In the progress of evolution, the biosynthetic capacity has been lost in the most highly evolved species. The inability of the guinea pig may be explained by the possible independent occurrence of a mutation. The flying mammals are considered to be more near, in an evolutionary sense, to the primates. The absence of biosynthetic capacity in this species supports such a contention.

The failure of the guinea pig, the flying mammals, monkey, and man to synthesize ascorbic acid is due to a common defect, namely, the absence of the terminal enzyme L-gulonolactone oxidase (*3, 16*). This in turn may be attributed to the loss of the gene or the capacity of the gene responsible for synthesizing the enzyme. Whereas the biosynthetic capacity started in the amphibians which evolved roughly about 330 to 340 million years ago, the gene mutation leading to loss of the capacity probably took place in the common ancestor of man and other primates about 25 million years ago (*17*). The mutation leading to the loss of such an essential gene was, however, neutral (*18*) and not lethal. The mutants did not become extinct because the environment furnished the vitamin and the species continued to survive.

Based on the nature of the evolutionary processes, Pauling (*19*) has put forward arguments suggesting that for optimum health the daily intake of ascorbic acid for an adult man should be 2.3 g, which could be increased to 9 to 10 g in some ailments. This is about 30 to 130 times the dose of 75 mg recommended by the Food and Nutrition Board of the National Research Council, of the United States. According to Pauling, the fact that most animals continue to synthesize ascorbic acid indicates that the need for the vitamin is greater than the amount provided by the usually available foodstuff. Pauling further considered that, although man has lost the biosynthetic ability, yet the need for ascorbic acid is similar to that of other mammals capable of synthesizing the vitamin. However, in experiments with guinea pigs, we did not get any extra beneficial effect of large doses of ascorbic acid on growth and maintenance of the animals fed a fortified wheat diet with adequate intake of protein (*20*). There are some reports (*21, 22*) that large doses of ascorbic acid are beneficial in a variety of stressful situations. We have indicated that the beneficial effect of large doses of ascorbic acid may be due to its ability to detoxify the excess histamine produced or released in stress conditions (*15*). In the guinea pig, a maximum detoxification effect was obtained with a dose of 50 mg of ascorbic acid per kilogram of body weight per day, which is approximately five times the normal need of these animals (*15*). On the other hand, when the guinea pigs were fed a low protein, high cereal diet, a daily intake of 0.3 g, or more, of ascorbic acid per kilogram of body weight was toxic as revealed by inhibition of growth and early mortality (*20*).

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Encoding of Geographic Dialects in the Auditory System of the Cricket Frog

Abstract. *The frequency sensitivity of the auditory nervous system of cricket frogs* (Acris) *varies geographically. This variation is closely matched to the spectral energy in their mating calls, thus enabling them to respond preferentially to the calls of their local dialect.*

The cricket frog gets its name from the cricket-like sound of the male's mating call. The two species that comprise this genus (*Acris*) are both found in the United States: *A. gryllus* occurs in the southeastern part, while *A. crepitans* is found throughout most of the country east of the Rockies. The two species are sympatric in the southeastern states from Virginia to Louisiana (*1*). Behavioral studies in the field have shown that females of each species respond preferentially to the mating calls of their own species (*2*). Thus the species-specific mating call of the male and the selective response of the female provide a reproductive isolating mechanism between these two species in their zone of sympatry. Furthermore, not only are their mating calls species-specific, but they are also geographically specific. When the mating call of a local male *crepitans* and the mating call of a male *crepitans* from a different geographical locality are presented simultaneously through separate loudspeakers, a female *crepitans* will respond preferentially to the mating call from her local population. By studying the response of female *crepitans* to synthetic calls, Capranica and Nevo (*2*) have identified the signal characteristics in the male's call that permit the female's recognition of calls from her own local population. This report presents evidence that the auditory nervous system of the cricket frog is "tuned" to the local dialect and thereby provides a major basis for the female's selective response to the calls of males of her own population. Our evidence is based on electrophysiological recordings from cells in the medullary auditory nuclei (*3*) of 17 cricket frogs obtained from New Jersey, South Dakota, and eastern Kansas.

Adult cricket frogs, about 1 inch (2.5 cm) in length and 1.5 to 2.0 g in weight, were anesthetized with Dial-urethane (Ciba) (3 μl/g); exposure of the medulla was made by a ventral approach through the roof of the mouth. Temperature of the animals was maintained between 22° and 25°C. Acoustic stimuli were delivered by a PDR-10 earphone enclosed in a sealed housing which was fitted around the animal's eardrum to provide a closed stimulus system. Stimuli consisted of tone bursts controlled by an electronic switch (Grason-Stadler 820E); sound intensity was monitored by a condenser microphone (B & K 4134) sealed in the earphone housing. Indium-filled pipettes with platinum-black tips (*4*) were used in recording the spike activity of single cells.

Isolation of single nerve cells in these small frogs was difficult. We typically observed multi-unit activity as the electrode was advanced through the auditory nucleus in the medulla. We succeeded in clear isolation of 66 units: the frequency and threshold sensitivity of the background multi-unit activity closely matched the response properties of the single cells that were isolated. We therefore are confident that the response characteristics of the single units which we present are representative of the auditory sensitivity of cricket frogs from the different geographical populations.

Two distinct types of single units were found in the medullary nuclei of cricket frogs (*5*). One type was sensitive to low-frequency tones and the other type was sensitive to high-frequency tones. From previous studies of the bullfrog's auditory system (*6*), we believe that the units tuned to low frequencies derive their input from the auditory nerve fibers that innervate the amphibian papilla in the inner ear, while the units tuned to high frequencies derive their input from fibers from the basilar papilla. In this respect, amphibians are exceptional among vertebrates in having two separate and anatomically distinct auditory receptor organs. Each organ is tuned to a separate frequency region which presumably provides the basis for the bimodal frequency sensitivity of anurans in general (*7*).

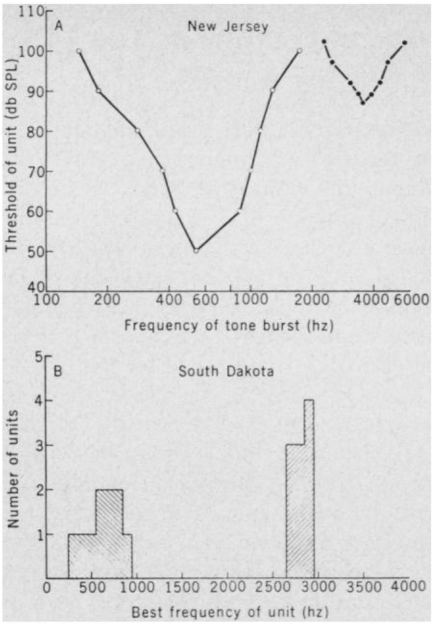

Fig. 1. (A) Tuning curves of a low-frequency unit, best frequency 550 hz, and of a high-frequency unit, best frequency 3550 hz, in *Acris crepitans* from New Jersey (0 db SPL = 0.0002 dyne/cm^2). (B) Histogram of best frequencies for 20 low- and high-frequency units in *A. crepitans* from South Dakota.

Figure 1A shows representative tuning curves (plot of neuron's threshold versus tonal frequency) for units of each type in *A. crepitans* from New Jersey. The low-frequency unit has its best frequency at 550 hz, whereas the high-frequency unit has its best frequency at 3550 hz (*8*). Note that the tuning curves are disjoint and do not overlap even at high sound intensities. The disjoint nature of their frequency sensitivities permitted ready classification of different units as either of low-frequency type or of high-frequency type. We found that the best frequencies of the low-frequency units were distributed over the range 200 to 1000 hz, while *all* of the high-frequency units had their best frequencies narrowly clustered around 3500 to 3550 hz. Furthermore, while the low-frequency units had moderately sensitive thresholds of 35 to 60 db SPL (sound pressure level), *all* of the high-frequency units had relatively poor thresholds around 75 to 80 db SPL. These conclusions are based on the single units that we could isolate as well as the multi-unit background activity that we routinely monitored: all of the auditory (single and multi-unit) activity that we observed in the New Jersey animals was tuned to these two frequency and threshold ranges.

The signal characteristics of a typical mating call from an adult male *crepitans* recorded in New Jersey consists of a sequence of clicks having a stereo-

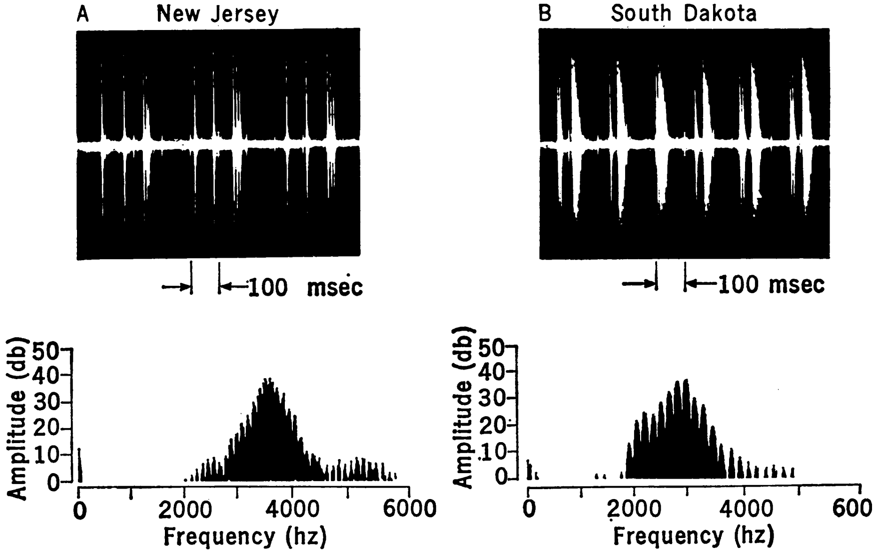

Fig. 2. (A) The mating call of an adult male *A. crepitans* from New Jersey consists of a repeated sequence of clicks. The spectrographic section shows the distribution of energy in a single sequence; this distribution remains the same throughout the call. (B) Mating call of an adult male *A. crepitans* from South Dakota. Details are the same as in (A).

typed temporal pattern. The energy in each click has a distinct spectral peak centered around 3550 hz (Fig. 2A). This frequency is a characteristic feature of the mating calls of male *crepitans* in New Jersey: the spectral peaks in the calls of different males, or of the same male at different times, usually show a standard deviation of less than 100 hz from the mean of 3550 hz. A comparison with the sensitivity of the high-frequency units indicates that these units are all selectively tuned to the spectral peak in the male's calls.

Suppose we consider a different population of *Acris crepitans*. Figure 1B shows a histogram of best frequencies for the medullary units that we were able to isolate in *crepitans* from South Dakota. The histogram is bimodal and emphasizes the disjoint frequency sensitivity of the two types of units. Those units sensitive to low frequencies, tuned to the range of 200 to 1000 hz, had thresholds distributed between 35 and 60 db SPL. These units were very similar to the low-frequency units in *crepitans* from New Jersey. The high-frequency (both single and multi) units were all narrowly tuned, not to 3550 hz as in New Jersey *crepitans*, but rather to 2900 hz. All of these high-frequency units, as in the New Jersey animals, were fairly insensitive, with thresholds at their best frequencies around 75 to 80 db SPL.

A representative mating call of an adult male *crepitans* from South Dakota (Fig. 2B) consists of a sequence of clicks with a temporal pattern that is characteristic of the males from this geographic population. The energy in each click of this call has a spectral peak, not at 3550 hz as in New Jersey *crepitans*, but instead at 2900 hz. The location of the spectral peak in the mating calls of male *crepitans* from South Dakota matches the narrow tuning of the high-frequency unit activity in this population of cricket frogs.

These results suggest that, where a shift in spectral energy in the mating call occurs throughout the geographical range, the tuning of the high-frequency units follow this shift. We pursued this conclusion with studies of *crepitans* from eastern Kansas. The high-frequency units from this population were all narrowly tuned around 3750 hz. The mating calls of males in eastern Kansas have a spectral peak narrowly centered around this same frequency.

Cricket frogs breed in very dense populations in which the distance between calling males is but 1 to 2 m. Behavioral studies in the field reveal that the acoustic intensity of a calling male at a distance of 1 m is typically about 95 db SPL. By employing synthetic mating calls in auditory discrimination tests in the field, Capranica and Nevo (*2*) found that female *crepitans* would no longer respond to a stimulus of this intensity if the spectral peak was shifted by as little as 500 hz either above or below the mean for the local population, even though the temporal pattern of clicks was appropriate for that population. We suggest, therefore, that the matched frequency sensitivity of the high-frequency units—and of the basilar papilla from which they presumably derive—provides a basis of such selective response behavior. Our finding that the high-frequency units all have rather high threshold may therefore have a special significance (*9*). The relative insensitivity of the high-frequency units permits spectral peak detection, narrowly tuned to the dominant frequency in the local call but not stimulated by neighboring frequencies of lower amplitude nor by the dominant frequencies of other dialects.

The sensitivity of low-frequency units in *crepitans* does not show any apparent variation with geographic population. Discrimination studies in the field involving filtered mating calls and synthetic calls indicate that the presence of high-frequency energy alone enabled the female to recognize the male's call. This result contrasts with earlier studies of the bullfrog (*Rana catesbeiana*). The bullfrog requires simultaneous presence of low- and high-frequency sound energy, exciting both basilar and amphibian papillae, to evoke his mating call (*10*).

The role of the low-frequency units in the cricket frog's auditory behavior requires further clarification (*11*). In studies of auditory discrimination involving synthetic calls, the addition of low-frequency energy in the range of 200 to 1000 hz to an effective stimulus inhibited the female's response. When this low-frequency energy was removed by filtering, the female readily approached the sound source. Since large species of frogs and toads typically produce low-frequency energy in their mating calls, and since these species are potential predators for the cricket frog, the low-frequency sensitivity—presumably from the amphibian papilla—may function to alert the cricket frog of nearby danger. This function would be consistent with the moderately sensitive thresholds of the low-frequency units.

The frequency sensitivities of the receptor organs in the inner ear of the cricket frog (*12*) provide a mechanism for discrimination of local dialects based on the spectral energy in the mating call. Only those signals which pass this peripheral filter can affect neural activity in higher auditory centers. Another feature of the dialect is the stereotyped temporal pattern of clicks within the call; this pattern varies with geographic population. The field studies of Capranica and Nevo (*2*) indicate that the pattern of clicks is a very important feature for call recognition among these animals. The manner in which this temporal information is extracted by the central auditory system is unknown.

One of the interesting aspects of this study is the question of how geographical dialects are maintained, namely, are the distinctive characteristics of a male's mating call and the corresponding matched auditory sensitivity of members of his local population determined by genetic mechanisms, or by environmental influence, or are they learned? Further experiments hopefully will reveal the factors that can lead to the maintenance of geographical variation in communicatory signals. Such studies could also provide insight into how vocal dialects might originate.

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12. Matched sensitivity of the high-frequency units to the spectral peak in the local male's mating call was also found in *Acris gryllus*. For example, high-frequency units in the medulla of *gryllus* from Georgia are all narrowly tuned around 3600 hz. The mating calls of male *gryllus* in Georgia have a *distinct* spectral peak near this same frequency. The sensitivities of the low-frequency units in *gryllus* are similar to those of *crepitans*, namely, distributed over the range of 200 to 1000 hz. This sensitivity is consistent with the hypothesis that the amphibian papilla provides a warning function for these tiny species. The mating cells of sympatric *crepitans* in Georgia have their spectral peaks centered around 4050 hz. Furthermore, the temporal patterns of clicks in the calls of the two species are very different, so that species-specific recognition involves both spectral and temporal signal characteristics (*2*). We did not explore the possibility of geographical variation in the auditory system of *A. gryllus*, although the characteristics of their calls also vary geographically (*2*).
13. The electrophysiological studies were conducted while R.R.C. and L.S.F. were members of the technical staff, Bell Telephone Laboratories, Murray Hill, New Jersey. Recording and analysis of mating calls were supported, in part, by grants GB-18836 to R.R.C. and GB-3167 to E.N. from the National Science Foundation.

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Flexible System for the Diagnosis of Schizophrenia: Report from the WHO International Pilot Study of Schizophrenia

Abstract. *Behavioral data on a large patient group were collected by investigators from nine countries in the International Pilot Study of Schizophrenia, sponsored by the World Health Organization. The data on half the group were analyzed to derive a system of 12 signs and symptoms for the identification of schizophrenia, as this disorder is diagnosed in many centers throughout the world. The findings were replicated with the other half of the patient group. The criteria constitute an operational method for identifying patients who would be commonly considered schizophrenic in many centers.*

Despite the efforts of many investigators over the years to develop methods for identifying patients with schizophrenia, widely accepted criteria for the differential diagnosis of this disorder have not been established. Regardless of their basic assumptions about schizophrenia, clinician and investigator alike require a system for classifying their patients in order to organize their concepts and to provide the basis for generalizations relevant to management, treatment, prognosis, or interpretation of biochemical, demographic, genetic, or other research data. Traditionally, signs and symptoms have been the key criteria in defining psychiatric classification (*1*).

In this report we present the results of an investigation to determine which clinical criteria are widely used as discriminating for schizophrenia. Such an investigation is possible with data collected by psychiatrists from nine countries in the International Pilot Study of Schizophrenia (IPSS) sponsored by the World Health Organization. This report, based on some of those data, defines an applicable, operational, and flexible system for identifying patients as schizophrenic. In a large cohort of patients, 12 signs and symptoms were found to be especially discriminating between schizophrenia and other psychiatric disorders. A diagnostic system based on these 12 variables was comparably effective in a second patient cohort.

The IPSS is a transcultural psychiatric investigation of 1202 patients in nine countries—Colombia, Czechoslovakia, Denmark, India, Nigeria, China, the Soviet Union, the United Kingdom, and the United States. It was designed as a pilot study to lay scientific groundwork for future international epidemiological studies of schizophrenia and other psychiatric disorders (*2*).

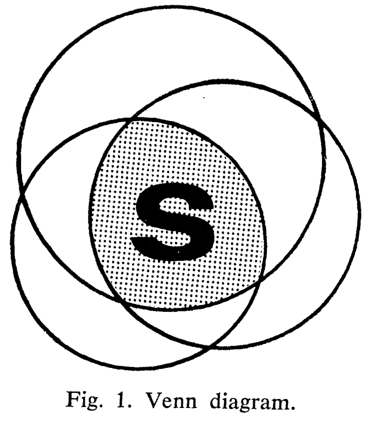

Fig. 1. Venn diagram.

The same inclusion and exclusion criteria were used for patients evaluated in all nine countries (*3*). The collaborating investigators in each nation used a standardized interview schedule, the Present State Examination (PSE), to elicit and record reliable sign and symptom data in patients recently hospitalized with evidence of psychosis (*2*). Standard psychiatric history and social description interviews were also used (*2*). After these interviews, psychiatrists in each country diagnosed their patients by using the categories of the International Classification of Disease (*4*).

Table 1 presents the diagnoses of the 1202 patients evaluated in the nine countries. Of these patients, 81 were dropped from this data analysis since their diagnoses could have been considered as belonging to either the schizophrenic or the nonschizophrenic group (see Table 1). The remaining 1121 patients were randomized by country to form cohorts A and B, each containing approximately 405 patients diagnosed schizophrenic and 155 patients diagnosed other than schizophrenic.

The PSE, used for rating patients' signs and symptoms, contains 360 items. For this study, the scoring of each item was either 0 (absent) or 1 (present). Missing information and ratings of questionably present were scored 0. The 360 individual items and the combinations of 55 items defining clinically relevant symptom groups were then arrayed as 415 overlapping variables. An analysis of variance was then carried out between patients diagnosed schizophrenic and nonschizophrenic in cohort A. The 150 strongest discriminators were inspected to eliminate the weaker of the overlapping variables. The surviving 69 variables are all statistically significant discriminators. They cover a broad range of psychopathology, including symptoms believed characteristic of schizophrenia by many psychodiagnostic schools.

The schizophrenic and nonschizophrenic patients in cohort A were then used as criteria for a stepwise discriminate function analysis (DFAn) to determine the interrelated discriminating power of the 69 variables. The 12 most discriminating symptoms were then made into a 12-point differential diagnostic system (*5*). The degree of discrimination between schizophrenia