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Electrophoretic Survey of Eastern North American *Allonemobius* (Orthoptera: Gryllidae): Evolutionary Relationships and the Discovery of Three New Species[1]

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ABSTRACT Cryptic species are common in the singing Orthoptera, and systematists have come to rely heavily on distribution, ecology, life cycle, and especially song characteristics both to recognize species and to work out their evolutionary relationships. Eastern North American ground crickets in the genus *Allonemobius* have been very well studied in the above manner, and the taxonomy of the group appeared secure. However, electrophoretic evidence presented here indicates that what has been called *A. allardi* in the past consists of three species and what has been called *A. fasciatus* consists of two species. The evolutionary history of the eastern North American members of the genus is reconstructed, using three different methods for estimating phylogenetic trees from genetic distance matrices. The two species best suited for speciation studies appear to be *A. fasciatus* and *A. socius*.

The time scale of most speciation events prevents their direct study. Consequently, evolutionary biologists have come to rely heavily on the examination of closely related populations and species to answer questions regarding the onset of reproductive isolation (e.g., Ayala et al. 1974, Kawanishi and Watanabe 1981). This situation has placed a premium on systematic work which examines the evolutionary relationships of closely related taxa, and on techniques which allow the elucidation of these relationships. An especially powerful tool for the study of closely related species is gel electrophoresis (Avise 1974). The usefulness of gel electrophoresis lies in its ease of application and its ability to distinguish phenotypic differences that reflect differences at the level of the gene even between morphologically similar (cryptic) species. In addition to its value as a taxonomic tool, when one or more diagnostic loci separate two species, electrophoresis can be used to study patterns of breeding in the field. This can be a powerful means of distinguishing cryptic sympatric species (e.g., Grassle and Grassle 1977) and of analyzing hybrid zones (e.g., Hunt and Selander 1973).

Cryptic species have been a source of great consternation for morphological systematists studying the singing Orthoptera. This is especially true for crickets (Gryllidae). As a result, systematists have come to rely heavily on characters such as song, ecology, and life cycle both to distinguish species and to construct phylogenies (Alexander 1962, Walker 1964). The genus *Allonemobius* in the subfamily Nemobiinae has proved particularly recalcitrant to taxonomic analysis by traditional morphological techniques. In 1913, after an extraordinarily detailed study of museum specimens, Hebard recognized four species in what he called the "subgenus" *Allonemobius: A. fasciatus, A. griseus, A. maculatus,* and *A. ambitiosus*. In addition, he recognized three subspecies—a southern and a western subspecies of *A. fasciatus* (*socius* and *abortivus*) and a southern subspeices of *A. griseus* (*funeralis*). Later, Fulton (1930, 1931) described another morphologically well-defined species, (*A. sparsalus*), and another subspecies of *A. fasciatus*, *tinnulus*. He also showed that the southern subspecies of *A. fasciatus* was not limited to the southern United States, but also occurred in the north (Fulton 1931, 1937). Although the results published by Fulton (1931, 1933, 1937) clearly demonstrated that the three eastern subspecies of *A. fasciatus* were all distinct species, he never recognized them with formal nomenclature. Subsequently, some taxonomists resisted recognizing these groups as separate taxa because of difficulty in distinguishing pinned specimens by using morphological characters. To remedy this situation, Alexander and Thomas (1959) formally raised the eastern subspecies of *A. fasciatus* to species status (naming them *fasciatus, tinnulus*, and *allardi*) and pointed out morphological characters that could be used to identify pinned adults.

The close relationship of these three species, as evinced by their morphological similarity and the ability of *A. allardi* and *A. tinnulus* to produce fertile hybrids in the laboratory (Fulton 1933), indicate that they are well-suited for speciation studies. However, before starting work designed to answer questions regarding the evolution of reproductive isolation, it is important to obtain a clear picture of evolutionary relationships. After all, the evolutionary history of a group sets the context in which questions regarding the evolution of reproductive isolation must be framed. Based on similarities in song and morphology and the ability to produce fertile hybrids in the laboratory, Alexander and Thomas (1959) suggested that *A. allardi* and *A. tinnulus* were sister species and that *A. fasciatus* was more remotely related to these two taxa. Later, Alexander (1962) went even further and suggested, based solely on similarities in calling songs, that *A. allardi*, *A. tinnulus*, and *A. griseus* make up one distinct unit of the genus *Allonemobius*, whereas *A. fasciatus* and *A. maculatus* make up two other distinct units. Adding to the confusion is the relationship of northern and southern *A. fasciatus* and the relationship of northern and southern *A. allardi*. Fulton (1931, 1937) noted that *A. allardi* and *A. fasciatus* from

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coastal North Carolina are found in somewhat different habitats than their northern song counterparts. To determine whether this difference was merely a response to climatic and environmental differences or whether it represented some evolutionary divergence, Fulton (1937) crossed individuals of both species from Ohio and North Carolina. For both species he obtained some offspring from the north-south crosses and concluded that the northern and southern forms were at least close relatives. However, the ability of *A. allardi* and *A. tinnulus* to form fertile hybrids in the laboratory, although none have ever been found in nature, makes it clear that tests of the sort conducted by Fulton are of limited usefulness in establishing relationships.

In the present study I use horizontal starch gel electrophoresis to examine the amount of genetic divergence among members of the *A. fasciatus* complex, as well as *A. griseus* and *A. maculatus*. In addition, I explore levels of divergence between northern and southern populations of *A. allardi* and between northern and southern populations of *A. fasciatus*. The results obtained are used to construct a phylogeny for *Allonemobius* and to clear up the uncertainty regarding the relationship of southern *A. allardi* and *A. fasciatus* to their northern song counteraprts.

Materials and Methods

My collecting localities for *A. allardi*, *A. fasciatus*, *A. tinnulus*, and *A. griseus* are shown in Fig. 1. A sample of *A. maculatus* from Iowa was provided by Wayne Richter of the University of Iowa. Field-collected individuals were cared for in the manner described by Harrison (1979). At the laboratory the populations were censused and then frozen and stored at −80°C until used for electrophoresis. Populations were sampled over the course of 3 years: 1979, 1980, and 1981. Most field collections were made when crickets were adult.

Using horizontal starch gel electrophoretic techniques adapted from Selander et al. (1971) and Harrison (1977), I examined genetic variation at 17 loci coding for soluble enzymes and one locus coding for a protein of unknown function. Details about technique, such as the gel and electrode buffers used with a particular enzyme, can be found in Howard (1982).

Individuals that were homozygous at a particular locus generally displayed a single band. Individuals having two bands, in the case of monomeric enzymes, or three bands, in the case of dimeric enzymes, were interpreted as being heterozygous. Different alleles were designated by different letters of the alphabet. For anodally migrating enzymes, the faster a band, the closer to the beginning of the alphabet was its letter designation. Cathodally migrating enzymes were designated by the same system, but in the opposite direction. Laboratory crosses have demonstrated that observed intraspecific variation is genetic (Howard 1982).

Several methods exist for estimating genetic distance or genetic similarity between populations based on electrophoretic data (e.g., Nei 1972, 1978, Rogers 1972). The most widely used method in the literature is that of Nei (1972). Recently, however, Farris (1981) has criticized the use of this measure in phylogenetic analysis because it does not satisfy the triangle inequality and hence is not a metric. Rogers' distance measure, though, is a metric and is appropriate for phylogenetic analysis (Swofford 1981). I calculated Nei's genetic distance so that I could compare my results with a large amount of previous work. I calculated Rogers' distance (1972) for use in constructing phylogenetic trees.

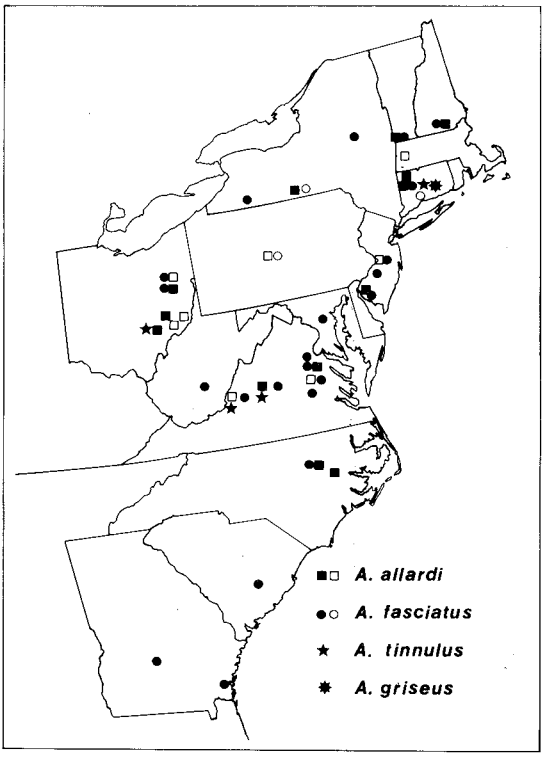

FIG. 1. Location of collection sites for *A. fasciatus*, *A. allardi*, *A. tinnulus*, and *A. griseus*. The distinction between open and closed symbols is explained in the text.

Results

Soon after I began my electrophoretic survey it became evident that the *A. fasciatus* complex is in need of revision. Electrophoretic comparisons indicate that northeastern and southeastern *A. fasciatus* populations are genetically distinct, and that what has been called *A. allardi* consists of at least three distinct groups of populations—one widely distributed in the northeastern United States and two occurring in the southeastern United States. In the case of *A. fasciatus*, there is essentially a fixed difference between northern and southern populations at one locus (*hexokinase*) and region-specific alleles at three others: *isocitrate dehydrogenase-1*, *glutamic-oxalacetic transaminase*, and *peptidase-3*. (*Glutamic-oxalacetic transaminase* could not be consistently scored in all the species surveyed in this study; hence, data regarding this enzyme could not be used in the construction of a phylogeny for eastern *Allonemobius*. Howard [1982] discusses the differences between northern and

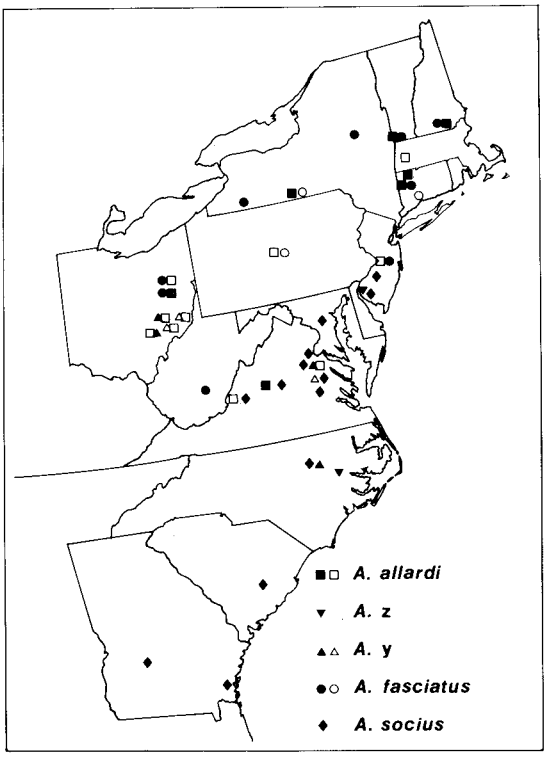

FIG. 2. Distributions of *A. allardi*, *A.* z, *A.* y, *A. fasciatus*, and *A. socius* in the eastern United States based on electrophoretic evidence. The distinction between open and closed symbols is explained in the text.

southern populations of *A. fasciatus* at this locus.) There are also substantial allele frequency differences at several other loci. The amount of differentiation between northern and southern populations as measured by Nei's genetic distance ranges from 0.130 to 0.235, a level of differentiation commensurate with that found between some previously described *Allonemobius* species and that found between closely related species of field crickets (Harrison 1977, 1979). Within each region genetic distances between geographically separated populations are small, ranging from 0.003 to 0.044 northern populations and from 0.002 to 0.031 for southern populations. The concordance of the patterns of differentiation over several loci and the abruptness of the discontinuity indicates that northeastern and southeastern forms of *A. fasciatus* are genetically isolated from one another and should be regarded as specifically distinct. Further evidence in support of this contention is that in areas of sympatry, despite variable levels of hybridization, both forms tend to remain distinct (Howard 1982, unpublished data). Figure 2 shows the ranges of the two species based on populations which were examined as part of this study.

Based on the locality of the type specimen (Pennsylvania) (Alexander and Thomas 1959), it seems that the name *A. fasciatus* should be applied to the northeastern species. Individuals from central Pennsylvania are genetically similar to the northeastern species (Howard, unpublished data). It also appears, from a description of a Georgia specimen by Scudder in 1877, that the name *A. socius* should be given to the southern species. This topic will be treated more extensively in a formal taxonomic revision of the *A. fasciatus* complex being prepared by Furth and Howard.

The situation with *A. allardi* resembles that with *A. fasciatus* except that here there are three species rather than two. The most abundant of these species (*A. allardi* based on the locality of the type of specimen) occurs in the northeastern United States, where it is found in dry grasslands. It is replaced in the southern United States by two species, which I will refer to as *A.* y and *A.* z until formal names are chosen by Furth and Howard. *A.* y is also a dry grassland inhabitant, but *A.* z occurs in moist grassy areas. Although morphologically indistinguishable, these species are genetically quite distinct. There is at least one diagnostic locus separating each of them. I have collected *A. allardi* and *A.* y together in Ohio and Virginia, without uncovering any evidence of hybridization. Four diagnostic loci separate these species so hybrids or backcrosses would be easy to spot. Because of habitat differences, I have never collected *A.* z together with *A. allardi* or *A.* y. But the range of *A.* z overlaps the ranges of *A. allardi* and *A.* y. Despite this range overlap, there is no sign of hybridization or introgression between *A.* z and either of the other two species. The lack of gene flow among populations which must often come in contact with one another clearly indicates that *A. allardi*, *A.* y, and *A.* z are all on different evolutionary pathways and should be considered separate species. Figure 2 shows the ranges of the three species based on collections from 1979, 1980, and 1981.

Table 1 summarizes allele frequency data from 40 populations of eight eastern North American species of *Allonemobius*. The location of the populations used to construct this table are shown in Fig. 1 (*A. tinnulus* and *A. griseus*) and Fig. 2 (*A. allardi*, *A.* z, *A.* y, *A. fasciatus*, and *A. socius*). They are the populations marked by solid symbols. Populations marked with open symbols were not used because of small sample sizes or incomplete electrophoretic profiles. Space limitations prohibit publishing allele frequencies from each population surveyed, but this information, along with detailed information about individual loci, is available in Howard (1982). In calculating mean allele frequencies for a species, all populations were given equal weight regardless of sample size. In cases where populations had been sampled for 2 consecutive years, the two samples were treated as separate populations. This approach seems justified by the fact that the genetic distance between populations collected from the same locality on consecutive years was sometimes greater than the genetic distances between these populations and geographically separated conspecific populations. Not included in Table 1 are the loci which were monomorphic: *acid phosphatase, general protein, α -glycerophosphate dehydrogenase, isocitrate dehydrogenase*-2, and *tetrazolium oxidase*. In a few instances, alleles listed in Table 1 do not appear to be represented in any species surveyed. These are alleles that do occur rarely in the genus

Table 1. Mean allele frequencies in populations of eight eastern North American *Allonemobius* species[a]

Allele		all	tin	z	y	fas	soc	gri	mac
Alp	n	24	31	18	13	33	24	28	30
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—
	c	—	—	—	—	—	—	—	1.00
Est-1	n	60	38	18	15	56	52	38	44
	b	—	—	—	—	0.02	0.01	—	—
	c	—	—	—	—	0.21	0.01	—	0.02
	d	—	—	—	—	—	0.01	—	—
	e	0.01	0.08	—	0.02	0.12	0.21	0.95	—
	f	0.01	—	—	0.02	—	0.02	—	—
	g	0.16	0.88	—	0.16	0.63	0.73	0.05	—
	h	0.15	—	—	0.63	—	—	—	0.98
	i	0.65	0.03	0.66	0.02	0.01	0.02	—	—
	j	0.02	0.02	—	0.02	0.01	—	—	—
	k	0.01	—	—	0.11	—	—	—	—
	l	—	—	0.34	0.02	—	—	—	—
Est-2	n	37	22	18	12	35	44	36	42
	b	—	—	—	—	—	0.01	—	—
	c	0.06	0.20	—	0.14	0.46	0.03	—	—
	d	—	—	—	—	—	0.01	—	—
	e	0.67	0.72	0.48	0.86	0.35	0.53	0.67	—
	f	—	—	—	—	—	—	—	—
	g	—	—	—	—	0.03	0.01	—	—
	h	0.06	0.03	0.44	—	0.12	0.37	0.33	—
	i	—	—	—	—	0.01	—	—	—
	j	—	0.01	0.07	—	0.02	0.01	—	—
	k	0.21	0.04	—	—	0.01	0.02	—	—
	l	—	—	—	—	—	0.02	—	1.00
Hk	n	53	36	18	15	50	52	38	32
	b	—	—	—	—	—	—	—	0.16
	c	0.01	0.95	—	—	—	—	—	0.84
	d	—	—	—	1.00	—	—	—	—
	e	0.99	0.05	0.88	—	—	—	—	—
	f	—	—	—	—	1.00	0.02	—	—
	g	—	—	—	—	—	—	1.00	—
	h	—	—	0.12	—	—	0.98	—	—
Idh-1	n	54	36	18	15	53	49	38	44
	b	0.02	0.21	—	—	—	—	—	—
	c	0.34	0.14	—	—	—	0.21	—	—
	d	0.61	0.66	—	—	—	0.44	—	—
	e	0.02	—	—	—	—	0.21	—	—
	f	—	—	—	—	—	—	—	—
	g	—	—	1.00	0.25	—	—	1.00	—
	h	—	—	—	0.75	—	—	—	—
	i	—	—	—	—	1.00	0.12	—	1.00
Me	n	23	22	7	15	27	24	32	44
	b	—	—	—	—	—	—	0.97	—
	c	1.00	1.00	1.00	1.00	1.00	1.00	0.03	—
	d	—	—	—	—	—	—	—	1.00
Mdh	n	62	38	18	14	53	42	38	44
	b	1.00	1.00	1.00	1.00	—	—	1.00	1.00
	c	—	—	—	—	1.00	1.00	—	—
Pep-1	n	20	18	3	3	20	16	22	28
	b	0.83	0.96	1.00	1.00	—	—	—	—
	c	—	—	—	—	0.99	0.99	1.00	1.00
	d	0.17	0.04	—	—	0.01	0.01	—	—
Pep-2	n	46	37	18	11	42	48	16	32
	b	—	0.01	—	—	—	—	—	1.00
	c	—	—	1.00	0.22	0.07	0.36	—	—
	d	1.00	0.98	—	0.89	0.93	0.64	—	—
	e	—	0.01	—	—	—	—	1.00	—
Pep-3	n	53	38	18	15	53	50	38	44
	b	—	—	—	—	—	0.01	—	—
	c	—	—	—	—	0.12	0.99	—	—
	d	—	—	—	—	—	—	—	—
	e	—	—	—	1.00	—	—	1.00	1.00
	f	—	—	—	—	0.88	0.01	—	—
	g	1.00	1.00	1.00	—	—	—	—	—

Table 1. Continued.

Allele		all	tin	z	y	fas	soc	gri	mac
6-Pgd	n	38	15	18	13	39	42	2	28
	b	—	0.02	—	—	0.06	0.08	—	—
	c	0.98	0.98	1.00	1.00	0.94	0.90	1.00	—
	d	0.02	—	—	—	—	0.02	—	1.00
Pgm	n	20	19	7	12	26	20	38	44
	b	—	—	—	—	—	—	—	1.00
	c	—	—	—	—	—	—	1.00	—
	d	1.00	1.00	1.00	1.00	1.00	1.00	—	—
Pgi	n	62	42	18	15	56	53	38	44
	b	0.01	0.04	—	0.02	0.01	—	—	—
	c	—	—	—	—	—	0.01	—	—
	d	0.29	0.28	0.02	0.78	0.03	0.06	—	—
	e	0.01	0.04	—	—	0.01	—	—	—
	f	—	—	—	—	—	—	—	—
	g	0.67	0.63	0.98	0.20	0.82	0.87	0.90	1.00
	h	—	—	—	—	—	—	—	—
	i	0.01	—	—	—	0.06	0.06	0.10	—
	j	—	—	—	—	0.04	—	—	—
	k	0.01	—	—	—	0.02	—	—	—
	l	—	—	—	—	—	—	—	—

[a]n Represents mean number of loci sampled in a population.

Allonemobius, but were not present in any populations used in this study. They are included to retain the scoring system of Howard (1982). Sample sizes for populations of *A.* y and *A.* z are small. This reflects the relative scarcity of these species in nature. However, because the values of Rogers' D and Nei's D are fairly insensitive to the presence or absence of rare alleles, estimates from small sample sizes are reliable.

Rogers' and Nei's genetic distances between the eight *Allonemobius* species surveyed are displayed in Table 2. These distances represent the mean of between population measures. Despite differences in absolute values, both measures of genetic distance agree closely with regard to relative levels of differentiation. Every population surveyed as part of this study could be unambiguously assigned to a particular species, and there is no overlap between intraspecific and interspecific genetic distances. To aid in visualizing the genetic discontinuity among species in the *A. fasciatus* complex, Fig. 3 displays a phenogram constructed for 17 populations, using the unweighted pair-group method of cluster analysis (UPGMA [Sokal and Michener 1958]). Note how tightly conspecific populations cluster compared with the distance separating different species.

Many methods exist for estimating phylogenetic trees from distance matrices (e.g., Fitch and Margoliash 1967, Farris 1972, Sneath and Sokal 1973, Felsenstein 1981) and there is considerable disagreement over which approach yields "better" trees (Farris 1972, Prager and Wilson 1978, Swofford 1981). The UPGMA method of cluster analysis is probably the most widely used method among biochemical systematists. It is a phenetic method, clustering groups on the basis of overall similarity. Its advantage over other methods is that it is fast and easy to calculate. Its disadvantage is that any phenogram generated can be regarded as an estimate of an evolutionary tree only if rates of evolutionary change along different phyletic lines are sufficiently homogeneous (Farris 1972, Swofford 1981). Two methods of constructing phylogenetic trees from genetic distance data which do not operate under the assumption of homogeneity of rates of divergence are the Fitch-Margoliash procedure (Fitch

Table 2. Mean Rogers' and Nei's genetic distances among North American *Allonemobius* species based on electrophoretic comparison of 18 loci; Rogers' genetic distances are shown above the diagonal, Nei's below the diagonal

	all	tin	z	y	fas	soc	gri	mac
all	—	0.138	0.176	0.262	0.345	0.323	0.510	0.538
tin	0.106	—	0.241	0.254	0.321	0.303	0.512	0.507
z	0.144	0.238	—	0.312	0.396	0.354	0.447	0.534
y	0.260	0.256	0.338	—	0.394	0.390	0.458	0.491
fas	0.400	0.362	0.492	0.480	—	0.208	0.510	0.478
soc	0.353	0.322	0.411	0.461	0.188	—	0.481	0.519
gri	0.714	0.707	0.595	0.584	0.717	0.660	—	0.492
mac	0.759	0.692	0.770	0.661	0.648	0.721	0.674	—

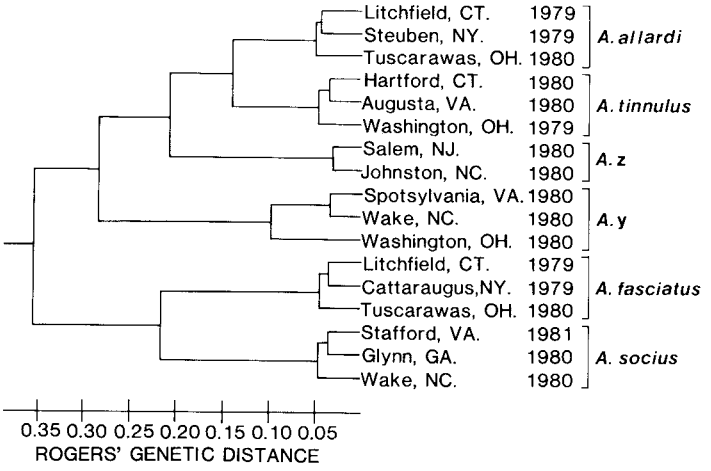

FIG. 3. Estimation of a phylogenetic tree for 17 populations of eastern North American *Allonemobius* based on UPGMA clustering of a Rogers' genetic distance matrix. Populations are designated by county and state where collected.

and Margoliash 1967) and the distance Wagner procedure (Farris 1972).

I constructed phylogenetic trees for the genus *Allonemobius* by using Rogers' distance matrix (Table 2) and all three of the methods above. The distance Wagner tree and the Fitch-Margoliash tree are identical in topology. They differ from the UPGMA tree in only one respect, the choice of a sister species for *A. allardi*. The UPGMA tree is shown in Fig. 4. To visualize the topology of the distance Wagner tree and the Fitch-Margoliash tree, interchange *A. tinnulus* and *A.* z on the UPGMA tree.

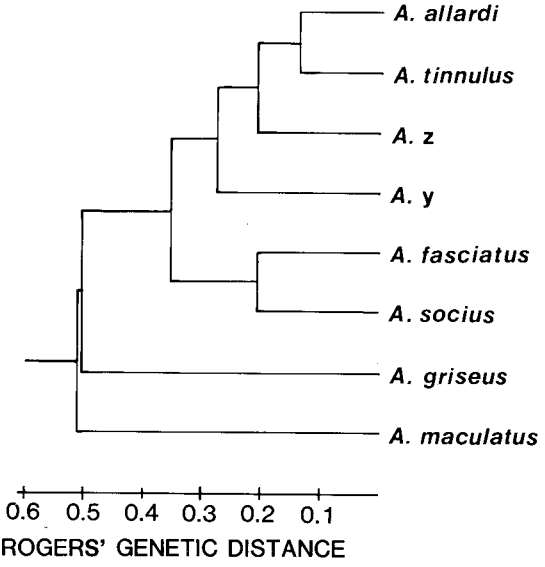

FIG. 4. Estimation of a phylogenetic tree for eastern *Allonemobius* based on UPGMA clustering of the Rogers' genetic distance matrix shown in Table 2.

Discussion

The most intriguing result of the present study is the discovery of three additional species in the *A. fasciatus* complex. This discovery was not totally unexpected. Forty-five years ago, Fulton (1937) suspected on the basis of habitat differences that there may have been some evolutionary divergence between northern and southern populations of *A. allardi* and *A. fasciatus*. However, subsequent investigators, most notably Alexander and Thomas (1959), found no evidence of divergence, and even Fulton did not suspect that southern *A. allardi* might consist of not one but two distinct species.

An important question raised by the discovery of these species is whether *A. allardi* and *A. fasciatus* share the same male calling songs as their southern replacements. In his extensive work on this group, Fulton (1931, 1933, 1937) never noted a calling song difference between *A. fasciatus* and *A. socius*. In the case of *A. allardi*, he did notice some intraspecific variation in calling song. At one locality in North Carolina and two localities in South Carolina, rather than singing with a continuous rapid trill characteristic of northern *A. allardi*, crickets broke their songs into phrases a few seconds in duration (Fulton 1937). However, some specimens from one of these localities did sing normally in the laboratory, making an interpretation of Fulton's findings difficult. Alexander and Thomas (1959) recorded calling songs of what they presumed to be *A. fasciatus* from localities in Ohio, Kentucky, West Virginia, Mississippi, Louisiana, and Florida and did not report a difference among songs from these areas. Moreover, these investigators compiled extensive listening records covering every county in Ohio over the course of 25 years and never noted hearing more than three types of calling songs corresponding to those of *A. allardi*, *A. fasciatus*, and *A. tinnulus*. Nevertheless, *A. socius* and *A.* y do occur in southeastern Ohio (Howard 1982). More recently, Walker

(personal communication) has detected a southeastern *A. allardi* with a faster trill than northeastern *A. allardi*. This fast-trilling *A. allardi* seems to correspond to *A.* y. In a paper currently under review, I confirm Walker's findings and show that all species of the *A. fasciatus* complex have distinct songs, although the differences among them are sometimes subtle.

Unlike Fulton, I did not perceive a habitat difference between *A. fasciatus* and *A. socius*. Both are typically associated with wet, grassy areas such as low-lying pasture. Rarely, individuals of both species are found in drier areas normally inhabited by *A. allardi* or *A.* y. The difference between our observations may be accounted for by the relative scarcity of *A.* y in the southern United States. Unlike *A. allardi*, the dry grassland inhabitant of the northern United States which often achieves tremendous population densities, *A.* y is relatively rare. The scarcity of *A.* y in dry grasslands enables the few *A. socius* males singing in those areas to be heard. This ease of detection may have misled Fulton into believing that *A. socius* lives in a wider range of habitats than *A. fasciatus*. *A.* z, the other southern species that resembles *A. allardi* in morphology and male calling song, appears to represent what Fulton called southern *A. allardi*. I have only collected this species from wet, grassy habitats such as land adjacent to marshes. The patterns of habitiat utilization and geographic distribution described above, in conjunction with phylogenetic considerations, suggest that geographic isolation or the evolution of habitat differences may be responsible for *Allonemobius* species diversity.

The amount of genetic differentiation between possible sister species in the *A. fasciatus* complex as measured by Nei's D ranges from 0.106 to 0.188. This level of differentiation is intermediate with respect to the amount found between sister species in a variety of other insect groups. For example, it is less than the genetic distance reported between subspecies of the *Drosophila willistoni* complex (Ayala et. al. 1974), similar to the genetic distance between sister species of the *Drosophila mulleri* complex (Zouros 1973), but greater than the genetic distance between sister species of field crickets (Harrison 1977, 1979) and sister species of Hawaiian *Drosophila* (Sene and Carson 1977).

Geographic variation in allele frequencies appears remarkably limited within the three species for which extensive data are available (*A. allardi*, *A. fasciatus*, and *A. socius*). With very few exceptions, the most common allele at a polymorphic locus is the same in all conspecific populations, even those separated by hundreds of kilometers (Howard 1982).

Three methods of estimating phylogenetic trees from a Rogers' genetic distance matrix (see above) give trees indicating that there are at least four distinct units in the genus *Allonemobius*. Two are represented by *A. maculatus* and *A. griseus*, which are morphologically distinct from the other six species as well as each other. The third is represented by *A. fasciatus* and *A. socius*. *A. allardi*, *A.* z, *A. tinnulus*, and *A.* y make up the final unit. These groupings agree well with phylogenetic relationships suggested by hybridization studies, and similarities in morphology and song. Thus far, I have found no morphological characters that are consistently useful in distinguishing *A. fasciatus* from *A. socius*. Similarly, it is not possible at present to separate *A. allardi*. *A. tinnulus*. *A.* y. amd *A.* z on purely morphological grounds. However, *A. fasciatus* amd *A. socius* can be easily distinguished from the other four species by differences in head banding intensity, ovipositor length, and stridulatory vein size (Alexander and Thomas 1959, Howard, unpublished date). *A. allardi* and *A. tinnulus* have been successfully crossed in the laboratory, as have *A. fasciatus* and *A. socius* (Fulton 1933, 1937, Howard 1982, unpublished data). Both sets of crosses yield fertile hybrids. However, crosses involving *A. fasciatus* or *A. socius* and either of the other two species have consistently failed (Fulton 1931, 1933, 1937, Howard, unpublished data). *A.* y and *A.* z have not yet been tested in interspecific crosses.

The only disagreement among the three phylogenetic trees involves the choice of a sister species for *A. allardi*. By the Fitch-Margoliash tree and the distance Wagner tree. *A.* z is the sister species of *A. allardi*. But by the UPGMA tree, *A. tinnulus* is the sister species of *A. allardi*. The uncertainty over the correct sequence of speciation events which gave rise to *A. allardi*, *A. tinnulus*, and *A.* z may render them less suitable for speciation studies than *A. fasciatus* and *A. socius*. The latter are also the only species that hybridize to a measurable extent in areas of sympatry (Howard 1982), a further suggestion of recent divergence and an attractive feature if one is interested in studying the evolution of reproductive isolation.

The discovery of three new species in the *A. fasciatus* complex underscores the importance of systematic studies as a prelude to evolutionary and ecological investigations. Not only does such work help to eliminate confounding factors in subsequent studies, but as in this case, it may also suggest new questions and point the direction of future research.

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