

SYSTEMATICS OF THE *RANA* *PIPIENS* COMPLEX: PUZZLE AND PARADIGM

David M. Hillis

Department of Zoology, University of Texas, Austin, Texas 78712

INTRODUCTION

Frogs of the genus *Rana* are among the most commonly studied vertebrates in laboratories worldwide. In primary schools, children often learn about reproduction and development by watching the fertilization of *Rana* eggs and the growth of the resulting embryos into tadpoles. In secondary schools, many young biologists-to-be are introduced to anatomy and physiology by dissecting pithed *Rana*. In college, students may encounter *Rana* in endocrinology, comparative anatomy, vertebrate physiology, and embryology classes. In addition, ongoing laboratory research on *Rana* spans virtually all fields of biology. So it is both surprising and unfortunate that study of the natural history and systematics of *Rana* has lagged far behind experimental laboratory studies. As this deficiency gradually has been corrected, the changes in interpretation of past laboratory studies have been profound. In Europe, the common edible frog ("*R. esculenta*") is now known to be a hybridogenetic form (113), a finding that will considerably alter the conclusions of much of the developmental, genetic, and physiological work conducted with this species. In North America, "the frog" in experimental studies usually has been a leopard frog, *R. pipiens*. Unfortunately, past studies that referred to *R. pipiens* may have involved any of a complex of approximately two dozen different species, which in spite of their morphological similarity are genetically, physiologically, and behaviorally quite distinct (26, 44, 56).

The *R. pipiens* complex has served through the years as a textbook example in discussions of systematics for several distinct reasons. Until the 1960s, *R. pipiens* was commonly cited in introductory biology and evolution textbooks

(e.g. 14, 23, 46, 59) as an example of reproductive isolation by distance and of a polytypic species. As field studies demonstrated that numerous species were involved, many of which could be found in sympatry exhibiting distinctive reproductive behaviors, the *R. pipiens* complex became an example of sibling species and the need for thorough systematic studies (e.g. 2). In addition, the *R. pipiens* complex now serves as an example of phylogenetic reconstruction using nontraditional characters (17). Unfortunately, past systematic problems with the *R. pipiens* complex have caused many biologists to abandon leopard frogs as research animals (8), even though phylogenetic information about the complex now provides a study system of considerably greater value for comparative investigations.

During much of the history of systematic investigations of the *R. pipiens* complex, the primary question concerned delimiting the species boundaries. Three distinct periods of research can be identified (Figure 1). Before and into the 1920s, taxonomists generally underappreciated geographic variation and polymorphism, and then described numerous morphological variants of leopard frogs as distinct species. This began to change in 1920 when Boulenger (4) first monographed New World frogs of the genus *Rana* and synonymized numerous described species of leopard frogs. This trend was strongly reinforced in the 1940s, when systematists began to emphasize the biological species concept and the polytypic species concept (84). Reproductive compatibility became the central criterion for species recognition, and great morphological variation was not considered surprising within a species. The number of recognized species in the *R. pipiens* complex was reduced considerably (Figure 1), with most species subsumed under the name *R. pipiens*. The most recent phase began in the 1960s, when field studies started to demonstrate areas of sympatry between morphologically distinct forms of leopard frogs, with little or no hybridization (62, 63, 96). Vocalizations were found to be distinct among species (56), biochemical analyses demonstrated considerable genetic differentiation (44, 94, 102), reproductive studies showed allochronic breeding seasons for many sympatric pairs (29, 39), and phylogenetic analyses showed that some frogs called *R. pipiens* were more closely related to other recognized species than to other frogs called *R. pipiens* (44). Some of the names available for leopard frogs were once again applied to distinct species, and many new species were described (Figure 1). These three periods can be divided into the thesis of typological species (prior to the 1940s), the reaction to typology or antithesis of polytypic species (1940s to 1960s), and the synthesis of evolutionary species (1960s to present).

Although recognition and description of the species in the *R. pipiens* complex continue to date, much of the systematic attention has turned to attempts to understand evolutionary relationships and the biogeographic history of the group. Contributions from morphology, karyology, and molecular

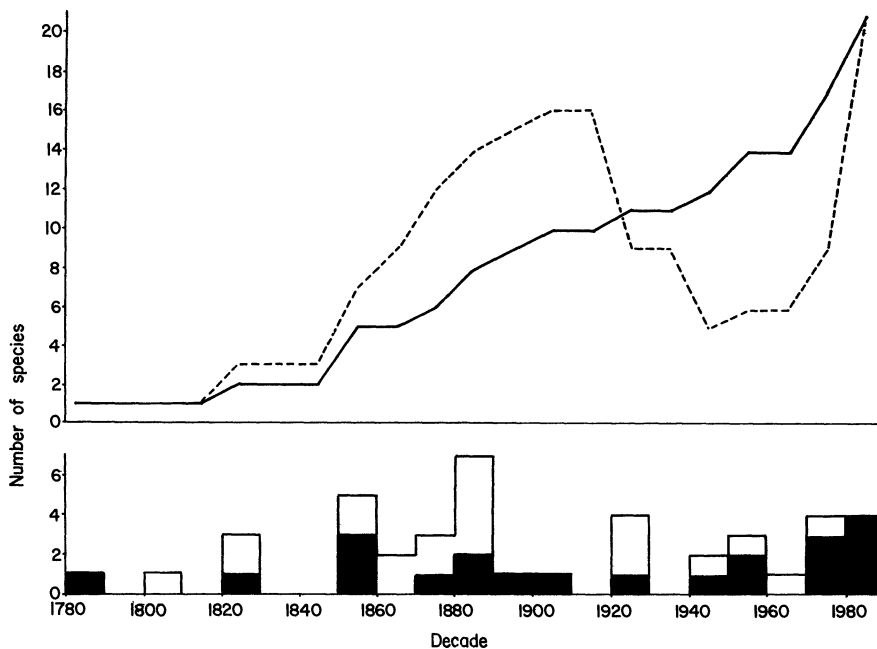


Figure 1 Description and synonymization of species in the *Rana pipiens* complex. The upper graph shows the number of species recognized during each decade (dotted line) and the cumulative number of species described that are recognized today. The lower bar graph shows descriptions of species of in the *R. pipiens* complex by decade; solid bars denote species currently recognized, and open bars denote current synonyms.

biology have begun to provide a phylogenetic framework from which to study and understand the biology of leopard frogs. Although far from complete, the current phylogenetic understanding of leopard frogs provides a means of interpreting a diversity of biological data. A new era of research involving leopard frogs is now possible, in which many biological phenomena can be studied in an evolutionary context in the field as well as the laboratory.

THE SPECIES PROBLEM

Thesis (before the 1940s): Typological Species

From 1782 when *R. pipiens* was described (109) into the 1920s, many morphological varieties of leopard frogs were described as distinct species (Figure 1). As was typical for the time, little regard was given to geographical variation or to the relationships of these forms in the species descriptions, largely because the concepts of geographic variation and evolution were foreign to the authors over much of this period. Even after widespread

acceptance of evolutionary change, most taxonomists continued operating under a typological species concept.

In 1825, LeConte described *R. palustris* (52). Because this species is broadly sympatric with *R. pipiens*, it was generally recognized as distinct through periods of synonymization. The species now known as *R. sphenoccephala* was first recognized as distinct in 1826 (38). Although this species is about as distinct morphologically from *R. pipiens* as is *R. palustris*, areas of sympatry were not found between *R. pipiens* and *R. sphenoccephala* until the 1970s, and for nearly a century and a half authors alternatively synonymized and recognized *R. sphenoccephala* as a distinct species.

Exploration of the southwestern United States and Middle America in the second half of the nineteenth century produced an explosion of descriptions of species in the *R. pipiens* complex (Figure 1). Many of these new names were synonyms, but many of the species recognized today were described during this period. Although some of the large, distinctive members of the complex living on the Mexican Plateau and in the southern United States have been recognized as distinct since their description, the smaller leopard frogs described during this period were later synonymized under *R. pipiens*.

Toward the end of the nineteenth century, systematists began to doubt the distinctiveness of the described species of leopard frogs. Cope (12) placed several species (including *R. sphenoccephala*) as subspecies of *R. virescens* (*R. pipiens*) in 1889, although Dickerson recognized *R. sphenoccephala* as distinct again in 1906 (16). Boulenger monographed the New World *Rana* in 1920 (4) and recognized that little evidence supported the description of numerous species of Middle American leopard frogs.

The typological species period continued through the 1920s, however, with the description of *R. burnsi*, *R. kandiyohi*, *R. noblei*, and *R. miadis* (3, 107, 117). The first two of these names were applied to distinctive phenotypes of *R. pipiens*, later shown to be produced by single mutant genes (71, 115). The name *R. noblei* was applied to a specimen of *R. pipiens* with incorrect locality data (108), and *R. miadis* was named based on a distinctive leopard frog on Little Corn Island, Nicaragua (3). Only the last is recognized as a distinct species today (24).

Antiithesis (1940s to 1960s): Polytypic Species

The developing ideas of widespread, polytypic species of leopard frogs were solidified in 1942 with the publication of Mayr's *Systematics and the Origin of Species* (58). Considerable variation was accepted within what was considered a polytypic species (73), since sympatric populations of the various forms were not well documented (84). The central criterion of Mayr's biological species concept was reproductive compatibility, and Moore (74–80) provided considerable evidence that most geographically separated forms of

the *R. pipiens* complex could produce offspring in artificial crosses. Although a few authors were never completely convinced (e.g. 5, 6, 119), the vast majority of systematists accepted this all-inclusive view of *R. pipiens*.

Moore (73) reviewed morphological variation of leopard frogs in North America in 1944 and concluded that supposed "diagnostic characters were . . . invalid when samples from many localities were studied." Unfortunately, Moore (73) used state boundaries to form his samples, and many samples contained more than one species; thus, diagnostic characters did not appear as such. For instance, his Texas sample contained at least *R. berlandieri* and *R. sphenoccephala*, and may have contained *R. blairi* as well. In pooling samples to compare morphometric proportions, Moore (73) apparently combined samples of *R. blairi* and *R. sphenoccephala* into his *R. sphenoccephala* sample and *R. pipiens* and *R. sphenoccephala* into his *R. pipiens* sample (although the *R. pipiens* sample may have included just *R. sphenoccephala*). Moore did discover some additional morphological features that were later found to be diagnostic of some species (e.g. presence versus absence of vestigial oviducts in males), but because of the lumping of samples, these characters did not appear to him to warrant the recognition of multiple species.

Given the apparent lack of uniform morphological differentiation noted by Moore (73), the reproductive compatibility tests proved decisive. The observation that some geographically distant populations of leopard frogs, previously considered distinct species, were reproductively compatible seemed to confirm the finding of one variable, widespread species (74). Acceptance of a widespread *R. pipiens* required hypotheses of adaption to local environments and reproductive isolation by distance to account for the reproductive incompatibilities observed (see Laboratory Crosses, below). The morphological data from studies of North American leopard frogs were extrapolated to Mesoamerican leopard frogs, even though no morphological study of leopard frogs was attempted on specimens from south of the United States during this time. Thus, the prevailing view became that *R. pipiens* extended from Canada to Panama, with each population adapted to local conditions. These populations were thought to represent a reproductive continuum, although specimens from distant localities or distinct habitats might be reproductively incompatible as a result of local adaptations. The same view of highly variable, continuous geographic variation was also applied to *R. areolata* and its relatives (58, 86)—a view that still has its proponents (1).

Synthesis (1960s to present): Multispecies Complex

Evidence that species diversity of leopard frogs was underestimated began to accumulate at a rapid rate in the 1960s. This was partly a result of development and application of new systematic methods and partly a result of detailed field studies in which contact zones were located and studied. The new

evidence came from many sources, and each new study suggested the same result: "*R. pipiens*" consisted of many distinct species.

MORPHOLOGY Analysis of morphological variation on a finer geographic scale indicated abrupt changes in suites of morphological characters that had been smoothed over by combining samples across species boundaries in previous studies. In Texas, McAlister (61) found three parapatric "morpho-units" (later to be recognized as *R. berlandieri*, *R. blairi*, and *R. sphenocéphala*). In Colorado, Post & Pettus (95) noted two distinct "character complexes" (*R. pipiens* and *R. blairi*). In Arizona, Mecham (63) studied two morphologically distinct forms of leopard frogs in the White Mountains. On a finer scale than studied by Moore (73), the geographic variation of leopard frogs seemed to be much clearer.

As the distinctiveness of leopard frog boundaries became clearer, the number of known diagnostic morphological characters rapidly increased. Morphological variation of leopard frogs was complicated, but close scrutiny showed clear distinctions. Morphological variation in the Plains states was relatively straightforward (20, 57, 66, 89), and by the early 1970s the patterns became clear in the eastern and northern United States (88). The patterns of variation were more complicated in the southwestern United States (because of a greater number of species), and the species in this area were not fully resolved until a decade later (28, 63, 90, 92, 93). Morphological variation of leopard frogs in Middle America is still not fully resolved, although notable progress has been made since the 1970s (27, 41, 45, 103, 104). Although many species are considered "cryptic," every recognized species in the *R. pipiens* complex can be identified by morphology alone. Morphological differences among species include differences in pigmentation, form of the dorsolateral folds, male oviducts, body proportions, and osteology (20, 27, 41, 45, 66, 88, 92, 93).

In recent years, larval morphology of leopard frogs has been studied in greater detail, and many species in the *R. pipiens* complex can now be identified at this life stage. To date, the tadpoles of eight species have been diagnosed (40, 45, 49, 110). Many of the species are readily distinguishable, and future work in this area is likely to be fruitful. Numerous differences have been reported in body form, pigmentation patterns, labial teeth, oral papillae, mouth shape, musculature, and the lateral line system (40, 45, 49, 110). Differences in eggs have been occasionally noted for some species, but to date no one has carefully analyzed egg morphology in the group.

VOCALIZATIONS In the 1960s, Littlejohn & Oldham (56) independently found two contact zones between species of leopard frogs with highly distinct mating calls. Working in north-central Texas, Oldham found two acoustically

differentiated forms (which he called Southern and Western), with very few intermediate (hybrid) calls at sympatric locations. Littlejohn found an additional call type (Eastern) in sympatry with the Southern type call in south-central Texas. The two investigators located a fourth call type (Northern) in Colorado and South Dakota. Several features of these calls were diagnostic, including call duration, pulse rate, pulse duration, and pulse rise time. Littlejohn & Oldham (56) considered these data to indicate species differentiation, but they did not attempt to apply formal names to their four taxa. Brown & Brown (7) provided supporting data from Illinois, and Pace (88) added data on call differentiation from the eastern United States. Mecham (65) extended the studies of mating calls into the southwestern United States and Mexico, and noted that some Mexican species recognized as distinct (*R. dumni*, *R. megapoda*, and *R. montezumae*) had mating calls that were very similar to frogs called *R. pipiens* (later named *R. chiricahuensis*) in the southwestern United States.

The data from vocalization behavior were convincing. Combined with other information they resulted in the partitioning of "*R. pipiens*" in the United States east of the Rocky Mountains. In 1973, the various authors (66) who had detailed vocalization behavior of leopard frogs in the central United States described *R. blairi* (Western form) and applied the previously synonymized names *R. berlandieri* to the Southern form and *R. sphenocéphala* to the Eastern form. The Northern form was found to be the true *R. pipiens*. At the same time, they noted two other distinct call types, the Lowland form and a new Southern form, both found in Arizona and adjacent Mexico. Differentiation of advertisement calls became the basis or partial basis for the description or removal from synonymy of many species of leopard frogs. In Mexico, *R. forreri* was recognized and *R. magnaocularis* described (27), and in the United States *R. chiricahuensis* (Arizona Southern form) and *R. yavapaiensis* (Lowland form) were described (92, 93).

All sympatric leopard frogs studied to date have differentiated advertisement calls, and numerous authors have suggested that these calls serve as premating isolating mechanisms (7, 29, 30, 34, 39, 56, 65, 88). However, little work has been conducted showing discrimination by the frogs between homo- and heterospecific calls. Oldham (87) studied the response of *R. berlandieri* and *R. sphenocéphala* to each others' calls, but found little response except by a few female *R. sphenocéphala* to calls of their own species. Gamba & Littlejohn (31) found evidence that the so-called "mating trill" of *R. berlandieri* functions (at least in part) to announce occupied space to nearby males. However, Kruse (50), investigating in the zone of sympatry between *R. blairi* and *R. pipiens*, noted positive phonotaxis of female *R. pipiens* to conspecific calls, negative phonotaxis of female *R. pipiens* to *R. blairi* calls, and no response by female *R. pipiens* to calls from a hybrid individual.

Despite the differentiation in vocalizations among sympatric members of the *R. pipiens* complex, many allopatric species have quite similar calls. For instance, the calls of members of the *R. montezumae* group in Mexico and the United States are broadly overlapping in the various parameters studied, including dominant frequency, pulse number, pulse rate, pulse duration, and pulse rise (65). Likewise, call differentiation among allopatric members of the *R. berlandieri* group is not dramatic, although many species are otherwise highly differentiated (25).

CONTACT ZONES AND ISOLATING MECHANISMS The *R. pipiens* complex consists of two major, broadly sympatric phylogenetic groups, the Alpha and Beta divisions (see Phylogeny and Biogeography, below). Although some species in both divisions have been confused in the past with *R. pipiens*, the greater part of the confusion has involved parapatrically distributed species of the Beta division. Partly because of the broad sympatry, many of the Alpha division species have long been recognized as distinct. The most convincing evidence of the distinctiveness of the various species confused with *R. pipiens* came when various combinations of these frogs were found together in contact zones, often with little or no hybridization.

One of the first contact zones to attract interest actually involved an Alpha division and a Beta division species: *R. chiricahuensis* and *R. pipiens*. These two species were collected in sympatry in 1942 in Arizona by Wright, who was not comfortable in calling them both *R. pipiens*: "These frogs are a queer lot. Are they all one [species]?" (119, p. 517). Mecham (62, 63) reported this area as a contact zone between two reproductively isolated forms of "*R. pipiens*" in 1968. The first case of sympatry between two Beta division species was reported in 1967 (96), between *R. pipiens* and what was later named *R. blairi*, in eastern Colorado. Three additional contact zones were reported shortly thereafter, involving the various combinations of *R. berlandieri*, *R. blairi*, and *R. sphenoccephala* in Texas (56). In all of these zones of sympatry, hybridization was either uncommon or absent.

Of species in the *R. pipiens* complex 30 pairs are now known to occur sympatrically (Table 1). Half of these cases are between an Alpha and a Beta division species, 13 are between two Beta division species, and only 2 are between two Alpha division species. Natural hybrids have been reported between 9 of these 30 species pairs. It is interesting that nearly half of the cases of hybridization have involved an Alpha species and a Beta species, and only one case of hybridization (*R. blairi*-*R. pipiens*) involves sister species (44). Although the study of hybrid zones was thought paramount to the study of speciation (see references in 118), most hybrid zones in the *R. pipiens* complex actually involve relatively distantly related species and therefore cannot be directly involved in the speciation process. Considering that this

phenomenon also has been reported in a group of parapatric lizards for which phylogenetic information is available (42), a critical review of the parapatric model of speciation is warranted. Suggestions of parapatric speciation in the *R. pipiens* complex (88) appear not to be generally applicable.

Both pre- and postmating isolating mechanisms have been studied extensively in the *R. pipiens* complex (Table 1). Most authors have suggested one or a combination of two or three premating isolating mechanisms to account for the absence or reduction of hybridization among leopard frogs. Because known advertisement calls of all sympatric species are distinct (Table 1), vocalization behavior may be the most important premating isolating mechanism within the *R. pipiens* complex. Littlejohn (53–55) suggested that one or more elements of the mating call of frogs must differ by a factor of approximately two in order for interspecific differentiation to be effective. However, cases are documented in which anurans discriminate between calls that differ by as little as 25% in pulse rate or 10% in dominant frequency (31a). The calls of all sympatric pairs in the *R. pipiens* complex differ by at least a factor of two in pulse rate, call duration, or dominant frequency, with the exception of *R. chiricahuensis*-*R. pipiens*, *R. palustris*-*R. areolata*, and *R. palustris*-*R. capito*. The first of these pairs of species produces hybrids in sympatry, although the latter two pairs do not.

A second major premating isolating mechanism for members of the *R. pipiens* complex is temporal differences in the breeding seasons. This mechanism has been suggested as an important component in reducing hybridization between eight of the pairs in Table 1. As evidence of the importance of this mechanism as an isolating mechanism, displacement of breeding seasons in sympatric populations compared to allopatric populations has been noted for *R. berlandieri*, *R. chiricahuensis*, and *R. sphenoccephala* (30, 39). Frost (26) reported that in sympatric populations of *R. forreri* and *R. magnaocularis*, neither species maximizes potential feeding/fat-storage opportunities with respect to breeding opportunities, even though virtually the entire year is used by breeding in one or the other species. He further suggested that the nearly asynchronous breeding seasons of the two species could best be understood in the context of reproductive character displacement via temporal competitive exclusion. Breeding data are not available for many of the sympatric species pairs, and comparative data from sympatric and allopatric populations are lacking for most species.

Spatial isolation through breeding habitat separations has also been reported as an important isolating mechanism in the *R. pipiens* complex. Most species in the complex prefer either lentic or lotic habitats; relatively few species are generalists in breeding habitat preferences. Correlated with these preferences are differences in tadpole morphologies (40, 45). Lentic breeding species have tadpoles with short tails with high fins and weakly developed

Table 1 Known occurrences of sympatry among species of the *R. pipiens* complex. "Percentage hybrids" column gives the range of estimates of hybrids in various sympatric populations. Reported isolating mechanisms are indicated by a "+"; mechanisms that do not contribute to isolation are indicated by a "-". A "+/-" in the call column indicates the advertisement calls are differentiated in the two species, but there is less than a two-fold difference in the measured parameters; a "+/-" in the hybrid incompatibility column indicates a one-way incompatibility (the reciprocal cross produces offspring); unknown data are indicated by "?".

Species pair	Percentage hybrids	Isolating mechanisms					References
		Call	Temporal	Habitat	Hybrid incompatibility	Hybrid inferiority	
<i>berlandieri-sphenocephala</i>	0-86	+	+	+	-	+	39, 48, 56, 61, 82, 101, 102
<i>berlandieri-blairi</i>	0-9	+	+	+	-	+	39, 56, 64, 89, 91, 97
<i>berlandieri-chiricahuensis</i>	0	+	?	-	-	+	97, unpub. data
<i>berlandieri-spectabilis</i>	0	?	?	-	-	+	45, 81, 99, 102, unpub. data
<i>blairi-pipiens</i>	0-70	+	+	+	-	+	18, 20, 32a, 50, 51, 57, 96
<i>blairi-sphenocephala</i>	0-5	+	+	+	-	+	20, 39, 64, unpub. data
<i>blairi-areolata</i>	0	+	-	-	-	+	13, unpub. data
<i>blairi-chiricahuensis</i>	0	+	-	-	-	+	28, 30, 97
<i>blairi-palustris</i>	0	+	-	+	-	+	64, unpub. data
<i>chiricahuensis-yavapaiensis</i>	0-3	+	-	-	-	+	30, 90, 92, 94

<i>chiricahuensis-piapiens</i>	0-9	+/-	+	-	-	+	+	30, 36, 62, 63, 90, 94
<i>dummi-neovolcanica</i>	0	+	?	?	+	?	?	65, 124
<i>forreri-spectabilis</i>	0-14	?	?	+/-	+	+	+	45, 102
<i>forreri-taylori</i>	0	?	?	?	-	?	?	41
<i>magnaocularis-forreri</i>	0	+	+	+/-	+	+	+	25-27, 29
<i>megapoda-neovolcanica</i>	0	+	?	?	+	?	?	41, 65, 124
<i>montezumae-spectabilis</i>	0	+	?	?	+	?	?	41, 65, 124
<i>montezumae-tlaloci</i>	0	?	?	?	-	?	?	41
<i>neovolcanica-forreri</i>	0	?	?	+/-	+	+	+	45, 81
<i>neovolcanica-spectabilis</i>	0	?	?	-	+	+	+	45, unpub. data
<i>palustris-areolata</i>	0	+/-	-	-	+	+	+	64, 67, 77
<i>palustris-capito</i>	0	+/-	?	?	+	?	?	41, 88
<i>palustris-piapiens</i>	0-5	+	+	-	-	+	+	37, 67-69, 72, 75, 77, 81, 88
<i>palustris-sphenocephala</i>	0-20	+	+	-	-	+	+	64, 75, 81, 102
<i>piapiens-areolata</i>	0	+	-	-	+	+	+	67, 72, 77, 112
<i>piapiens-sphenocephala</i>	0	+	-	-	-	+	+	70, 74, 81, 88
<i>sphenocephala-areolata</i>	0	+	-	-	-	?	?	13, 60, 64
<i>sphenocephala-capito</i>	0	+	-	?	-	?	?	41, 88
species a-species b	0	?	?	?	-	?	?	41
species d-species e	0	?	?	?	?	?	?	41

musculature, whereas lotic species have tadpoles with long, muscular tails and low fins (40). Although only a few species are completely restricted to their preferred habitats, these preferences contribute to reproductive isolation of many sympatric pairs in the *R. pipiens* complex (Table 1). For instance, although *R. berlandieri* will breed in ponds in central Texas, much of the breeding of this species takes place in streams, whereas the partially sympatric *R. sphenoccephala* breeds almost exclusively in lentic situations (39). Thus, hybrids between these two species occur primarily in the latter habitats. Because numerous streams in the zone of sympatry between these two species have been dammed into ponds by humans, hybridization has been promoted (39). Although the habitat separation between *R. berlandieri* and *R. sphenoccephala* is incomplete, other species are completely isolated by habitat. For instance, two broadly sympatric species on the Mexican Plateau, *R. montezumae* and *R. spectabilis*, may never breed in mixed choruses. In the montane areas of sympatry, male *R. montezumae* call from floating positions in lakes and ponds, well away from shore, whereas *R. spectabilis* breeds primarily in the streams leading into the lakes or occasionally in marshy areas around the lake shore (45). Another kind of breeding habitat preference effectively isolates the species *R. areolata* and *R. palustris* in areas of sympatry; *R. areolata* breeds in pools in open fields, whereas *R. palustris* breeds in wooded areas. Soil substrate preferences partially isolate sympatric populations of *R. blairi* and *R. pipiens*. In Nebraska, the former species primarily occupies loess soil and the latter species primarily occupies sandy soil (57). In Colorado, construction of artificial ponds has resulted in penetration by *R. pipiens* into the former *R. blairi* habitat; during dry periods hybridization in these artificial situations is extensive (32a).

Postmating isolating mechanisms primarily have been studied through artificial crossing experiments (see Laboratory Crosses, below). Although only a few hybrid combinations produce no viable offspring, virtually all hybrids between species of the *R. pipiens* complex exhibit reduced viability and fertility (Table 1). Field data indicate that hybrids do not survive as well and that their reproductive fitness is considerably lower than that of parental species (30, 48). Kocher & Sage (48) sampled a cohort of *Rana* through metamorphosis at a site of hybridization between *R. berlandieri* and *R. sphenoccephala*, and they found that although the samples of tadpoles contained a large number of hybrids, very few hybrids survived metamorphosis.

Although many hybrid zones between members of the *R. pipiens* complex have been studied, the contact zone between *R. berlandieri* and *R. sphenoccephala* has been studied in the most detail (39, 40, 48, 56, 101). Despite fairly well-developed premating isolating mechanisms (39), these two species hybridize; in some areas (especially recently created habitats) hybridization is extensive (39, 48, 101). Detailed allozymic transects through the hybrid zone

suggest that the zone is stable in position, that linkage disequilibrium is high, and that larval and post-metamorphic samples show a strong shift from hybrid toward parental genotypes through time, presumably because of differential mortality (48). Thus, despite the extensive hybridization in some mixed populations of these two species, the hybrid zone appears to be a genetic sink; gene flow between the two species is minimal or nonexistent (101).

KARYOLOGY The karyotypes of all members of the *R. pipiens* complex studied to date are very similar (11, 15, 22, 29, 33, 36, 47), so relatively few cytogenetic investigations have been conducted on the group. All of the species studied have 13 pairs of metacentric chromosomes, with a nucleolus organizer region present on the long arm of chromosome pair 10. Karyotypic evolution throughout the genus *Rana* is highly conservative (35, 85), and studies incorporating banding techniques are needed to clarify chromosomal evolution in this group (105, 106).

Chromosomal analyses have been useful in studying the effects of hybridization. Reduced bivalent formation in meiotic chromosomal spreads of hybrids has been used as an index of genetic incompatibility and reduced fertility (29, 30, 92). Triploid hybrids have been reported between *R. pipiens* and *R. chiricahuensis*, and it has been suggested that these triploid hybrids may be more viable than diploid hybrids (36).

LABORATORY CROSSES Extensive artificial crossing experiments have been conducted among members of the *R. pipiens* complex, in part because of the ease with which *Rana* reproductive systems can be manipulated. Early crossing experiments were considered evidence of reproductive isolation through adaptation to local environments. Moore (74–80) formulated the hypothesis that an uninterrupted series of reproductively connected populations of leopard frogs spanned the range of the complex, from Canada through Central America, but populations from distinct ecological (especially temperature) regimes were often reproductively isolated if artificially brought together. Frogs from similar latitudes (e.g. Vermont and Wisconsin, USA) were found to be reproductively compatible, whereas frogs separated by similar distances but of different latitudes (e.g. Vermont and Florida, or Vermont and Texas) showed developmental abnormalities in hybrid offspring. Furthermore, frogs that bred in similar temperature regimes but were of very different geographical origins (e.g. Vermont and Colorado, or even Vermont and montane Costa Rica) were often more reproductively compatible than were frogs that were close geographically but of different temperature regimes (e.g. montane Oaxaca versus lowland Veracruz, Mexico). Under the assumption that all the frogs involved belonged to a single, widespread species, Moore (74–80) concluded that adaptation to local environments

(especially developmental temperature) resulted in reproductive incompatibility between frogs from distinct habitats.

A somewhat different picture began to emerge when Moore (81, 82) conducted androgenetic haploid hybridization experiments among leopard frogs. In these experiments, female nuclei were removed from fertilized ova; the male genome then controlled development of the female cytoplasm. These experiments revealed that much genetic divergence had been masked in the diploid hybridization experiments (81, 82). As the data from other areas began to show distinct discontinuities among leopard frogs, rather than a series of clinally varying populations, alternate explanations for the diploid hybridization data became apparent (84). It is now clear that the reproductive incompatibilities were found among species, whereas complete reproductive compatibility was found among populations within species. For instance, the crosses among frogs from Vermont, Wisconsin, and Colorado were all among populations of *R. pipiens* (sensu stricto), whereas the other crosses in the above examples were interspecific crosses. To add confusion to the picture, some species are highly incompatible with all other leopard frogs (in particular, *R. berlandieri* and *R. forreri*; 25, 29, 45, 92), whereas others show few incompatibilities with most other leopard frogs, whether closely or distantly related. Thus, the hybrids between *R. taylori* from Costa Rica and *R. pipiens* from Vermont (79, 116) developed much more normally than the crosses between *R. spectabilis* from Oaxaca and *R. berlandieri* from Veracruz (81), even though the latter two species are more closely related than the former pair (44).

Eighty-one interspecific combinations among members of the *R. pipiens* complex have been experimentally hybridized, or approximately one tenth of the possible crosses (Table 2). From these crosses, three major conclusions can be drawn. The first is that the vast majority of interspecific crosses within the *R. pipiens* complex produce offspring capable of metamorphosis. The exceptions mostly involve *R. berlandieri* and *R. forreri*. In particular, all interspecific combinations tested using female *R. forreri* were found to be lethal (25, 29, 45, 92). The embryos of crosses involving female *R. forreri* exhibit exogastrulation, and subsequently fail to neurulate properly (29). In contrast, the reciprocal crosses (using male *R. forreri*) produce some offspring, although of lower fitness than controls. The phenomenon of lethality in crosses with female *R. forreri* extends even to crosses with its sister species, *R. berlandieri* (25).

A second conclusion that can be drawn from the crossing data is that virtually all interspecific crosses among members of the *R. pipiens* complex produce hybrids with measurably lower fitness than the conspecific controls. Parameters of lower fitness include developmental abnormalities, delayed development, lower embryonic and larval survivorship, lower survivorship

Table 2 Laboratory crosses between species of the *R. pipiens* complex. Key to results: A. Development appears normal (many combinations not raised beyond larvae); B. hybrid inferiority as indicated by increased mortality, delayed development, developmental defects, and/or reduced fertility; C. severe hybrid inferiority, as indicated by high mortality or sterility of hybrids; and D. hybrid incompatibility (development ceases prior to free-living larvae). Females are shown in rows, males in columns.

	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	References
<i>a. areolata</i>	A	D	A	-	-	-	-	AB	A	CD	A	A	-	A	-	-	13, 60, 64, 77
<i>b. berlandieri</i>	CD	A	BC	C	-	B	B	CD	CD	-	B	CD	BC	BC	-	-	13, 25, 60, 61, 64, 74-76, 81, 97, 99, 114
<i>c. blairi</i>	AB	BC	A	CD	-	-	-	A	A	-	-	-	-	B	-	-	13, 28, 64, 97
<i>d. chiricahuensis</i>	-	-	C	A	-	-	D	-	-	-	C	-	-	-	-	C	28, 92, 97
<i>e. dummi</i>	-	-	-	-	A	-	-	-	-	-	BC	-	D	-	-	-	83
<i>f. forreri</i>	-	D	-	-	-	A	D	-	D	-	-	-	-	-	-	D	25, 29, 45, 92
<i>g. magnaocularis</i>	-	B	-	-	-	B	A	-	B	-	-	-	-	-	-	C	25, 29, 45, 92
<i>h. megapoda</i>	-	-	-	-	-	-	-	A	-	-	-	CD	-	-	-	-	83
<i>i. montezumae</i>	-	CD	B	-	-	-	-	-	A	-	-	-	-	B	-	-	64
<i>j. neovolcanica</i>	-	-	-	-	-	B	B	-	A	-	D	-	-	-	-	-	45, 81
<i>k. palustris</i>	A	B	A	-	-	-	-	B	C	-	A	B	-	A	-	-	64, 67-69, 72, 75, 77, 81, 100
<i>l. pipiens</i>	A	CD	B	-	-	B	B	AB	-	BC	AB	A	-	BC	B	B	67-69, 70, 72, 74-76, 77, 79, 81, 83, 92, 112, 114, 116
<i>m. spectabilis</i>	-	CD	-	B	-	B	-	-	-	B	-	B	A	B	-	-	45, 81, 99
<i>n. sphenoccephala</i>	AB	BC	-	B	-	-	-	A	AB	-	-	AB	B	A	-	-	13, 60, 61, 64, 70, 74, 75, 81, 82
<i>o. taylori</i>	-	-	-	-	-	-	-	-	-	-	-	B	-	-	A	-	92
<i>p. yavapaiensis</i>	-	-	-	C	-	-	BC	-	-	-	C	-	-	-	A	-	116

through metamorphosis, lower tolerance to environmental stress, and lower fertility (13, 25, 29, 30, 45, 60, 61, 64, 69, 70, 74–83, 92, 97, 99, 114).

The third conclusion from these data is that reproductive compatibility provides a poor indication of phylogenetic relatedness in the *R. pipiens* complex. Some species (e.g. *R. forreri*) are highly incompatible with all tested species regardless of relatedness. Other species are highly compatible with most other species, including those quite distantly related (79, 116). Finally, some species show greater reproductive incompatibility with closely related species than with distantly related species. For instance, crosses between *R. spectabilis* and the relatively closely related *R. berlandieri* are much less successful than between *R. spectabilis* and the more distantly related *R. pipiens* or *R. sphenoccephala*, or even than the quite distantly related *R. chiricahuensis* (Table 2). There is no consistent pattern, however, and apparently no way to extract phylogenetic information from reproductive compatibility data. These data suggest that reproductive compatibility is a poor criterion for either grouping or ranking taxa.

MOLECULAR ANALYSES Most of the known morphological differences among leopard frogs are autapomorphic, so morphology provides few clues to the relatedness among species of the *R. pipiens* complex. This led to the erroneous idea that these species have diverged very recently. When the molecular revolution in systematics began in the 1960s, leopard frogs were among the first organisms studied (32, 102). As biochemical data accumulated for leopard frogs, it became apparent that they are genetically highly diverse species that have maintained a conservative morphology (44). For comparison, the level of allozymic divergence among some species of the *R. pipiens* complex is approximately three times greater than the intergeneric divergence among the human and great ape genera *Homo*, *Gorilla*, *Pan*, and *Pongo* (9, 44). This genetic diversity provides abundant data for species diagnosis, study of hybridization, and phylogeny reconstruction.

Most of the biochemical analyses conducted on the *R. pipiens* complex concern protein electrophoresis. Studies of blood proteins have been used extensively to diagnose species of the complex and their hybrids (18, 27, 28, 32, 37, 51, 89, 90, 94). Allozymic analyses have provided greater resolution of species boundaries and the variation needed to study hybrid zones in detail (21, 44, 48, 91, 98, 101, 102). Allozymic study of a hybrid zone between *R. berlandieri* and *R. sphenoccephala* first emphasized the phenomenon of the presence of many rare alleles in hybrid zones (101). Allozymic analyses of laboratory-reared hybrids have provided an extensive bank of gene linkage data (19, 120–123). Primarily as a consequence of these latter studies, approximately 110 protein loci are regularly surveyed among leopard frogs (123), more than for any other vertebrates except humans. Most of these loci

are variable within the *R. pipiens* complex, and these data provide a powerful data set for reconstructing the evolutionary history of these species (44).

A few other kinds of biochemical analyses have been applied in a more limited fashion to the *R. pipiens* complex. Immunological comparisons of albumins have been made (10), and the ribosomal RNA genes have been surveyed among some of the species (43). However, neither of these techniques has provided nearly as much resolution of species or phylogeny as have allozymes, although both techniques provide useful information on the relationships of the *R. pipiens* complex to other groups of *Rana* (41, 43). Both the immunological and ribosomal gene studies suggest that the *R. pipiens* complex is related to a group of Neotropical *Rana* that contains the *R. tarahumarae* and *R. palmipes* species groups (43).

PHYLOGENY AND BIOGEOGRAPHY

Information on the phylogeny of the *R. pipiens* complex comes primarily from biochemical data, although a few morphological characters are also informative (41, 43, 44). Phylogenetic analysis reveals two distinct groups in the *R. pipiens* complex, which have been termed the Alpha and Beta divisions (44). These two divisions are broadly sympatric; within each division the species are distributed parapatrically (Figures 2 and 3). The New World *Rana* radiation is believed to date to the mid-Eocene (10), when a land connection between North America and Eurasia was disrupted. The oldest fossil *Rana* material from the New World dates to the Miocene (111), although the lack of older material is not surprising considering the poor fossil record of anurans. If one accepts the mid-Eocene date for origins of New World *Rana*, then a uniform rate of biochemical divergence since that time would suggest an Oligocene origin for the *R. pipiens* complex. The alternative, of course, is that the rates of divergence have not been uniform. Paleontologists have been hesitant to assign fossil material to current species groups of *Rana*, although it appears likely that the origins of the present day species groups predate all the New World *Rana* fossil material.

The Alpha and Beta divisions are each comprised of two distinct species groups—one North American and one Mesoamerican clade (Figures 2 and 3). In the Alpha division, one species group (the *R. montezumae* group) is found from Arizona south through the Sierra Madre Occidental to the southern Mexican Plateau; the other Alpha division group (the *R. areolata* group) is found in the eastern United States and Canada (Figure 2). In the Beta division, the dichotomy of two species groups geographically parallels that of the Alpha division, except that both Beta division groups (the *R. pipiens* and *R. berlandieri* groups) are more widely distributed than their Alpha division counterparts. Two narrow zones of contact now exist between members of the

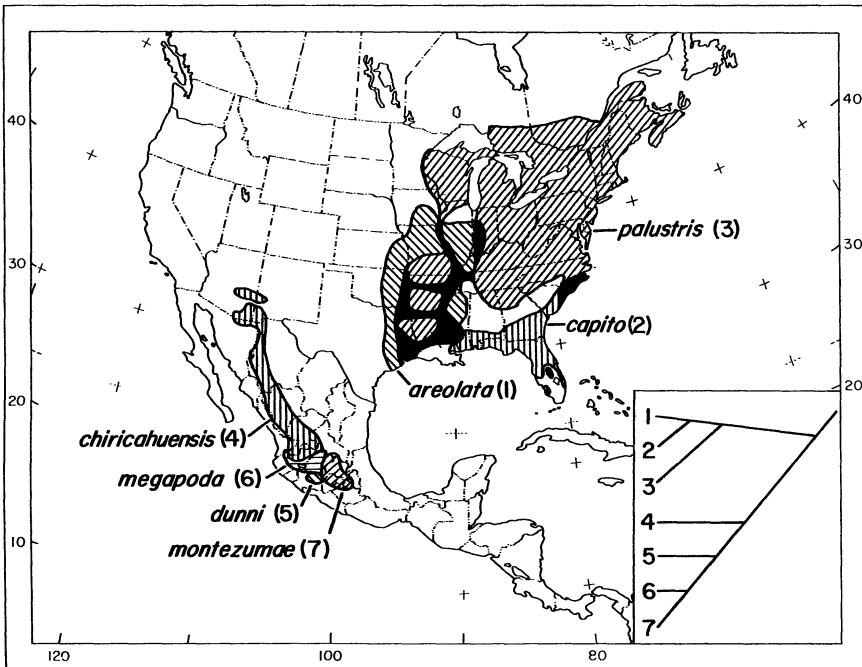


Figure 2 Distribution and phylogeny of species of the Alpha division of the *Rana pipiens* complex. Black areas represent zones of sympatry of two or more species.

R. pipiens group and a member of the *R. berlandieri* group: one involves *R. blairi* and *R. berlandieri* in west-central Texas, and the other involves *R. sphenoccephala* and *R. berlandieri* in east-central Texas (44).

In both the Alpha and Beta division, the ranges of species are much more extensive within the North American than the Mesoamerican clade (Figures 2 and 3). This is probably a result of the much greater diversity of habitats throughout Mesoamerica, as compared to North America. Within both the Alpha and Beta Mesoamerican species groups a dichotomy exists between species on the Mexican Plateau (Alpha: *R. dunni*, *R. megapoda*, and *R. montezumae*; Beta: *R. neovolcanica*, *R. spectabilis*, and *R. ilaloci*) and species in the Sierra Madre Occidental (Alpha: *R. chiricahuensis*; Beta: *R. magnaocularus* and *R. yavapaiensis*). The distributions of the two Mesoamerican species groups differ primarily in the presence of an additional group of species in the Beta division, namely a coastal and lower Central American group (*R. berlandieri*, *R. forreri*, and probably *R. miadis* and *R. taylori*).

The phylogenetic data for the *R. pipiens* complex, although incomplete, provide a framework for the reinterpretation and expansion of experimental studies of leopard frogs. Studies of behavior, ecology, physiology, and

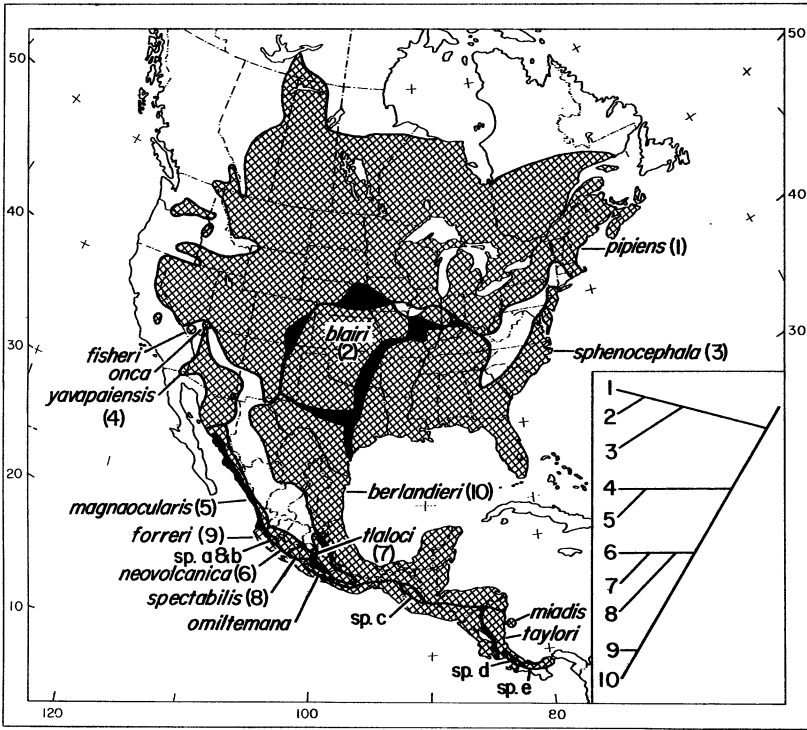


Figure 3 Distribution and phylogeny of species of the Beta division of the *Rana pipiens* complex. Black areas represent zones of sympatry of two or more species. The placement of *R. fisheri* and *R. onca* in this division is arbitrary.

morphology can be conducted among members of the *R. pipiens* complex and interpreted in a comprehensive and cohesive manner. Studies of speciation can be concentrated on sister taxa. Given the diversity of vocalizations present among species of the *R. pipiens* complex, the group would be ideal for a study of the evolution and divergence of advertisement calls. Virtually any aspect of interspecific biological evolution requires information on evolutionary history; the stage is now set for a return to prominence of the *R. pipiens* complex in discussions of evolutionary theory.

CONSERVATION

Some species and many populations of leopard frogs are threatened with extinction. Destruction of habitat has been paramount in the loss of most populations and species, although over-harvesting of *R. pipiens* for laboratory

study may have depleted some populations of this species. The majority of threatened species are Mesoamerican, although two species from the deserts in the southwestern United States (*R. onca* and *R. fisheri*) are now believed to be extinct. These two species were lost before even their systematic status was clarified; it is unknown to which division they belonged, and their taxonomic status is not clear (24). One species of leopard frog (*R. tlaloci*) inhabits the remnants of the great lakes that once existed in the Valley of Mexico, where Mexico City now stands, and is now in extreme danger of extinction (45). Populations of another species with a limited distribution in the Sierra Madre del Sur of Mexico (*R. omiltemana*) have been severely reduced as a result of lumber operations. An undescribed species in the Mexican state of Jalisco appears to have been driven to extinction even before its description. Everywhere, the fragile aquatic habitats of leopard frogs are being reduced or eliminated. Clearly, efforts are needed to combat and reverse this trend before a valuable and irreplaceable natural resource is lost forever.

CONCLUSIONS

The *R. pipiens* complex consists of approximately 27 living or recently extinct species. Although morphological divergence among species in the complex has not been great, the group is otherwise quite diverse. Previous uses of the *R. pipiens* complex as a model for reproductive adaptations to local environments and reproductive isolation by distance were confounded by unrealized systematic diversity. Studies of morphology, vocalizations, contact zones, isolating mechanisms, karyology, laboratory crosses, and biomolecules all support a view of numerous evolutionarily distinct lineages of leopard frogs. There are two primary divisions (Alpha and Beta) of the complex broadly sympatric in North and Middle America; species within these divisions are distributed in a parapatric fashion with narrow contact zones in which some hybridization may occur. Hybridization is limited by several pre- and postmating isolating mechanisms, the most important of which are differences in vocalization behavior and breeding seasons, as well as marked hybrid inferiority. Reproductive compatibility provides a poor, and in several cases misleading, picture of evolutionary history in the group. On the other hand, diversity of genetically determined biomolecules among the species provides a key to the reconstruction of phylogeny, which in turn provides a framework for the understanding of numerous biological processes that can be studied using the complex. However, endangerment of many of the species through destruction of habitat needs to be curtailed in order to preserve this important biological resource.

ACKNOWLEDGMENTS

This work has been supported by National Science Foundation grant BSR 8657640. John Frost has been instrumental in encouraging, supporting, and collaborating in my studies of this group. I thank Brian Crother, Rafael de Sá, Michael Dixon, William Fesperman, and Michael Ryan for suggestions on various drafts of this manuscript.

Literature Cited

1. Altig, R., Lohoefer, R. 1983. *Rana areolata*. *Cat. Am. Amphib. Rept.* 324:1-4
2. Baker, J. J. W., Allen, G. E. 1982. *The Study of Biology*. Reading, Mass: Addison-Wesley. 4th ed.
3. Barbour, T., Loveridge, A. 1929. Vertebrates from the Corn Islands: reptiles and amphibians. *Bull. Mus. Comp. Zool.* 69:138-46
4. Boulenger, G. A. 1920. A monograph of the American frogs of the genus *Rana*. *Proc. Am. Acad. Arts Sci.* 55:413-80
5. Bragg, A. N. 1949. The status of leopard frogs of Oklahoma. *Wassman Club Collect.* 7:211-14
6. Bragg, A. N. 1950. Further notes on range extensions of *Salientia*. *Proc. Okla. Acad. Sci.* 31:42-44
7. Brown, L. E., Brown, J. R. 1972. Call types of the *Rana pipiens* complex in Illinois. *Science* 176:928-29
8. Brown, L. E. 1973. Speciation in the *Rana pipiens* complex. *Am. Zool.* 13:73-79
9. Bruce, E. J., Ayala, F. J. 1979. Phylogenetic relationships between man and the apes: electrophoretic evidence. *Evolution* 33:1040-56
10. Case, S. M. 1978. Biochemical systematics of members of the genus *Rana* native to western North America. *Syst. Zool.* 27:299-311
11. Corcoran, M. F., Travis, J. 1980. A comparison of the karyotypes of the frogs *Rana areolata*, *Rana spinocephala*, and *Rana pipiens*. *Herpetologica* 36:296-300
12. Cope, E. D. 1889. The Batrachia of North America. *Bull. US Nat. Mus.* 34:1-525
13. Cuellar, H. S. 1971. Levels of genetic compatibility of *Rana areolata* with members of the *Rana pipiens* complex (Anura: Ranidae). *Evolution* 25:399-409
14. Curtis, H. 1968. *Biology*. New York: Worth
15. Di Bernardino, M. A. 1962. The karyotype of *Rana pipiens* and investigation of its stability during embryonic differentiation. *Dev. Biol.* 5:101-26
16. Dickerson, M. C. 1906. *The Frog Book*. New York: Doubleday, Page
17. Duellman, W. E., Trueb, L. 1986. *Biology of Amphibians*. New York: McGraw-Hill
18. Dunlap, D. G. 1978. Hemoglobin phenotypes in the frogs *Rana pipiens*, *Rana blairi*, their hybrids and a backcross. *Comp. Biochem. Physiol.* 62B:167-73
19. Dunlap, D. G. 1982. Linkage analysis of the transferrin, albumin, and hemoglobin loci in leopard frogs. *J. Hered.* 73:247-48
20. Dunlap, D. G., Kruse, K. C. 1976. Frogs of the *Rana pipiens* complex in the northern and central plains states. *Southwest. Nat.* 20:559-71
21. Dunlap, D. G., Platz, J. E. 1981. Geographic variation of proteins and call in *Rana pipiens* from the northcentral United States. *Copeia* 1981:876-79
22. Earley, E. M. 1971. Karyotypes of fifteen anuran species, with particular reference to secondary constrictions. PhD thesis. Tulane Univ., New Orleans
23. Ehrlich, P. R., Holm, R. W. 1963. *The Process of Evolution*. New York: McGraw-Hill
24. Frost, D. R., ed. 1985. *Amphibian Species of the World: A Taxonomic and Geographical Reference*. Lawrence, Kan: Allen
25. Frost, J. S. 1982. Functional genetic similarity between geographically separated populations of Mexican leopard frogs (*Rana pipiens* complex). *Syst. Zool.* 31:57-67
26. Frost, J. S. 1983. Comparative feeding and breeding strategies of a sympatric pair of leopard frogs (*Rana pipiens* complex). *J. Exp. Zool.* 225:135-40

27. Frost, J. S., Bagnara, J. T. 1976. A new species of leopard frog (*Rana pipiens* complex) from northwestern Mexico. *Copeia* 1976:332-38
28. Frost, J. S., Bagnara, J. T. 1977. Sympatry between *Rana blairi* and the southern form of leopard frog in southeastern Arizona (Anura:Ranidae). *Southwest. Nat.* 22:443-53
29. Frost, J. S., Bagnara, J. T. 1977. An analysis of reproductive isolation between *Rana magnaocularis* and *Rana berlandieri forreri* (*Rana pipiens* complex). *J. Exp. Zool.* 202:291-306
30. Frost, J. S., Platz, J. E. 1983. Comparative assessment of modes of reproductive isolation among four species of leopard frogs (*Rana pipiens* complex). *Evolution* 37:66-78
31. Gambs, R. D., Littlejohn, M. J. 1979. Acoustic behavior of males of the Rio Grande leopard frog (*Rana berlandieri*): an experimental analysis through field playback trials. *Copeia* 1979:643-50
- 31a. Gerhardt, H. C. 1988. Acoustic properties used in call recognition by frogs and toads. In *The Evolution of the Amphibian Auditory System*, ed. B. Fritsch, M. J. Ryan, W. Wilczynski, T. E. Hetherington, W. Walkowiak, pp. 455-83. New York: Wiley
32. Gilliespie, H., Crenshaw, J. W. 1966. Hemoglobin variation in *Rana pipiens* (Amphibia: Anura). *Copeia* 1966:889-93
- 32a. Gillis, J. E. 1975. Characterization of a hybridizing complex of leopard frogs. PhD thesis. Colo. State Univ., Fort Collins
33. Gomez, E. 1983. Comparison of gross karyotypes among several species of the *Rana pipiens* complex (Anura: Ranidae). MA thesis, Univ. Kans., Lawrence
34. Greding, E. J. 1972. Call specificity and hybrid compatability between *Rana pipiens* and three other *Rana* species in Central America. *Copeia* 1972:383-85
35. Green, D. M. 1983. Evidence for chromosome number reduction and chromosomal homosequentiality in the 24-chromosome Korean frog *Rana dybowskii* and related species. *Chromosoma* 88:222-26
36. Green, D. M., Delisle, D. M. 1985. Allotripleidy in natural hybrid frogs, *Rana chiricahuensis* X *Rana pipiens* from Arizona: chromosomes and electrophoretic evidence. *J. Herpetol.* 19:385-90
37. Hardy, J. D. Jr., Gillespie, J. H. 1976. Hybridization between *Rana pipiens* and *Rana palustris* in a modified natural enviroment. *Bull. Md. Herp. Soc.* 12: 41-53
38. Harlan, R. 1826. Descriptions of several new species of bacrachian reptiles, with observations on the larvae of frogs. *Am. J. Sci. Arts* 10:53-55
39. Hillis, D. M. 1981. Premating isolating mechanisms among three species of the *Rana pipiens* complex in Texas and southern Oklahoma. *Copeia* 1981:312-19
40. Hillis, D. M. 1982. Morphological differentiation and adaptation of the larvae of *Rana berlandieri* and *Rana sphenoccephala* (*Rana pipiens* complex) in sympatry. *Copeia* 1982:168-74
41. Hillis, D. M. 1985. Evolutionary genetics and systematics of New World frogs of the genus *Rana*: an analysis of ribosomal DNA, allozymes, and morphology. PhD thesis. Univ. Kansas, Lawrence
42. Hillis, D. M. 1985. Evolutionary genetics of the Andean lizard genus *Pholidobolus* (Sauria:Gymnophthalidae): phylogeny, biogeography, and a comparison of tree construction techniques. *Syst. Zool.* 34:109-26
43. Hillis, D. M., Davis, S. 1986. Evolution of ribosomal DNA: Fifty million years of recorded history in the frog genus *Rana*. *Evolution* 40:1275-88
44. Hillis, D. M., Frost, J. S., Wright, D. A. 1983. Phylogeny and biogeography of the *Rana pipiens* complex: a biochemical evaluation. *Syst. Zool.* 32:132-43
45. Hillis, D. M., Frost, J. S. 1985. Three new species of leopard frogs (*Rana pipiens* complex) from the Mexican Plateau. *Occ. Pap. Mus. Nat. Hist. Univ. Kans.* 117:1-14
46. Keeton, W. T. 1967. *Biological Science*. New York: Norton
47. Kiley, S., Wohnus, J. F. 1968. Chromosomal analysis of *Rana pipiens* and *Bufo americanus* and their hybrid. *Cytogenetics* 7:78-90
48. Kocher, T. D., Sage, R. D. 1986. Further genetic analyses of a hybrid zone between leopard frogs (*Rana pipiens* complex) in central Texas. *Evolution* 40:21-33
49. Korky, J. K. 1978. Differentiation of the larvae of members of the *Rana pipiens* complex in Nebraska. *Copeia* 1978: 455-59
50. Kruse, K. D. 1981. Phototactic responses of female northern leopard frogs (*Rana pipiens*) to *Rana blairi*, a presumed hybrid, and conspecific mating trills. *J. Herpetol.* 15:145-50
51. Kruse, K. C., Dunlap, D. G. 1976. Serum albumins and hybridization in two species of the *Rana pipiens* complex

- in the north central United States. *Copeia* 1976:394-96
52. LeConte, J. 1825. Remarks on the American species of the genera *Hyla* and *Rana*. *N. Y. Ann. Lyc. Nat. Hist.* 1:278-82
 53. Littlejohn, M. J. 1960. Call discrimination and potential reproductive isolation in *Pseudacris triseriata* females from Oklahoma. *Copeia* 1960:370-71
 54. Littlejohn, M. J. 1965. Premating isolation the *Hyla ewingi* complex (Anura:Hylidae). *Evolution* 19:234-43
 55. Littlejohn, M. J., Loftus-Hills, J. J. 1968. An experimental evaluation of premating isolation in the *Hyla ewingi* complex (Anura:Hylidae). *Evolution* 22:659-63
 56. Littlejohn, M. J., Oldham, R. S. 1968. *Rana pipiens* complex: mating call structure and taxonomy. *Science* 162:1003-5
 57. Lynch, J. D. 1978. The distribution of leopard frogs (*Rana blairi* and *Rana pipiens*) (Amphibia, Anura, Ranidae) in Nebraska. *J. Herpetol.* 12:157-62
 58. Mayr, E. 1942. *Systematics and the Origin of Species*. New York: Columbia Univ. Press
 59. Mayr, E. 1963. *Animal Species and Evolution*. Cambridge: Harvard Univ.
 60. McAlister, W. H. 1961. Artificial hybridization between *Rana a. areolata* and *R. p. pipiens* from Texas. *Tex. J. Sci.* 13:423-26
 61. McAlister, W. H. 1962. Variation in *Rana pipiens* Schreber in Texas. *Am. Midl. Nat.* 67:335-63
 62. Mecham, J. S. 1968. Studies on evolutionary effects of isolation in the *Rana pipiens* complex. *Yrbk. Am. Philos. Soc.* 1968:314-16
 63. Mecham, J. S. 1968. Evidence of reproductive isolation between two populations of the frog, *Rana pipiens*, in Arizona. *Southwest. Nat.* 13:35-44
 64. Mecham, J. S. 1969. New information from experimental crosses on genetic relationships within the *Rana pipiens* species group. *J. Exp. Zool.* 170:169-80
 65. Mecham, J. S. 1971. Vocalizations of the leopard frog, *Rana pipiens*, and three related Mexican species. *Copeia* 1971:505-16
 66. Mecham, J. S., Littlejohn, M. J., Oldham, R. S., Brown, L. E., Brown, J. R. 1973. A new species of leopard frog (*Rana pipiens* complex) from the plains of the central United States. *Occ. Pap. Mus. Texas Tech Univ.* 18:1-11
 67. Moore, B. C. 1941. Androgenetic frog hybrids. *Anal. Rec.* 81(Suppl.2):83-84
 68. Moore, B. C. 1950. The development of reciprocal androgenetic frog hybrids. *Biol. Bull.* 99:88-111
 69. Moore, J. A. 1941. Developmental rate of hybrid frogs. *J. Exp. Zool.* 86:405-22
 70. Moore, J. A. 1941. Developmental rate of hybrids between *Rana pipiens* and *Rana sphenocephala*. *Proc. Soc. Exp. Biol. Med.* 47:207-10
 71. Moore, J. A. 1942. An embryological and genetical study of *Rana burnsi* Weed. *Genetics* 27:408-16
 72. Moore, J. A. 1943. Corresponding genes in spotted frogs. *J. Hered.* 34:2-7
 73. Moore, J. A. 1944. Geographic variation in *Rana pipiens* Schreber of eastern North America. *Bull. Am. Nat. Hist.* 82:345-70
 74. Moore, J. A. 1946. Incipient intraspecific isolating mechanisms in *Rana pipiens*. *Genetics* 31:304-26
 75. Moore, J. A. 1946. Hybridization between *Rana palustris* and different geographical forms of *Rana pipiens*. *Proc. Natl. Acad. Sci. USA* 32:209-12
 76. Moore, J. A. 1947. Hybridization between *Rana pipiens* from Vermont and eastern Mexico. *Proc. Natl. Acad. Sci. USA* 33:72-75
 77. Moore, J. A. 1949. Patterns of evolution in the genus *Rana*. In *Genetics, Paleontology, and Evolution*, ed. G. L. Jepson, G. G. Simpson, E. Mayr, pp. 315-38. New Jersey: Princeton Univ. Press
 78. Moore, J. A. 1949. Geographic variation of adaptive characters in *Rana pipiens* Schreber. *Evolution* 3:1-24
 79. Moore, J. A. 1950. Further studies on *Rana pipiens* racial hybrids. *Am. Nat.* 84:247-54
 80. Moore, J. A. 1955. Abnormal combinations of nuclear and cytoplasmic systems in frogs and toads. *Adv. Genet.* 7:139-82
 81. Moore, J. A. 1965. Diploid and haploid hybridization of different populations of the *Rana pipiens* complex. I. Experiments with females from Mexico. *J. Exp. Zool.* 165:1-20
 82. Moore, J. A. 1965. Diploid and haploid hybridization of different populations of the *Rana pipiens* complex. II. Experiments with females from Oklahoma. *J. Exp. Zool.* 165:461-74
 83. Moore, J. A. 1966. Hybridization experiments involving *Rana dunnii*, *Rana megapoda*, and *Rana pipiens*. *Copeia* 1966:673-75
 84. Moore, J. A. 1975. *Rana pipiens*—the changing paradigm. *Am. Zool.* 15:837-49
 85. Morescalchi, A. 1973. Amphibia. In *Cytotaxonomy and Vertebrate Evolu-*

- tion, ed. A. B. Chiarelli, E. Capanna, pp. 233-347. New York: Academic Press
86. Neill, W. T. 1957. The status of *Rana capito stertiens* Schwartz and Harrison. *Herpetologica* 13:47-52
 87. Oldham, R. S. 1974. Mate attraction by vocalization in members of the *Rana pipiens* complex. *Copeia* 1974:982-84
 88. Pace, A. E. 1974. Systematic and biological studies of the leopard frogs (*Rana pipiens* complex) of the United States. *Mus. Zool. Misc. Publ. Univ. Mich.* 184:1-140
 89. Platz, J. E. 1972. Sympatric interaction between two forms of leopard frog (*Rana pipiens* complex) in Texas. *Copeia* 1972:232-40
 90. Platz, J. E. 1976. Biochemical and morphological variation of leopard frogs in Arizona. *Copeia* 1976:660-72
 91. Platz, J. E. 1981. Suture zone dynamics: Texas populations of *Rana berlandieri* and *Rana blairi*. *Copeia* 1981:733-34
 92. Platz, J. E., Frost, J. S. 1984. *Rana yavapaiensis*, a new species of leopard frog (*Rana pipiens* complex). *Copeia* 1984:940-48
 93. Platz, J. E., Mecham, J. S. 1979. *Rana chiricahuensis*, a new species of leopard frog (*Rana pipiens* complex) from Arizona. *Copeia* 1979:383-90
 94. Platz, J. E., Platz, A. L. 1973. *Rana pipiens* complex: hemoglobin phenotypes of sympatric and allopatric populations in Arizona. *Science* 179:1334-36
 95. Post, D. D., Pettus, D. 1966. Variation in *Rana pipiens* (Anura: Ranidae) of eastern Colorado. *Southwest. Nat.* 11:467-82
 96. Post, D. D., Pettus, D. 1967. Sympatry of two members of the *Rana pipiens* complex in Colorado. *Herpetologica* 23:323
 97. Purcell, J. W. 1968. Embryonic temperature adaptations in southwestern populations of *Rana pipiens*. MS thesis. Texas Tech Univ., Lubbock
 98. Pytel, B. A. 1986. Biochemical systematics of the eastern North American frogs of the genus *Rana*. *Herpetologica* 42:273-82
 99. Ruibal, R. 1955. A study of altitudinal races in *Rana pipiens*. *Evolution* 9:322-38
 100. Rugh, R. 1935. Pituitary-induced sexual reactions in the Anura. *Biol. Bull.* 68:74-81
 101. Sage, R. D., Selander, R. K. 1979. Hybridization between species of the *Rana pipiens* complex in central Texas. *Evolution* 33:1069-88
 102. Salthe, S. N. 1969. Geographic variation of the lactate dehydrogenases of *Rana pipiens* and *Rana palustris*. *Biochem. Genet.* 2:271-303
 103. Sanders, O. 1973. A new leopard frog (*Rana berlandieri brownorum*) from southern Mexico. *J. Herpetol.* 7:87-92
 104. Sanders, O., Smith, H. M. 1971. Skin tags and ventral melanism in the Rio Grande leopard frog. *J. Herpetol.* 5:87-92
 105. Schmid, M. 1978. Chromosome banding in Amphibia. II. Constitutive heterochromatin and nucleolus organizer regions in Ranidae, Microhylidae, and Rhacophoridae. *Chromosoma* 68:131-48
 106. Schmid, M. 1980. Chromosome evolution in Amphibia. In *Cytogenetics of Vertebrates*, ed. H. Müller, pp. 4-27. Basel: Birkhäuser
 107. Schmidt, K. P. 1925. New Chinese amphibians and reptiles. *Am. Mus. Novit.* 175:1-13
 108. Schmidt, K. P. 1953. A checklist of North American amphibians and reptiles. Sixth ed. Chicago: Univ. Chicago Press
 109. Schreber, H. 1782. Beitrag zur Naturegeschichte der Frosche. *Der Naturforscher* 18:182-93
 110. Scott, N. J. Jr., and R. D. Jennings. 1985. The tadpoles of five species of New Mexican leopard frogs. *Occ. Pap. Mus. Southwest. Biol.* 3:1-21
 111. Tihen, J. A. 1951. Anuran remains from the Miocene of Florida, with a description of a new species of *Bufo*. *Copeia* 1951:230-35
 112. Ting, H. P. 1951. Diploid, androgenetic, and gynogenetic haploid development in anuran hybridization. *J. Exp. Zool.* 116:21-57
 113. Uzzell, T., Günther, R., Berger, L. 1977. *Rana ridibunda* and *Rana esculenta*: a leaky hybridogenetic system (Amphibia Salientia). *Proc. Acad. Nat. Sci. Philadelphia* 128:147-71
 114. Volpe, E. P. 1954. Hybrid inviability between *Rana pipiens* from Wisconsin and Mexico. *Tulane Stud. Zool.* 1:111-23
 115. Volpe, E. P. 1955. A taxo-genetical analysis of the status of *Rana kandiyoichi*. *Weed. Syst. Zool.* 4:74-82
 116. Volpe, E. P. 1957. Embryonic temperature adaptations in highland *Rana pipiens*. *Am. Nat.* 91:303-10
 117. Weed, A. C. 1922. New frogs from Minnesota. *Proc. Biol. Soc. Washington* 35:107-10
 118. White, M. J. D. 1900. *Modes of Speciation*. San Francisco: W. H. Freeman

119. Wright, A. H., Wright, A. A. 1949. *Handbook of Frogs and Toads*. Ithaca, NY: Comstock. 3rd ed.
120. Wright, D. A., Richards, C. M. 1982. Peptidase isozymes of the leopard frog *Rana pipiens*: properties and genetics. *J. Exp. Zool.* 221:283-93
121. Wright, D. A., Richards, C. M. 1983. Two sex-linked loci in the leopard frog *Rana pipiens*. *Genetics* 103:249-61
122. Wright, D. A., Richards, C. M., Nace, G. W. 1980. Inheritance of enzymes and blood proteins in the leopard frog, *Rana pipiens*: three linkage groups established. *Biochem. Genet.* 18:591-616
123. Wright, D. A., Richards, C. M., Frost, J. S., Camozzi, A. M., Kunz, B. J. 1983. Genetic mapping in amphibians. Isozymes: *Curr. Top. Biol. Med. Res.* 10:287-311
124. Zweifel, R. G. 1957. A new frog of the genus *Rana* from Michoacán, Mexico. *Copeia* 1957:78-83