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CHROMOSOMAL EVOLUTION IN *RHOGEESSA* (CHIROPTERA: VESPERTILIONIDAE): POSSIBLE SPECIATION BY CENTRIC FUSIONS

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Summary.—Within the *Rhogeessa tumida-parvula* complex, there are seven cytotypes involving diploid numbers of 30, 32, 34, 42, 44, and 52. Based on G-band analysis, the 30, 32, 34, and 44 forms differ from each other by centric fusions (13 different fusions were identified). Two $2n = 32$ samples (one from Belize and one from Nicaragua) having essentially identical standard karyotypes differed from each other by eight fusion events. Cytotypes are allozymically distinct; however, other studies indicate that the cytotypes are morphologically very similar if not indistinguishable (LaVal, 1973; Baker, 1984). G-band chromosomal data are best interpreted as indicating that, within this complex, several species exist which should be maintained by a post-mating isolating mechanism resulting from meiotic problems in F_1 individuals.

Mus musculus (Capanna et al., 1977; Capanna, 1982; White, 1978) and the *Rhogeessa tumida-parvula* complex are unusual among mammalian species thus far studied in their pattern of chromosomal variation and presumptive mode of speciation. Strong evidence exists suggesting that in both groups populations differing by multiple centric fusions are biological species. It appears that the population and reproductive biology of *Mus* and *Rhogeessa* are quite different and any model that attempts to explain the mode of speciation in these two diverse taxa, must be compatible with their different biological characteristics.

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The pattern of chromosomal variation in *Mus musculus* is sufficiently distinctive among vertebrates that it has served as the primary data set for two models of speciation (Capanna et al., 1977; White, 1978; Capanna, 1982) as well as a source of considerable debate (Bickham and Baker, 1980; John, 1981). The discovery of another mammalian genus that displays a similar pattern of chromosomal evolution provides additional insight into the biology of organisms proposed to have speciated by extensive centric fusions of acrocentric chromosomes.

Bats (Order Chiroptera) are generally characterized as having a conservative rate of chromosomal evolution (Baker, 1970, 1979; Baker et al., 1982; Bush et al., 1977). Few chromosomal races have been described (Baker, 1979), and closely related species often have indistinguishable G- and C-banded karyotypes (Baker

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and Bickham, 1980). There are, however, a few species which markedly differ from this pattern (Baker, 1981; Greenbaum, 1981; Baker and Bickham, 1980). As described below, the most striking case involves bats of the *tumida-parvula* complex of the genus *Rhogeessa.*

Within the *Rhogeessa tumida-parvula* complex, there are six described cytotypes involving diploid numbers ($2n$) of 30, 32, 34, 42, 44, and 52 (Bickham and Baker, 1977; Honeycutt et al., 1980). *R. parvula,* which has been recognized by classical morphological studies (LaVal, 1973), has $2n = 44$ with no reported intraspecific chromosomal variation (Bickham and Baker, 1977). The remaining five cytotypes are from samples which, at the start of this study, were identified as *R. tumida* (Fig. 1). However, LaVal (1973), Bickham and Baker (1977), and Honeycutt et al. (1980) hypothesized that *R. tumida,* as currently recognized (Hall, 1981), is a composite of several morphologically indistinguishable species. In fact, at least two cytotypes ($2n = 42$ and 34) are sympatric, and, in a sample of thirty individuals (19 of the $2n = 34$ cytotype and 11 of the $2n = 42$ cytotype) from a single locality, no hybrids were found. These data have served as the basis for the description of the $2n = 42$ form as a distinct species, *R. genowaysi* (Baker, 1984).

Bickham and Baker (1977) suggested that the primitive karyotype for the genus *Rhogeessa* consisted of $2n = 50$ with all autosomes being acrocentric. A karyotype like that proposed as primitive for *Rhogeessa* is found in several species of *Eptesicus.* Based on classical morphology, LaVal (1973) also suggested that *Rhogeessa* and *Eptesicus* shared a common ancestor after separating from the *Myotis* lineage. Therefore, our first efforts will be toward testing the hypothesis that a karyotype like that found in New World *Eptesicus* is intermediate between karyotypes found in *Rhogeessa* and that proposed as primitive for the family Vespertilionidae. A karyotype like that found in most species of *Myotis* is identical to that proposed as primitive for the family Vespertilionidae (Bickham, 1979*a*, 1979*b*). After determining the most probable primitive karyotype for outgroup comparisons, it should be possible to identify synapomorphic chromosomal rearrangements in order to define clades within the *tumida* group. Therefore, one of the goals of this study was to produce a hypothesis of evolutionary relationships based on a cladistical analysis of G-banded chromosomes and to test the proposed relationships with independent data from electrophoretic analyses. Ultimately, we sought to provide a synthesis which would result in a better understanding of the significance of chromosomal evolution in this variable complex of bats.

Materials and Methods

Chromosomal Data.—G- and C-banded and nondifferentially stained karyotypes were prepared from bone marrow (Lee and Elder, 1980; Baker et al., 1982) and in vitro cultured fibroblasts (Bickham and Baker, 1977). Numbering of chromosomes refers to proposed homology to the standard for *Myotis* (Bickham, 1979*a,* 1979*b*). Both *Eptesicus fuscus* and *Myotis* (Bickham, 1979*b*) were used as outgroups to determine plesiomorphic chromosomal conditions.

Electrophoretic Data.—Twenty-one presumptive loci coding for enzymes and serum proteins were assayed. These included malate dehydrogenase-1,2 (*Mdh-1,2*), lactate dehydrogenase-1,2 (*Ldh-1,2*), isocitrate dehydrogenase-1,2 (*Idh-1,2*), general protein-4 (*Gp-4*), 6-phosphogluconate dehydrogenase (*6-Pgd*), mannose-6-phosphate (*Mpi*; Nichols et al., 1973), phosphoglucose isomerase-1,2 (*Pgi-1,2*), phosphoglucomutase-1,2 (*Pgm-1,2*), peptidase-1 (*Pep-1,* substrate = glycyl-L-leucine), peptidase-2 (*Pep-2,* substrate = leucylglycylglycine), leucine aminopeptidase (*Lap*), esterase-1,2 (*Est-1,2*), alcohol dehydrogenase (*Adh*), glutamate oxalate transaminase-1 (*Got-1*), and α-glycerophosphate dehydrogenase (α-*Gpd*). Enzyme staining,

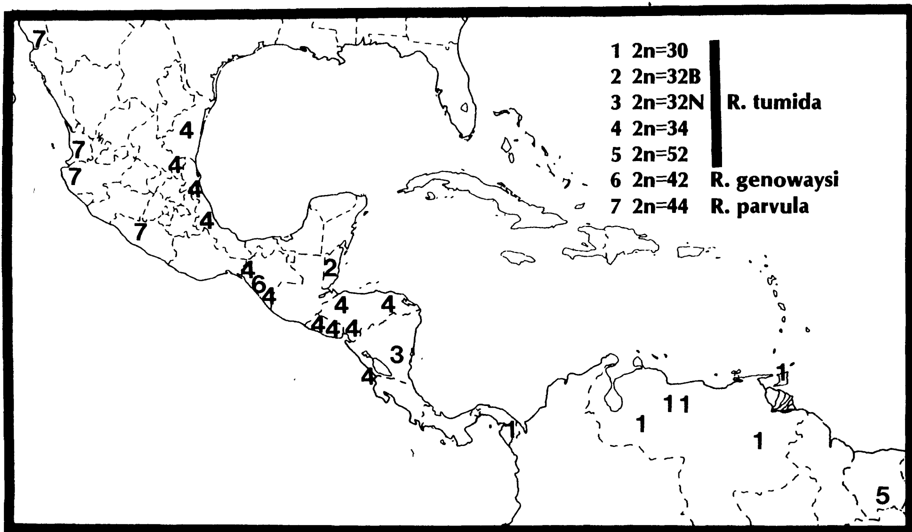

FIG. 1. Geographic distribution of cytotypes of the *Rhogeessa tumida-parvula* complex. Cytotypes mapped in this figure that are not included in the specimens examined are listed in Bickham and Baker (1977).

buffer systems, and electrophoretic techniques are essentially those of Selander et al. (1971). Loci were designated numerically with "*1*" being the most anodal isozyme and successively higher numbers being given to more cathodal loci. Allozymes at each of the loci were given letter designations with "*a*" denoting the most anodally migrating electromorph and "*b,*" "*c,*" etc. designating progressively less anodal allozymes. Allozyme similarities were determined through side-by-side comparisons of all variant electromorphs. Gene frequencies were then determined for each of the variants at each presumed locus. Using the allozyme frequencies, Rogers' *D* coefficients (Rogers, 1972) were determined for each pair of cytotypes. The resulting matrix of distance values were then subjected to the method of Fitch and Margoliash (1967) for the construction of a phylogenetic tree. Prior to the construction of a Fitch and Margoliash (1967) tree, we utilized the outgroup technique (Wake et al., 1978; Watrous and Wheeler, 1981) for determining primitive and derived character states. We chose *Eptesicus fuscus* as the outgroup taxon for our study. As discussed above, the genus *Eptesicus* has been hypothesized as the sister taxon to *Rhogeessa* (Bickham and Baker, 1977; LaVal, 1973). In order to designate primitive and derived conditions at each isozyme, we assumed any electromorph present both in *Eptesicus* and *Rhogeessa* to be primitive for the entire clade (Watrous and Wheeler, 1981). Once the primitive electromorph was identified, any other allozymes present in the *Rhogeessa* samples were designated as apomorphic (Hennig, 1966). Synapomorphic (shared derived) electromorphs were then used to construct a phylogenetic tree based upon the distribution of these character states (Watrous and Wheeler, 1981). Autapomorphic (unique derived) characters were not used in phylogeny reconstruction but are useful in comparisons of rates of biochemical evolution.

Specimens Examined.—In addition to specimens reported in Bickham and Baker (1977), standard karyotypes were examined from the following localities. If G- and C- bands also were prepared, then a "(G and C)" follows that locality.

Rhogeessa parvula: ($2n = 44$) 8 mi S of Alamos, Sonora, MEXICO (G and C)
R. genowaysi: ($2n = 42$) 23.6 mi NW of Huixtla, Chiapas, MEXICO
R. tumida: ($2n = 52$) Sipaliwini Airstrip, Nickerie, SURINAME
($2n = 34$) 31.2 mi SE of Tuxtepec, Oaxaca, MEXICO
23.6 mi NW of Huixtla, Chiapas, MEXICO (G and C)
Cinco Cerros, Chiapas, MEXICO
Acuna, Tamaulipas, MEXICO
Ojo de Auga, Veracruz, MEXICO
Ciudad Valles, San Luis Potosi, MEXICO
Rancho Pago Pago. San Luis Potosi, MEXICO
Lancertilla Botanical Gardens, Atlantida, HONDURAS
2.6 mi W, 10.8 mi S of Jico Galan, Valle, HONDURAS
5 mi N, 1 mi W of San Juan del Sur, Rivas, NICARAGUA
($2n = 32B$) Amergris Caye, 9.75 mi S of San Pedro, Belize District, BELIZE (G and C)
St. John's College, Belize City, BELIZE
Burrell Boom Village, Belize District, BELIZE (G and C)
($2n = 32N$) Rama, Zelaya, NICARAGUA
($2n = 30$) 45 km SW of Calabozo, Guarico, VENEZUELA
Hato, Guarico, VENEZUELA
Masguaral, Guarico, VENEZUELA
18 km NE of El Manteco, Bolivar, VENEZUELA
8 km SW of St. Barbara, Barinas, VENEZUELA (G and C)
Cana, Darien, PANAMA
Eptesicus fuscus: Memphis, Hall County, Texas, USA

RESULTS

Chromosomal Variation in Rhogeessa.—G- and C- bands were determined for the $2n = 30$, 32, 34, and 44 cytotypes. Banding analyses revealed that the geographically disjunct (Belize and Nicaragua, Fig. 1) populations of the $2n = 32$ cytotype possessed distinctly different G-banded karyotypes. In this paper, the Belize sample is referred to as $2n = 32B$ and the Nicaraguan samples as $2n = 32N$. Table 1 shows the biarmed chromosomes shared by two or more cytotypes as identified by G-band pattern (nomenclature follows, Bickham, 1979*a*, 1979*b*). Unique biarmed chromosomes (autapomorphs) included: $2n = 44$, 3/1, 25/5; $2n = 34$, 10/3; $2n = 32B$, 10/9, 14/2; $2n = 32N$, none; $2n = 30$, 15/2. All cytotypes had 25 pairs of arms ($FN = 50$), so any arm not listed in Table 1 or the above list of unique biarmed chromosomes, was present as an acrocentric (plesiomorphic) chromosome. Figure 2 illustrates the proposed evolutionary relationships of these five cytotypes based on a cladistic analysis of the G-band data. Autosomal C-bands were restricted to the centromeric regions.

Allozyme Variation in Rhogeessa.—Four of the loci examined (*Mdh-2, Ldh-1, Gp-4,* and *Pep-1*) were monomorphic across all populations. Allozyme data for the remaining 17 polymorphic isozymes, along with sample sizes used in the electrophoretic portion of our study are given in Table 2. Character states for which the primitive electromorph could be determined were used to construct the phylogeny presented in Figure 3.

Nei's genetic identity values (Nei, 1972) for each of the taxon pairs are given in Table 3. By subjecting coefficients of Rogers' distance (Rogers, 1972) to the Fitch and Margoliash (1967) method for construction of phylogenetic trees, we arrived at the phylogeny pictured in Figure 4. This represents our "best" tree based upon the criteria that there be no negative branch lengths along any lineage and that the resulting phylogeny have the lowest possible F-value (Prager and Wilson, 1978) of any of the trees tested.

DISCUSSION

Chromosomal Variation in Rhogeessa.—The karyotype found in some species of *Eptesicus* (proposed as primitive for *Rhogeessa*) differs from the karyotype

TABLE 1. Biarmed autosomes (fusion products) occurring in cytotypes of *Rhogeessa*. Chromosomal arms identified and numbered according to *Myotis* standard. Fis? = possible fission event.

Cytotype	Shared biarmed autosomes											
$2n = 44$	20/18	16/17										
$2n = 34$	20/18	16/17	23/3	22/12	21/19	14/9	Fis?	11/1	15/4			
$2n = 32$B	20/18	16/17	23/3	22/12	21/19	Fis?	13/8	11/1	15/4			
$2n = 32$N	20/18	16/17	23/3	22/12	21/19	14/9	13/8			10/4	5/1	11/7
$2n = 30$	20/18	16/17	23/3	22/12	21/19	14/9	13/8			10/4	5/1	11/7

proposed as primitive for the family (that of *Myotis*) by fissions in the three biarmed chromosomes, 1/2 3/4 and 5/6, producing six acrocentric pairs and by an inversion in the biarmed pair 16/17 changing it to an acrocentric (Bickham, 1979*b*). Pair 16/17 is found in its biarmed condition in all cytotypes of *Rhogeessa* thus far examined; therefore, it is most parsimonious to assume that the *Rhogeessa* clade split from the *Eptesicus* clade prior to the inversion in the 16/17 which is an autapomorph for the *Eptesicus* clade. All *Rhogeessa* thus far examined share one other biarmed pair (20/18) resulting in a working hypothesis that the primitive karyotype for the *Rhogeessa* cytotypes thus far G-banded, was a $2n = 48$, $FN = 50$ with two biarmed autosomal pairs plus a biarmed X chromosome. Pair 16/17 is shared with many other taxa of vespertilionids but 20/18 is a synapomorphy for the genus *Rhogeessa*. The $2n = 44$ clade shares no other synapomorphic fusions with other cytotypes, but it has two autapomorphies (3/1 and 25/5). The other four cytotypes examined all share three synapomorphic fusions (23/3, 22/12, and 21/19) which strongly suggest a common ancestry for these four cytotypes after they diverged from the $2n = 44$ cytotype. At this point, their common ancestor had a $2n = 42$ and an FN = 50. The common ancestor of the $2n = 30$, 32N, 32B, and 34 cytotypes also may have shared two other fusions (14/9 and 13/8 with reversal fissions of the 14/9 in the $2n = 32$B form and 13/8 in the $2n = 34$ form). If these fusions were shared in a common ancestor, then its $2n$ would equal 38. This phylogenetic scheme is illustrated in Figure 2.

An alternative way of explaining the pattern of chromosomal evolution would be for 14/9 and 13/8 to have fused independently on separate lineages. The same number of events is required in both explanations. The major point here is, as in other chromosomal studies (Robbins and Baker, 1981; Baker et al., 1983; Rogers, 1983), either convergent events or reversals have occurred within the chro-

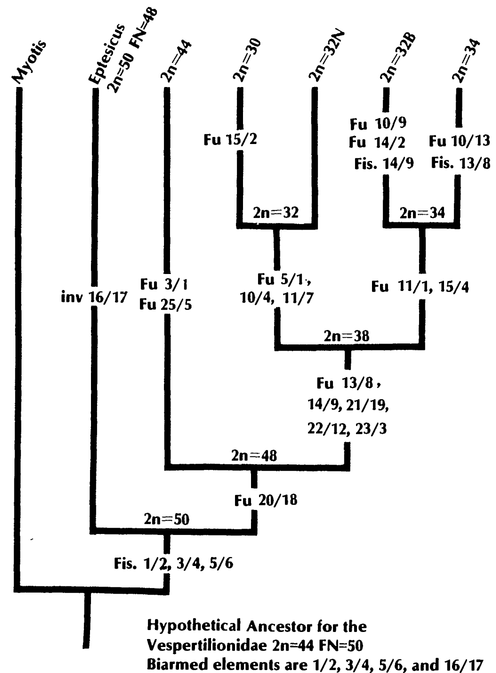

FIG. 2. Cladogram based on G-banded chromosomal data using *Myotis velifer* and *Eptesicus fuscus* as outgroups. Justification for using the *Myotis*-like karyotype for the primitive are presented in Bickham (1979*b*). An alternative means of explaining the events of chromosomal evolution is discussed in the text. Fu = fusion, Fis = fission, Inv = inversion.

TABLE 2. Allozyme designations and frequencies for the six *Rhogeessa* cytotypes and for *Eptesicus fuscus*. For cytotypes in which more than one allele is present, the number in parentheses immediately following the allele designation is the frequency of that allele. Isozyme and allozyme designations are identified in the text. Sample sizes are given in the column designated *N*. Asterisk (*) designates loci for which the primitive electromorph could be determined.

Species Cytotype	*N*	Isozymes																
		*Mdh-1**	*Ldh-2*	*Idh-1*	*Idh-2**	*6-Pgd*	*Mpi*	*Pgi-1*	*Pgi-2*	*Pgm-1**	*Pgm-2*	*Pep-2**	*Lap**	*Est-1*	*Est-2*	*Adh*	*Got-1**	*α-Gpd**
R. parvula 2*n* = 44	2	*a*	*b*	*b*	*a*	*a*	*e*	*a*	*a*	*b*	*a*	*d*	*a* (0.75) *b* (0.25)	*i*	*h* (0.50) *i* (0.50)	*f*	*a*	*c*
R. genowaysi 2*n* = 42	2	*c*	*b*	*c*	*b*	*i*	*h* (0.75) *j* (0.25)	*b*	*b*	*b*	*b*	*c*	*a*	*b* (0.25) *f* (0.75)	*d* (0.25) *h* (0.75)	*d*	*b*	*d*
R. tumida 2*n* = 34	10	*c*	*b*	*a* (0.06) *b* (0.94)	*b*	*c* (0.10) *e* (0.40) *f* (0.30) *g* (0.10) *h* (0.10)	*d* (0.15) *f* (0.30) *g* (0.05) *i* (0.15)	*b*	*b*	*b*	*b*	*a* (0.35) *c* (0.65)	*a* (0.90) *c* (0.10)	*b* (0.50) *f* (0.40) *g* (0.10)	*b* (0.10) *c* (0.30) *d* (0.20) *f* (0.10) *h* (0.10) *i* (0.20)	*a* (0.10) *b* (0.20) *c* (0.70)	*b*	*b* (0.15) *d* (0.60) *f* (0.35)
2*n* = 32B	6	*c*	*b*	*b*	*b*	*b* (0.33) *d* (0.33) *f* (0.17) *g* (0.17)	*c*	*b*	*b*	*b*	*b*	*a* (0.58) *c* (0.42)	*a*	*b* (0.25) *d* (0.25) *e* (0.08) *f* (0.25) *h* (0.17)	*d* (0.33) *e* (0.17) *h* (0.33) *j* (0.17)	*b*	*b*	*e* (0.20) *f* (0.80)
2*n* = 30	4	*c*	*b*	*b*	*b*	*f* (0.25) *g* (0.25) *h* (0.50)	*d*	*b*	*b*	*a* (0.25) *b* (0.75)	*b*	*b*	*a*	*e* (0.50) *h* (0.50)	*i*	*e*	*b*	*a* (0.50) *e* (0.50)
2*n* = 52	1	*b*	*b*	*c*	*a*	*h*	*a*	*b*	*b*	*b*	*b*	*a*	*a*	*a* (0.50) *c* (0.50)	*i*	*e*	*b*	*d*
E. fuscus	1	*a*	*a*	*d*	*a*	*j*	*b*	*c*	*c*	*b* (0.50) *c* (0.50)	*c*	*a*	*a*	*j*	*a*	*g*	*b*	*e*

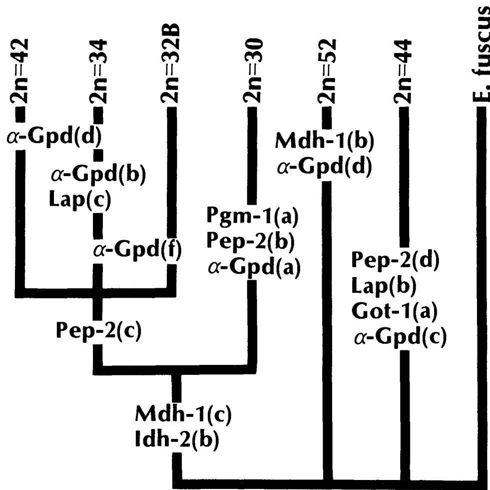

FIG. 3. Cladogram based on electrophoretic data using *Eptesicus fuscus* as the outgroup. The origin of *α-Gpd(d)* cannot be placed in the cladogram as a single event without requiring convergent events at other loci.

mosomal data set. No matter which alternative proves to be true, several aspects of chromosomal evolution in this complex of bats are worthy of note.

First, within the *tumida-parvula* complex, comparative chromosomal banding data suggest that several species are present which might be expected to be minimally maintained by a postmating isolating mechanism resulting from meiotic problems if F_1's are produced (Table 4). An example of such speciation is the $2n = 32$B cytotype when compared to the $2n = 32$N cytotype. That these two essentially identical standard karyotypic forms probably represent different species is the most unexpected result obtained in this study. The $2n = 32$B sample has a karyotype that differs by eight chromosomal rearrangements from that of the $2n = 32$N sample. Two chain multivalents (one involving five chromosomes and one with seven) would result at meiosis of an F_1 hybrid, most probably leading to hybrid sterility. In fact, if segregation is random, the probability of a normal gamete being produced is 0.0098. Table 4 contains the calculated hypothetical meiotic configurations that would result in potential F_1's from crosses between cytotypes for which G-band chromosomal data are available. Based on potential meiotic problems, it would appear that in Middle America alone, the $2n = 44$, $2n = 34$, $2n = 32$B, and $2n = 32$N cytotypes all represent samples from reproductively isolated lineages. Additionally, the $2n = 42$ and $2n = 34$ forms are sympatric in Chiapas, Mexico, and there is no evidence of hybridization. These five species, plus the morphologically distinct *Rhogeessa mira* described by LaVal (1973) (for which no karyotypic data exist) bring the total number of species that occurs in Middle America in the *tumida-parvula* complex to six. Theoretically, the chromosomal differences between the $2n = 32$N and the South American $2n = 30$ cytotype should not result in reproductive isolation (Table 4). The $2n = 32$N cytotype is most like the $2n = 30$ cytotype from South America, with which it shares three chromosomal synapomorphies (11/7, 10/4, and 5/1) and the $2n = 32$B cytotype is more like the $2n = 34$ cytotype from adjacent Mexico with which it shares two chromo-

TABLE 3. Coefficients of Nei's genetic identity (Nei, 1972) among the six *Rhogeessa* cytotypes and *Eptesicus fuscus.*

	$2n = 42$	$2n = 34$	$2n = 30$	$2n = 44$	$2n = 52$	$2n = 32$B	*E. fuscus*
$2n = 42$	1	0.81	0.66	0.36	0.64	0.32	0.74
$2n = 34$		1	0.79	0.42	0.66	0.37	0.87
$2n = 30$			1	0.41	0.67	0.35	0.76
$2n = 44$				1	0.41	0.36	0.42
$2n = 52$					1	0.41	0.60
$2n = 32$B						1	0.38
E. fuscus							1

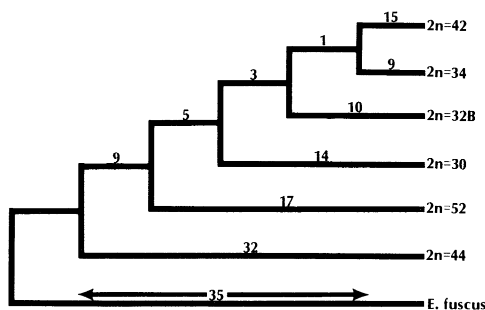

FIG. 4. Fitch-Margoliash tree for the cytotypes of *Rhogeessa* using *Eptesicus fuscus* as an outgroup. *F*-value was 3.6.

somal synapomorphies (11/1 and 15/4). Although the meiotic data for *Rhogeessa* are hypothetical, empirical data from *Mus musculus*, a species complex exhibiting a similar pattern of chromosomal variation, documents reduced fertility of F_1 hybrid males from populations with different multiple centric fusions (Capanna et al., 1977).

Second, all of the rearrangements identified by G-bands can be explained by fusions (or fissions), and it appears that karyotypic orthoselection as described by White (1975) is occurring in this group. Bickham and Baker (1977) noted from G-banding data that parsimony failed to predict the number of events required to relate standard karyotypes of the $2n = 30$ and the $2n = 34$ cytotypes. Examination of additional cytotypes by G-banding documents that this is the pattern for other cytotypes as well. Whereas, seven fusions could explain the evolution of the standard karyotypes of the $2n = 44$ cytotype to the $2n = 30$ form, nearly twice that many fusions (13) are required to explain the G-band homology. Clearly, this data set documents the potential danger of using standard karyotypic data to indicate evolutionary relationships or magnitude of chromosomal evolution between species.

Even though it may be that all $2n = 34$ populations are identical, the fact that two populations with similar standard karyotypes were so different (see $2n = 32$B and $2n = 32$N above) may indicate that other such local differences exist within the *tumida-parvula* complex. In this group of bats, any assumed homology based on standard karyotypes is at best tenuous.

Genic Variation in Rhogeessa.—Figure 3 represents the results of a locus-by-locus analysis of the allozyme data utilizing outgroup criteria. As can be seen, a clade comprised of the $2n = 30$, 34, and 42 cytotypes is formed on the basis of

TABLE 4. Hypothetical multivalent configurations that would result if normal pairing occurred in meiosis in potential F_1's between cytotypes. Numbers in parentheses are the expected fertilities if segregation from multivalents greater than three is random.

	Parental type			
	$2n = 44$	$2n = 34$	$2n = 32$B	$2n = 32$N
Parental type				
$2n = 34$	6 III 1 V chain (0.0625)			
$2n = 32$B	7 III 1 V chain (0.0625)	1 VII chain (0.0156)		
$2n = 32$N	6 III 1 VI chain (0.03125)	1 VI chain 1 V chain (0.00195)	1 V chain 1 VII chain (0.00098)	
$2n = 30$	7 III 1 VI chain (0.03125)	1 V chain 1 VII chain (0.0098)	1 V chain 1 VI ring (0.00195)	1 III (1.0)

two synapomorphic elements (no samples of 32N individuals were examined in the electrophoretic portion of our study). Further, within this clade the $2n$ = 32B, 34, and 42 cytotypes form a lineage relative to the $2n$ = 30 cytotype. This series of relationships has the following congruencies with the karyotypic data: 1) the $2n$ = 30, 32B, and 34 cytotypes form a common evolutionary clade distinct from the $2n$ = 44 sample, and 2) the $2n$ = 32B cytotype falls within a clade containing the $2n$ = 34 form.

Because the locus-by-locus analysis of our data could determine the primitive character states for only 11 of the 21 isozymes examined, (including loci monomorphic across all populations; see Table 2 and Results section) we also analyzed cytotype relationships utilizing the Fitch and Margoliash (1967) method of tree reconstruction. This method incorporates all of the data from the electrophoretic portion of our study using Rogers' *D* values (Rogers, 1972). The resulting phylogeny, Figure 4, gives a greater level of resolution in terms of dichotomous groupings. When one compares this phylogeny with that given by the outgroup method (Fig. 3), it is apparent that major incongruencies are absent. The $2n$ = 30, 32B, 34, and 42 cytotypes still form a clade relative to the $2n$ = 52 and 44 samples and *Eptesicus.* In addition, the $2n$ = 32B populations are found along the common lineage with the $2n$ = 34 and 42 cytotypes. This method also suggests an association of the $2n$ = 34 and 42 cytotype, a relationship not indicated by the outgroup method. Further, the $2n$ = 52 lineage is tied in strongly to the lineage which gave rise to all other cytotypes except $2n$ = 44. This would seem to indicate that the divergence of the lineage which gave rise to the $2n$ = 44 cytotype from the primitive $2n = 48$, $FN = 50$ stock was followed by a cladogenetic event with one lineage characterized by taxa with reduced diploid numbers (i.e., $2n$ = 30, 32, 34, and 42) and the other lineage composed of a taxon which possessed increased diploid and fundamental numbers (i.e., $2n = 52$, $FN = 52$). It is also consistent with the $2n$ = 44 cytotype being recognized as a species (*R. parvula*) distinct from the other cytotypes studied (*R. tumida* and *R. genowaysi*).

The electrophoretic findings support the conclusions generated from the chromosomal data in that allozymic differentiation is observed among the various cytotypes. Although this differentiation might indicate a level of reproductive isolation, samples from throughout the ranges of the various cytotypes are needed to substantiate this possibility. Thus, within our samples of these bats are chromosomally distinct groups which probably represent distinct biological species.

The chromosomal and allozymic data contrast with data on exomorphology and skull measurements (LaVal, 1973) where no measurements or other characters have been found to consistently distinguish *parvula, tumida,* and *genowaysi* (see fig. 6 in LaVal, 1973; and Baker, 1984). LaVal (1973) found no indication of the groupings documented by chromosomal and genic data, although he recognized extensive morphological variation and the probable polytypic nature of *R. tumida.* Additionally, in our sample of sympatric chromosomal forms (19 *tumida* with a $2n$ = 34 karyotype and 11 *genowaysi* with a $2n$ = 42), Baker (1984) found no cranial measurements that distinguish the groups as identified by chromosomes. Genically, these two chromosomal forms are distinguished by four fixed allelic differences.

It appears that *Mus musculus* and the *Rhogeessa tumida-parvula* complex are unusual among mammalian species thus far studied in their pattern of chromosomal variation and presumptive mode of speciation (White, 1978; Bickham and Baker, 1980; Capanna, 1982). Strong evidence exists suggesting that, in both groups, populations differentiated by multiple centric fusions are distinct biological species.

It is important to note that the reproductive biology of *Mus* and *Rhogeessa* are quite different. In *Mus,* individuals

mature sexually in three months and may have several litters of six or more young per year. In contrast, *Rhogeessa tumida* has a synchronized monoestrus breeding cycle, with individuals reaching sexual maturity at the age of one year and females bearing two young per year (based on observations of a colony in Belize, Tim McCarthy, pers. comm.). Such a low reproductive rate would also suggest a relatively long average life span for such a small mammal (about 5 grams as an adult). In both *Mus* and *Rhogeessa,* the chromosomal rearrangements are either the primary cause of reproductive isolation (White, 1978) or else a direct result of other biological features involved in the speciation process (Bickham and Baker, 1980). Any model that attempts to explain the mode of speciation in these two diverse mammalian taxa must be compatible with their extremely different biological characteristics.

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