore focuses on female choice behaviors and indeed female choice on leks can dramatically influence mate success in some species (Bradbury and Gibson 1983). The "female preference" model theorizes that female preference for clustered groups of males is a driver of lek formation, as groups allow females to compare multiple males at once (Balmford 1991). The "hot spot" model predicts that males aggregate at sites based on the number of visiting females (Bradbury et al. 1986; Westcott 1994). The "hotshot" model suggests that only a few males are highly attractive to females and leks form as less attractive males seek to better their chances of mating by associating with those few best males (Beehler and Foster 1988). Lastly, the "black hole" model involves no female preferences and instead proposes that males cluster territories in leks to retain females that would otherwise leave (Stillman et al. 1993). When females are mobile and move among territories at least once before mating, males in clustered territories can be more successful. Each of these

models makes predictions about the distribution of male quality across the lek (Tarof et al. 2005; Manica et al. 2020). For example, the hotshot model predicts that highest quality males would be in the center of the lek. The black hole model, on the other hand, would predict a random distribution of male quality across the lek. Patterns of variability in quality and location of males across leks thus determine the realized choices available to females.

Chorus aggregations of male gray treefrogs are often described as leks (Sullivan and Hinshaw 1992). At the chorus site, males establish short-term territories (often just one night) where they vocally advertise to attract females (Fellers 1979a). Males provide no discernable resources to females, and females choose partners based on criteria that offer them no direct benefits (Morris 1989). Furthermore, these treefrogs exhibit male-male aggressive interactions, alternative mating tactics, and female choice based on call characteristics, consistent with expectations of the hotshot model (Fellers 1979a, b). However, there is little evidence that females make choices by comparing all males in a chorus (Wells and Schwartz 1984; Gerhard and Klump 1988; Morris 1989). We hypothesized that male gray treefrogs (*Hyla versicolor*) in choruses are spatially arranged into smaller units within the chorus as a whole and that male advertisement call quality is associated with spatial positions within those smaller units. These smaller units may then represent the scale over which female choice of mates occurs in this species.

Patterns of variability in inter-male spacing and male behavioral interactions within leks, including anuran choruses, are difficult to discern. Aggregations may contain "clusters within clusters," for example, with subsets of the population interacting at local levels rather than across the entire aggregation (Bates et al. 2010; Bee et al. 2016). In gray treefrogs, males increase call duration and decrease call rate relative to their near neighbors to minimize overlapping (Schwartz et al. 2001, 2002). Females prefer long, non-overlapping calls (Wells 2007). Aggressive interactions also occur in anurans between neighbors in local groups (Wells 2007). For example, competing males lower call frequency during escalating aggressive interactions with close neighbors in the gray treefrog (Reichert and Gerhardt 2013a). Therefore, in leking anuran amphibians, social interactions between males are likely to occur in local groups that represent some subset of the chorus as a whole but whose spatial extent is unknown.

Choruses of frogs, then, can be described as collections of spatial point patterns where the patterns are defined by *x* and *y* coordinates for each frog in the landscape. Frogs also display other attributes related to their calling behavior, such as notes per call or calls per minute, that may be related to social interactions within a local group of neighbors (Schwartz et al. 2002). The identification of

which animals might together represent a local interacting group and whether groups contain patterns in the distribution of male quality are difficult problems to solve. We used two robust analytical approaches to describe the spatial distribution of male gray treefrogs in choruses and the relationships between their positions and advertisement call features. The first approach was the use of Ripley's K function to determine whether frog distribution within choruses demonstrated spatial randomness (Ripley 1977). The K functions can also indicate whether non-random choruses are clustered (points closer than expected) or dispersed (points farther apart than expected in a random pattern). The second approach was the use of two cluster analysis algorithms (the silhouette method and partitioning around medoids (Rousseeuw 1987; Kaufman and Rousseeuw 1990)), to determine the potential membership of frogs in particular clusters or local groups and whether patterns in male call quality might exist in those groups.

Methods and materials

Active breeding choruses of the Eastern gray treefrog, *Hyla versicolor*, were surveyed for the length of the breeding season, across 3 years. We recorded the advertisement calls of every calling male on every night of activity and mapped the spatial location of males in the chorus area. Calls were analyzed to extract their temporal characteristics. We then used spatial point pattern analyses and cluster analyses to determine the spatial structure of the aggregations and whether locations of males were related to call characteristics.

Sampling and call analysis procedures

Three vernal ponds, the usual chorus location of gray treefrogs, were monitored, using a different pond each year (2014–2016), at the University of Notre Dame Environmental Research Center in Gogebic County, Michigan, USA (Table 1). The average size of the ponds was 1350 m², and chorus size ranged from 2 to 19 males per night. We recorded male advertisement calls using SONY PCM-M10 recorders (Sony Electronics Inc., Park Ridge, NJ, USA;16-bit stereo LPCM file, 44.1-kHz sampling rate). Sound recordings were made for at least 2 min or for 20 successive calls (whichever came first) and from approximately 1 m from the focal frog. After recording, we measured body temperature in situ using a Raytek Raynger ST infrared thermometer (Fluke Process Instruments, Everett, WA, USA; Model RAYST20XBUSVB), measured snout-vent length (SVL) to the nearest millimeter, and toe-clipped frogs for permanent identification. The original calling site was flagged, and males returned. The following day, we recorded the coordinates of flags to the nearest centimeter using a permanent grid system of stakes laid out at 3-m intervals. Although males were occasionally re-recorded during the night, we used only the first recording of an evening for analysis. Many males were re-recorded on other nights, but the spatial analyses were limited to within-night comparisons, with the exception of the analysis of all calling site locations across a season. We rotated where in the pond area we began our recordings each evening to ensure that all areas were regularly searched during peak activity periods. It was not possible to record data blind because our study involved focal animals in the field.

Frogs were generally found calling from ground level and up to about 50 cm. They were not observed perching on our grid stakes. Perches were primarily found on shrubby

Table 1 Characteristics of chorusing sites and resident male gray treefrogs (*Hyla versicolor*) during three breeding seasons in northern Michigan, USA (means ± standard error of the mean)

	2014	2015	2016
Season duration	May 28–June 11	May 24–June 9	May 20–June 18
Site location	46°14′30″ N, 89°31′08″ W	46°14′29″ N, 89°31′7″ W	46°15′03″ N, 89°30′56″ W
Number of nights with callers	9	13	24
Number of nights with 8 or more callers	7	5	12
Total callers per season	84	98	211
Chorus size (males per chorus) *(median; range)*	9.3 ± 1.4 *(10; 2–15)*	7.5 ± 1.1 *(6; 4–14)*	8.8 ± 1.2 *(7; 2–19)*
Male snout-vent length (mm)	44.9 ± 0.3	46.0 ± 0.3	45.2 ± 0.2
Distance to nearest medoid (m)	9.1 ± 0.7	7.5 ± 0.7	4.5 ± 0.2
Nearest neighbor distance (m)	6.8 ± 0.5	6.4 ± 0.5	3.9 ± 0.3
Clusters per chorus[a]	2.29 ± 0.18	2.4 ± 0.25	2.42 ± 0.15
Site dimensions (*X, Y*; in meters)	28, 61	39, 38	33, 26

[a]Number of clusters determined by the silhouette method

and herb layers, in shallow water or marshy areas. Shrubs included speckled alder (*Alnus incana* ssp. *rugosa*), honeysuckles (*Lonicera xbella* and *L. canadensis*), northern bush honeysuckle (*Diervilla lonicera*), and leatherwood (*Dirca palustris*), while the herb layer was dominated in the open by sedges in the wettest areas (*Carex* species).

The sound analysis program RavenPro v1.4 (Cornell Bioacoustics Research Program, Ithaca, NY, USA) was used to analyze the first 10 advertisement calls of each recording. We measured call duration (s), intercall interval (s), notes per call and note period (s) directly from oscillograms and spectrograms of each call (as defined in (Kohler et al. 2017); Fig. 1). We measured the note period (time between the offset of a note to the offset of the following note) of five notes starting at the first peak note, and used this average as the note period for each call. The peak notes were defined as the first note at or nearly at the maximum amplitude reached during that call. The term "note" as used here refers to an acoustic unit within the call that corresponds to an individual contraction of trunk musculature and, therefore, can be thought of as both an acoustic unit and a biomechanical and energetic unit of the call (Wells and Taigen 1986; McLister et al. 1995; Girgenrath and Marsh 1997). The same call feature is often alternatively called a "pulse."

From these, we calculated call rate (calls/min), duty cycle, and note rate (notes/s). Call rate was computed as the instantaneous call rate for each call by dividing 60 s by the sum of each call duration and its subsequent intercall interval (= 60 s/call duration + intercall interval; (Kohler et al. 2017)). Duty cycle was computed by dividing call duration by call period (sum of each call duration and its subsequent intercall interval). Lastly, note rate was calculated by dividing the number of notes in a call by the call duration, measured from the onset of the first note to the offset of the last note. Variables thus determined from 10 calls per male were

then averaged to provide single values of call characteristics per male each night.

Ambient environmental temperature can alter both call morphology and call recognition in many amphibians, including gray treefrogs (Gerhardt 1978; Gayou 1984; Wells 2007; Reichert and Hobel 2018). We examined the relationships between temperature and measured call features using linear least squares regression, and used the resulting regression equations to adjust all measured call features for temperature variation within each year to 18 °C (average body temperature across all years). We then used the adjusted values to calculate derived call parameters (Supplementary Materials, Tables S1 and S2). For this and all further analyses, we used R version 3.5.0 (R Core Team 2019).

Spatial analysis procedures

The two primary methods of spatial analysis we used were point pattern analysis with Ripley's K function (Ripley 1977) and cluster analyses with the silhouette and partitioning around medoids clustering algorithms (Rousseeuw 1987; Schubert and Rousseeuw 2019). Coordinates for each male's position on each night were analyzed as individual point patterns. Most males were sampled only once so their positions were assumed to be stationary. Although most of the analysis utilized only individual nights, we did also analyze the distribution of males across all nights in each of the three seasons, to assess whether particular sites may be preferred.

We used a different pond in each year and the spatial extent of the choruses thus differed between years. The size of the spatial window used was fixed for all nights within a year by identifying the minimum and maximum *x* and *y* coordinates among all nights that year. This provided the dimensions of the chorus that were used for all nightly analyses (rounded to nearest whole number that included
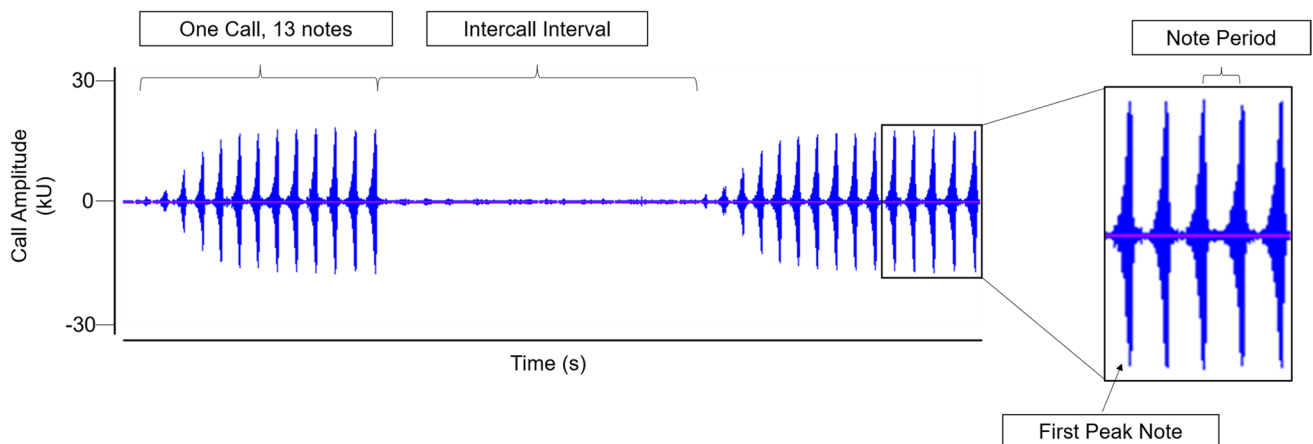
Fig. 1 Example of two typical advertisement calls of a male gray treefrog (*Hyla versicolor*). Waveform shows changes in the amplitude of the call in relative arbitrary units (kU). Inset shows a magnified view of five individual notes that make up a portion of the second call

all points; Table 1). Note that the dimensions of the spatial window were thus the same within one year but differed across years. Points representing individual frog positions were then arrayed within the window based on the night of recording and each frog's *x* and *y* coordinates (examples in Fig. 2A–C).

We analyzed the characteristics and distribution of male treefrogs in the chorus at several levels. In the simplest case, we examined whether males were distributed randomly, in a clustered (aggregated) pattern or a dispersed pattern. We used Ripley's K function for this purpose over individual nights and for cumulative spatial arrangements over each annual breeding season (Ripley 1977). This allowed us to examine the point patterns across multiple distance scales. The computed K function describes the number of points within a circle of radii sequentially larger from each focal event. For the analysis for cumulative spatial arrangements over an entire season, we pooled individual points across all nights within a season (see Fig. 2). For the analysis across separate nights, K functions were computed for individual point patterns (that is, for single nights) and then K functions themselves were secondarily pooled for particular groups of nights (not points across nights, in these cases; Fig. 3).

We used both homogeneous and inhomogeneous Poisson processes to generate 99 simulations of a theoretical K function distribution from a pattern showing complete spatial randomness (CSR; Diggle 2003). In the case of homogeneous processes, the underlying assumption is that the intensity of points (average number of points per unit area) is uniform across the spatial window. We used these analyses to determine if there were deviations from CSR across the entire extent of the space, irrespective of potential environmental

impediments to chorusing. These results are used to generate the dashed red line (e.g., Fig. 2) that corresponds to CSR.

On the other hand, for inhomogeneous processes, the assumption is that the intensity of points varies across the window (Baddeley et al. 2016). Again, 99 simulated point patterns are generated based on a Poisson distribution. However, the K functions are calculated in this case with a differing intensity of points across the window, similar to the intensity in the data itself. Thus, a local value for intensity is used for the inhomogeneous K functions, rather than the global value used for homogeneous K functions. The intensity of points is determined by a leave-one-out kernel smoothing algorithm (density.ppp in R; Baddeley et al. 2016). A kernel corresponds to a smaller "cell" within the window, where a focal event is not considered ("leave-one-out") but the intensity of points within the rest of the cell is calculated based on an isotropic gaussian function (Baddeley et al. 2016).

These analyses allowed us to generate theoretical K functions that considered differences in intensity across the spaces in our dataset. Kernel smoothed intensity functions for individual nights were examined for possible locations of local groups (R function density.ppp). Edge corrections were not applied, as the spatial extent of each chorus was identified and each spatial window contained all calling males, reducing the possibility of border effects (Baddeley et al. 2016). We conducted point pattern analyses using R package "spatstat" v1.61–0 (Baddeley et al. 2016).

Individual night K functions were also pooled, to determine whether the spatial structure of choruses varied depending on chorus size. Some nights displayed minimal calling activity, with a range of 1 to 7 calling males per night. The median number of calling males per night across

Fig. 2 Spatial distribution of calling sites used by male gray treefrogs at three different ponds in northern Michigan, presented as point patterns (**A**–**C**) and as homogeneous Ripley's K functions (**D**–**F**). Dots represent the position of calling males observed during the 2014 (**A**, *n*=84 sites), 2015 (**B**, *n*=98), and 2016 (**C**, *n*=211) breeding seasons, drawn to scale. Corresponding Ripley's K functions for these spatial distributions are shown (**D**–**F**). Each set of function values (K(r)) was computed over different radii (*r*, in meters). Red line shows the K function values expected if the pattern exhibited complete spatial randomness under homogeneous conditions
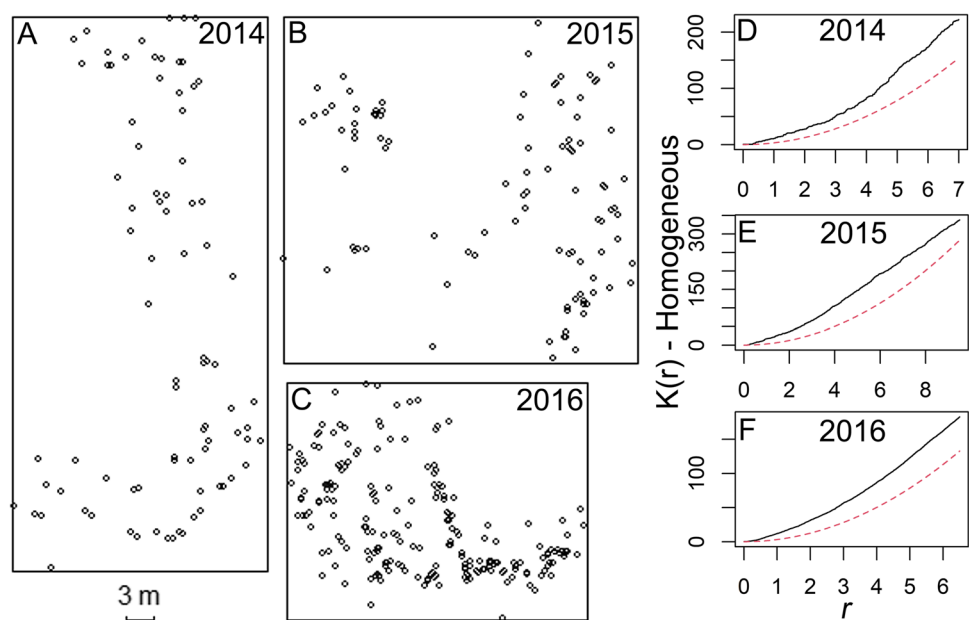
Fig. 3 Ripley's K function analyses of spatial point patterns in male gray treefrog choruses of different sizes. Pooled K-functions across multiple nights are shown for all nights of chorus activity (**A** and **D**, across all years, $n=46$ nights), for nights with lower levels of activity (**B** and **E**, 7 or fewer callers, $n=22$ nights), or nights with high levels of activity (**C** and **F**, 8 or more callers, $n=24$ nights). Homogeneous functions are shown in **A**–**C** and inhomogeneous functions in **D**–**F**. Red lines indicate K functions for a theoretical Poisson process representing complete spatial randomness under homogeneous and inhomogeneous assumptions. Shading around pooled functions represents the 95% confidence interval. Radius (r) in meters
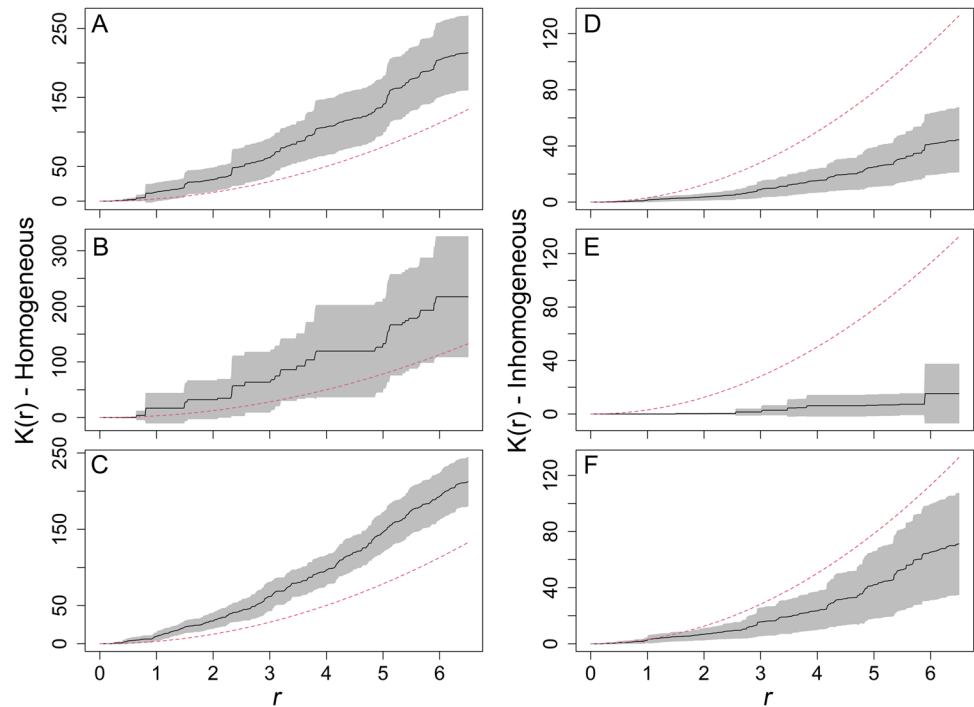
all years was 8 frogs (Table 1), so we compared pooled K functions for nights with 7 or fewer callers to nights with 8 or more callers (and a pooled function with all nights, for comparison). Confidence intervals (95%) were calculated for pooled K functions based on Baddeley et al (2016).

Exploration of the relationship between the spatial location of treefrog males and the characteristics of their advertisement calls utilized the nonhierarchical clustering algorithm, "partitioning around medoids" (PAM; Kaufman and Rousseeuw 1990). Cluster analysis consists of robust approaches for exploring data to find patterns (Jain 2010). Our approach to analyzing anuran choruses is new because, to our knowledge, clustering algorithms have not been previously used to identify spatial clusters in frog choruses. This is an unsupervised learning algorithm which is less sensitive to outliers than the common k-means cluster analysis method (Han et al. 2012). It was especially advantageous in our case because it minimized subjective judgements in creating clusters, it resulted in non-overlapping groupings of points, and it identified specific data points that were the most centrally located in clusters. This method thus provides an unbiased and objective approach to identify possible small social groups within larger aggregations. These analyses used only nights with active choruses of 8 or more calling males.

We created distance matrices for all males in each chorus using the Euclidian distance from each frog to all other frogs. Using these matrices, the silhouette clustering algorithm was used to determine the optimal number of clusters (Kaufmann and Rousseeuw 1987; Rousseeuw 1987; using R

package "factoextra" v1.0.5 (Kassambara and Mundt 2017)). When the optimal number of clusters was constrained to a maximum of 7, the silhouette method identified a range of 2 to 7 clusters, although some contained only individual frogs. The average cluster number with this method was 2.75 clusters (Table 1). We thus set the optimal number of clusters to three, so that for each night, frogs would be separated into either two or three clusters, and instances where clusters contained only one or two points would be minimized. We assigned frogs to clusters using the PAM algorithm and identified the medoids (Kaufmann and Rousseeuw 1987; Schubert and Rousseeuw 2019; using R package "cluster" v.2.0.7–1 (Maechler et al. 2021)). Medoids are the points (frogs) closest to the center of each cluster.

Relationships between each male's call characteristics and their spatial position within the chorus as a whole or in smaller clusters within choruses were determined primarily with chi-squared tests and linear least squares regression. First, we used chi-squared tests to determine if there was a relationship between medoid assignment and the minimum and/or maximum values of each measured and derived call parameter. This allowed us to determine if the single medoid in each cluster exhibited call features at the extremes, compared to all the other frogs in his cluster. For this analysis, we excluded 6 clusters where the assigned medoid had no or insufficient recorded call information and also excluded clusters containing only 1 or 2 frogs, resulting in a total of 45 clusters (all active choruses with 8 or more frogs calling).

Lastly, we computed the distance of each frog to the medoid assigned to his cluster (medoids themselves have a

distance of zero). Linear least squares regression was used to examine the relationships between call parameters and each frog's distance to their cluster's medoid. This was compared to a simple regression of all call parameters and nearest neighbor distance for all choruses (regardless of activity), without considering medoids or cluster membership (see Supplementary Information). All choruses were also used to determine if chorus size was significantly related to any call parameters. Regressions included study year as a fixed effects variable.

Results

Gray treefrogs were not randomly distributed across the landscape, but instead showed significant spatial clustering across nights within a season. Using an innovative approach, we identified distinct clusters of males within individual nightly choruses and then assigned males to a cluster and found the male located closest to the center of the cluster (the "medoid"). Medoids displayed advertisement call features that were significantly different from the other males within their clusters. Lastly, closely related aspects of chorus activity (chorus size and nearest neighbor distance) were also correlated with specific call features.

Deviations from random in the spatial patterns of frogs in a chorus

The distribution of male treefrog calling sites across three different ponds in three years showed a distinct deviation from complete spatial randomness (CSR; Fig. 2). Visual inspection of the point patterns indicates areas of the chorus that are relatively lacking in calling sites and others with clusters of sites at higher intensities within the pond areas (Fig. 2A–C). The homogeneous Ripley's K function shows the spatial scales at which calling site locations, from all nights per year, deviate from CSR (Fig. 2D–F). We found K function estimates in each year to be well above the theoretical estimates of K functions for simulated CSR. This indicates clustered patterns, while chorus K function estimates below the CSR estimate would indicate dispersed patterns. Interestingly, each year, the distribution of calling sites is clustered at all radii from about 1 m outward (Fig. 2D–F; examples of single nights in Fig. 4).

On the other hand, because some elements of frog location may be influenced by features such as the site topography, we also examined the choruses using inhomogeneous K functions. This function uses a different model to generate the expected value for CSR, based on the Poisson process (Baddeley et al. 2016). The model incorporates the differences in point intensity across the landscape. Pooled
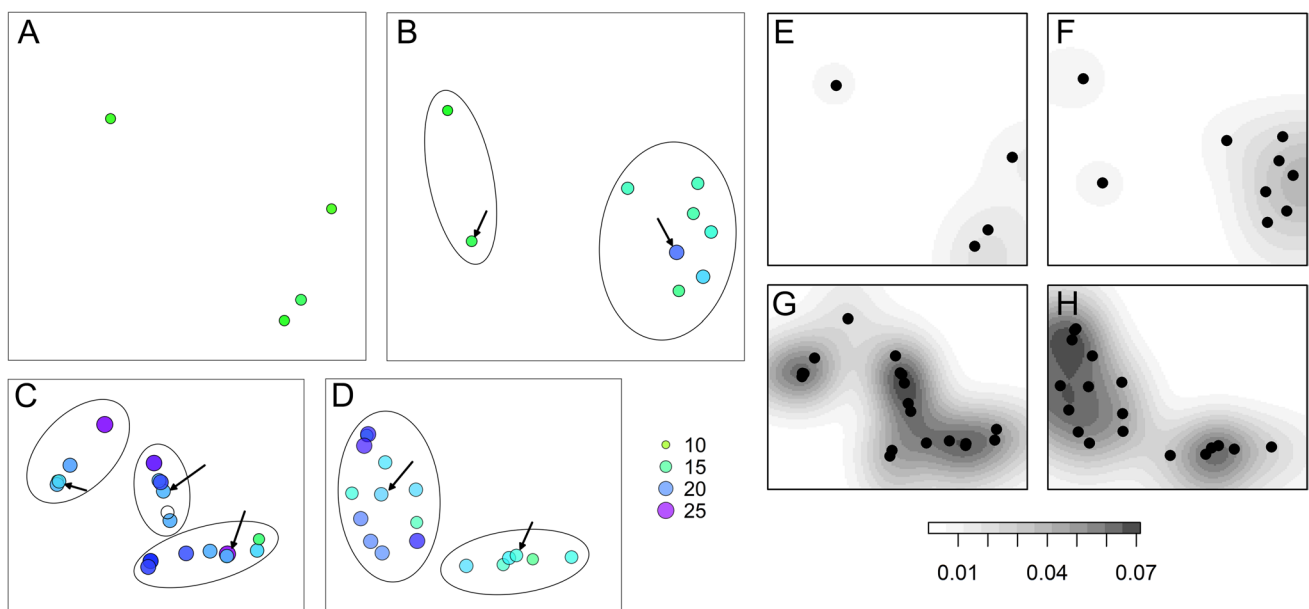
Fig. 4 Examples of the spatial distribution of male gray treefrog callers on four chorus nights (**A–D**). **A** May 29, 2015; chorus of 4 males. **B** May 25, 2015; chorus of 9 males. **C** May 26, 2016; chorus of 19 males. **D** June 9, 2016; chorus of 18 males. (Some points in C and D are obscured by other points.) Clusters to which each frog belongs, if any (determined by the PAM algorithm; indicated by ovals). Medoids (frogs closest to the center of the cluster) are indicated by arrows. Average number of notes per call for each male is indicated by the gradient for symbol size and color. **E–H** Examples of kernel smoothed intensity functions for the same four nights. This approach calculates a localized density for subsets of the chorus, with shades of gray representing estimated intensity values, expressed in points per unit area. It thus reflects patterns where point features (frogs) are concentrated

K functions for choruses of different sizes were compared for both homogeneous and inhomogeneous assumptions (Fig. 3). Overall, when choruses of all sizes are pooled, homogeneous K functions indicate that male treefrog callers are clustered across the spatial window, while inhomogeneous K functions indicate that they are dispersed (Fig. 3A, D).

These patterns of the distribution of male treefrogs in space are influenced by the number of calling males or chorus size. When choruses contained fewer than 8 calling males, both homogeneous and inhomogeneous K functions lacked the power to indicate clustered or dispersed patterns (Figs. 3B, E and 4A, E). In larger, more active choruses, on the other hand, with 8 or more calling males per night, the homogeneous K functions showed significant clustering (Fig. 3C). Plots of intensity functions for individual nights also show patterns with higher concentrations of calling males (Fig. 4B–D, F–H). The inhomogeneous K functions showed the opposite pattern. For choruses with 8 or more callers, males were dispersed at all radii above about 2 m, indicating that males were farther apart than would be expected from a random pattern (Fig. 3F).

Relationships between cluster membership, cluster positions, and call features

Active treefrog choruses, with 8 or more calling males each, exhibit two or three distinct clusters per chorus, based on the silhouette method for the analysis of spatial positions (Table 1). Using the optimal number of clusters, the PAM algorithm determined the cluster to which each

frog belonged, and identified one male in each cluster as a "medoid." A medoid is defined as the male with the least average dissimilarity (in this case, Euclidean distance to other males) compared to all the others in the cluster. This is an important distinction because the geometric center of each chorus may contain no frogs. Examples of cluster and medoid assignments are shown for three different choruses in Fig. 4B–D. The very small chorus in Fig. 4A does not have clusters assigned because the K function for this night did not indicate significant clustering.

Some features of the advertisement calls of male gray treefrogs were significantly correlated with the distance between males and the medoid assigned to the male's cluster (Table S3). We found significant negative correlations between the distance to medoid and the number of notes per call produced by males (Fig. 5A and Table S3). Importantly, our model with the distance to medoid and the year explained about 26% of the variability in notes per call that we observed. On the other hand, we found significant positive correlations between the distance of males to medoids and the instantaneous call rates (Fig. 5D and Table S3), as well as the intercall interval and note rate (Fig. 5B and Table S3). For intercall interval, however, there were significant distance by year interactions. Although call duty cycle was significant, the estimate was very small (− 0.00024; Table S3). In summary, as the distance of males from cluster medoids increased, the number of notes per call and call duration of males decreased overall, while call rate and note rate increased. There was

Fig. 5 Relationship between the distance from a calling male gray treefrog to the medoid assigned in his cluster (in meters) and four features of the advertisement calls ($n = 281$ frogs). Medoids themselves have a distance of zero. Linear least squares regressions with the 95% confidence interval indicated by shading and year as a covariate
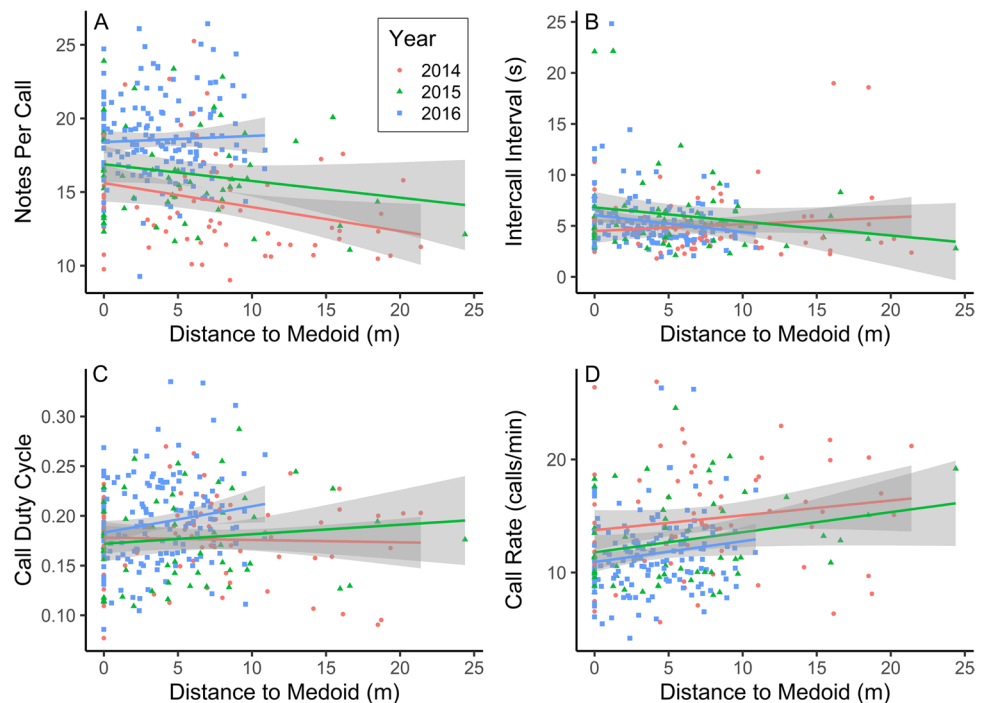
no significant correlation between distance to medoid and male snout-vent length ($F(5,287) = 1.48$, $p = 0.20$).

Medoids themselves displayed some call features that differed significantly from the other males in their cluster (Fig. 6 and Table S4). We used chi-squared analyses to predict the percent of medoids expected to have a call feature that was either the maximum in their cluster or the minimum value for that feature in their cluster. Medoids displayed the highest number of notes per call, the longest call durations, the longest intercall intervals, the longest call periods, and the highest note rates out of all the frogs in their individual

assigned clusters significantly more often than expected (Fig. 6A and Table S4). Similarly, medoid males were also more likely to have the lowest call rates and note periods, compared to the rest of their cluster (Fig. 6B and Table S4). Medoids thus occupy a relatively central spatial position in their clusters and display exceptional call features, compared to surrounding males. Medoids had neither the largest nor the smallest snout-vent lengths in their clusters (Table S4).

Chorus size and nearest neighbor distance were also significantly correlated with some call features in male treefrogs. There was a positive correlation between chorus
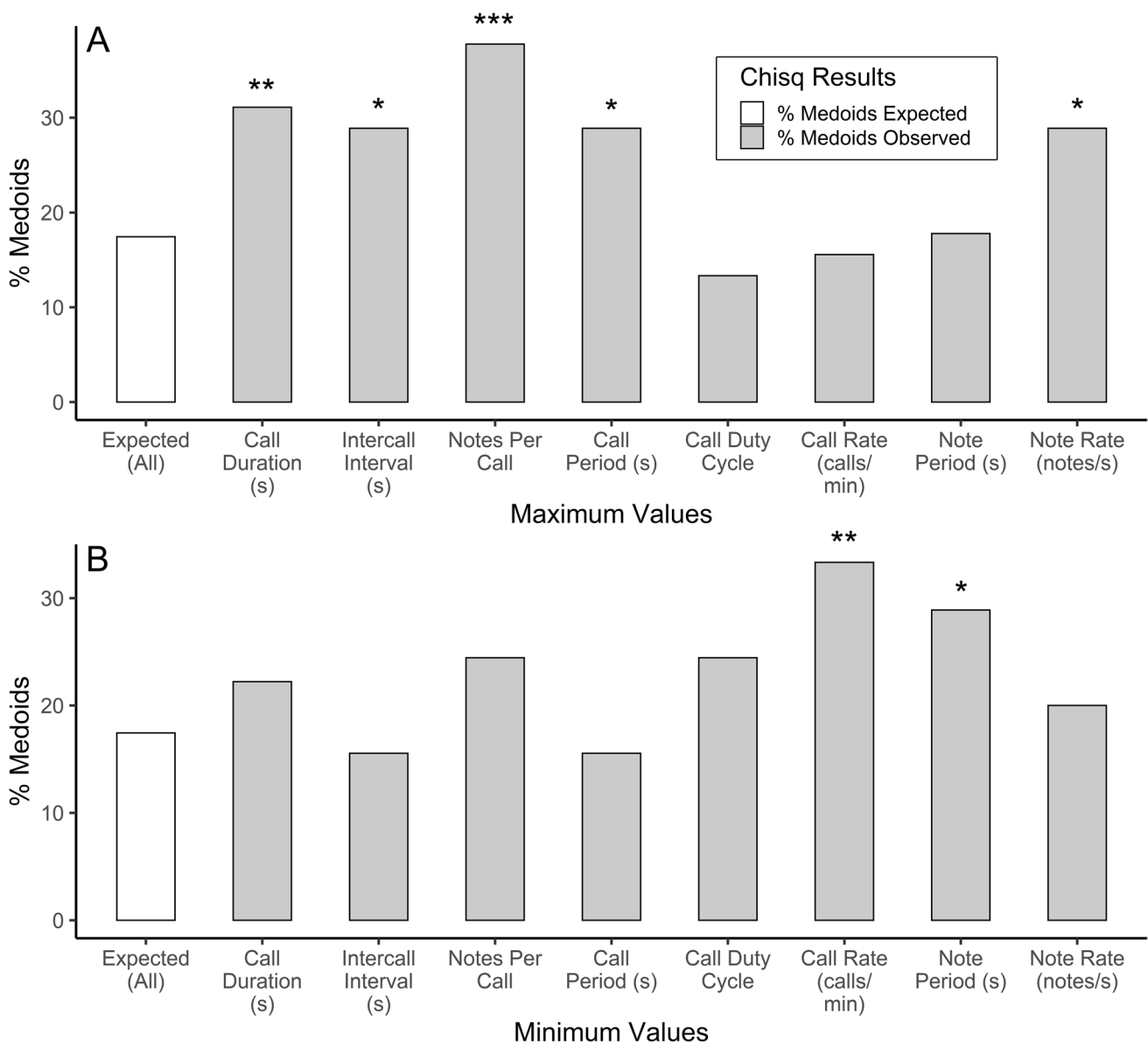
Fig. 6 Determination of whether male gray treefrog medoids possess the maximum (**A**) or minimum (**B**) values within their cluster for any call parameters. Chi-squared analyses predicted the expected percent of medoids that possess a value at the extreme (compared to other frogs in each cluster) and the percent actually observed ($n = 258$ frogs in 45 clusters). Clusters containing only 1 or 2 frogs were not included. Difference between expected and observed counts, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$

size (number of males calling on a given night) and both the average number of notes per call and closely associated call duration for all males on that night (Fig. 7A and Table S3). Chorus size and year explained about 52% of the variability in call duration that we observed. On the other hand, there was a negative correlation between chorus size and the average call rate (Fig. 7D and Table S3). Chorus size showed a significant positive correlation with snout-vent length of the frogs such that larger choruses contained larger males, on average (Table S3). This was, however, evident primarily in 2014 (Fig. 7C).

Nearest neighbor distance was significantly correlated with many call features (Table S3). Overall, the pattern followed that for distance to medoid. There was a significant negative correlation between distance to nearest neighbor and snout-vent length, such that smaller males were found at greater distances from their nearest neighbors (Table S3). It is important to note that these more general relationships for chorus size and nearest neighbor distance reflect the chorus activity as a whole and are not directly related to either cluster membership or medoid status.

Discussion

Breeding choruses of calling males in many anuran amphibians, such as the gray treefrog, are often described as leks because they lack resources of obvious use to females (Fellers 1979a; Sullivan and Hinshaw 1992; Jennions and Petrie 1997). However, key related features of leks, including the

distribution of males of different quality and female preferences for males in different spatial positions, are poorly understood. We established that gray treefrog choruses in three Michigan aggregations displayed non-random spatial distributions of males by using spatial point pattern analysis. In active advertising choruses, these non-random distributions indicate that males can be divided into smaller local groups. The advertisement call characteristics of males within local groups vary with their positions, relative to the males closest to the center, supporting the hypothesis that there are social influences on the calls of resident males. Specifically, males closest to the centers of local groups ("medoids" identified with the PAM method) were significantly more likely to possess call characteristics preferred by females, such as calls with more notes. There was a significant negative correlation between the distance of cluster males from their assigned medoids and notes per call, such that males farther from the center of clusters had less preferred calls. We thus identified possible leks on a spatial scale smaller than a single breeding assemblage that might represent the scale most salient to choosing females.

Characteristics of gray treefrog chorus cluster medoids

Male gray treefrogs that were assigned the status of a cluster "medoid" by the PAM algorithm differed in important ways from non-medoid members of their own cluster. Cluster medoids possessed advertisement call characteristics preferred by female treefrogs significantly more often

Fig. 7 Relationships between chorus size for male gray treefrogs (number of calling males per night) and the mean value for all participating frogs that night on three features of the advertisement call and frog snout-vent length ($n = 46$ nights). Linear least squares regressions with the 95% confidence interval indicated by shading and year as a covariate
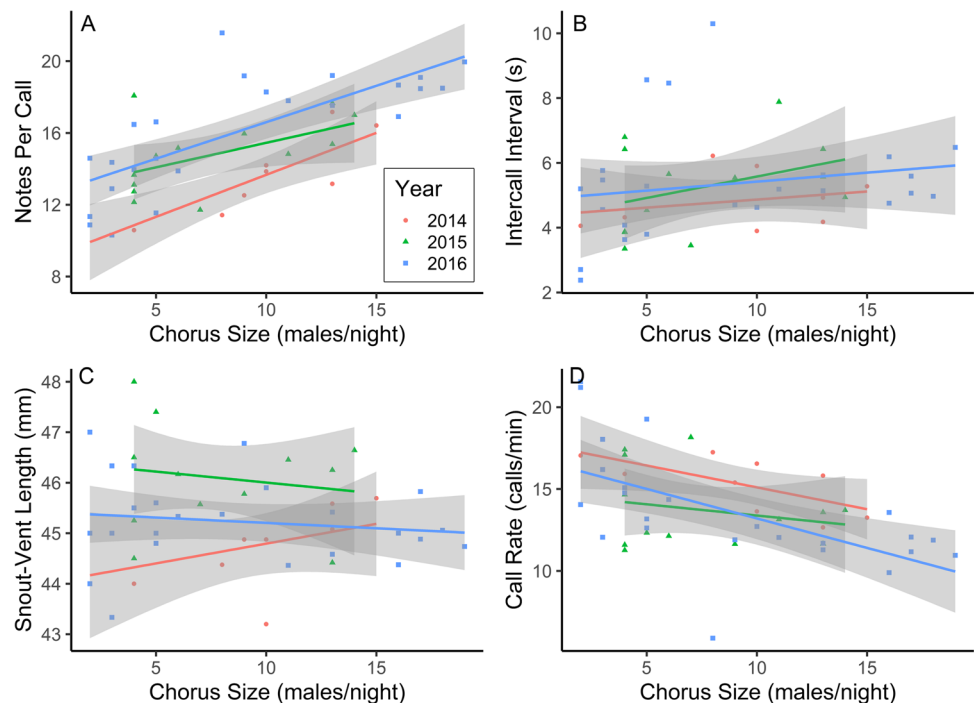
than expected by chance. Female anurans of many species, including gray treefrogs, are well known to prefer male calls with more notes per call, and thus longer call durations (Klump and Gerhardt 1987; Gerhardt et al. 1996, 2000; Bush et al. 2002; Bee 2008; Tárano and Fuenmayor 2013). Within individual clusters, about 38% of the medoids (the males we identified as being closest to the center of the local group) possessed the highest number of average notes per call, compared to the other members of the cluster (expected value less than 18%). Longer calls may serve as reliable indicators of genetic advantage and confer benefits to offspring of females that mate with such males (Klump and Gerhardt 1987; Welch et al. 1998; Doty and Welch 2001; Welch 2003; Forsman and Hagman 2006; Richardson et al. 2008).

Although anuran choruses are commonly described as leks because males defend no resources of value to females and females visit only to mate, how such anuran leks might compare with "classic" leks in other taxa is unclear (Bradbury and Gibson 1983). Our finding that cluster medoid males show particularly attractive call characteristics, compared to surrounding males, represents the first evidence that an anuran chorus might contain "hotshot" type leks (Beehler and Foster 1988). Hotshot models predict that males more centrally positioned in leks will produce more costly and/or more preferred courtship displays, while other lek models make no explicit predictions about spatial positions of males with particular qualities (Tarof et al. 2005; Manica et al. 2020). Critical to this finding was our ability to divide an entire breeding aggregation (a "chorus") into smaller local groups ("clusters") with an impartial technique. Thus, the spatial scale within which we considered relative male quality differed from previous work. Given chorus structures where smaller clusters are embedded within the chorus assemblage, efforts to find exceptional males in the center of the whole chorus (to support the hotshot model, for example) might not be fruitful. Instead, we suggest that criteria related to lek structure focus at a more local level within spatially defined clusters that together make up whole choruses.

The long calls observed for many gray treefrog medoids represent displays that are both preferred *and* more costly. The high metabolic costs of producing long calls usually result in a negative correlation between call duration and call rate. This means that as call duration increases, the intercall interval (silent period between calls) will also increase and the overall call rate will fall (Wells 2007). Low call rates for medoids therefore do not represent a preferred feature for female gray treefrogs, but it is likely a natural consequence of the long call durations, due to energetic constraints for males (Wells and Taigen 1986; Bevier 1997; Grafe 1997; McLister 2001).

On the other hand, we currently have no evidence that cluster medoids achieve significantly more mates than non-medoids, a core requirement for the hotshot model

(Bradbury and Gibson 1983; Hoglund and Alatalo 1995). The operational sex ratio at anuran choruses is typically highly skewed toward males (Sullivan and Hinshaw 1992). A few males thus achieve most of the matings each season and the majority do not mate. In one study of gray treefrogs, for example, only 14% mated at all, across three years (Bertram et al. 1996). However, male mate success is usually correlated simply with chorus attendance, and it is unknown if features such as male spatial position are related (Woodward 1982; Robertson 1986; Gerhardt et al. 1987; Arak 1988; Morris 1989; Sullivan and Hinshaw 1992).

Importantly, however, although medoid males displaying characteristics preferred by females were found more frequently than expected, this was not the case 100% (or even 50%) of the time. Our analysis was rather course-grained in considering only the male closest to the geometric center of the chorus and only whether that male had the highest or lowest values. We would not have identified cases where the nearest neighbor of a medoid, for example, who might thus also be very close to the center, showed such exceptional characteristics. In addition, choruses are highly dynamic with males entering or leaving continuously (Fellers 1979a). Our maps thus represent a "snapshot" of chorus structure and male attractiveness may be modulated by other call features such as amplitude or being the first to arrive at the chorus (Fellers 1979a; Brenowitz and Rose 1994; Stewart and Bishop 1994; Ringler et al. 2017).

Social interactions within gray treefrog chorus local clusters

Active male gray treefrog choruses could consistently be subdivided into two or three smaller clusters, or local groups, based on distance to neighbors in this study. We suggest that these smaller units represent the spatial scale over which inter-male competition occurs. This competition could be due to acoustic interactions or overt physical aggression, although the former is more common in anurans (Wells 2007). Although assignments to clusters were based solely on distance using the PAM method, both our chi-squared analysis and regressions on distance to medoid support a relationship between call characteristics, cluster membership, and medoid status. If males within identified clusters were not interacting with one another, we would not expect exceptional characteristics in cluster medoids, nor any relationship between distance from medoid and call characteristics. The temporal features of male anuran calls are significantly influenced by the calls of their neighbors (Schwartz et al. 2002). For example, male anurans may modulate call duration, complexity, rate, amplitude, or timing relative to neighbors, to increase the attractiveness of their calls (Schwartz 1987, 1993; Lopez et al. 1988; Greenfield and Rand 2000; Reichert and Gerhardt 2013b). Importantly,

cluster mates will hear cluster members at higher amplitudes, and amplitude can determine to which neighbor a male is most likely to respond vocally.

Male anurans respond vocally to the advertisement calls of their near neighbors. Alternation of calls with a single neighbor are common, when one male delays calling such that its call will then fall within the silent intercall interval of the neighbor and avoid acoustic interference, for example (Wells 2007). This can also occur within small groups of three males but in larger groups such alternation becomes impossible as the space between calls is not sufficient (Greenfield and Rand 2000). In experimental lab choruses of gray treefrogs, males are exquisitely sensitive to the number of other callers in the chorus (Schwartz et al. 2002). Males decreased the number of notes per call with the removal of each single caller from their chorus of eight. They also increased notes per call with the sequential re-additions. Interestingly, despite these changes, the relative rank of males based on notes per call changed little. Male treefrogs thus respond to neighbors in local groups of the sizes we identified as clusters here. In addition, the existence of stable hierarchies in experimental choruses supports our finding of clusters with high quality cluster medoids, which may represent dominant or high-ranking males, in local groups (Jennions and Petrie 1997).

Typically, "hotshot" leks possess higher quality males in the center and inferior males establish territories surrounding them (Bradbury and Gibson 1983). Given the strong preference of female gray treefrogs for male calls with more notes per call and thus longer durations in the lab, we have evidence for this pattern in natural choruses. Male gray treefrogs with less preferred call characteristics were distributed around medoids in a distance-dependent manner. We found that on average, as distance from the central medoid frog increased, the number of notes produced and call duration decreased, while call rate and note rate increased. On the other hand, the importance of notes per call for choice under field conditions may be more modest. Females do discriminate sharply against very short calls (6 notes or fewer), but such calls were rare in our choruses (Schwartz et al. 2001). In natural choruses, distance to males, acoustic interference, and predator avoidance, to name just a few obstacles, may take precedence (Deb and Balakrishnan 2014; Boyd and Gordon 2021). Lastly, the presence of inferior males around high quality males in a "hotshot-style" lek would also be supported if non-calling satellite males were arrayed outward from medoids, however satellites were also rare in our choruses, and we did not actively monitor their occurrence (Fellers 1979a).

The sampling behavior of female anurans is poorly understood, so it is currently unclear whether females may sample and choose within the clusters we identified. Certainly, it does seem unlikely that female gray treefrogs sample from

entire choruses, which may cover 500 to 1000 m^2 (Fellers 1979a, b; Godwin and Roble 1983; Morris 1989; Ptacek 1992; Sullivan and Hinshaw 1992). On the contrary, some observations show females mating with the first male they encounter or choosing one from the closest two (Fellers 1979b; Wagner and Sullivan 1992). The two most prominent decision-making theories for female mate choice are the minimum threshold rule (also called the sequential search rule) and the best-of-n rule (or pooled comparison rule) (Janetos 1980; Real 1990). Female treefrogs using the minimum threshold rule to choose a mate would be unlikely to preferentially mate with the medoids we identified. Overall, even the poorer quality males we recorded were still well above thresholds for female choice in lab situations (Schwartz et al. 2001; Boyd and Gordon 2021). However, preferential mating with cluster medoids could be accomplished if female gray treefrogs use a best-of-n method to make mate decisions when "n" is the cluster size. There is some evidence that females choose mates from small sets (proposed to be 3 to 5) of the closest males (Gerhardt and Klump 1988; Morris 1989; Telford et al. 1989; Stratman et al. 2021). Such sets could also be represented in the clusters we identified.

Identification of local groups in treefrog choruses

The non-random distribution of chorusing male frogs across the landscape has been recognized for many years, but statistical approaches to such distributions have been well developed only more recently (Wells 1977; Fellers 1979a; Wilczynski and Brenowitz 1988; Ovaska and Hunte 1992; Baddeley et al. 2016). Male treefrog choruses in our populations also showed non-random patterns of distribution. Some regions of the terrain were not used by males, resulting in homogeneous K functions displaying clustered patterns. We did not investigate why males avoided some landscape areas, although this could have been due to locations of desirable perches and calling sites with desirable acoustic features, thermal environment, or absence of predators, among other reasons (Wilczynski and Brenowitz 1988; Ptacek 1992; Bernal et al. 2007; Höbel and Barta 2014; Schwartz et al. 2016).

When male distributions were considered with inhomogeneous K functions, gray treefrog choruses showed significant dispersion within nightly choruses. Thus, within individual choruses, males are more regularly found somewhat farther from each other than would occur randomly. Maintenance of particular inter-individual distances results in non-random spacing here in gray treefrogs, and also in several other anurans (Robertson 1984; Telford 1985; Wilczynski and Brenowitz 1988; Gerhardt et al. 1989; Bourne 1992; Tárano 2009), although this is not universally the case (see for example, Ovaska and Hunte 1992). Amplitude of the calls of near neighbors can be used as a reliable spacing

device (Fellers 1979a; Brenowitz et al. 1984; Robertson 1984; Wilczynski and Brenowitz 1988; Brenowitz 1989; Gerhardt et al. 1989). Spacing between males can also be influenced by physical aggression (Wells 2007).

Of course, we have no proof that frogs themselves recognize "cluster mates." However, two lines of evidence support the hypothesis that members of a cluster may interact socially. The first evidence is our finding of differences in call features depending on medoid status and depending on the distance of non-medoids from their cluster's medoid. The second line is the well-described focus of male anurans on vocal interactions with nearest neighbors (Fellers 1979a; Wilczynski and Brenowitz 1988; Schwartz 1993; Burmeister et al. 1999; Reichert and Gerhardt 2013b). Frogs identify nearest neighbors based on the amplitude of their calls, which is reliably correlated with distance, and the PAM method depended in our analysis on Euclidean distances (Naguib and Wiley 2001).

Chorus size was significantly correlated with two closely related preferred features of the calls of male gray treefrogs. The size of the chorus varied across years with an average number of callers per night between 7.5 and 9.3 frogs. As the size of choruses increased, the number of notes per call and hence the call duration also increased. This association is typical for anuran choruses, including those of gray treefrogs, and likely results from increased inter-male competition (Wells 2007). The size of a chorus may have important consequences, as a correlation between chorus size and the number of females arriving has been reported for several anurans (Ryan et al. 1981; Tejedo 1992; Wagner and Sullivan 1992). Importantly, at our latitude the density of gray treefrog choruses was on the low side (mean of 1 frog/125 m^2) and choruses of 10 frogs/100 m^2 have been reported at lower latitudes (Ptacek 1992). We predict that such large choruses would contain more clusters per chorus although it is also possible that at high densities male distribution becomes random. Changes in calling behavior related to overall size or density of the chorus aggregation are not necessarily related to dynamics in smaller local clusters (Forester and Lykens 1986). Such changes can occur just based on overall chorus "noise" rather than interactions with specific neighbors (Bee 2012). In addition, of course, if there are biotic or abiotic restrictions on appropriate breeding locations, then increases in chorus size necessitate an increase in density and a decrease in nearest neighbor distance.

Conclusion

The spatial scales over which female gray treefrogs search for mates may maximize or restrict their ability to express their preferences. In addition, females navigating typical anuran chorus conditions, with a chaotic auditory environment in the dark, may well make tradeoffs. We sought to determine whether there were patterns to the spatial distribution of males that could influence female choice. For potential male mates, we found evidence of their spatial organization into smaller local groups within larger and more active chorus aggregations. Within these groups, males closest to the geometric centers ("medoids") displayed characteristics preferred by females significantly more often than non-medoid members of their own groups. This finding provides the first evidence that anuran choruses may contain "hotshot" type leks. However, whether females may preferentially mate with hotshot medoids or choose mates within the local clusters we identified is unknown. We suggest that examining inter-male competition and female mate choice in smaller objectively identified groups that make up anuran breeding aggregations could provide new insights.

Declarations

References

Alexander RD (1974) The evolution of social behavior. Annu Rev Ecol Syst 5:325–383

Arak A (1988) Female mate selection in the natterjack toad - active choice or passive attraction. Behav Ecol Sociobiol 22:317–327

Baddeley A, Rubak E, Turner R (2016) Spatial point patterns: methodology and applications with R. Chapman and Hall/CRC Press, London

Balmford A (1991) Mate choice on leks. Trends Ecol Evol 6:87–92

Bates ME, Cropp BF, Gonchar M, Knowles J, Simmons JA, Simmons AM (2010) Spatial location influences vocal interactions in bullfrog choruses. J Acoust Soc Am 127:2664–2677

Bee MA (2008) Parallel female preferences for call duration in a diploid ancestor of an allotetraploid treefrog. Anim Behav 76:845–853

Bee MA (2012) Sound source perception in anuran amphibians. Curr Opin Neurobiol 22:301–310

Bee MA, Reichert MS, Tumulty J (2016) Assessment and recognition of rivals in anuran contests. Adv Stud Behav 48:161–249

Beehler BM, Foster MS (1988) Hotshots, hotspots, and female preference in the organization of lek mating systems. Am Nat 131:203–219

Bernal XE, Rand AS, Ryan MJ (2007) Sexual differences in the behavioral response of tungara frogs, *Physalaemus pustulosus*, to cues associated with increased predation risk. Ethology 113:755–763

Bertram S, Berrill M, Nol E (1996) Male mating success and variation in chorus attendance within and among breeding seasons in the gray treefrog (*Hyla versicolor*). Copeia 1996:729–734

Bevier CR (1997) Utilization of energy substrates during calling activity in tropical frogs. Behav Ecol Sociobiol 41:343–352

Bonachea LA, Ryan MJ (2011) Localization error and search costs during mate choice in Tungara frogs, *Physalaemus pustulosus*. Ethology 117:56–62

Bourne GR (1992) Lekking behavior in the neotropical frog *Ololygon rubra*. Behav Ecol Sociobiol 31:173–180

Bowyer RT, McCullough DR, Rachlow JL, Ciuti S, Whiting JC (2020) Evolution of ungulate mating systems: Integrating social and environmental factors. Ecol Evol 10:5160–5178

Boyd SK, Gordon NM (2021) Auditory and distance cues interact to modulate female gray treefrog preferences for male advertisement calls. Behav Ecol Sociobiol 75:95

Boyko AR, Gibson RM, Lucas JR (2004) How predation risk affects the temporal dynamics of avian leks: Greater sage grouse versus golden eagles. Am Nat 163:154–165

Bradbury J, Gibson R (1983) Leks and mate choice. In: Bateson P (ed) Mate choice. Cambridge University Press, Cambridge, pp 109–138

Bradbury J, Gibson R, Tsai IM (1986) Hotspots and the dispersion of leks. Anim Behav 34:1694–1709

Brenowitz EA (1989) Neighbor call amplitude influences aggressive behavior and intermale spacing in choruses of the Pacific treefrog (*Hyla regilla*). Ethology 83:69–79

Brenowitz EA, Rose GJ (1994) Behavioral plasticity mediates aggression in choruses of the Pacific treefrog. Anim Behav 47:633–641

Brenowitz EA, Wilczynski W, Zakon HH (1984) Acoustic communication in spring peepers – Environmental and behavioral aspects. J Comp Physiol 155:585–592

Brockmann HJ, St Mary CM, Ponciano JM (2018) Discovering structural complexity and its causes: Breeding aggregations in horseshoe crabs. Anim Behav 143:177–191

Burmeister S, Konieczka J, Wilczynski W (1999) Agonistic encounters in a cricket frog (*Acris crepitans*) chorus: Behavioral outcomes vary with local competition and within the breeding season. Ethology 105:335–347

Bush SL, Gerhardt HC, Schul J (2002) Pattern recognition and call preferences in treefrogs (Anura: Hylidae): a quantitative analysis using a no-choice paradigm. Anim Behav 63:7–14

Deb R, Balakrishnan R (2014) The opportunity for sampling: the ecological context of female mate choice. Behav Ecol 25:967–974

Diggle P (2003) The statistical analysis of spatial point patterns, 2nd edn. Hodder Education Publishers, London

Doty GV, Welch AM (2001) Advertisement call duration indicates good genes for offspring feeding rate in gray tree frogs (*Hyla versicolor*). Behav Ecol Sociobiol 49:150–156

Duraes R, Loiselle BA, Parker PG, Blake JG (2009) Female mate choice across spatial scales: influence of lek and male attributes on mating success of blue-crowned manakins. Proc R Soc Lond B 276:1875–1881

Evans JC, Votier SC, Dall SRX (2016) Information use in colonial living. Biol Rev 91:658–672

Fellers GM (1979a) Aggression, territoriality, and mating behavior in North American treefrogs. Anim Behav 27:107–119

Fellers GM (1979b) Mate selection in the gray treefrog, *Hyla versicolor*. Copeia 1979:286–290

Forester DC, Lykens DV (1986) Significance of satellite males in a population of spring peepers (*Hyla crucifer*) Copeia. 1986:719–724

Forsman A, Hagman M (2006) Calling is an honest indicator of paternal genetic quality in poison frogs. Evolution 60:2148–2157

Gayou DC (1984) Effects of temperature on the mating call of *Hyla versicolor*. Copeia 1984:733–738

Gerhardt HC (1978) Temperature coupling in the vocal communication system of the gray tree frog, *Hyla versicolor*. Science 199:992–994

Gerhardt HC, Daniel RE, Perrill SA, Schramm S (1987) Mating behavior and male mating success in the green treefrog. Anim Behav 35:1490–1503

Gerhardt HC, Diekamp B, Ptacek M (1989) Inter-male spacing in choruses of the spring peeper, *Pseudacris (Hyla) crucifer*. Anim Behav 38:1012–1024

Gerhardt HC, Dyson ML, Tanner SD (1996) Dynamic properties of the advertisement calls of gray tree frogs: Patterns of variability and female choice. Behav Ecol 7:7–18

Gerhardt HC, Klump GM (1988) Masking of acoustic signals by the chorus background noise in the green tree frog - a limitation on mate choice. Anim Behav 36:1247–1249

Gerhardt HC, Tanner SD, Corrigan CM, Walton HC (2000) Female preference functions based on call duration in the gray tree frog (*Hyla versicolor*). Behav Ecol 11:663–669

Gibson RM (1996) Female choice in sage grouse: The roles of attraction and active comparison. Behav Ecol Sociobiol 39:55–59

Girgenrath M, Marsh RL (1997) In vivo performance of trunk muscles in tree frogs during calling. J Exp Biol 200:3101–3108

Glos J, Wegner F, Dausmann KH, Linsenmair KE (2008) Oviposition site selection in an endangered Madagascan frog: Experimental evaluation of a habitat model and its implications for conservation. Biotropica 40:646–652

Godwin GJ, Roble SM (1983) Mating success in male treefrogs, *Hyla chrysoscelis* (Anura, Hylidae). Herpetologica 39:141–146

Grafe U (1997) Use of metabolic substrates in the gray treefrog *Hyla versicolor:* Implications for calling behavior. Copeia 1997:356–362

Greenfield MD, Rand AS (2000) Frogs have rules: Selective attention algorithms regulate chorusing in *Physalaemus pustulosus* (Leptodactylidae). Ethology 106:331–347

Han J, Kamber M, Pei J (2012) Data Mining: Concepts and Techniques, 3rd edn. Morgan Kaufmann Publishers Inc, San Francisco

Höbel G, Barta T (2014) Adaptive plasticity in calling site selection in grey treefrogs (*Hyla versicolor*). Behaviour 151:741–754

Hoglund J, Alatalo RV (1995) Leks. Princeton University Press, Princeton

Jain AK (2010) Data clustering: 50 years beyond K-means. Pattern Recog Lett 31:651–666

Janetos AC (1980) Strategies of female mate choice - a theoretical analysis. Behav Ecol Sociobiol 7:107–112

Jennions MD, Petrie M (1997) Variation in mate choice and mating preferences: a review of causes and consequences. Biol Rev 72:283–327

Jiguet F, Arroyo B, Bretagnolle V (2000) Lek mating systems: a case study in the little bustard *Tetrax tetrax*. Behav Process 51:63–82

Johnson JR, Semlitsch RD (2003) Defining core habitat of local populations of the gray treefrog (*Hyla versicolor*) based on choice of oviposition site. Oecologia 137:205–210

Jones TM, Quinnell RJ, Balmford A (1998) Fisherian flies: benefits of female choice in a lekking sandfly. Proc R Soc Lond B 265:1651–1657

Kassambara A, Mundt F (2017) factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.5, https://CRAN.R-project.org/package=factoextra

Kaufmann L, Rousseeuw PJ (1987) Clustering by means of medoids. In: Dodge Y (ed) Statistical Data Analysis Based on the L1 Norm. Elsevier, Amsterdam, pp 405–416

Kaufman L, Rousseeuw PJ (1990) Finding Groups in Data: An Introduction to Cluster Analysis. John Wiley & Sons, New York

Klump GM, Gerhardt HC (1987) Use of non-arbitrary acoustic criteria in mate choice by female gray tree frogs. Nature 326:286–288

Kohler J, Jansen M, Rodriguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rodel MO, Vences M (2017) The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. Zootaxa 4251:1–124

Lopez PT, Narins PM, Lewis ER, Moore SW (1988) Acoustically induced call modification in the white-lipped frog, *Leptodactylus albilabris*. Anim Behav 36:1295–1308

Macedo RH, Podos J, Graves JA, Manica LT (2018) Breeding clusters in birds: ecological selective contexts, mating systems and the role of extrapair fertilizations. Anim Behav 143:145–154

Mackenzie A, Reynolds JD, Brown VJ, Sutherland WJ (1995) Variation in male mating success on leks. Am Nat 145:633–652

Maechler M, Rousseeuw PJ, Struyf A, Hubert M, Hornik K (2021) cluster: Cluster analysis basics and extensions. R package version 2.1.1, https://CRAN.R-project.org/package=cluster

Manica LT, Graves JA, Podos J, Macedo RH (2020) Hidden leks in a migratory songbird: mating advantages for earlier and more attractive males. Behav Ecol 31:1180–1191

McLister JD (2001) Physical factors affecting the cost and efficiency of sound production in the treefrog *Hyla versicolor*. J Exp Biol 204:69–80

McLister JD, Stevens ED, Bogart JP (1995) Comparative contractile dynamics of calling and locomotor muslces in 3 hylid frogs. J Exp Biol 198:1527–1538

Meuche I, Brusa O, Linsenmair KE, Keller A, Prohl H (2013) Only distance matters - non-choosy females in a poison frog population. Front Zool 10:16

Morris MR (1989) Female choice of large males in the treefrog *Hyla chrysoscelis* - the importance of identifying the scale of choice. Behav Ecol Sociobiol 25:275–281

Naguib M, Wiley RH (2001) Estimating the distance to a source of sound: mechanisms and adaptations for long-range communication. Anim Behav 62:825–837

Nekola JC, Kraft CE (2002) Spatial constraint of peatland butterfly occurrences within a heterogeneous landscape. Oecologia 130:53–61

Ovaska K, Hunte W (1992) Male mating behavior of the frog *Eleutherodactylus johnstonei* (Leptodactylidae) in Barbados, West Indies. Herpetologica 48:40–49

Perez DM, Backwell PRY (2019) Male spacing and female choice in a fiddler crab. Behav Ecol 30:1769–1774

Ptacek MB (1992) Calling sites used by male gray treefrogs, *Hyla versicolor* and *Hyla chrysoscelis*, in sympatry and allopatry in Missouri. Herpetologica 48:373–382

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/

Real L (1990) Search theory and mate choice.1. Models of Single-Sex Discrimination. Am Nat 136:376–405

Reichert MS, Gerhardt HC (2013a) Gray tree frogs, *Hyla versicolor*, give lower-frequency aggressive calls in more escalated contests. Behav Ecol Sociobiol 67:795–804

Reichert MS, Gerhardt HC (2013b) Socially mediated plasticity in call timing in the gray tree frog, *Hyla versicolor*. Behav Ecol 24:393–401

Reichert MS, Hobel G (2018) Phenotypic integration and the evolution of signal repertoires: a case study of treefrog acoustic communication. Ecol Evol 8:3410–3429

Richardson C, Lena JP, Joly P, Lengagne T (2008) Are leaders good mates? A study of call timing and male quality in a chorus situation. Anim Behav 76:1487–1495

Ringler M, Szipl G, Hodl W, Khil L, Kofler B, Lonauer M, Provin C, Ringler E (2017) Acoustic ranging in poison frogs–it is not about signal amplitude alone. Behav Ecol Sociobiol 71:114

Ripley BD (1977) Modeling spatial patterns. J Roy Stat Soc B Met 39:172–212

Robertson JGM (1984) Acoustic spacing by breeding males of *Uperoleia rugosa* (Anura, leptodactylidae). J Compar Ethol 64:283–297

Robertson JGM (1986) Male teritoriality, fighting and assessment of fighting ability in the Australian frog, *Uperoleia rugosa*. Anim Behav 34:763–772

Rousseeuw PJ (1987) Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. J Comput Appl Math 20:53–65

Rudolf VHW, Rodel MO (2005) Oviposition site selection in a complex and variable environment: the role of habitat quality and conspecific cues. Oecologia 142:316–325

Ryan MJ, Tuttle MD, Taft LK (1981) The costs and benefits of frog chorusing behavior. Behav Ecol Sociobiol 8:273–278

Schubert E, Rousseeuw PJ (2019) Faster *k*-medoids clustering: Improving the PAM, CLARA, and CLARANS algorithms. In: Amato G, Gennaro C, Oria V, Radovanović M (eds) Similarity Search and Applications. SISAP 2019. Lecture Notes in Computer Science, vol 11807. Springer International Publishing, Cham, pp 171–187

Schwartz JJ (1987) The function of call alternation in anuran amphibians: A test of three hypotheses. Evolution 41:461–471

Schwartz JJ (1993) Male calling behavior, female discrimination and acoustic interference in the neotropical treefrog *Hyla microcephala* under realistic acoustic conditions. Behav Ecol Sociobiol 32:401–414

Schwartz JJ, Buchanan BW, Gerhardt HC (2001) Female mate choice in the gray treefrog (*Hyla versicolor*) in three experimental environments. Behav Ecol Sociobiol 49:443–455

Schwartz JJ, Buchanan BW, Gerhardt HC (2002) Acoustic interactions among male gray treefrogs, *Hyla versicolor*, in a chorus setting. Behav Ecol Sociobiol 53:9–19

Schwartz JJ, Gerhardt HC (1989) Spatially mediated release from auditory masking in an anuran amphibian. J Comp Physiol A 166:37–41

Schwartz JJ, Hunce R, Lentine B, Powers K (2016) Calling site choice and its impact on call degradation and call attractiveness in the gray treefrog, *Hyla versicolor*. Behav Ecol Sociobiol 70:1–19

Stewart MM, Bishop PJ (1994) Effects of increased sound level of advertisement calls on calling male frogs, *Eleuthrodactylus coqui*. J Herpetol 28:46–53

Stillman RA, Clutton-Brock TH, Sutherland WJ (1993) Black holes, mate retention, and the evolution of ungulate leks. Behav Ecol 4:1–6

Stratman KD, Oldehoeft EA, Hobel G (2021) Woe is the loner: Female treefrogs prefer clusters of displaying males over single "hotshot" males. Evolution 75:3026–3036

Sullivan BK, Hinshaw SH (1992) Female choice and selection on male calling behavior in the gray treefrog *Hyla versicolor*. Anim Behav 44:733–744

Tárano Z (2009) Structure of transient vocal assemblages of *Physalaemus fischeri* (Anura, Leiuperidae): Calling site fidelity and spatial distribution of males. S Am J Herpetol 4:43–50

Tárano Z, Fuenmayor E (2013) Mate choice based on acoustic features in Johnstone's whistling frog *Eleutherodactylus johnstonei*: an experimental approach. S Am J Herpetol 8:52–59

Tarof SA, Ratcliffe LM, Kasumovic MM, Boag PT (2005) Are least flycatcher (*Empidonax minimus*) clusters hidden leks? Behav Ecol 16:207–217

Tejedo M (1992) Large male mating advantage in natterjack toads, *Bufo calamita* - Sexual selection or energetic constraints. Anim Behav 44:557–569

Telford SR (1985) Mechanisms and evolution of inter-male spacing in the painted reedfrog (*Hyperolius marmoratus*). Anim Behav 33:1353–1361

Telford SR, Dyson ML, Passmore NI (1989) Mate choice occurs only in small choruses of painted reed frogs *Hyperolius marmoratus*. Bioacoustics 2:47–53

Wagner WE, Sullivan BK (1992) Chorus organization in the Gulf Coast toad (*Bufo valliceps*) - Male and female behavior and the opportunity for sexual selection. Copeia 1992:647–658

Welch AM (2003) Genetic benefits of a female mating preference in gray tree frogs are context-dependent. Evolution 57:883–893

Welch AM, Semlitsch RD, Gerhardt HC (1998) Call duration as an indicator of genetic quality in male gray tree frogs. Science 280:1928–1930

Wells KD (1977) The social behavior of anuran amphibians. Anim Behav 25:666–693

Wells KD (2007) The ecology and behavior of amphibians. University of Chicago Press, Chicago

Wells KD, Schwartz JJ (1984) Vocal communication in a neotropical treefrog, *Hyla ebraccata* - Advertisement calls. Anim Behav 32:405–420

Wells KD, Taigen TL (1986) The effect of social interactions on calling energetics in the gray treefrog (*Hyla versicolor*). Behav Ecol Sociobiol 19:9–18

Westcott DA (1994) Leks of leks - A role for hotspots in lek evolution. Proc R Soc Lond B 258:281–286

Whitney CL, Krebs JR (1975) Spacing and calling in Pacific treefrogs, *Hyla regilla*. Can J Zool 53:1519–1527

Wilczynski W, Brenowitz EA (1988) Acoustic cues mediate inter-male spacing in a neotropical frog. Anim Behav 36:1054–1063

Woodward B (1982) Male persistence and mating success in Woodhouse's toad (*Bufo woodhousei*). Ecology 63:583–585