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CYTOTYPES AND MORPHOMETRICS OF TWO PHYLLOSTOMATID BATS, MICRONYCTERIS HIRSUTA AND VAMPYRESSA PUSILLA

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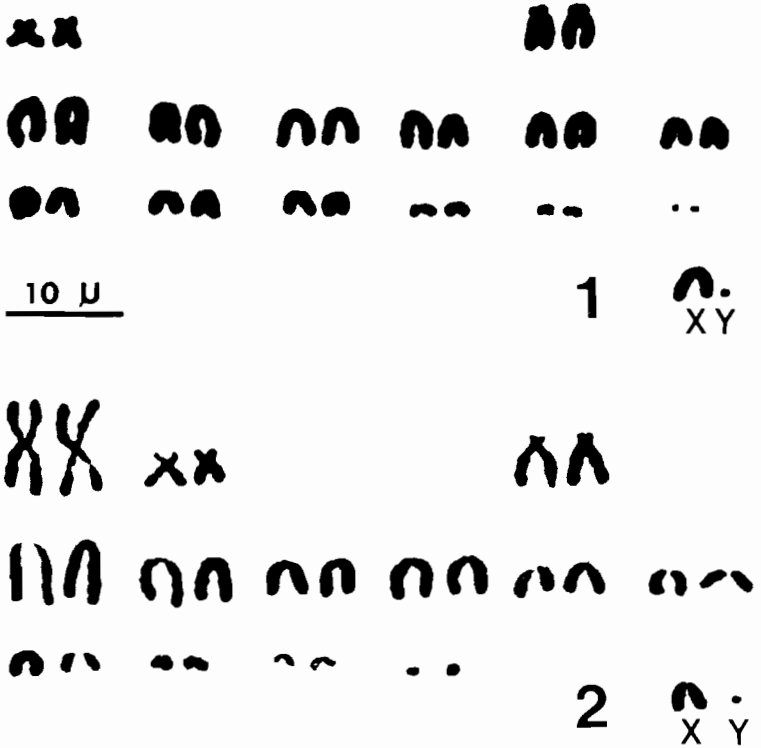
As pointed out by Jackson (1971) and Patton (1972), the key to understanding mechanisms of chromosomal evolution lies in studies of chromosomal variation within populations that can hybridize. Hybrids between cytotypes contain the answer to problems of meiotic pairing and reduced fertility, as well as information concerning the nature of zones of contact. However, before detailed studies can be initiated, chromosomal races and hybrid zones must be located. Few chromosomal races have been described for bats (Baker, 1970*a*). Within the family Vespertilionidae, five species are known to have such races (Baker, 1970*a*) and races have been reported in two species of the family Phyllostomatidae.

We here describe chromosomal races of two additional species of phyllostomatids (*Vampyressa pusilla* and *Micronycteris hirsuta*) and discuss morphological variation associated with these races.

METHODS AND MATERIALS

Specimens were collected from natural populations and karyotypic preparations were made at temporary field laboratories by *in vivo* bone marrow culture after injection with Velban (Baker, 1970*b*). At least 15 spreads were examined from each individual. Fundamental number (*FN*) is defined as the number of arms of the autosomal complement; the terms metacentric, submetacentric, subtelocentric, and acrocentric are used as defined by Patton (1967).

External and cranial measurements used in the morphometric analyses were taken as described by Baker *et al.* (1972). All statisti-



FIGS. 1-2.—Representative karyotypes of male *Micronycteris hirsuta*: 1, $2N=30$; 2, $2N=28$.

cal procedures were performed on the IBM 360-50 computer at Texas Tech University. The univariate analysis program that was used yielded standard statistics (mean, range, standard deviation, standard error of the mean, variance, and coefficient of variation), and employed a single classification analysis of variance (F-test, significance level .05) to test for significant differences between or among the means of the groups (Sokal and Rohlf, 1969). When means were found to be significantly different, the Sums of Squares Simultaneous Test Procedure (SS-STP) was used to determine the maximally nonsignificant subsets.

RESULTS

Micronycteris hirsuta

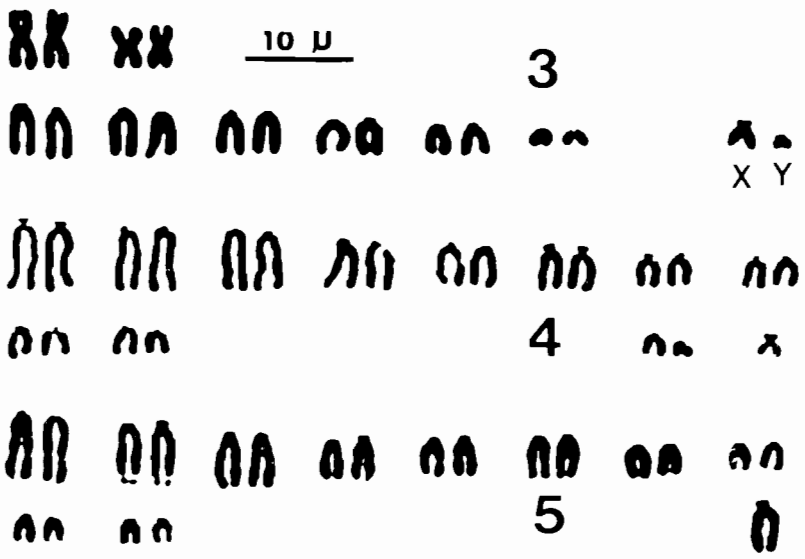
One cytotype of *M. hirsuta* has $2N=30$ and $FN=32$ (Fig. 1). The autosomes consist of one medium-sized pair of submetacentrics, one

TABLE 1.—*Standard statistics for one external and seven cranial measurements for two samples of* Micronycteris hirsuta. *Results of* F-*test* (*.05 level of significance*) *are given in right-hand column. See text for geographic origin of specimens in each sample.*

Sample	N	Mean (range) ± 2SE	CV	Significance level
		Length of forearm		
A	4	42.5(42.0-43.0) ± 0.43	1.0	*
B	5	41.1(39.6-42.5) ± 0.99	2.7	
		Greatest length of skull		
A	4	23.9(23.8-24.2) ± 0.19	0.8	*
B	5	23.2(22.8-23.5) ± 0.26	1.2	
		Condylobasal length		
A	4	20.3(19.8-20.8) ± 0.43	2.1	ns
B	5	19.8(19.4-20.2) ± 0.29	1.7	
		Zygomatic breadth		
A	4	11.5(11.3-11.7) ± 0.17	1.5	ns
B	5	11.3(10.8-11.9) ± 0.42	4.1	
		Mastoid breadth		
A	4	10.3(10.0-10.4) ± 0.19	1.9	ns
B	5	10.1(9.9-10.4) ± 0.17	1.9	
		Postorbital breadth		
A	4	5.0(4.9-5.1) ± 0.12	2.3	ns
B	5	4.8(4.6-5.0) ± 0.14	3.3	
		Length of maxillary toothrow		
A	4	9.1(8.9-9.1) ± 0.10	1.1	ns
B	5	9.0(8.6-9.3) ± 0.25	3.1	
		Breadth across upper molars		
A	4	7.4(7.3-7.5) ± 0.10	1.4	ns
B	5	7.3(7.0-7.6) ± 0.21	3.3	

pair of subtelocentrics, and 12 pairs of acrocentric elements. The X is a medium-sized acrocentric element and the Y is a minute acrocentric. Specimens having this karyotype are from Honduras and Nicaragua. The other cytotype has $2N=28$ and $FN=32$ (Fig. 2). There is one large pair and one medium-sized pair of submetacentrics, one subtelocentric pair, and 10 pairs of acrocentric elements. The X is a medium-sized acrocentric and the Y is a minute acrocentric. The specimens having this karyotype are from Trinidad.

One external and seven cranial measurements of two samples of *M. hirsuta* were tested for significant differences (Table 1). One sample consisted of four specimens from Trinidad and the other of



FIGS. 3-5—Representative karyotypes of *Vampyressa pusilla* and *Mesophylla macconnelli*: 3, male *V. pusilla*, $2N=18$; 4, male *V. pusilla*, $2N=23$; 5, male *M. macconnelli*.

five individuals from Honduras and Nicaragua. In two measurements, length of forearm and greatest length of skull, the sample from Trinidad averaged significantly larger than did the sample from Central America. In the other five cranial measurements, specimens from Trinidad averaged larger, but only slightly so. Therefore, bats from the limited samples of *M. hirsuta* can be distinguished on the basis of several characteristics.

Vampyressa pusilla

One cytotype of *V. pusilla* has $2N=18$ and $FN=20$ (Fig. 3). Two pairs of autosomes are metacentric or submetacentric and the remaining six pairs are acrocentric. The sex chromosome system appears to be the classical XX/XY. The X is a small subtelocentric element and the Y has a small second arm and is the smallest chromosome of the complement. This cytotype was found in specimens from Honduras and Nicaragua. The other cytotype of *V. pusilla* has $2N=24$ in females, and $2N=23$ in males. The FN is 22 if all autosomes are considered uniarmed. As can be seen in Fig. 4, two pairs of autosomes have a distinct but small second arm. The complement contains no metacentric or submetacentric autosomes and in males there are three chromosomes that do not resemble any other element in size

TABLE 2.—*Standard statistics for one external and eight cranial measurements for four samples of* Vampyressa pusilla. *Results of* F*-test (.05 level of significance) are given in right-hand column. See text for geographic origin of specimens in each sample.*

Sample	N	Mean (range) ± 2SE	CV	Significance level
		Length of forearm		
A	25	31.4(29.3-33.2) ± 0.42	3.3	ns
B	3	30.4(29.8-31.5) ± 1.07	3.0	
C	12	31.4(29.7-32.9) ± 0.54	3.0	
D	7	30.7(29.9-31.2) ± 0.41	1.7	
		Greatest length of skull		
A	36	18.4(17.5-19.3) ± 0.16	2.7	ns
B	4	18.3(17.9-19.0) ± 0.50	2.7	
C	14	18.5(17.7-19.1) ± 0.24	2.5	
D	7	18.3(17.8-18.6) ± 0.22	1.6	
		Zygomatic breadth		
A	32	10.7(10.0-11.3) ± 0.11	2.8	ns
B	4	10.5(9.8-10.9) ± 0.50	4.7	
C	12	10.7(10.0-11.2) ± 0.18	3.0	
D	7	10.7(10.3-10.9) ± 0.20	2.5	
		Postorbital breadth		
A	36	4.6(4.3-5.0) ± 0.05	3.3	ns
B	4	4.7(4.4-5.2) ± 0.34	7.2	
C	14	4.7(4.3-4.9) ± 0.10	4.2	
D	7	4.7(4.6-4.8) ± 0.05	1.5	
		Breadth of braincase		
A	35	8.2(7.5-8.7) ± 0.09	3.2	ns
B	4	8.3(8.0-8.7) ± 0.31	3.8	
C	14	8.1(7.7-8.5) ± 0.12	2.8	
D	7	8.2(7.7-8.4) ± 0.20	3.2	
		Mastoid breadth		
A	34	9.1(8.6-9.6) ± 0.08	2.7	ns
B	4	9.1(8.8-9.5) ± 0.29	3.2	
C	14	9.1(8.9-9.4) ± 0.08	1.7	
D	7	9.1(8.8-9.4) ± 0.15	2.2	
		Length of maxillary toothrow		
A	36	5.8(5.4-6.3) ± 0.07	3.5	ns
B	4	5.6(5.5-5.8) ± 0.13	2.2	
C	14	5.9(5.4-6.3) ± 0.15	4.8	
D	7	5.8(5.7-6.1) ± 0.12	2.8	
		Palatal length		
A	35	8.3(7.7-9.0) ± 0.12	4.1	ns
B	4	8.3(7.7-8.6) ± 0.39	4.7	
C	14	8.6(8.0-9.4) ± 0.20	4.4	
D	7	8.4(7.9-8.8) ± 0.26	4.2	

TABLE 2.—*Continued.*

		Breadth across upper molars		
A	36	7.7(7.1-8.3) ± 0.08	3.1	ns
B	4	7.6(7.5-7.7) ± 0.12	1.5	
C	14	7.8(7.0-8.3) ± 0.20	4.8	
D	6	7.7(7.5-8.0) ± 0.14	2.3	

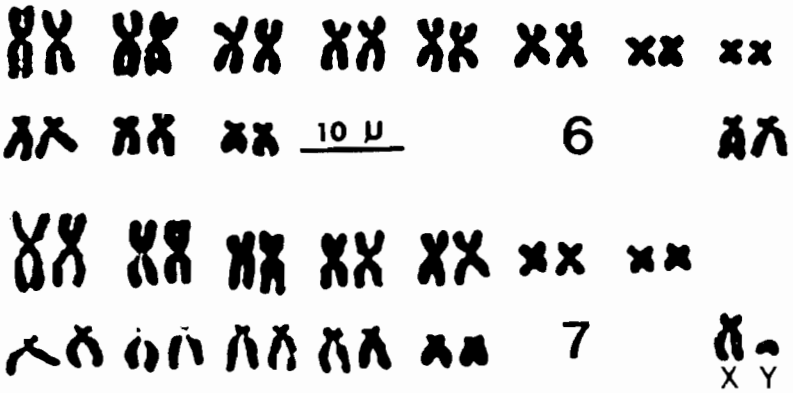
or morphology. Clearly, the sex-determining mechanism in this cytotype is not XX/XY. Specimens having this cytotype are from Colombia.

One external and eight cranial measurements of four samples of *V. pusilla* were tested for significant differences (Table 2). The samples consisted of specimens from South America (A), the Darién of Panamá (B), central and western Panamá (C), and Nicaragua (D). No significant differences were found among the means of these samples for any of the nine measurements (Table 2). In four measurements (greatest length of skull, length of maxillary toothrow, palatal length, and breadth across upper molars), specimens from central and western Panamá had the largest mean value. Specimens from the Darién of Panamá had the largest mean value for breadth of braincase. Two or more samples shared the largest mean value for the remaining four measurements; in fact, all four samples had the same mean value for mastoid breadth. These data indicate no demonstrable differences between individuals in these samples on the basis of the morphometric data employed. We have examined carefully external and cranial features of these specimens for other morphometric and qualitative characters that might be useful in distinguishing them but have found none.

DISCUSSION

The chromosomal races of *Micronycteris hirsuta* can be derived by a single centric fusion-fission involving two large pairs of acrocentrics and a pair of metacentrics. Such chromosomal alterations do not generally interfere with meiosis and should not result in a reduction in fertility, at least from the standpoint of chromosomal homology.

The origin of the races of *Vampyressa pusilla* is less obvious. The most parsimonious routes would require three events to derive the diploid numbers in females (18 $\longleftrightarrow$ 24) and one of these must alter the sex determining system. It would seem likely that such chromosomal divergence would be accompanied by demonstrable morphological divergence. If this has been the case, we have been unable to detect it.



FIGS. 6-7.—Representative karyotypes of *Vampyressa*: 6, female *V. brocki*; 7, male *V. nymphaea*.

When the diploid numbers and fundamental numbers of *V. pusilla* are compared with the range of values of the other stenodermine species that have been studied (Baker, 1973), the diploid number (of the Central American cytotype) and the fundamental number (of the South American cytotype) are the lowest found in the subfamily. The nearest diploid values are found in *Mesophylla macconnelli* ($2N=21$ in males, 22 in females, Fig. 5), *Vampyressa brocki* ($2N=24$, Fig. 6), *Vampyressa nymphaea* ($2N=26$, Fig. 7), and species of *Chiroderma* ($2N=26$). The only stenodermine species that has a fundamental number approximating that of *V. pusilla* ($FN=20$ and 22) is *M. macconnelli* ($FN=24$).

Baker (1973) hypothesized that the primitive karyotype of stenodermines had a diploid number of 30 or 32 and a fundamental number or 56 or 60. He suggested that the genera *Chiroderma*, *Vampyressa*, *Mesophylla*, and *Ectophylla* form a line of evolution within the stenodermines, with *V. pusilla* and *M. macconnelli* being the most derived (from a karyotypic standpoint) species. The South American cytotype of *V. pusilla* and the karyotype of *M. macconnelli* both have the same type of sex-determining system. The diploid number of the male is one less than that of the female. This system was probably derived by translocating the Y chromosome to one of the autosomes. A similar system is found in a species of mongoose, *Herpestes auropunctatus* (Fredga, 1964). This system probably is derived (rather than primitive) relative to that of *Chiroderma*, *V. nymphaea*, and *V. pusilla* (Central American cytotype), which have the classical XX/XY. If the most parsimonious routes are taken, *M. macconnelli* and the South

American cytotype of *V. pusilla* evolved from a common ancestor after diverging from a *V. pusilla* stock having the Central American cytotype. However, the degree of divergence between *V. pusilla* and *M. macconnelli* in cranial morphology and dental features would argue against such evolutionary affinities. Therefore, we feel the most logical explanation is that *V. pusilla* and *M. macconnelli* evolved from a common stock that had the derived six-determining system (where the Y has probably been translocated to an autosome), and that the Central American cytotype has undergone additional alterations that have resulted in a sex-determining system appearing as the classical XX/XY.

Even if the details of the above relationships are incorrect, it is highly probable that *V. pusilla* and *M. macconnelli* are more closely related to each other than *V. pusilla* is to either *V. nymphaea* or *V. brocki*. A close relationship between *V. pusilla* and *M. macconnelli* based on cranial and dental features has been suggested by Starrett and Casebeer (1968).

An interesting aspect of chromosomal variation in the two species studied is that there is greater morphological divergence in the one having the least chromosomal divergence. Even if morphological divergence within *M. hirsuta* is no more than would normally be expected within a single species, the lack of morphological divergence between the two cytotypes of *V. pusilla* is unique. In fact, the degree of divergence of *V. pusilla* cytotypes is greater than is generally characteristic of closely related species of bats, and it is possible that these chromosomal differences signal the completion of speciation. On the other hand, we can find no geographic variation in cranial and external characters that would justify even the recognition of subspecies of *V. pusilla*. If chromosomal analysis demonstrates genetic divergence of the two cytotypes, then recognition of subspecies using chromosomal characters would be justified. To do so, however, would raise a problem as to the karyotype of the holotypes and the allocation of museum material to the correct subspecific taxa, because subspecies cannot presently be recognized on the basis of gross morphology. We suggest that the species be considered monotypic until additional data are available.

Specimens Examined

Specimens examined are housed in the National Museum of Natural History (USNM), the Museum of Natural History, The University of Kansas (KU), and The Museum, Texas Tech University (TTU). Specimens used for karyotypic and morphometric studies are listed separately.

Specimens Examined for Karyotypic Studies

Micronycteris hirsuta.—TRINIDAD: Blanchisseuse, 4 (TTU). HONDURAS: 10.3 mi. SSW Dulce Nombre de Culmí, Olancho, 2 (TTU). NICARAGUA: 5 mi. N and 1 mi. W San Juan del Sur, Rivas, 3 (TTU); 9 mi. E Rama at Dos Bocas, Zelaya, 2 (TTU).

Vampyressa pusilla.—COLOMBIA: Restrepo, Upin Salt Mine, Meta, 7 (TTU); Leticia, Amazonas, 2 (TTU). HONDURAS: 10.3 mi. SSW Dulce Nombre de Culmí, Olancho, 3 (TTU). NICARAGUA: 3 km. NW Rama, Zelaya, 1 (TTU); 4.5 km. NW Rama, Zelaya, 5 (TTU); 9 mi. E Rama at Dos Bocas, Zelaya, 2 (TTU). COSTA RICA: Cariblanco, Alajuela, 2 (TTU).

Mesophylla macconnelli.—COLOMBIA: Leticia, Amazonas, 3 (TTU); Restrepo, Upin Salt Mine, Meta, 2 (TTU). TRINIDAD: various localties, 22 (TTU).

Vampryessa brocki.—COLOMBIA: Leticia, Amazonas, 3 (TTU).

Vampryessa nymphaea.—NICARAGUA: 3 km. NW Rama, Zelaya, 3 (TTU); 9 mi. E Rama at Dos Bocas, Zelaya, 2 (TTU).

Specimens Examined for Morphometric Studies

Micronycteris hirsuta.—*Sample A.* TRINIDAD: Blanchisseuse, 1 (TTU); Guayaguayare, 2 (TTU); Las Cueves, 1 (TTU). *Sample B.* HONDURAS: 10.3 mi. by rd. SSW Dulce Nombre de Culmí, Olancho, 1 (TTU). NICARAGUA: 5 mi. N, 1 mi. W San Juan del Sur, Rivas, 2 (TTU); 3 km. NW Rama, Zelaya, 1 (TTU); 9 mi. E Rama at Dos Bocas, Zelaya, 1 (TTU).

Vampyressa pusilla.—*Sample A.* COLOMBIA: Leticia, Amazonas, 2 (TTU); Santa Marta, Magdalena, 1 (USNM); Restrepo, Upin Salt Mine, Meta, 8 (TTU). ECUADOR: Río Bobonaza, Canelos, 1 (USNM). VENEZUELA: 2 km. SW Altamira, Barinas, 2 (USNM); Altamira, Barinas, 10 (USNM); La Copa, 4 km. NW Montalban, Carabobo, 12 (USNM). *Sample B.* PANAMA: Tacarcuna Village Camp, Darién, 4 (USNM). *Sample C.* CANAL ZONE: Barro Colorado Island, 2 (USNM). PANAMA: Río Changena, Bocas del Toro, 1 (USNM); Sibube, Bocas del Toro, 1 (USNM); Cerro Hoya, Los Santos, 9 (USNM); Caña, 2 (USNM). *Sample D.* NICARAGUA: 17 km. N, 15 km. E Boaco, Santo Rosa, 5 (KU); 2 km. N, 6 km. E Esquipulas, Matagalpa, 2 (KU).

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