plating technique for mammalian cells was essentially the beginning of quantitative biology using cultured mammalian cells, especially in radiobiology. Therefore, the availability of the presently described technique finally makes possible the same types of quantitative biological studies with cultured insect cells.

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Localization of sound producing animals using the arrival time differences of their signals at an array of microphones[1]

Irene Magyar, W.M. Schleidt and B. Miller

Department of Zoology, University of Maryland, College Park (Maryland 20742, USA), 10 October 1977

Summary. This method provides a reliable means to determine distance between vocalizing individuals and their locations through a prolonged period of time without disturbance to the communication process by the presence of an observer. The accuracy of this method was tested by varying the arrival time differences on the microphone grid for known locations in a random fashion. This test shows that a vocalizing animal within an area of 4 ha surrounding the microphone array can be localized with an accuracy of ± 1 m. The accuracy decreases with the distance from the central area and as a function of the geometry of the array. The location of an individual can be determined based on 5 vocalizations given in sequence.

When studying vocalizations used in long distance communication, such as bird song, the location of the communicants and the distance between them are among the most important variables. Although the locations may be determined by personal inspection or in reference to a grid of markers, the necessary presence of the observer is likely to disturb the animals and to influence the communication process under investigation. Furthermore, it is difficult and expensive in terms of time and effort to collect such measurements throughout and extended period of time (days or months) in sufficient number to warrent a quantitative analysis.

As an alternative to such direct observations, an acoustical method for the localization of vocalizing animals which minimizes disturbances in their environment has been designed[2]. It is based on the measurement of arrival time differences of the animal's signals at an array of microphones. Although a similar system has been designed to locate a vocalizing whale in a 3-dimensional space[3], Schleidt's was the 1st attempt to design a system for studies of acoustical communication in ground dwelling animals. Here, an evaluation of the accuracy of the method is presented along with data samples obtained with the array at a study site in Maryland and a determination of the most efficient means of analyzing real data samples.

Materials and methods. The microphone array was used to locate male bobwhite quail, *Colinus virginianus*. The bobwhite call is well suited to test this method since it carries over a considerable distance (several 100 m), it is usually repeated many times from the same location allowing repeat measurements for replications, and particular features of the call are highly individualistic within a population so that specific males can be recognized reliably from sonagrams (figure 1).

4 microphones (Uher M517 with a protective cover and wind screen) were set up in an array at the Chesapeake Bay Center for Environmental Studies. 3 of the microphones were in a straight line, spaced 50 m apart; the 4th was placed on a line perpendicular to the line formed by the 1st 3 and 50 m from the central microphone. The microphones were each connected to an individually shielded, twisted pair of wires in cables which were laid on the ground to a barn approximately 150 m from the nearest microphone. The cable terminal contained the line transformers converting the balanced microphone lines to the grounded input of a 4 channel tape recorder (Sony TC-654-4). The recording was monitored through headphones.

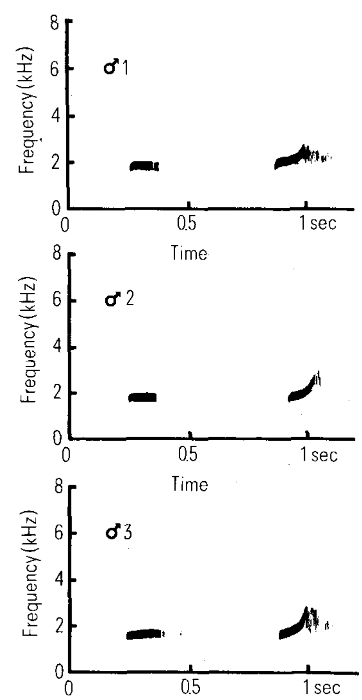

Fig. 1. Examples of bobwhite calls of males 1, 2, and 3 to show individual differences.

Given a vocalizing quail at the location x,y (figure 2), the sound will spread in a spherical wave front reaching M2 1st, M1 2nd, M0 3rd and M3 last. The differences in arrival time of the signal between the 4 microphones (n-a, m-a, l-a)

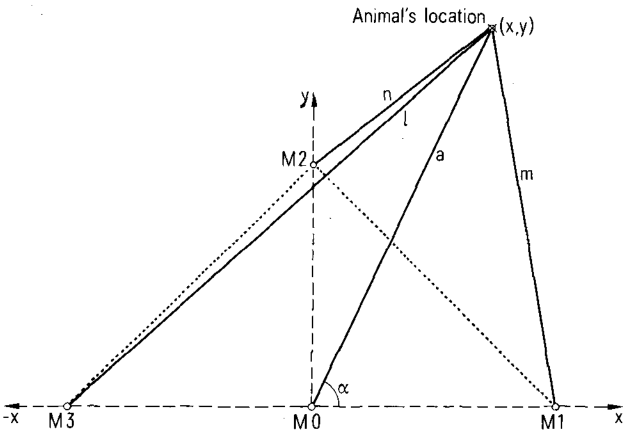

Fig. 2. Geometry of the microphone array used to determine the animal's location (x,y) in reference to the location of the microphones (M0, M1, M2, M3).

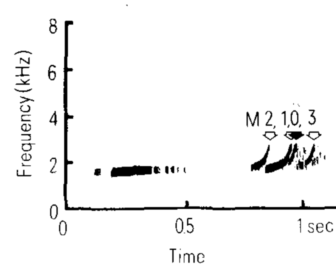

Fig. 3. Sonagram of 1 bobwhite call of male 3 as its signal arrives sequentially at the 4 microphones.

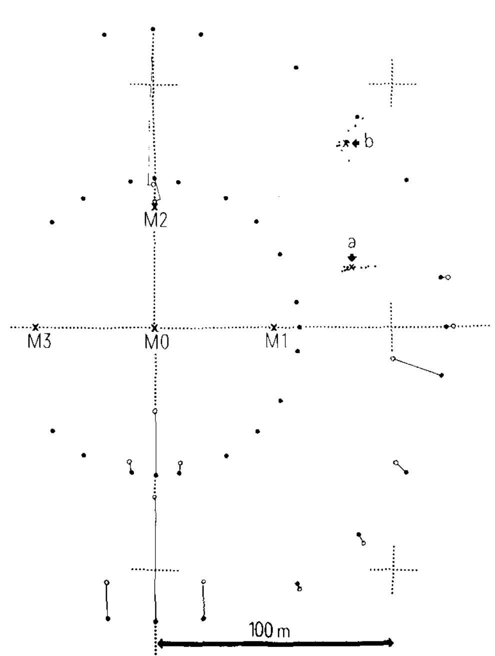

Fig. 4. Microphone array with target areas (large solid dots) of 1 m radius. Open circles represent median locations of scatter if outside the target area. The clusters of small dots at a and b represent locations calculated from actual arrival time differences for birds a and b, respectively; x indicates the median location within each cluster.

are a function of the speed of sound and the geometry of the array. These differences are used to calculate the values for x,y in reference to the array.

The actual measurements of the arrival time differences are made in the laboratory by playing the recordings on the tape recorder, mixing the signals from 2 or more channels (in a Uher stereo mixer 500) and recording it on a Kay-Elemetrics sonagraph (6061B). Each vocalization is identified to individual and the arrival time differences between the channels are measured with a caliper in $\frac{1}{100}$ mm (figure 3). A computer program, which has been specifically written for this task, converts these values to the x,y coordinates of the study area.

Results and discussion. The accuracy of the localization varies depending on the distance from the center of the array and the angle, alpha, from the x axis. This was determined empirically by varying the arrival time differences for known locations randomly over a range of ± 0.3 mm and comparing the median value of the resulting scatter of x,y points with the actual location. As shown in figure 4, The test points (solid dots) arranged in concentric circles represent a target area of 1 m radius each. In most cases, the median of the scatter fell within the target area. This location is indicated by an open circle connected by a line to its target area. The areas which show the greatest deviations are those where the arrival time differences of a large number of points (located mainly on the axes of the grid) vary only slightly from one another, usually well within the range with which the differences can be measured. Since these areas are known to be less accurate than others of the grid, individuals with computed x,y values falling in one of these error prone areas can be rechecked by comparing the arrival time differences measured with the arrival time differences calculated for the computed x,y location, the order of arrival of the vocalizations to the various microphones (which changes along the y axis and the diagonals) with the order of arrival for the calculated locations, and the relative intensities of the vocalizations at the various microphones to the relative intensities expected at the computed location. These 3 tests are sufficient to locate the individual in question with satisfactory accuracy.

The points marked a and b represent the median locations of the scatter obtained from 10 successive calls given by 2 individuals. The scatter is of the same order of magnitude as that obtained in our test program assuming a ± 0.3 mm error in reading the arrival time differences of the sonagrams. Within the central area of approximately 4 ha the resolution of this method is within ± 1 m.

A comparison was then made between the median x,y value obtained from the 10 locations generated from the arrival time differences of 10 successive calls and the x,y location generated if a single input consisting of the median value of 10 arrival time difference was used. Also, a test was run to determine if a difference existed beteen the x,y location generated from the median value of 10 arrival time differences as opposed to the x,y location generated from using the median value of arrival time differences from 5 successive calls. A Wilcoxon Sign Rank test shows that no significant difference exists in either case; i.e. the median value of arrival time difference from 5 successive calls can be used to determine the x,y location of a vocalizing individual.

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