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GEOGRAPHIC VARIATION IN LARVAE OF THE *RANA PIPIENS* GROUP IN NEBRASKA

by

John K. Korky

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TITLE

Geographic Variation In Larvae Of The Rana Pipiens Group In Nebraska

BY

John K. Korky

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GEOGRAPHIC VARIATION IN LARVAE OF THE *RANA PIPIENS* GROUP IN NEBRASKA

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The geographic variation in larvae of the *Rana pipiens* group of frogs in Nebraska is described and analyzed. A total of 540 larvae from eighteen localities were individually examined with respect to twenty-nine varying characters. The samples were analyzed with regard to intralocality, intraspecific, and interspecific variability by means of statistical analysis.

Statistical techniques employed in the analysis include univariate comparisons, multivariate multiple linear regression analysis, and factor analytic procedures. These programs were utilized to assess the genetic divergence of the putative sibling species of the *Rana pipiens* complex in Nebraska, *Rana pipiens*, and *Rana blairi*.

The results of the analyses show that a relatively small amount of the total variability in the data may be attributed to interspecific variation. This may be due to two factors; one, the two species have only recently diverged from a common ancestor, and, therefore, time has not permitted sufficient differences to accrue that we perceive as interspecific variation and; two, interspecific competition among tadpoles in nature is essentially non-existent, and

as a result there is little, if any, selective pressure for the evolution of interspecific variation.

The vast majority of variability within the data is attributable to a high degree of local adaptation by the species to suites of local selective regimes. Presumably, this is a consequence of populations becoming spatially stable resulting in reduced gene flow, permitting adaptation to local conditions to occur so that subpopulations in different habitats have partially independent evolutionary pathways.

The larvae of Rana pipiens and Rana blairi, hitherto undifferentiated, are distinguished mainly by the configuration of the body and position of the oral disc.

Finally, a vicariant speciation model is proposed that may account for the present distribution of members of the Rana pipiens complex.

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INTRODUCTION

Geographic variation may be defined as, "the occurrence of differences among spatially segregated populations of a species (Mayr, 1963).

The consummate importance of the investigation of this phenomenon cannot be overstated. Its study led to the eradication of the typological species concept with that concept's emphasis on the eidos. Its existence was known to Linné and other early investigators in the nascent field of systematics, and its observation ultimately led both Wallace and Darwin along their inspired paths to their discourses on organic evolution. Ultimately, it has formed the cornerstone for many of our modern conceptions concerning the nature of species and speciation.

Geographic variation appears to be a quasi-universal phenomenon among sexually reproducing organisms, and may be absent or greatly reduced only when certain infrequent concomitant circumstances are in evidence. The character states that may exhibit geographic variation cover the gamut of external to internal, morphological to physiological, and the literature is robust with such documentations, many of which are biometric compilations. These studies have led to some generalized conclusions of note, not the least of which is, that geographic variation is a resultant of the adaptive response of different populations of a species to their respective

microgeographic habitats, and has a genetic basis with the exception of cases of environmental induction. The degree of difference between populations may be scored on a continuum from almost absolute identity to disparity, but in actuality no two populations can be identical on the molecular level for obvious reasons.

Recent technological advances have provided the systematist with novel approaches to the quantification of geographic variation. Gould and Johnston (1972) examine the application of multivariate analysis and electrophoretic techniques to the study of geographic variation on a most subtle level, and predict with unquestionable certainty the far-reaching effects these techniques will have on systematics and such orthodox dogma as the biological species concept. Although their application of such approachs to systematics is relatively innovative, it can, using a sensitive enough method to detect differences among members of a population, be traced conceptually to Mayr (1942, p. 33) and perhaps to the viniculturist who has discerned such refined differences all along.

Leopard frogs (Rana pipiens, sensu lato) have historically been subject to intensive scrutiny by a host of investigators. By the 1930's and 1940's it was well known that leopard frogs exhibited great geographic variation. Related to this great variation, one must keep in mind the fact that these frogs have a greater distribution than any other species of frog, one that transcends many different ecogeographic areas, hence the amount

of geographic variation is not surprising. The various populations range from northern Canada to the western Panama Canal Zone and from the Atlantic Ocean to western America or even to the Pacific Ocean. Hence a species of even moderate adaptive plasticity, given a casual relationship of adaptive response and range of environments exists, would be expected to show considerable local adaptation. One must then conclude that the leopard frog is an organism with great plasticity based on the large amount of geographic variation documented in the literature.

More contemporary research on the nature of geographic variation in leopard frogs was initiated by Moore (1939), and was pursued by many others subsequently. The following broad generalizations may be advanced from the available body of research: leopard frogs consist of many distinctive local populations; morphologically distinctive phena may be syntopic at a given locality; characteristics may vary clinally over a broad area in some cases and in others may change abruptly; the systematics of the group remains in a state of flux [Platz and Platz (1973) and Pace (1974)]. Littlejohn and Oldham (1968) demonstrated that four essentially allopatric forms were present in the central United States, and were divisible on the basis of mating call types and adult morphology. Two of these forms are present in Nebraska. They are the northern leopard frog, Rana pipiens Schreber,and the plains leopard frog, Rana blairi Mecham, Littlejohn,Oldham, Brown and Brown. Conant (1975) terms the ranges of these two species as almost mutually exclusive. More recent investigations,to be

discussed below, have provided a composite picture of the distribution of leopard frogs in the northcentral Great Plains, and particularly of the two aforesaid species in Nebraska.

Fittingly, Moore (1975) summarizes the state of present investigations into the Rana pipiens complex paradigm and his prediction regarding the future designation of new species in the southwest is borne out by the work of Frost and Bagnara (1976), and no doubt others as yet to surface.

While considerable attention has focused on the adult frogs of this complex, little has centered on their larvae beyond diploid and haploid hybridizations to attempt to discern degrees of relatedness. The overall lack of nomenclatural certainty that investigations have shown with respect to this group of frogs as a whole is pointed out by Tucker (1976), and the paucity of our knowledge regarding their larvae is revealed by Wassersug (1976). The latter state of affairs warrants careful attention since any attempt to discern the population dynamics of members of this complex must address itself to the question of selection upon the larval stages as well as adult stages, since the larvae are subject to selective pressure also, and consequently differences in fitness of the larvae contribute to differential survival and affect the genetic structure of descendant populations.

Essentially then, this study is one involving the assessment of genetic divergence between putative sibling species of the R. pipiens complex within Nebraska by means of an analysis of geographic variation of their larvae. Such a study entails an

analysis of variation and covariation of morphological characters on three hierarchic levels. Firstly, there must be an analysis of variation within each locality (intralocality variation); secondly, there must be an analysis of variation between localities of the same species (intraspecific variation); and thirdly, there must be an analysis of variation between localities of the two species (interspecific variation). Finally, variation in characters on the various hierarchic levels should be related to certain environmental and geographic features where practical, and the adaptive significance of the patterns of geographic variation should be rigorously examined and evaluated from the standpoint of ecogeographic theories that seek to explain the phenomenon of local adaptation as mediated by genetic mechanisms and selection pressures.

It is the intent of this study to proceed along the above lines of investigation in examining geographic variation in the relatively "homogeneous" northcentral Great Plains.

THE DISTRIBUTION OF THE *RANA PIPIENS* GROUP IN NEBRASKA

The distribution of adult leopard frogs of the *Rana pipiens* group in Nebraska has been recently defined by Dunlap and Kruse (1976) and Lynch (in press).

The occurrence of *R. blairi* in Nebraska (Fig. 1) covers the south-central and south-eastern portions of the state. It does not occur west of the confluence of the North and South Platte Rivers, except for an isolate in Garden County above Lake McConaughy. The species also occurs on the lower reaches of the Loup, Elkhorn, and Niobrara Rivers. An isolate also occurs on the upper Elkhorn River in western Holt County.

Rana pipiens is found north of the Platte River (Fig. 2), but is essentially absent along the North Platte River in the panhandle. The species is present along Lodgepole Creek to its eastern terminus in the South Platte River. *R. pipiens* is found in the upper and lower portions of the Loup Rivers but not in the middle stretches of those rivers. The species is found commonly along the Niobrara and Elkhorn drainages, and the Missouri River. In central Nebraska, the occurrence of *R. pipiens* is closely associated with the presence of the Nebraska sandhills.

Lynch (in press) reports on the fragmented zone of sympatry in eastern Nebraska and states it is quite narrow "generally

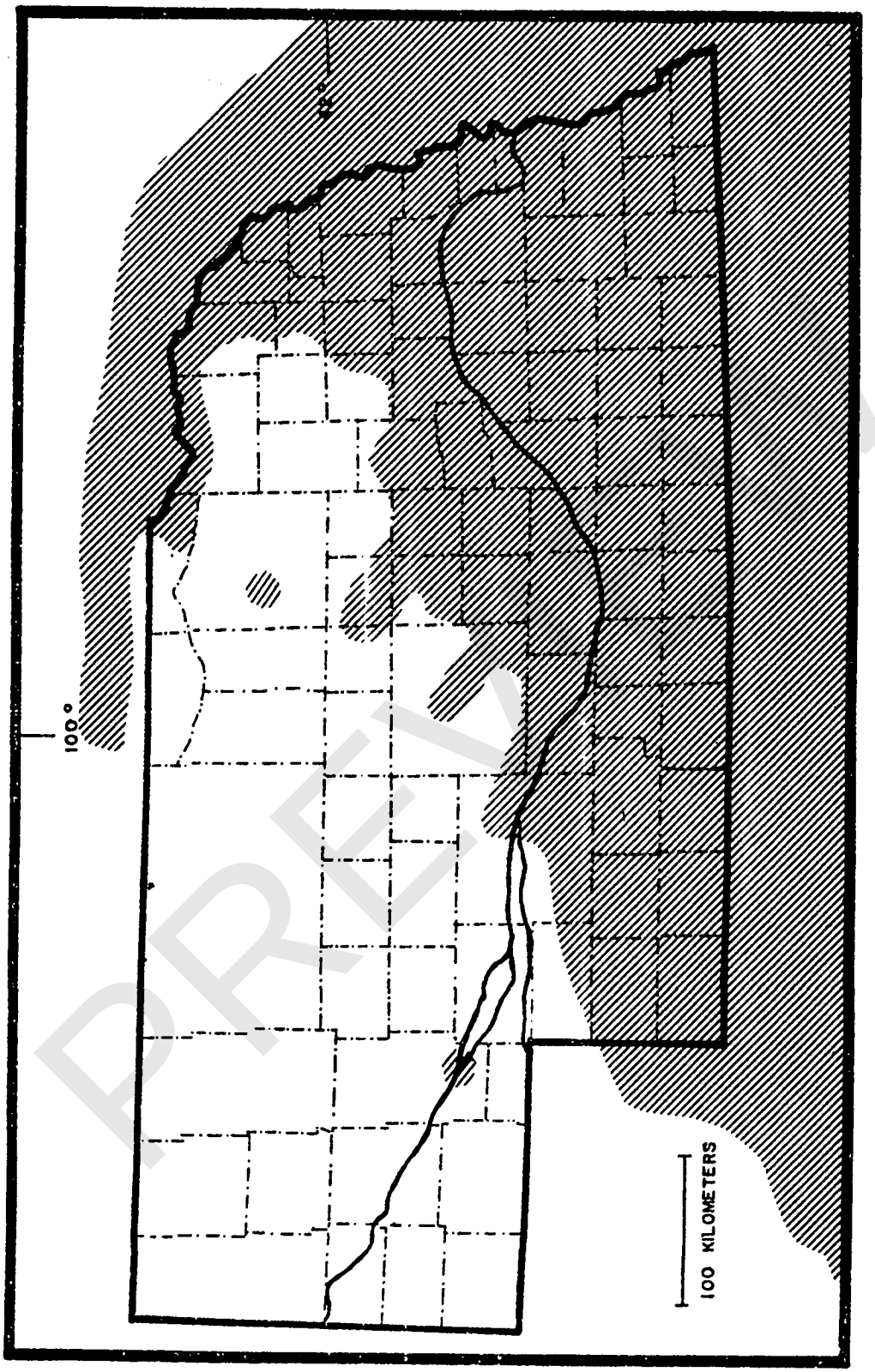

Fig. 1. Distribution of Rana blairi in Nebraska, taken from Lynch (in press).

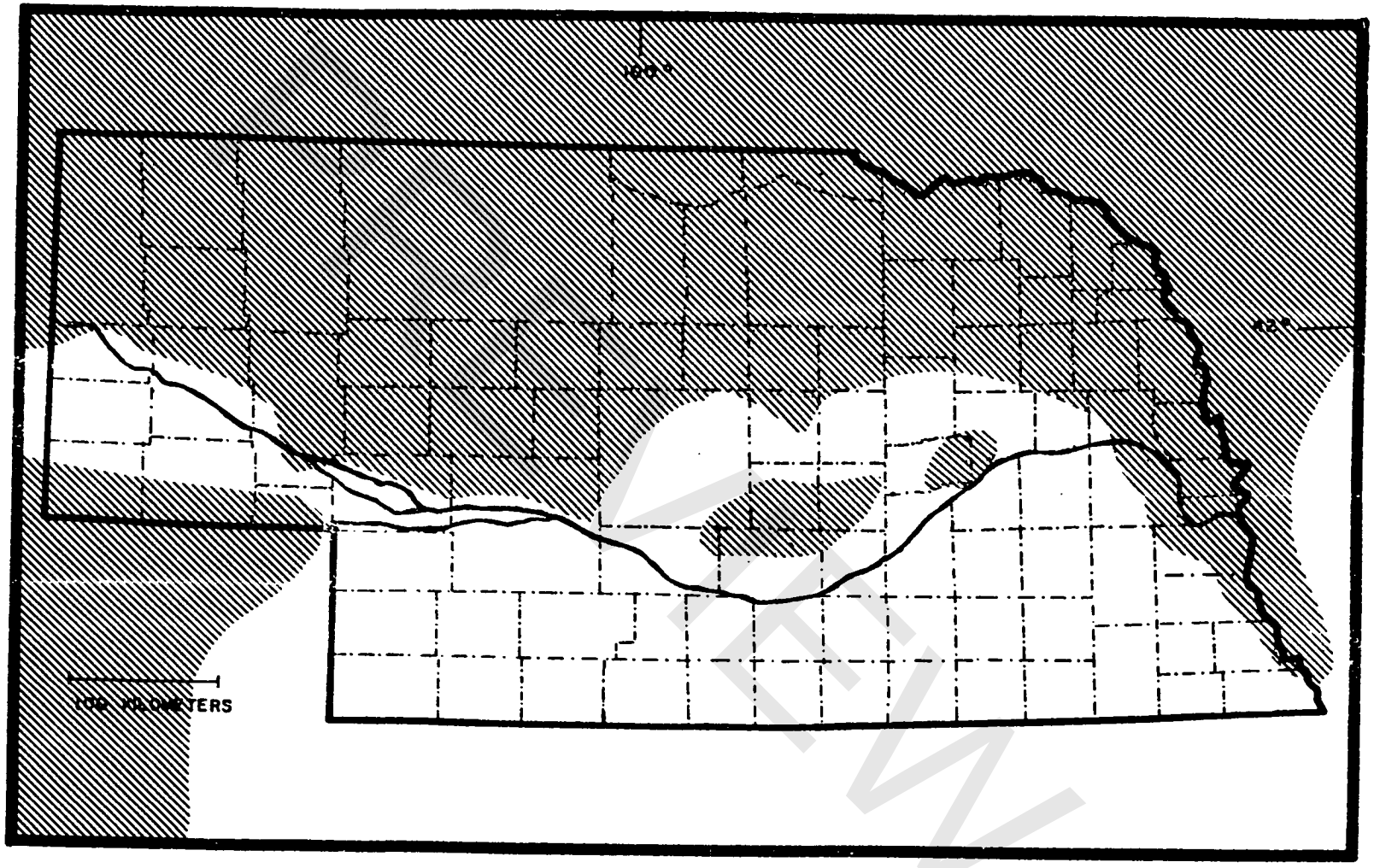

Fig. 2. Distribution of Rana pipiens in Nebraska, taken from Lynch (in press).

some 10-13 km in width but up to 32 km wide" (Fig. 3). He suggests that in streams with a heavy silt burden, R. blairi is predominant; and where streams are silt free, R. pipiens predominates; and streams with intermediate silt transport levels support both species. Thus substrate selection would seem to operate as a means of ecologic isolation between the two species. The isolation between the two species is however, not absolute. Lynch (in press) estimates that in northeast Nebraska, within the largest zone of sympatry, although hybridization is infrequent, hybrids constitute generally less than 5% of the pooled populations.

While these recent studies have dealt extensively with the adult frogs of the Rana pipiens group in the northcentral Great Plains they have largely ignored the larvae of the species present, although larvae have been taken at some of the localities. Therefore, the remainder of this study will center on the larvae of this group in Nebraska.

Methods and Materials

A total of 540 larvae in different stages of development is available from eighteen different localities. Larvae were obtained in the summers 1973-1976, through the efforts of myself, fellow students and Dr. John D. Lynch. Collecting sites were determined in some cases by records of either larvae or transformed frogs having been previously taken in the area, and in others by trial and error. Larvae were collected either by seining or by

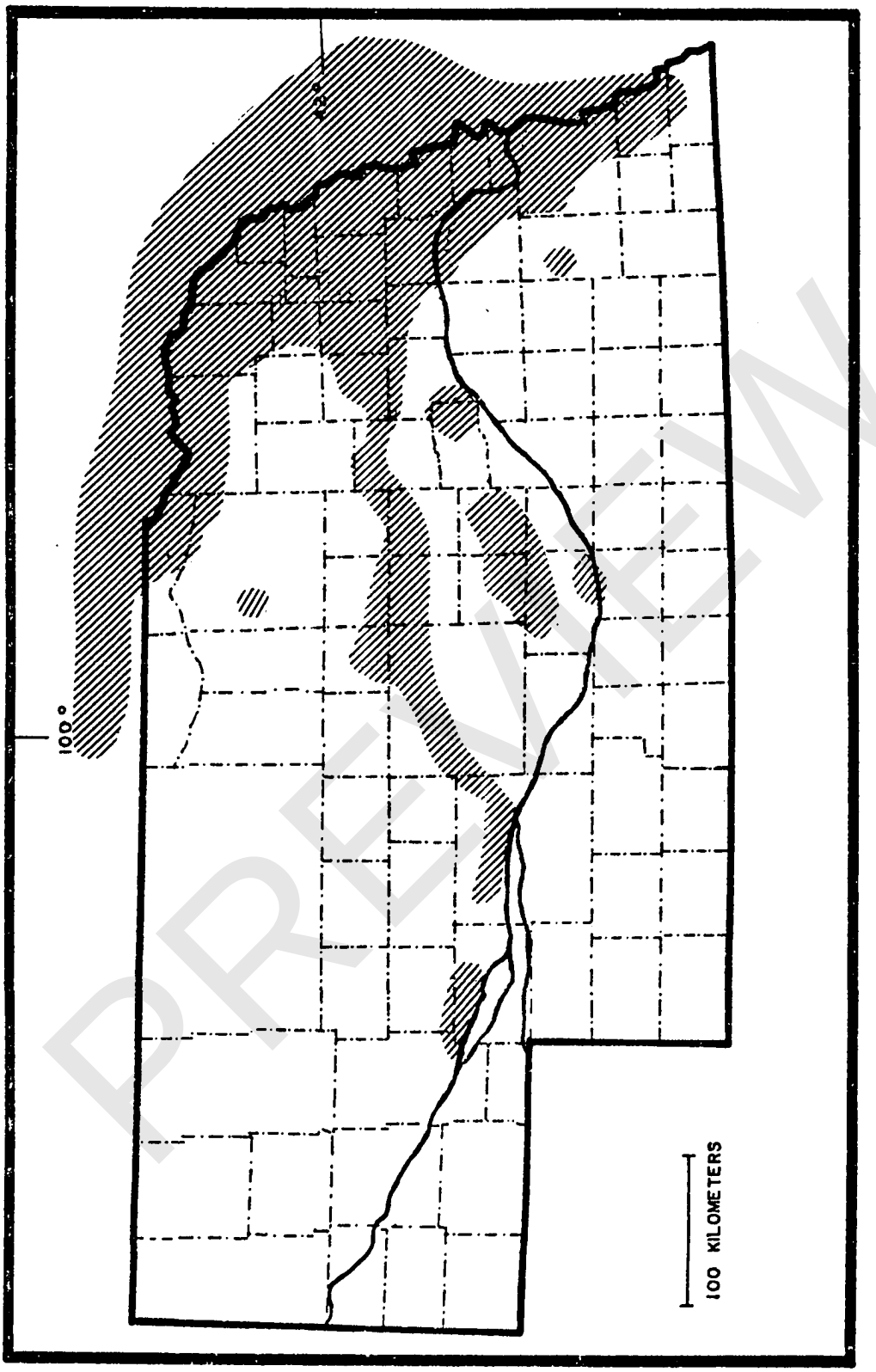

Fig. 3. Distribution map, zone of sympatry of R. pipiens and R. blairi, taken from Lynch (in press).

using small, fine-mesh hand nets. Larvae were killed and preserved in 10 per cent formalin, and are now in the collection of the University of Nebraska State Museum (UNSM).

Specimens were measured with calipers and staged according to the method of Gosner (1960). Altig's (1970) descriptions of characters and larval terminology are followed here. Unqualified statements pertaining to size refer to total length. Tooth-row measurements were made with a binocular dissecting microscope and calibrated ocular micrometer.

Sample size was determined according to the methods of Sokal and Rohlf (1969) and Cochran and Cox (1957), resulting in 30 tadpoles per locality being statistically satisfactory.

Structure of the Data

Some pertinent considerations regarding the structure of the data are warranted at this time. Each of the 540 larvae examined in this study was scored with regard to twenty-nine varying characters that presumably represent a random sampling of the genome. Thus the raw data set consists of 15,660 bits. In all cases individuals at a given locality are produced by cross mating and are therefore not genetically identical. Consequently, different genotypes would be represented within one locality, and it would then follow that different localities are genetically different from each other. However, owing to the general nature of oviposition in the various species of leopard frogs, i.e., a single female may deposit a clutch of some 2000 to 6000 eggs at one time