in peripheral target tissues (*13*, *14*). The neuronal nuclear concentration of the label may, therefore, in analogy to the estradiol induction of a specific genetic expression in uterine nuclei (*15*), represent the site of a positive "feedback" rather than that of a direct inhibition.

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Rana pipiens Complex: Mating Call Structure and Taxonomy

Abstract. *Geographic variability in call structure indicates that four largely allopatric populations of leopard frogs are present in the central United States. These forms appear to maintain their distinctness in narrow zones of sympatry, and most adult males can be separated morphologically. It is suggested that the four forms represent distinct species and that the idea of gradual clinal variability in one wide-ranging species is wrong.*

Attempts to classify leopard frogs (*Rana pipiens*) by adult morphology and artificial diploid hybridization tests have led to general acceptance of the idea of one wide-ranging, variable species, with levels of genetic incompatibility between populations directly related to geographic separation (*1*, *2*). The example has since become widely used as a classical model for geographic speciation in which the effect of distance on rates of gene flow and the consequent development of reproductive isolation are shown (*3*). More recent studies (*4*, *5*) found further evidence of geographic variability but left the evolutionary interpretation unchanged. Other studies (*6*, *7*) recognized two distinct, largely allopatric forms (the CF and DF complexes) in leopard frogs of northern Colorado with a narrow zone of overlap, about 5 miles (8 km) wide.

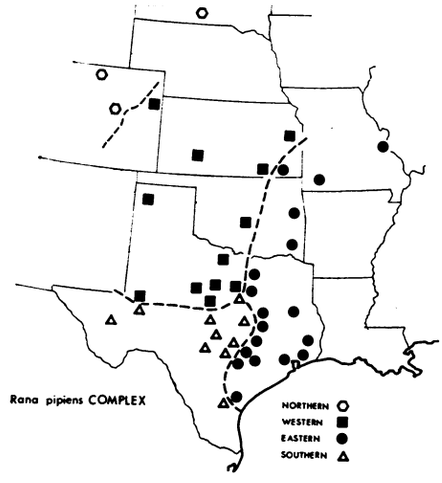

Fig. 1. Distribution of call types of the *Rana pipiens* complex in the central United States and approximate positions of replacement zones.

In 1963 Oldham found two distinctive mating call types (*8*), (Western and Southern), which overlap and maintain their distinctness in a narrow east-west zone across north-central Texas. In 1966 Littlejohn found a third distinctive call type (Eastern) in central Texas. This form is largely allopatric to the other two but has a narrow north-south zone of overlap with the Southern form in central Texas. In Johnson County, Texas, all three populations occur in a mosaic distribution, with two forms at some breeding sites.

Finally, mating calls of leopard frogs in the northern United States are also distinctive. Field work with D. Pettus and D. D. Post (Colorado State University) enabled us to establish that this fourth (Northern) call group is equivalent to the CF complex, and the Western call race to the DF complex. Figure 1 shows a provisional map of the distribution of the four call types in the central United States.

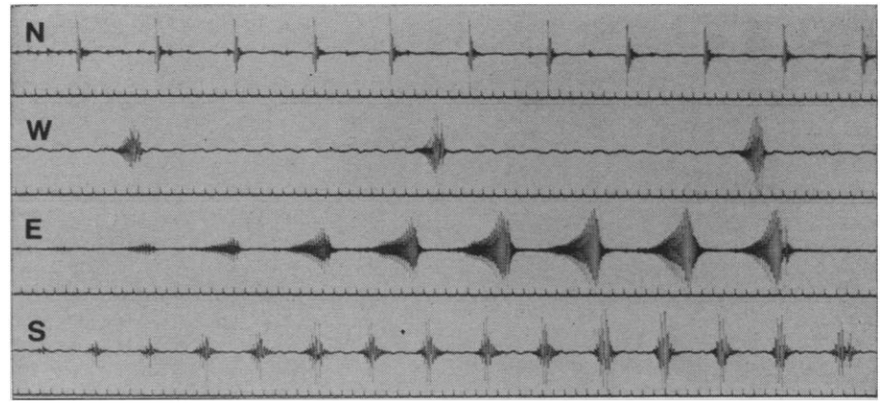

Fig. 2. Oscillograms of the mating calls of the *Rana pipiens complex*. (*N*) Northern (incomplete call): Larimer County, Colorado, effective temperature 12.5°C; (*W*) Western: Cowley County, Kansas, effective temperature 17.2°C; (*E*) Eastern: Johnson County, Texas, effective temperature 17.5°C; (*S*) Southern: Johnson County, Texas, effective temperature 16.3°C. Time marker indicates 10-msec intervals.

Table 1. Selected characteristics of presumed mating calls of call types of the *Rana pipiens* complex. Mean values are given with ranges in parentheses.

Call type	Sample size (individuals)	Call duration (sec)	Pulse rate (No./sec)	Pulse duration (msec)	Pulse rise time* (msec)
Northern †	4	3.75 (3.30–4.73)	13.7 (12.9–14.6)	17.8 (16–20)	2.0 (2)
Western ‡	7	0.66 (0.48–0.89)	5.6 (4.6– 6.8)	27.0 (23–35)	9.1 (7–11)
Eastern §	7	0.41 (0.31–0.52)	14.8 (14.3–15.3)	39.4 (33–50)	24.3 (20–30)
Southern ‖	7	0.64 (0.47–0.83)	28.2 (26.0–31.3)	19.1 (16–22)	9.7 (9–11)

* Interval for pulse envelope to increase from 10 percent to 90 percent of peak amplitude. † One from Elbert County, and three from Larimer County, Colorado. ‡ Two from Brown County, one from Dallam County, three from Johnson County, and one from Martin County, Texas. § All from Bastrop County, Texas. ‖ All from Travis County, Texas.

Mating calls were tape recorded at breeding sites (*9*), and, where possible, individuals were collected after recording and their buccal or cloacal temperatures measured (*10*). Call samples of the four forms are presented in Table 1. These were selected on three bases: (i) that the samples were from areas near, or within, the contact zones; (ii) that effective temperatures of the Eastern, Western, and Southern forms were all between 20° and 25°C to minimize possible temperature effects; and (iii) that background noise levels were low enough to permit accurate determination of call characteristics. For the Northern form the few recordings available were made between 12° and 16°C.

A call consists of a sequence of pulses, with each sequence separated by a silent period many times greater than the longest interval between pulses. The four calls differ most strikingly in duration, and in the temporal characteristics of the pulses within them (Fig. 2, Table 1). Sometimes calls are produced in groups, the first call being longest. In such cases call duration was estimated from the first call in the group (*11*). All of the calls have energy distribution mainly between 400 and 2500 hz. There is little evidence of harmonic structure in the relatively short pulses of the Northern, Western, and Southern types. The longer pulses of the Eastern form have slow rise times and a rich harmonic content (*12*).

Two morphological characters allowed identification of most collected adult males of the four call types. These were found to be of greatest diagnostic value (*6*) and were also noticed by McAlister (*5*). The dorsolateral folds (*6*) may be continuous (Northern and Eastern) or displaced medially toward the posterior (Western and Southern). Vestigial oviducts may be present (Northern and Southern) or absent (Western and Eastern). While the presence or absence of vestigial oviducts seems absolute, the dorsolateral folds are more variable, with diagnostic value of about 95 percent.

Recordings were obtained at four localities where the Eastern and Southern forms overlap in Texas. The largest sample was from Ottine, Gonzales County, and included 32 individuals of the Eastern type, 9 Southern, and 2 intermediate in pulse duration and pulse rate, presumed to represent hybrids. A sample of calls of 15 individuals from 20 miles (32 km) east of Austin, Travis County, included 3 Eastern, 11 Southern, and 1 probable hybrid (based on an intermediate pulse duration). In the Cleburne area, Johnson County, all calls heard or recorded were referable to one call type or the other. Of 13 individuals from Big Lake, Welder Wildlife Refuge, San Patricio County, 8 were of the Eastern type, 1 Southern, and 4 probable hybrids (based on intermediate pulse rates). The zone of overlap is about 5 miles (8 km) wide in Travis and adjacent counties.

Contacts between the Western and Southern types were found at five localities in north central Texas (*13*) and the zone of overlap is about 10 miles (16 km) wide, at most. Three probable hybrids (based on intermediate pulse rates) were detected in a series of recordings which included calls of at least 25 individuals of each parental form; otherwise, the call types maintain their distinctness in sympatry. Contacts between the Western and Eastern types were located at two stations in Johnson County, Texas, and no intermediates were detected. We have no indications of accentuation of call differences (reinforcement) in the zones of sympatry.

The behavior of the Eastern, Western, and Southern types in sympatry with apparently limited hybridization as well as the morphological differences among adult males would seem to be indicative of species differentiation at least in the areas under consideration (*14*). This conclusion may also be extended to the Northern form, for its divergence is of a similar degree. While this taxonomic interpretation rests largely on an acoustical character, such behavioral attributes have already proved to be of immense value in resolving taxonomic problems among soniferous groups of animals (*15*). At any rate one finding is unequivocal: at least four largely allopatric and distinctive entities are present within the central North American component of the *R. pipiens* complex; and where they have been studied, the replacements occur sharply over narrow zones of sympatry and not through gradual clinal variation. This interpretation does not differ greatly from the conclusions of some earlier natural historians (*16*). Since no topotypic calls or specimens were examined, and the nature of interactions in other contact areas is yet to be determined, it seems inappropriate to attempt applying formal names to the four taxa at this time.

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9. Several types of microphones and tape recorders were used in this investigation, but variations in their characteristics are not believed to have affected the comparisons made here.
10. If an animal was not collected, water or wet bulb air temperature was used, depending on the calling site, as these values are close to cloacal temperatures. In some cases, only dry bulb air and water temperature were available, and the quantitative use of associated recordings was restricted to situations in which the differential was less than 3°C. The value used is termed the effective temperature.
11. Call duration and pulse rate were derived from audiospectrograms of two or three calls of each individual (Kay model 6061-A Sona-Graph). Pulse characteristics were obtained from cathode ray oscillograms of one call of each individual.
12. There is a possibility that this difference could be an artifact of the method of spectral analysis. See W. A. Watkins, in *Marine Bio-Acoustics*, W. N. Tavolga, Ed. (Pergamon, Oxford, 1967), vol. 2.
13. Coleman area, Coleman County; 12 miles (19.2 km) west of Brownwood, and Brown-

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Human-Mouse Somatic Cell Hybrids with Single Human Chromosome (Group E): Link with Thymidine Kinase Activity

Abstract. *Mouse somatic cells lacking thymidine kinase were mixed in culture with human diploid cells lacking hypoxanthine guanine phosphoribosyl transferase, and hybrid cells were isolated and maintained in a selective medium containing hypoxanthine, aminopterin, and thymidine. The hybrid cells at the time of isolation had karyotypes consisting predominantly of mouse chromosomes but with one human chromosome, a submetacentric member of group E, apparently giving thymidine kinase to the hybrid cell. However, after long-term propagation in the selective medium this chromosome has been lost, although cells continue to show thymidine kinase activity as demonstrated by the incorporation of ^{3}H-thymidine into DNA in the hybrid cell. The hybrid cells have only mouse electrophoretic variants for glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and malate dehydrogenase, suggesting that the human genetic loci for these enzymes are not represented in the hybrid genome and may be unlinked to that for thymidine kinase.*

Weiss and Green have described human mouse hybrids containing a partial complement of human chromosomes (*1*). One of the human chromosomes was apparently associated with thymidine kinase activity, since this chromosome was present only in cells able to grow in medium containing hypoxanthine, aminopterin, and thymidine (HAT), which selects for the presence of the enzyme, and not in cells maintained in medium containing 5-bromodeoxyuridine (BUDR), which selects for cells with thymidine kinase deficiency.

We have mixed mouse somatic cells lacking thymidine kinase with human diploid cells lacking hypoxanthine guanine phosphoribosyl transferase and have isolated hybrid cells as a result of their ability to grow in selective medium which kills the mutant parent cells (*2*). The human parent cells were fibroblasts obtained by skin biopsy from a male with Lesch-Nyhan syndrome, and were isolated in this laboratory. They lack hypoxanthine guanine phosphoribosyl transferase (*3*), are resistant to 2 amino 6-mercaptopurine (10 μg/ml), and are unable to grow in medium containing HAT. These diploid cells have 46 chromosomes; chromosomes of groups E (Nos. 16 to 18) and F (Nos. 19 and 20) (*4*) are easily distinguished in morphology from chromosomes of the parental mouse cell.

The mouse parent, a subline of L-M mouse fibroblasts [LM (TK$^-$)] clone 1-D, was provided by Dr. M. Weiss. This clone of mouse fibroblasts was isolated by Kit *et al.* (*5*) and is resistant to BUDR (30 μg/ml) by virtue of its thymidine kinase deficiency. Clone 1-D has 52 (49 to 56) chromosomes with ten metacentric and submetacentric chromosomes, including the distinctive submetacentric marker D chromosome. The remaining chromosomes are telocentric (Table 1).

Hybrid cultures were initiated by mixing together 2×10^6 mouse and 2×10^6 human cells, in a 1-ml volume of glucose-free Hanks' solution. Ultraviolet-irradiated Sendai virus was added to one-half of the suspension of mixed cells to enhance contact between cells (*6*). The cell suspension that was not treated with virus and the Sendai-treated cell suspension were each plated into a 100-mm Falcon plastic petri dish, and the mixed populations were maintained in growth medium (minimum essential medium, 1 percent nonessential amino acids, 10 percent fetal calf serum) for 48 hours, at which time the medium was changed to HAT [growth medium + hypoxanthine ($1 \times 10^{-4}M$), aminopterin ($4 \times 10^{-7}M$), and thymidine ($1.6 \times 10^{-5}M$) + glycine ($3 \times 10^{-6}M$)]. Both mouse and human parental cells showed toxicity with cessation of growth in selective medium. After a latent period of 4 weeks HAT-resistant clones were first observed, and only in the petri dish containing ultraviolet-irradiated Sendai-treated cells. Twelve independent clones were isolated from this petri dish during the 6th week after mating. Eight clones have survived propagation in HAT for at least 6 months, and have been observed in regard to karyotype and enzyme characteristics.

Karyotypes of the hybrid cells during the course of propagation were prepared according to methods previously described (*7*). From earliest observations the hybrid cells had a karyotype consisting of almost the entire mouse genome with only one or two human chromosomes. Table 1 shows the characteristics of the karyotype of the mouse parent, clone 1-D, and of the hybrid cells close to the time of isolation. Four of the 21 hybrid cells analyzed had no apparent human chromosome. The remaining cells had included in their genome a chromosome morphologically similar to a submetacentric member of the human group E (Fig. 1). There were four cells which had, in addition to the previously described E group chromosome, one to two other chromosomes, morphologically different from those normally seen in clone 1-D. These presumably human chromosomes were, with one exception, also submetacentric members of group E. That the total numbers of chromosomes in the hybrid cells were essentially the same as those of the mouse parent is attributable to the loss in the hybrid cells of a mouse telocentric chromosome.

The association of the human submetacentric E group chromosome with thymidine kinase activity was suggested by our inability to find this chromosome in 116 hybrid cells presumably lacking thymidine kinase since they had survived transfer to media containing BUDR. Because the hybrid clones were isolated by virtue of their ability to grow in HAT, we assumed that these clones were thymidine kinase positive.