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Sex Chromosomes in Teiid Whiptail Lizards (Genus *Cnemidophorus*)

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INTRODUCTION

Morphologically recognizable sex-correlated chromosomes have been demonstrated recently in iguanid lizards. Gorman and Atkins ("1966" [1967], 1968) reported them for species of *Anolis,* and Cole, Lowe, and Wright (1967) reported them for *Sceloporus.* In both of these iguanid genera, however, the majority of the species analyzed lack readily recognizable heteromorphic "pairs" of chromosomes. The present paper reports an X-Y [XY(♂):XX(♀)] sex chromosome mechanism in teiid lizards of the genus *Cnemidophorus* (*C. tigris*), most species of which lack readily recognizable heteromorphic pairs of chromosomes.

METHODS AND MATERIALS

Chromosomes of bone marrow cells were examined on slides prepared by means of Patton's (1967) hypotonic citrate, air-dried technique, slightly modified (Lowe, Wright, and Cole, 1966). A total of approximately 159 cells from 16 individuals of *C. tigris* (13 males and three

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females) were examined. Each lizard utilized is catalogued in the herpetological collection at the University of Arizona (U.A.Z. Nos. 18122, 18506, 18546, 18554, 18749, 18752, 18970, 20412, 21645, 21857, 25239–25242, 25432, and 25433).

The particular chromosomes considered in the present paper are those of Set I (following Lowe and Wright, 1966a, 1966b) occurring in *C. tigris,* as the heteromorphic pair is a member of this Set. The Set I chromosomes (nos. 1–3) are quickly recognized as the very largest in the karyotype; they are metacentric or submetacentric. Also, within Set I each chromosome is accurately and individually recognized as follows (fig. 7): number 1, clearly the largest chromosome, metacentric to submetacentric; number 2, clearly the second largest chromosome, metacentric to submetacentric, and with a terminal satellite (marker) on one arm; number 3, clearly the smallest of the three, submetacentric.

As the heteromorphism in the number 3 chromosomes of males results from a difference in arm ratios, it may not be obvious to those investigators who are not cytologically oriented and familiar with variation in chromosome lengths that appear in cell spreads. Furthermore, many cytologists have reported findings similar to those presented here, without describing pertinent details of precisely how their conclusions were reached; consequently, in many cases, their readers cannot have a high level of confidence in the reported results. We, therefore, in addition to examining 159 cells from 16 individuals, have quantified particular data and treated them statistically, using techniques similar to those of Rothfels and Siminovitch (1958) and Patau (1960). Our detailed analyses were carried forward with the subspecies *C. tigris maximus* (see Lowe and Asplund, In press).

Ten cells from a male and 10 from a female were selected and photographed. Selection of each cell was based only on the measurability of the Set I chromosomes, and not on the degree of heteromorphism or homomorphism. Such a selection results in an analysis that includes variation in appearance of the chromosomes (due to differential coiling and artifacts in preparation, as well as heteromorphism), so that the results are least likely to describe the differences as being greater than they actually are. Indeed, the results may thus describe the differences as being smaller than they actually are. We emphasize that if we had selected cells on the basis of the heteromorphic or homomorphic appearance of the Set I chromosomes (particularly number 3), the results of our statistical analyses would have been at significantly higher confidence levels, which could have been misleading in regard to the variation that actually occurs in the morphological appearance of chromosomes.

Each Set I chromosome was measured (to the nearest tenth of a millimeter) on a printed enlargement of each of the 20 cells with vernier calipers. Both chromatids of each arm were measured, and the mean was taken to represent the length of that arm; the centromere (all chromosomes) and the satellite and secondary constriction (number 2 chromosomes) were excluded from measurements because of their variability in stretching.

The measurements for each cell were standardized so that they would be comparable. The total length of all Set I chromosome arms was determined for each cell. The measurement of each arm of each chromosome was converted to its percentage of the total Set I length. The total length of each chromosome is the sum of these converted values. These are the length values referred to throughout the present paper, unless stated otherwise.

The procedure of converting the measurement of a particular chromosome arm into an expression of its percentage length of the total complement is necessary if the arm lengths are to be comparable from cell to cell (within and between each sex). This common practice by cytogeneticists results in tertiary data that must be used if statistical tests are to be subsequently made. Nevertheless, such tests should provide a reasonable approximation of the relative mean distributions of the parameters, provided that their distributions are normal. Moreover, it should be noted that detailed quantification serves, in the present case (tables 1 and 2; figs. 1–6), to verify the heteromorphism that is already discernible by the usual "qualitative" methods (fig. 7).

For the present problem we used one additional procedure to increase our confidence; we biased our data ("weighted") against our hypothesis. As stated above, the hypothesis of heteromorphism was arrived at while critically examining cells with the microscope, before the photographs and measurements were made. The hypothesis was further supported after the photographs were printed, for each author examined the 20 photographs without checking their labels and accurately sorted them into two separate piles of 10 each, based on a comparison by eye of the two number 3 chromosomes in each cell. In this manner we were able to recognize the "odd" number 3 chromosome (the Y) of the male because of its apparently low arm ratio (length of long arm/length of short arm) relative to that of the other number 3 chromosome in the same cell. Because we could recognize the difference between the number 3 chromosomes in males, our measurements for them were recorded as pertaining to either the 3*A* or the 3*B* chromosome. The number "3" designates the individual chromosome (no. 3) and the letter (*A* or

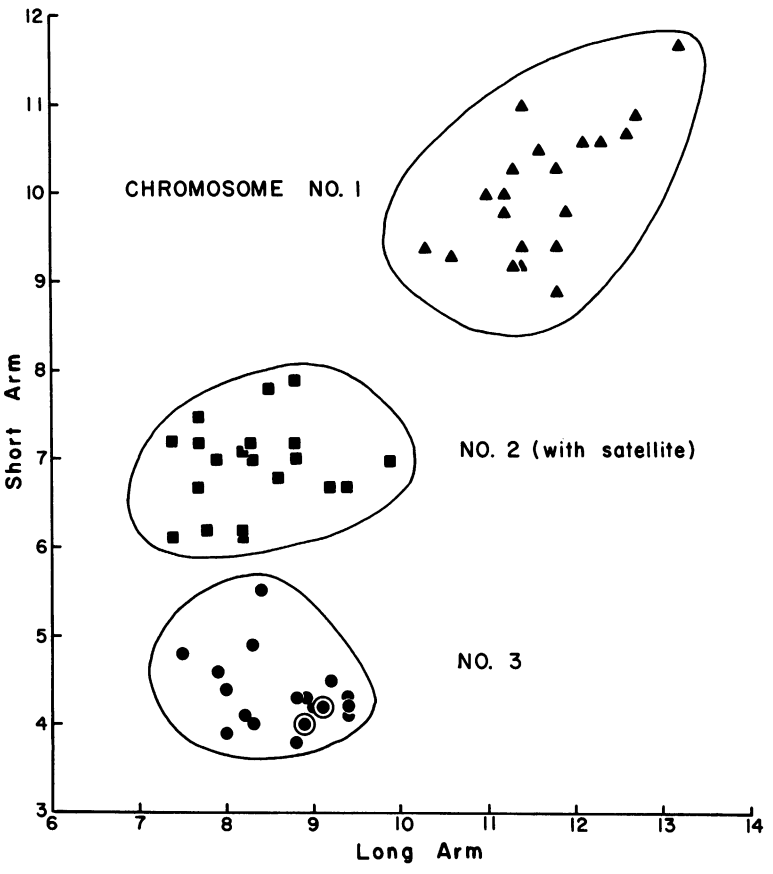

FIG. 1. Karyogram of the Set I chromosomes of 10 cells from a female of *Cnemidophorus tigris maximus*. Length (axes) is expressed as a percentage of the total length of the Set I complement, calculated independently for each cell. Note that chromosome no. 3 forms a distinct group as do nos. 1 and 2.

B) designates the relative arm ratio; *A* is the chromosome with the higher ratio (X), and *B* is the chromosome with the lower ratio (Y). We followed the same procedure in recording the measurements for the other chromosomes in each cell, thus "weighting" the data to produce a tendency to indicate heteromorphism in each pair of each sex. Thus we performed the statistical analyses comparing the chromosomes within each sex with this "ratio weighting factor" operating against our hypothesis.

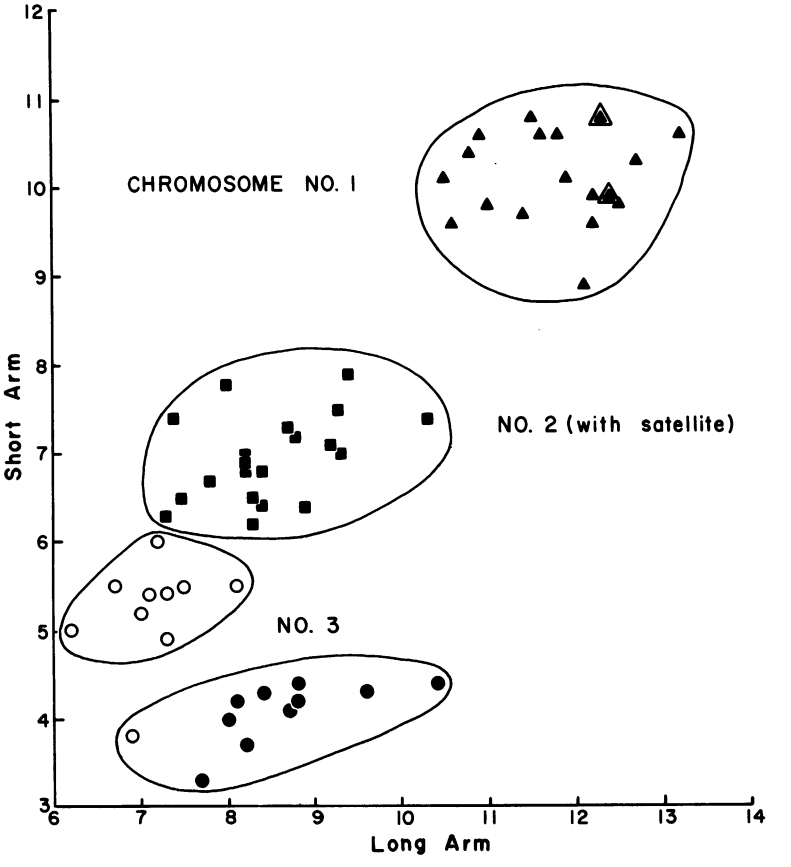

FIG. 2. Karyogram of the Set I chromosomes of 10 cells from a male of *Cnemidophorus tigris maximus*. Length (axes) as in figure 1. Note that chromosome no. 3 tends to fall into two groups and nos. 1 and 2 are grouped as in the female (fig. 1).

RESULTS AND CONCLUSIONS

A partial karyogram (Patau, 1960) illustrating the Set I chromosomes of the 10 female cells combined (fig. 1) demonstrates the ease with which the chromosomes can be individually recognized in *C. t. maximus*. The number 1 and number 2 chromosomes are unquestionably distinct. The number 2 and number 3 chromosomes also separate out nicely, although one number 3 is in an intermediate position. There is no question as to the proper identification of this individual, for the two

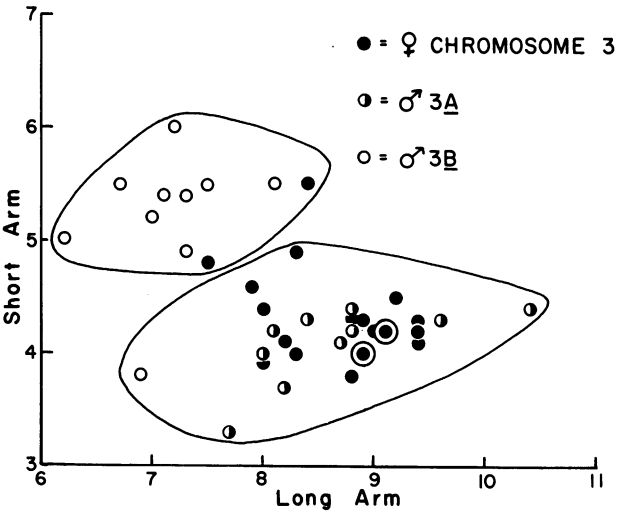

FIG. 3. Karyogram of the no. 3 chromosomes of 10 cells from a male *Cnemidophorus t. maximus* and 10 cells from a female (same individuals plotted in figs. 1, 2). Note that the male 3*B* chromosomes tend to form a group of their own, which is verified by statistical analysis (see text).

number 2 chromosomes in the same cell are easily recognized by their secondary constrictions and satellites.

A partial karyogram illustrating the Set I chromosomes of the 10 male cells combined (fig. 2) similarly shows the ease with which the number 1 chromosomes are distinguished from the number 2 chromosomes, and indicates that both the number 1 and number 2 chromosomes are grouped similarly in both sexes (compare figs. 1 and 2). In the males, however, chromosome number 3 (fig. 2) separates nicely into two groups, with only one "mistake" (5%) among the 20 points plotted.[1]

A partial karyogram illustrating only the number 3 chromosomes of both sexes together (fig. 3) also shows the tendency for two groups, with only three "mistakes" (7.5%) among the 40 points plotted. "Mistakes" on the magnitude of 7.5 per cent in the karyogram are not surprising, because the groups are delimited somewhat arbitrarily; nor are they disturbing, as the apparent dichotomy is verified by the statistical analyses discussed below.

Considering that the data for each chromosome in each cell were re-

[1] We emphasize that such "mistakes" do occur in random samples of cells, and that it is a random sample which is being reported here.

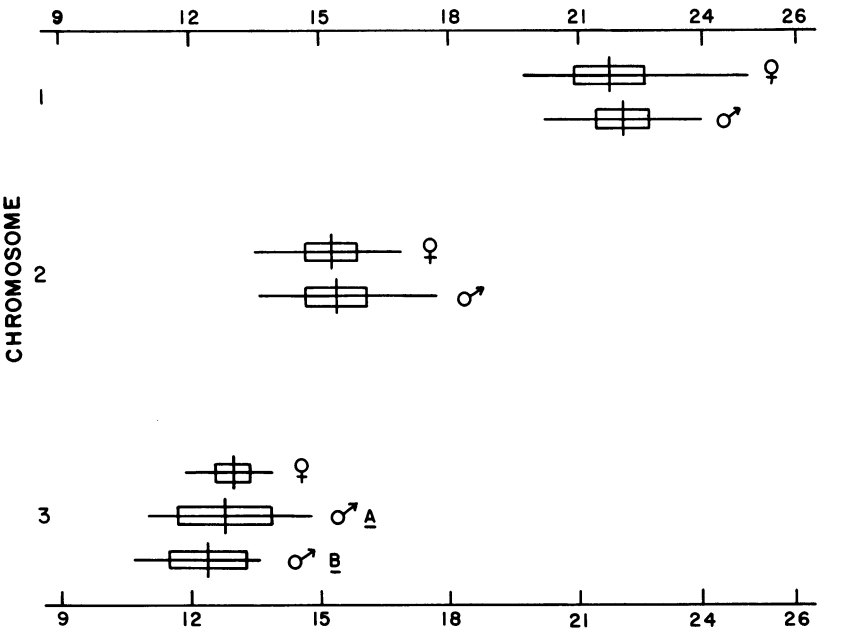

FIG. 4. Comparison of the total lengths of the Set I chromosomes of 10 cells from a male *Cnemidophorus t. maximus* and 10 cells from a female (same individuals plotted in figs. 1–3). Horizontal line designates range, vertical line designates mean, and rectangle represents 99% confidence interval. Note no significant difference between sexes for any particular pair and no heteromorphism in no. 3 of males (see table 2).

corded in a paired fashion (the *A* chromosome of each homologous pair being the one with the highest arm ratio), all the cells for a given sex were analyzed by means of paired comparison *t*-tests (see Simpson, Roe, and Lewontin, 1960). The conclusions indicated by these tests (table 1) are that at the 99 per cent confidence level there is no significant difference in the total length of the *A* and *B* chromosomes of number 3 in males, whereas there is a highly significant difference in both the length of the long arm and the length of the short arm of these same chromosomes (table 1, last column). Furthermore, there is no significant difference at the 99 per cent level between the *A* and *B* chromosomes of numbers 1, 2, and 3 in females and numbers 1 and 2 in males for any of the characteristics tested.

The paired-comparison *t*-test could not be used for comparing the chromosomes between sexes because the observations are not paired for

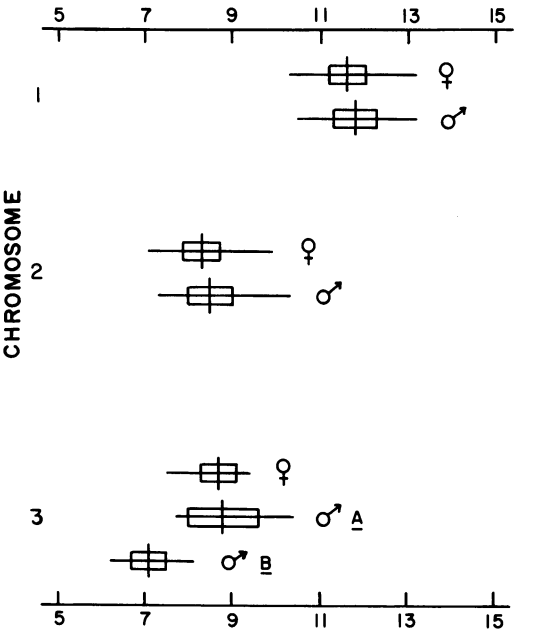

FIG. 5. Comparison of the lengths of the long arms of the Set I chromosomes of *Cnemidophorus t. maximus.* Individuals and symbols are the same as in fig. 4. Note that the male 3*A* chromosome is indistinguishable from the female no. 3 (=X), whereas the male 3*B* (=Y) has a significantly shorter long arm (see table 2).

such an analysis. Therefore, we used Student's *t*-tests (see Simpson, Roe, and Lewontin, 1960) for these comparisons. Since we concluded from the comparisons within each sex (above) that the female *A* and *B* chromosomes of each pair were homomorphic for each characteristic, these data were pooled for each chromosome. The data for the *A* and *B* chromosomes were pooled also for the homomorphic male pairs (nos. 1 and 2). The results of these analyses (figs. 4–6; table 2) indicate the following at the 99 per cent confidence level: (1) chromosome 1 is the same for each characteristic in both sexes; (2) chromosome 2 is the same for each characteristic in both sexes; (3) chromosome 3 is the same total length in females as it is in males (both *A* and *B*; fig. 4; table 2); (4) chromosome 3*A* of males has a long arm the same length as that in chromosome 3 of females, whereas 3*B* of males has a shorter long arm (fig. 5; table 2); and (5) chromosome 3*A* of males has a short arm the same length as that in chromosome 3 of females, whereas 3*B* of males has a longer short arm (fig. 6; table 2).

TABLE 1

RESULTS OF PAIRED COMPARISON *t*-TESTS USED TO ANALYZE EACH PAIR OF SET I CHROMOSOMES[a] WITHIN BOTH SEXES OF *Cnemidophorus tigris maximus*

Chromosomes / Characteristics	Females: 1*A* compared to 1*B* ($n = 9$)[b]	Females: 2*A* compared to 2*B* ($n = 9$)	Females: 3*A* compared to 3*B* ($n = 9$)	Males: 1*A* compared to 1*B* ($n = 9$)	Males: 2*A* compared to 2*B* ($n = 9$)	Males: 3*A* compared to 3*B* ($n = 9$)
Total Length	$t = -0.48$	$t = 1.16$	$t = -0.16$	$t = -0.84$	$t = 1.07$	$t = 1.95$
	$0.7 > P > 0.6$	$0.3 > P > 0.2$	$0.9 > P > 0.8$	$0.5 > P > 0.4$	$0.4 > P > 0.3$	$0.1 > P > 0.05$
Long Arm Length	$t = 0.60$	$t = 2.66$	$t = 1.19$	$t = 0.42$	$t = 2.56$	$t = 7.10$
	$0.6 > P > 0.5$	$0.05 > P > 0.02$	$0.3 > P > 0.2$	$0.7 > P > 0.6$	$0.05 > P > 0.02$	$P < 0.001$
Short Arm Length	$t = -1.49$	$t = -2.02$	$t = -2.42$	$t = -2.62$	$t = -0.46$	$t = -8.97$
	$0.2 > P > 0.1$	$0.1 > P > 0.05$	$0.05 > P > 0.02$	$0.05 > P > 0.02$	$0.7 > P > 0.6$	$P < 0.001$

[a] The *A* chromosome of each pair is that with the highest arm ratio (long arm/short arm). Note the highly significant differences (99% confidence level) in the long arm and short arm lengths of the male 3*A* (=X) and 3*B* (=Y) chromosomes, whereas their total lengths are not significantly different.

[b] n = degrees of freedom.

TABLE 2

RESULTS OF STUDENT'S *T*-TESTS USED TO ANALYZE EACH PAIR OF SET I CHROMOSOMES BETWEEN BOTH SEXES OF *Cnemidophorus tigris maximus* AND THE NUMBER 3 CHROMOSOMES WITHIN MALES[a]

Characteristics	♀Chromosome #1 (N = 20)[b]	♂Chromosome #1 (N = 20)	♀Chromosome #2 (N = 20)	♂Chromosome #2 (N = 20)	♀Chromosome #3 (N = 20)	♂Chromosome #3*A* (N = 10)	♂Chromosome #3*B* (N = 10)
Total Length	21.7 ± 0.28	22.0 ± 0.20	15.3 ± 0.20	15.4 ± 0.24	13.0 ± 0.14	12.8 ± 0.33	12.4 ± 0.28
	$t = -0.83$		$t = -0.32$		$t = 0.69$		
	$0.5 > P > 0.4$		$0.8 > P > 0.7$		$0.5 > P > 0.4$		
					$t = 2.28$		
					$0.05 > P > 0.02$		
						$t = 0.97$	
						$0.4 > P > 0.3$	
Long Arm Length	11.6 ± 0.14	11.8 ± 0.17	8.3 ± 0.14	8.5 ± 0.17	8.7 ± 0.14	8.8 ± 0.24	7.1 ± 0.14
	$t = -0.85$		$t = -0.89$		$t = -0.41$		
	$P \approx 0.4$		$0.4 > P > 0.3$		$0.7 > P > 0.6$		
					$t = 7.98$		
					$P < 0.001$		
						$t = 6.35$	
						$P < 0.001$	

TABLE 2 (*Continued*)

Characteristics	♀Chromosome #1 (N = 20)[b]	♂Chromosome #1 (N = 20)	♀Chromosome #2 (N = 20)	♂Chromosome #2 (N = 20)	♀Chromosome #3 (N = 20)	♂ Chromosome #3*A* (N = 10)	♂Chromosome #3*B* (N = 10)
Short Arm Length	10.0 ± 0.17	10.1 ± 0.10	6.9 ± 0.14	7.0 ± 0.10	4.3 ± 0.10	4.1 ± 0.10	5.2 ± 0.17
		$t = -0.50$		$t = -0.63$		$t = 1.26$	
		$0.7 > P > 0.6$		$0.6 > P > 0.5$		$0.3 > P > 0.2$	
						$t = -4.83$	
						$P < 0.001$	
							$t = -5.81$
							$P < 0.001$

[a] For male chromosome no. 3, the *A* chromosome is that with the highest arm ratio (long arm/short arm). Note the highly significant differences (99% confidence level) in the long arm and short arm lengths of the male 3*B* (=Y) and the female 3 (=X) chromosomes, whereas the male 3*A* (=X) chromosome is not significantly different from the female 3 (=X) chromosome.

[b] N = sample size.

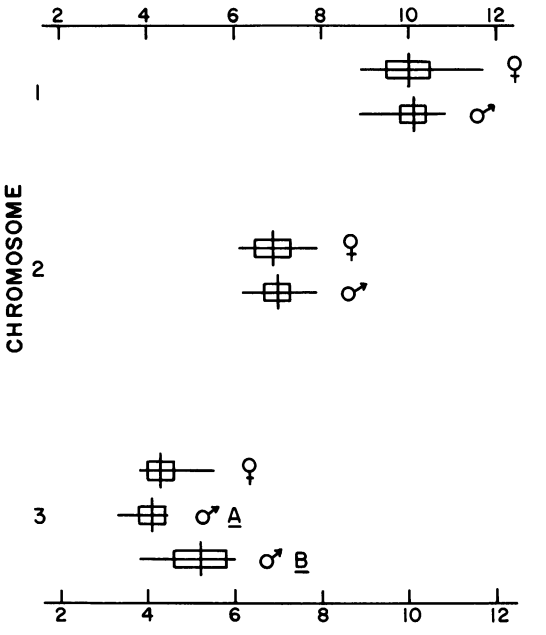

FIG. 6. Comparison of lengths of the short arms of the Set I chromosomes of *Cnemidophorus t. maximus.* Individuals and symbols are the same as in fig. 4. Note that the male 3*A* chromosome is indistinguishable from the female no. 3 (=X), whereas the male 3*B* (=Y) has a significantly longer short arm (see table 2).

Because the 3*A* chromosome in males is morphologically the same as both number 3 chromosomes in females, and the 3*B* chromosome in males is different, the male is the chromosomally heteromorphic sex and we designate this as an XY(♂):XX(♀) system (fig. 7). Furthermore, as the X and Y are of the same length and they differ in that the Y has a relatively shorter long arm and a relatively longer short arm, we suggest that this difference in centromere position results from an unequal pericentric inversion. This inversion may have occurred rather recently and probably the primitive condition is that which is found in females today, as most species of *Cnemidophorus* lack such heteromorphic pairs of chromosomes.

The heteromorphic chromosomes are not peculiar to *Cnemidophorus tigris maximus,* on which the measurements and analyses are based. Indeed, the heteromorphism is evident in specimens of *C. tigris* from localities widely scattered throughout the range of the species (fig. 7). This, together with the apparent absence of readily recognizable sex chromo-

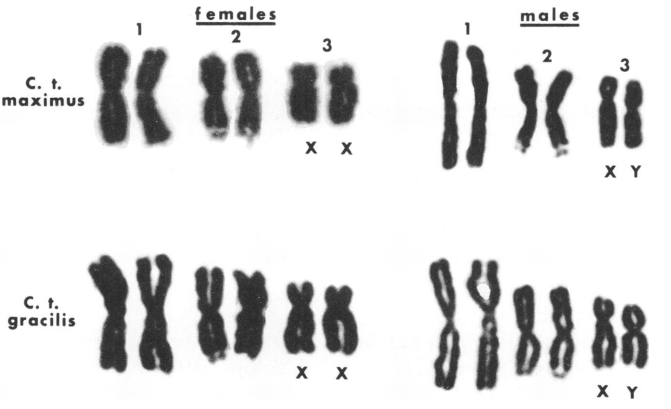

FIG. 7. "Pasties" of the Set I chromosomes (nos. 1–3) from two females and two males of *Cnemidophorus tigris,* representing two subspecies. Note heteromorphism in position of the centromere in the male pair no. 3. Specimens illustrated are U.A.Z. Nos. 18970 and 21857 (*maximus*), and 20412 and 25239 (*gracilis*).

somes in most of the species of the genus, constitutes additional evidence for the close relationship of *maximus* and *tigris,* as reported by Lowe and Asplund (In press).

The sex chromosome of *Cnemidophorus tigris* (specifically, the X) can be identified in the karyotypes of the all-female parthenogenetic species that originated by means of hybridization between *C. tigris* and other species (Lowe and Wright, 1966a, 1966b), and presently this factor is playing a large role in the investigation of the origins of these species and the mode of sex determination in *Cnemidophorus.* For example, cells of a diploid parthenogenetic species, *Cnemidophorus neomexicanus,* possess the X chromosome of *C. tigris,* which is evident in the illustration of its karyotype presented by Lowe and Wright (1966b, p. 83, fig. 2). Also illustrated in the same figure is the haploid karyotype of the two parental species, *C. tigris* and *C. inornatus;* in the haploid karyotype of *C. tigris* the Y chromosome was inadvertently illustrated instead of the X. In another example (Lowe, Wright, Cole, and Bezy, In press), the Y chromosome of *C. tigris* is clearly recognizable in a tetraploid male individual that resulted from hybridization between *C. tigris* (diploid and bisexual) and *C. sonorae* (triploid and unisexual).

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