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Acoustic interactions among male gray treefrogs, *Hyla versicolor*, in a chorus setting

Abstract We assembled groups of up to eight male gray treefrogs, *Hyla versicolor*, in an artificial pond and examined vocal behavior under conditions of different chorus size. Males avoided call overlap when calling in groups of two, but not in larger choruses. The pattern of interference failed to reveal selective attention based on inter-male separation, and males in close spatial proximity tended to overlap calls more than more widely separated individuals did. Males were sensitive to the removal of males from or the addition of males to an aggregation, often responding to a change of just one individual with shifts in call duration and rate. Nevertheless, males tended to maintain their relative position in a hierarchy based on call duration following changes in chorus size. We hypothesize that adjustments in gross temporal properties are a response to the increased probability of call interference in larger choruses. Finally, we calculated repeatabilities of call duration, calling rate, and pulse effort within nights using sliding-analysis windows of 30 s to 29.5 min. The change in repeatabilities with window duration suggests that a female could best distinguish among males by assessing their performance for between 4 and 14 min, depending on the measure of calling behavior. However, because the magnitude of change was relatively small, our data do not lend strong support to the hypothesis that there is an optimum time period over which females should assess the calling of males in order to maximize their ability to discriminate among males.

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Introduction

Males of many species of anuran amphibians aggregate at breeding sites where they advertise vocally to attract females for mating. Within these choruses, males may respond to the calls of their neighbors in ways that may help them maintain or increase their relative attractiveness. For example, males may elevate the rate, complexity, duration, and intensity of their calls (Lopez et al. 1988; Wells 1988; Gerhardt et al. 1994; Schwartz 2001). Males may also shift the timing of their advertisement calls or call elements to reduce the potential for acoustic interference (Narins and Zelick 1988; Klump and Gerhardt 1992; Schwartz 1993; Grafe 1996) or to increase the likelihood that their signals will lead rather than follow those of their neighbors (Greenfield and Rand 2000). Male signaling is not always confined to advertisement calling but may also involve the use of aggressive calls. When agonistic exchanges occur between nearby males, they may escalate to physical encounters in which rivals wrestle with one another, presumably to achieve sole access to a region around a calling site (Wells and Schwartz 1984; Wagner 1989; Brenowitz and Rose 1994). Clearly, the acoustic environment in a dense chorus is complex, and background noise, acoustic interference, changing social conditions and vocally responsive competitors may make the challenge of attracting a mate more difficult than under low-density conditions.

The gray treefrog, *Hyla versicolor*, forms choruses in and near ponds in central Missouri from late April to mid July. Males produce an advertisement call that is composed of a series of, on average, about 14–18 pulses (Gerhardt et al. 1996). The within-call delivery rate and shape of the pulses are "static" temporal features (Gerhardt 1991) and mediate discrimination by females of conspecific males from males of the putative diploid progenitor of *H. versicolor*, *H. chrysoscelis* (Ptacek et al.

1994; Keller and Gerhardt 2001) in areas of syntopy (Gerhardt and Doherty 1988). Females discriminate strongly against pairs of calls in which the pulse pattern within calls is obscured by overlap (Schwartz 1987), perhaps explaining why neighboring individuals usually tend to alternate vocalizations (see below). The number of pulses in advertisement calls is a "dynamic" feature and may vary both within and among males in the chorus. In particular, males increase call duration in response to the calls of other males in the chorus and during playback experiments (Wells and Taigen 1986). However, longer calls tend to be delivered at a reduced rate, relative to shorter calls, such that there are usually only small changes in pulse effort (number of pulses per call ×calling rate) in response to acoustic stimulation. In arena-based two-stimulus tests of choice, females discriminated in favor of higher calling rates. Although preference-strength depended upon the absolute values of the alternatives, females also preferred calls of longer duration and selectively approached calls that are as little as 10% longer (i.e., 2 pulses for calls about 20 pulses long; Gerhardt et al. 2000). Moreover, females discriminated in favor of longer calls relative to shorter calls when these were broadcast at pulse efforts that were equal (Schwartz et al. 2001) or nearly so (Klump and Gerhardt 1987; Gerhardt et al. 1996).

Until fairly recently, information on acoustic communication among male anurans was based upon natural observations of individuals, playback tests to single frogs, and stereo recordings of pair-wise vocal interactions. While such data can be valuable, these methods do not provide information about vocal interactions within groups of more than two males. Thus researchers may miss important behaviors specifically related to the problems of communication within a chorus setting. In this study, we used a multi-channel call-monitoring system (e.g. Brush and Narins 1989; Passmore et al. 1992; Schwartz et al. 1995) to examine the vocal behavior of male *H. versicolor* in choruses of up to eight individuals within an artificial pond inside a screened enclosure. This venue was used because movement of subjects rendered previous attempts to monitor males in the field unsuccessful. Another advantage was that, in our artificial pond, we could be confident that we were monitoring all vocal interactions. In different locations within and among natural choruses of treefrogs, the size and densities of male aggregations may vary. The size of and local densities within choruses also may shift as males enter amplexus or cease calling for other reasons. By working in an enclosure, we could control male spacing and easily manipulate chorus size, and so explore how the vocal behavior of males responds to these changes. Manipulations of chorus size, rather than the utilization of different choruses of different sizes, also allowed us to examine the responses of specific males to density changes, as well as the impact of shifts in chorus size on calling relationships.

We focused on aspects of male calling that should influence the ability of individuals to attract females: call timing and the two components of pulse effort, call pulse number and calling rate. Information on call timing allowed us to determine the level of acoustic interference that occurred among males in the choruses, data previously unavailable for *H. versicolor*. In particular, we asked how chorus size and the spatial relationships among males influenced the relative timing of calls. Information on call duration (i.e., pulse number) and calling rate allowed us to determine how shifting levels of competition within the chorus, as a consequence of our manipulations of chorus size, influenced the relative calling performance of males. We also characterized levels of within- and among-male variability in these dynamic features over a range of time scales. In particular, we estimated "repeatabilities" (also called the intraclass correlation; Zar 1984). An analysis of repeatability is important for three main reasons. First, it estimates the potential for a female to choose a mate that truly differs from other males in a call or calling property. For this to be so, variation of this property within males should not be greater than the variation among males. Second, it indicates how much a female may gain in the accuracy of her assessment by considering additional samples of male calls. Third, repeatability sets an upper bound for heritability on the measured trait (Boake 1989; Falconer and Mackay 1996). We also determined how shifting levels of competition within the chorus influence the relative calling performance of males. Aspects of male vocal behavior that we address here (e.g., call plasticity, repeatability of calling performance, call timing) have been studied before (Wells and Taigen 1986; Gerhardt 1991; Sullivan and Hinshaw 1992; Runkle et al. 1994; Gerhardt et al. 1996; H.C. Gerhardt and G.M. Klump, unpublished data). However, by obtaining a complete record of calling from males of known position, often following small changes in chorus size, we could examine acoustic interactions and calling patterns in a fashion that revealed subtle details of vocal behavior and the extraordinary sensitivity of males to their sound environment.

Methods

General procedures

We performed all tests in an octagonal artificial pond (5.2 m=circumscribed diameter) located within a screened enclosure in a greenhouse at the University of Missouri (see Schwartz et al. 2001 for a complete description). Acoustic foam wedges (Soundcoat) stacked along the greenhouse walls surrounding the enclosure reduced sound echoes, while opaque sheets of plastic blocked light from the pond area and visually isolated the frogs from the researchers. We positioned a directional microphone (Azden ECZ-660) 25–35 cm away from each of our subjects and each subject was confined within a screen cage on a cinderblock perch along the periphery of the pond. Each perch was 1.9 m from the two adjacent perches. This distance was close to the minimum that would allow calls from different males to be consistently distinguished by our call-monitoring system, and was within the range of inter-male separations between interacting males in the field (personal observations). Vocal activity was monitored using custom software running on an Amiga 600 computer, linked through

its parallel port to a custom eight-channel hardware module that received signals from each microphone (see Schwartz 1993; Schwartz et al. 1995 for more details). The computer sampled the eight data lines of the parallel port at 100 Hz, and the eight-channel interface was set to produce sound-triggered voltage transitions sufficient to resolve individual pulses within the calls of *H. versicolor*. The computer automatically stored data to disk at either 10-min or 5-min intervals. Raw data files were analyzed using additional custom software that extracted information on the timing relationships (additional details are provided below) of the subjects' calls and the components of their pulse efforts. We used PC-SAS for statistical analyses.

We captured males at a pond in the Baskett Wildlife Area in Ashland, Missouri. The frogs were freeze-branded for individual identification (Donnelly et al. 1994) and released in the artificial pond approximately 30 h before testing. Although it was only possible to monitor a maximum of 8 males, we typically placed 20–30 of them in our enclosure to select a subset that would reliably call. Each afternoon (between 1530 and 1630 hours CDT) we exposed the frogs to a simulated rainstorm, lasting 30–40 min, delivered via a sprinkler system above the artificial pond, because this increased the likelihood that males would call in the evening. For the same reason, each night (between 2000 and 2100 hours CDT) before our tests we broadcast a recording of a natural chorus of gray treefrogs from a pair of speakers located just outside the pond. Once at least eight males were calling in a sustained and vigorous fashion, we removed silent individuals and placed callers on their caged perches. A few additional calling males were left undisturbed, so they could be used to replace chosen subjects that failed to resume calling following handling. If not needed, these potential replacements were removed and all our subsequent work in a night was confined to the entire set and subsets of the initial group of remaining males. Once the vocal activity of the caged frogs had stabilized, we ended the broadcast of the recorded chorus and waited 20–30 min before beginning our experiments. Not all tests of calling performance or repeatability began with eight males because some of the males had stopped calling by the time experiments began.

For our analyses, we treated males within the same chorus as independent, because each individual occupied a unique position in the chorus and so was subject to acoustic conditions that differed in fine details from those of its neighbors.

Call timing

We monitored call timing in ten choruses. We began all tests with eight males and, after each of two 10-min monitoring periods, reduced the size of the chorus by 50%. Thus, for each of ten tests, we acquired 10 min of data for a group of eight, four, and finally two individuals. We sequentially, rather than randomly, reduced chorus sizes because removing males and subsequently returning them to the chorus decreased the likelihood that they would call again. The particular males that we removed, however, were chosen randomly, with the constraint that the first four individuals were removed from alternate positions (e.g., 1, 3, 5, and 7) and the next two individuals were removed from positions on opposite sides of the octagonal pond (e.g., 6 and 2). The data were analyzed to determine the percentage of male calling time that was free from acoustic interference as a function of chorus size and separation between individuals. We also compared observed levels of overlap with those to be expected by chance. For each 10-min data file, this expectation was obtained by randomizing the original time-ordered list of call durations and inter-call intervals of each male in the chorus and then recalculating call overlap. The resulting level of overlap for each pair of males was then compared with the actual level. This process was repeated 100 times, and a result in which actual overlap was less than that in the randomized data set 95 or more times was considered statistically significant. This general approach was advocated by Popp (1989) and used by Brush and Narins (1989) and Schwartz (1993) in their studies of anuran chorusing.

Calling performance

Data on call duration and calling rate obtained during manipulations of chorus size allowed us to compare the relative calling performance of competing males. As previously described, in one set of manipulations, we changed chorus sizes in steps of 50% (from eight to four to two). However, for this analysis, we used data from 12 nights rather than 10 because we included data from 2 additional nights when initial chorus size was 7 rather than 8 males. After we had acquired 10 min of data at each of the three chorus sizes, we attempted to reverse the pattern of density change by adding the removed males back into the chorus (to their former positions). This increasing series of chorus sizes controlled for any effect that time of night might have had on the calling behavior of the frogs. Not all males called in the increasing density series, probably because of the disturbance associated with removal and handling. In a second set of density manipulations ($n=4$ nights), we modified chorus size 1 male at a time and began tests with initial chorus sizes of 6–8 males. In these tests, we set our computer to acquire data for 5-min rather than 10-min periods and only removed subjects from the choruses. Removed individuals were selected randomly.

Repeatability analysis

We used the complete calling records from different choruses of five to eight males obtained on ten different nights to calculate repeatabilities of pulses/call, calling rate, and pulse effort during each night. Repeatability was calculated as (groups MS–error MS)/(groups MS+($n-1$)×error MS), where n is the number of observations per group=male (see Zar 1984 for equation used for our calculation of n for unequal ns), and ranges from –1 to 1. A repeatability of 1 indicates no within-male variation for the trait in a sample while a repeatability of –1 indicates no among-male variation. To facilitate our analyses and statistical comparisons, we calculated calling rate and pulse effort on a call-by-call basis: for calling rate, we used the reciprocal of each call period, and for pulse effort we formed the ratio of pulses per call and call period. The initial pulse-effort values were converted to pulses per minute and subsequently averaged to provide an estimate of pulse effort at each chorus size. Because we had data on nearly the entire duration of chorus activity for each night, and had calculated calling performance on a fine time scale, we could also determine whether repeatabilities changed as a function of the time period over which females might compare males. No published estimates of this time period are available for this species or any other anuran, but by varying our analysis time, we could estimate the minimum time needed to distinguish among males statistically and how such discrimination might, in principle, be improved as the duration of the assessment period increased. We used "assessment windows" of 30 s to 29.5 min, and assumed a female could start listening during any inter-call interval during the evening. Thus, for each evening's data set, we calculated a series of repeatabilities for each "assessment window", and successive calculations in this series were based on calling in partially overlapping blocks of time, unless there was a gap in chorusing longer than the duration of the window. We then averaged the mean values from different nights to obtain an overall estimate of the repeatability for that potential time period of female evaluation.

Results

Call timing

Manipulations of chorus size had a significant effect on the proportion of an individual's calling time that was free from acoustic interference by other members of the chorus (Kruskal-Wallis test, data pooled from all choruses:
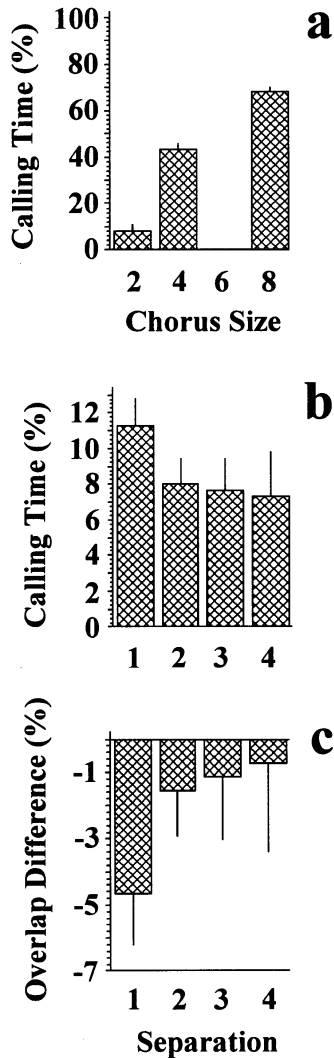
Fig. 1 a Calling time (mean %+2 SE of total time producing sound, *n*=10 choruses) of males of *Hyla versicolor* that was obstructed by the calls of any other male in the chorus for choruses of different sizes in the greenhouse pond. **b** Calling time of males that was overlapped by the calls of other males as a function of separation (in terms of calling-site positions in the artificial pond; *n*=10 choruses of 8 frogs each). **c** The difference between expected and observed call overlap among pairs of males as a function of separation. A negative value indicates that there was more overlap than expected. The expected level of acoustic interference was obtained by randomizing listings of the call durations and inter-call intervals of each male 100 times and then recalculating call overlap. The magnitude of the difference between the mean expected and observed overlap was calculated as: 100×(Expected overlap[i][j]–Observed overlap[i][j])/Calling time[i]. Here, male [i] is the "interfering" male whose calls follow and overlap those of the "leading" male [j]

χ^2=106.3, *P*<0.0001; χ^2>6.64, *P*<0.03 for each of ten choruses). Males of *H. versicolor* were less likely to have portions of their advertisement calls overlapped by the vocalizations of another individual in smaller groups than in larger groups (Fig. 1a). Proximity of individuals also influenced the chance that males would overlap one another's calls, although this effect was significant only when data from all choruses were pooled. Immediate

neighbors in the chorus were more likely to exhibit interference than males who did not call from adjacent positions (i.e., those separated by two or more calling sites; Fig. 1b; Kruskal-Wallis test, data pooled from all choruses: χ^2=47.7, *P*<0.0001; χ^2>9.04, *P*<0.023 for just two of ten choruses). Thus "selective avoidance" of interference with nearest neighbors was not evident. Furthermore, by randomizing the calling data, we found that observed acoustic overlap among the closest neighbors in the pond in eight-male choruses was greater than expected by chance (Fig. 1c). In particular, only 11.25% of 160 of these pair-wise interactions among neighbors (*n*=10 choruses) showed significantly less than expected interference. Males demonstrated active avoidance of overlap only after six individuals had been removed from the pond. For such 2-male choruses, actual interference was significantly less than expected for 70% (14/20) of pair-wise interactions. Values for 4- and 8-male choruses were 10.8% (13/120) and 17.3% (96/560), respectively. Results were qualitatively similar if we consider just the two "focal" males that were present at all chorus sizes [4-male choruses: 10% (6/60), 8-male choruses: 15% (21/140)].

Calling performance

In each of the 12 choruses in which we performed manipulations of chorus size by 50%, each removal of males resulted in a reduction in call duration (pulses per call) for each of the 2 focal males present at all densities (r_s=0.664–0.914, mean r_s=0.835, median r_s=0.848, *P*<0.0001 for each of 24 males). For 5 of the 12 choruses that we monitored during this set of manipulations, at least 6 males called following the final reintroduction of removed males. In these five choruses, the reduction in call duration after removals of individuals was followed by an increase in the number of pulses per call for each focal male with each of the two subsequent increases in chorus size (Fig. 2, r_s=0.357–0.870, mean r_s=0.688, median r_s=0.745, *P*<0.0001 for each of ten males). Males also decreased pulse number in response to removals of one male at a time. The first removal of a male in each of all four choruses in which we performed this manipulation was certainly the most difficult change for the remaining males to detect. Nevertheless, following the first removal we observed a decline in the mean number of pulses per call by 22 of the 25 remaining males in the 4 choruses: each of the males in 3 tests and a majority of males (4 of 7) in 1 test. For three of the four focal males in these four choruses, a drop in the average pulses per call accompanied each reduction by one male (Fig. 3, r_s=0.366–0.855, mean r_s=0.705, median r_s=0.800, *P*<0.0001 for each of four males). Although all males altered the duration of their calls during these manipulations, their ranking relative to other chorus males with respect to call duration changed little (50% chorus size changes, mean change in rank between sequential chorus sizes=0.203, SE=0.040, *n*=123 possible changes in rank; one-male reductions, x̄=0.383,
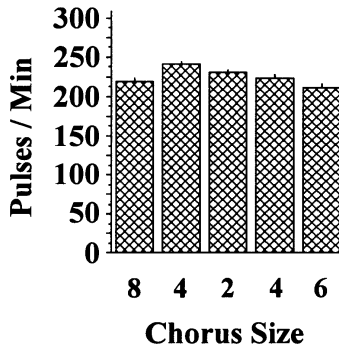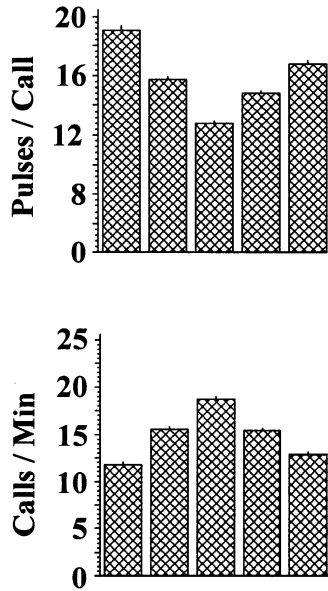
Fig. 2 Calling behavior of males (mean+2 SE, *n*=5 pairs of focal males in 5 choruses) during manipulations of chorus size in steps of 50% and subsequent reintroductions. Not all males called after the final reintroduction
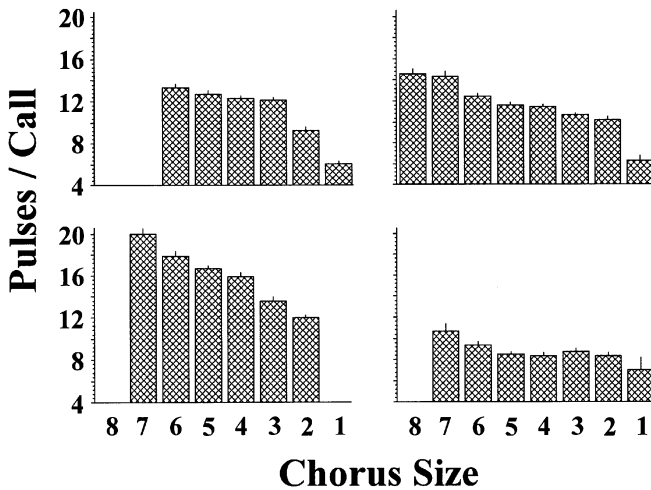

Fig. 3 Call pulse number of the focal male (mean+2 SE) during manipulations of chorus size in steps of 1 in each of 4 choruses (clockwise from *upper left*: chorus 62, 63, 67, 65)
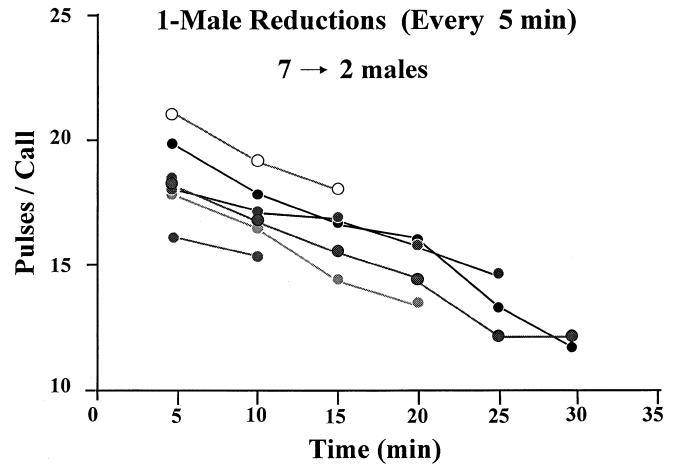

Fig. 4 Call pulse number (mean over 5-min intervals) of males in one chorus during manipulations of chorus size in steps of one male. Note the tendency for the remaining males to maintain their approximate ranking following removals. *Lines* join the data points to aid in visual inspection only, and so do not accurately indicate calling behavior in the intervening time intervals
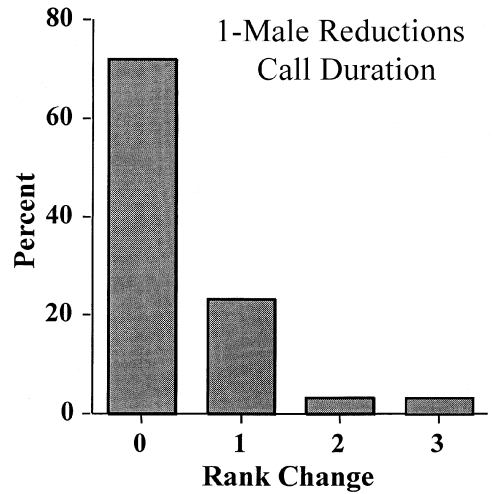

Fig. 5 Percent of total opportunities for a change in rank for call duration (*n*=81 opportunities in 4 choruses) observed between successive 1-male reductions in chorus size in which the observed change in rank was by 0, 1, 2 or 3 positions

SE=0.074, *n*=81; Figs. 4, 5). For example, the male that gave the longest calls at the start of the experiment typically continued to give the longest calls at the chorus sizes he experienced.

Changes in calling rate responded to the density manipulations in the opposite fashion; that is, the calling rate of the remaining individuals increased after we removed males from the pond. During the reductions of chorus size in steps of 50%, there was only 1 instance in which a focal male failed to increase his calling rate (r_s=–0.162 to –0.803, mean r_s=–0.570, median r_s=–0.598, $P<0.01$ for each of 24 males). Calling rate of focal males declined when males were returned to the choruses (Fig. 2, r_s=-0.311 to –0.764, mean r_s=–0.577,
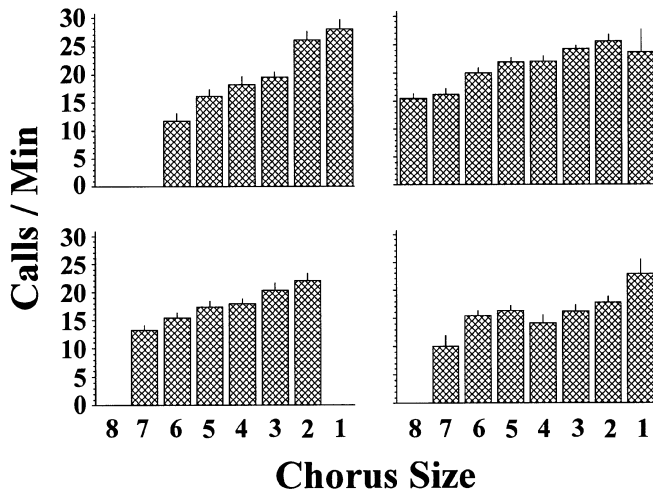
Fig. 6 Calling rate of the focal male (mean+2 SE) during manipulations of chorus size in steps of 1 in each of 4 choruses (clockwise from *upper left*: chorus 62, 63, 67, 65)

Fig. 7 Pulse effort of the focal male (mean+2 SE) during manipulations of chorus size in steps of 1 in each of 4 choruses (clockwise from *upper left*: chorus 62, 63, 67, 65)
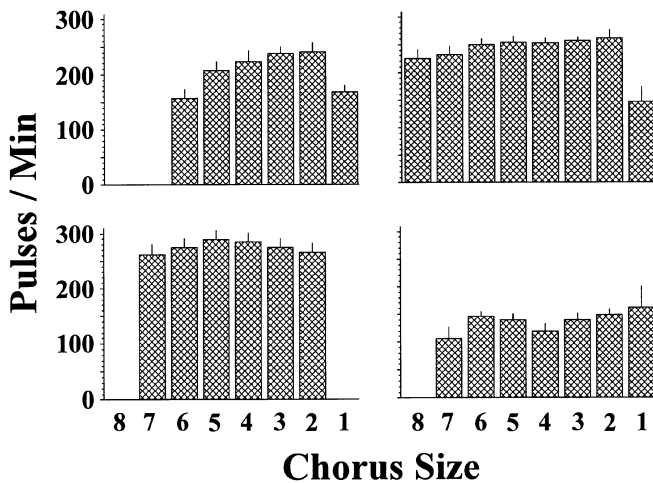
median r_s=–0.561, $P<0.0001$ for each of ten males). In the single-male removal series, all but two of the removals (test 63: decline from two males to one male, test 67: decline from five males to four males) resulted in an increase in the calling rate of the focal male (Fig. 6, r_s=–0.302 to –0.655, mean r_s=–0.532, median r_s=–0.585, $P<0.0001$ for each of four males).

In contrast to the changes observed in the number of pulses per call and calling rate, changes in pulse effort were less pronounced and showed no consistent directional pattern (e.g., declining or increasing) with respect to chorus size. For example, during the 5 of 12 tests using 50% manipulations in which at least 6 males called following the final reintroduction of removed males, the percentage range in pulse effort [100×(largest–smallest)/smallest] of the two focal males was always less than 34% (test 52=10.35%, test 53=12.89%, test 54=19.09%,

test 58=33.33%, test 60=14.68%; Fig. 2). With the one-male reduction series, results were generally similar. However, for two of the three tests in which the focal male continued to call when isolated, pulse effort declined substantially following the final removal (Fig. 7).

Repeatability analysis

In Fig. 8, we show graphical examples of three measures of calling behavior for two different choruses, and the corresponding measures of repeatability when calculated over 30-s or 29.5-min time widows. It is obvious that there can be considerable variation both among and within males in each measure of vocal behavior and that, over the course of an evening, males may change their relative positions in performance rank and begin or end their calling at different times. The nightly patterns of calling behavior are particularly clear for chorus I where we were fortunate to have captured not only the first stages of chorusing but also its termination 6.5 h later. Note that those periods of time when an individual is exhibiting large changes in vocal behavior (i.e., near the start or end of calling activity) are relatively brief when compared with the intermediate phase of more stable calling.

Repeatabilities for pulses/call were high [mean of all means (n=590) over all 10 choruses for all "assessment windows"=0.676, n=7,963,228 repeatabilities] although there was a slight decline with increasing duration of the assessment window (Fig. 9, top row). Although the variance in repeatabilities at any given time interval was low (Fig. 9, middle row), individual values for repeatability ranged from –0.8 to 1.0 when all time intervals and all choruses are considered. Therefore, there was a small chance that a female could be listening at a time when repeatability, and thus the potential for discrimination, was low. Perhaps the time window for assessment has been shaped by selection to maximize females' potential to discriminate while at the same time minimize the variance in this potential. Figure 9 (bottom row) shows the data on the mean and variance in repeatability re-scaled so that all values fall between 0 and 100% of their maximum values. When standard errors are considered, the intersection point at 7 min 30 s suggests that an assessment window of between 4 and 11 min would best simultaneously satisfy the aforementioned dual goals. That is, relative to females with other assessment windows, females that compare the call durations of males for 4–11 min would not only, on average, be better able to discriminate but they would also reduce their risk of not being able to discriminate at any particular time in an evening.

Repeatabilities of instantaneous calling rate (1.0/inter-call interval) and pulse effort (pulses per call/inter-call interval) were lower than those for pulses/call (mean of all means over all ten nights for all "assessment windows"=0.363 for calling rate and 0.243 for pulse effort, Fig. 9), although the range in all values for repeatability was comparable (calling rate: -0.617 to 1.0; pulse effort: –0.773 to 1.0).

Fig. 8 Calling behavior of males in 2 choruses (chorus I: *n*=6 males, chorus II: *n*=8) monitored in the artificial pond. Data are mean values (pulses/call) or totals (calls, pulses) for individuals calculated over successive 10-min blocks. The perch position of each male is indicated numerically (*1–8*) and the mean of the repeatabilities, calculated as described in the text, is given for assessment time windows of 30 s and 29.5 min above each plot. *Lines* join the data points to aid in visual inspection only, and so do not accurately indicate calling behavior in the intervening time intervals
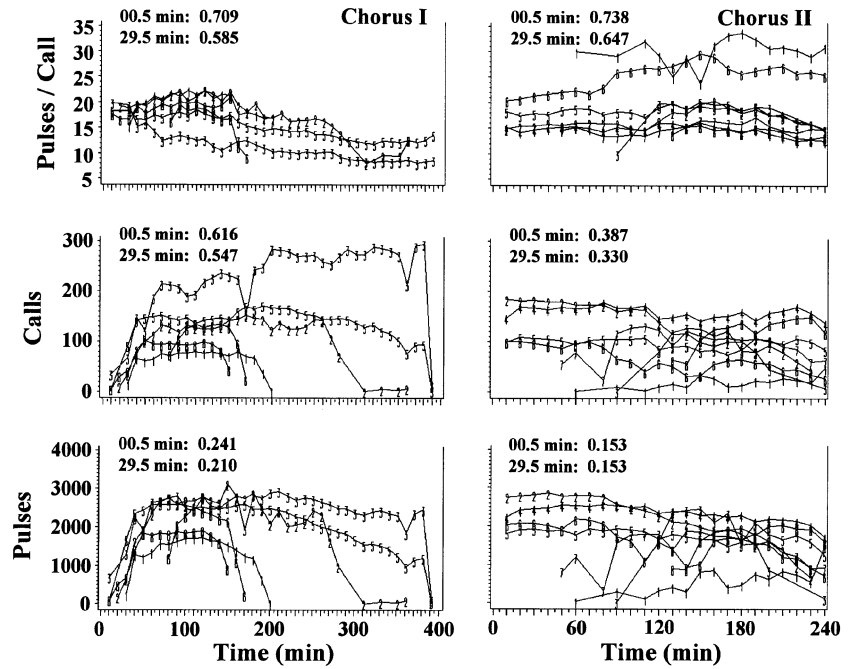

Fig. 9 Repeatability of pulses/call, calling rate, and pulse effort when calculated using sliding assessment time windows ranging from 30 s to 29.5 min. Data values are mean repeatabilities (*top panels*) for each chorus averaged for 10 choruses (±2 SE) and variance in repeatabilities (*middle panels*) for each chorus averaged for 10 choruses (±2 SE). The *bottom panels* show the mean and variance in repeatabilities re-scaled so that all values fall between 0 and 100% of the maximum value. The numerical sequence of the *left* and *right ordinates* are reversed so that the crossing point gives the best simultaneous high mean and low variance for repeatability
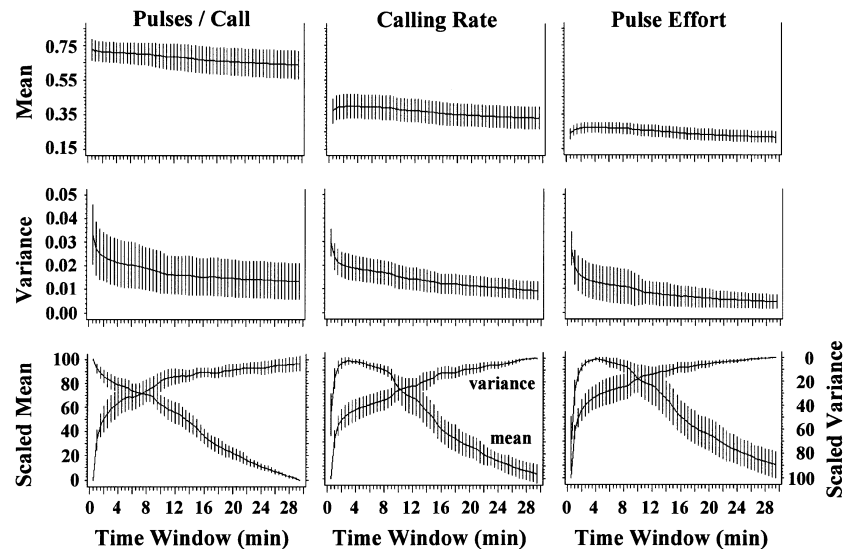
Following an initial small rise, for both measures of performance there was a slight drop in mean repeatability as the duration of the assessment window increased, and also the variance in repeatabilities was quite small (Fig. 9). When the re-scaled means and variances are plotted together, the curves cross at 10 min, suggesting an assessment window of about 9–14 min when using calling rate and 8–14 min when using pulse effort.

Discussion

Our data confirm earlier findings that illustrate the sensitivity of male treefrogs, and in particular males of *H. versicolor*, to their acoustic environment. The length of calls, call rate and pattern of acoustic interference were influenced by our density manipulations and males responded to the subtle changes accompanying reductions in chorus size by just one individual. The relationships among males for measures of calling performance remained relatively stable, even when choruses changed in size; however, call overlap among males was greater in larger aggregations. Males did not exhibit selective avoidance of interference with their closest neighbors in the chorus, although they did show an ability to reduce call overlap when only one other male was present. We focus in more detail on specific aspects of calling behavior below.

Call timing

A potentially serious problem for male anurans advertising vocally for a mate is acoustic interference which may mask or degrade calls or call elements. This is especially so in species such as *H. versicolor* in which functionally relevant fine-scale temporal information incorporated in pulse rate, shape, and duration (Gerhardt and Doherty 1988; Diekamp and Gerhardt 1995; Gerhardt and Schul 1999; J. Schul, unpublished data) can easily be obscured when calls overlap. Indeed, in both arena-based phonotaxis experiments (Schwartz 1987; Schwartz and Gerhardt 1995; Marshall and J.J. Schwartz, unpublished data) and female choice tests using calling males in the artificial pond (Schwartz et al. 2001), call interference impaired the ability of real or simulated males (i.e., speakers) to attract females. By shifting the relative timing of their calls, males may reduce the chance that calls overlap. However, timing shifts resulting in behavior such as call alternation will necessarily become less effective in this regard as more males join the chorus. Unless males compensate by lowering pulse effort with increases in chorus size, in larger choruses the opportunity for acoustic interference is greater than in smaller choruses. In gray treefrogs, the pulse effort of individual males remains about the same (Wells and Taigen 1986; this study), and the fraction of a male's pulse trains that remain free from overlap declines with increases in chorus size (Fig. 1a). Our data support field impressions and experimental expectations (Schwartz 1987; G.M. Klump and H.C. Gerhardt, unpublished data) that males tend to alternate calls in pair-wise interactions. However, statistically significant deviations from random expectation of overlap were absent in choruses with four or more males. Selective attention, in which males adjust the timing of calls so as to reduce interference with their nearest or loudest members of the chorus, is an option that has been documented in some anurans (Brush and Narins 1989; Schwartz 1993; Boatright-Horowitz et al. 2000; Greenfield and Rand 2000) and chorusing orthopteran insects (Minckley et al. 1995; Snedden et al. 1998). Our data, however, provide no empirical support for the use of this tactic by gray treefrogs. There are two possible explanations. First, the apparent absence of this phenomenon could be an artifact of the acoustics in our artificial pond in which males were regularly spaced at the same elevation. Second, selective attention may in fact be absent in *H. versicolor* because these frogs use other solutions that can partially offset the interference problem. For example, by shifting to longer calls delivered at lower rate in larger choruses, males increase the odds that, on average, more pulses per call are broadcast free of interference than otherwise would be the case (Schwartz et al. 2001; see below). Moreover, even when call overlap occurs, females of *H. versicolor* can exploit directional characteristics of their auditory system to help extract critical, fine temporal information (Schwartz and Gerhardt 1995).

Calling performance

We found that calling rate increased, call duration declined and pulse effort remained relatively stable during our manipulations of chorus size, results consistent with expectations based on the observations made by Wells and Taigen (1986). However, there are two intriguing aspects of our findings. The first is the very high sensitivity of males to chorus size, as reflected in their calling behavior. For example, in the one-step reductions, three of the four focal males present at all chorus sizes reduced their average call duration following every removal of a male from their chorus. Even the initial removal of a male elicited a decline in mean number of pulses per call by the vast majority of males. Whether males were responding directly to the change in the number of calling males, the change in the background noise levels in the chorus, or both is not certain. Perhaps detecting discrete calls is in itself unnecessary but, rather, any noise or other sounds that stimulate the auditory system of the frogs will elicit a change in call duration. This would not be surprising because, in a dense chorus, the extensive overlap of calls will obscure many of the fine temporal features of individual calls.

A second significant finding is that males reliably maintained their relative rank in the chorus for call duration during the manipulations. The fact that males respond fairly consistently to drops in chorus size with a decline in pulse number in part explains this result. However, our finding raises the intriguing question: why do males ranking lower for call duration maintain their relative performance rank at lower chorus sizes although they are capable of giving longer calls, as evidenced by their behavior at higher chorus sizes? One possibility is that if lower-ranking males failed to ratchet down their call duration following reductions in chorus size, previously higher-ranking males would detect this and elevate their call duration so as to restore their former rank for call duration. According to this scenario, such attempts by formerly lower-ranking males to "cheat" would not succeed. The behavior that we observed may also indicate that males have little to lose by maintaining their rank in the chorus. In fact, data from tests of female phonotaxis in the artificial pond and eight-speaker choice tests in the field suggest that this is so. Call duration explains only a small percentage of the variance in mating success. Moreover, in a chorus setting, females appear to discriminate strongly just against extremely short calls (e.g. six pulses long; see Schwartz et al. 2001) which are only rarely given by males. Strong discrimination against very short calls has also been demonstrated in two-choice tests, in which relative intensity of choice alternatives was used as an indicator of relative preference strength (Gerhardt et al. 2000).

The discrimination by females against very short calls, as well as against overlapped calls (Schwartz 1987; Schwartz and Gerhardt 1995; Schwartz et al. 2001), suggests an explanation for the use of particular

call durations by males. The call duration adopted by males in a chorus of a particular size may be such as to ensure that, on average, the number of pulses free from overlap *per call* is sufficiently high so as to avoid the very strong discrimination shown by females to very short calls. So, in a small chorus with little acoustic interference among males, individuals can use relatively short calls without seriously jeopardizing their opportunity of attracting a female, because few to none of their call pulses will be obscured by call overlap. However, in a large chorus, where the chance of call overlap is high, the same males must use longer calls (albeit delivered at lower rate) to maintain the same level of attractiveness. The vulnerability of calls to acoustic interference may therefore be the environmental factor (in contrast to changes in competition among males) that largely explains not only why males shift the two components of pulse effort in opposite directions, but also why they tend to maintain their relative ranking for calling performance. Data on acoustic overlap experienced by males from the same greenhouse-pond choruses but using calls of different duration support this "interference-risk hypothesis" (Schwartz et al. 2001). Interestingly, Ronacher et al. (2000) found that signal duration could reduce vulnerability to masking in the grasshopper, *Chorthippus biguttulus*. However, Kime (2001) found that increases in call complexity and length that often accompany acoustic stimulation of male tungara (*Physaleamus pustulosus*) and cricket frogs (*Acris crepitans*) failed to reduce susceptibility to masking during tests of female phonotaxis in these species.

The interference-risk hypothesis is consistent with proximate explanations (e.g., physical or physiological constraints) that determine the call duration that particular males tend to use at particular chorus sizes or background noise levels, as well as ultimate explanations (e.g., the call duration is an honest indicator of "good genes"; Welch et al. 1998). Indeed, if long calls at low rate are more difficult or expensive to give than short calls at high rate (Wells and Taigen 1986; Pough et al. 1992; Grafe 1997) – a result consistent with the reduced nightly chorus tenure of males producing long calls (Wells and Taigen 1986; Pough et al. 1992) – the tendency of males to maintain their position in the call duration hierarchy is easier to understand than it otherwise would be. Males may simply adjust the two components of their pulse effort to their long-term (e.g., entire evening) capability and the current acoustic conditions.

Repeatability

Our analysis of repeatability focused on within-night variation and so was somewhat different from analyses presented in previous studies of *H. versicolor* that used data from the same males recorded on different nights to calculate this measure (Gerhardt 1991; Sullivan and Hinshaw 1992; Runkle et al. 1994; Gerhardt et al. 1996).

Specifically, our purpose was to use repeatability to evaluate the potential that females have to use different measures of calling performance over a range of assessment periods to select a mate on a particular evening. Of course, this potential, based on patterns of within- and among-variation in male calling, will also be shaped by the innate abilities of females to discriminate. These abilities will be determined by characteristics of females' central and peripheral nervous systems (see Gerhardt et al. 1996 for discussion of this point), as well as the length of time females actually compare vocalizing males.

We found that repeatability for pulses/call was high ($\bar{x}=0.676$), as compared to the repeatability for calling rate ($\bar{x}=0.363$) and the repeatability for pulse effort ($\bar{x}=0.243$). This means that, relative to among-male variation, there was greater consistency in call duration for individual males than there was in calling rate and pulse effort. Our results suggest also that females could better use the number of pulses in the calls of males to distinguish among potential mates than, for example, how frequently those pulses are delivered.

In our previous study of female choice in *H. versicolor* (Schwartz et al. 2001), males calling in the artificial pond that were chosen by females had both longer calls and higher pulse efforts than males that were not chosen – although the magnitudes of the differences were small. The rate of calling, on its own, was not a significant factor in male success. Our analysis of the change in repeatability as a function of assessment time suggests that a period of anywhere between 4 and 14 min, depending on the calling performance measure, would best enable a female to distinguish among males. However, because we saw relatively little change in repeatability for any measure of calling performance as a function of the duration of our analysis window, it seems unlikely that there has been strong selection on assessment time for the purpose of maximizing the ability of females to distinguish among males. Rather, in this regard, it may make little difference how long a female listens to a group of males. So, for example, a female using pulses/call, which had a relatively high repeatability, to select among males could usually differentiate among them by listening for even just a minute or two, provided differences among the males were sufficiently great to be "meaningful" (Nelson and Marler 1990). Moreover, such a female would gain little in the accuracy of her assessment of male performance by sampling more than four to five calls per male for this purpose (see Falconer and Mackay 1996). Accordingly, other factors (e.g., predation risk, physiological aspects of oviposition) may exert a more profound impact on the time a female remains near a group of calling males than the need to discriminate among males based on calling performance.

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