1973

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The primary objectives of this study were: (1) to provide quantitative descriptions of the chromosomes of the domestic swine *(Sus scrofa:* 2n=38), the European wild swine (2n=36) and the domestic-European wild hybrid (2n=37); and (2) to define chromosomal differences between the three karyotypes.

The diploid (2n) chromosome number of the domestic swine has been shown to be 38 (Ruddle, 1961; Makina *et al.*, 1962; McConnell *et al.*, 1963). The European wild swine of the Tellico Wildlife Management Area of Tennessee has been shown to have a diploid chromosome number of 36 (Rary *et al.*, 1968). European wild swine with 36 chromosomes crossed with 38 chromosome domestic swine have produced fertile 37 chromosome offspring (UT-AEC, 1967 unpublished data).

McConnell *et al.*, (1963) and Ruddle (1965) have quantitatively analyzed the somatic chromosomes from a small number of cells of the domestic swine (2n=38). McConnell utilized chromosome measurements taken from a total of 21 cells, 13 female and 8 male, from the second passage of pig kidney tissue cultures.

Establishment of a "definition" of a karyotype of a particular species provides a normal or control for such studies as quantitative analysis of chromosome breakage by ionizing radiations and other cytogenetic studies which would directly involve chromosome morphology. Establishment of a "definition" of human karyotype, for example, has proved to be of great value in analysis of many "diseases" and abnormalities that are directly related to chromosome abnormalities (Chu, 1964; Hall, 1964).

MATERIALS AND METHODS

The strain of domestic swine used in this study was the Pitman-Moore (Weaver and McKean, 1965) miniature which were selected for use from the University of Tennessee Agricultural Research Laboratory herd in Oak Ridge, Tennessee. The European wild swine were trapped in the Tellico Wildlife Management Area of Eastern Tennessee (Rary *et al.*, 1968). The European

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wild-domestic hybrids were selected from the progeny of planned matings between domestic and European wild swine.

A variation of the leukocyte culture technique of Moorhead *et al.*, (1960) was used to obtain suitable metaphase spreads for karyotype preparation. The culture medium consisted of the following ingredients: 7.4 ml of Medium 199 (Hyland; Grand Island), 2.0 ml of fetal calf serum, 0.2 ml of saline contain 2,000 units of penicillin and 2,000 mcg of streptomycin, and 0.4 ml of phytohemagglutinin (Nowell, 1960). Blood samples were drawn from the external jugular vein of the swine by means of heparinized syringes with 18 gauge, 1½ inch needles. One ml of the whole blood was placed in 3-oz prescription bottles which contained 10 ml of the prepared culture media. The cultures were then placed, flat side down, in an incubator (at 37°C) and left undisturbed for the desired incubation time. Colchicine (0.11 ml of 10-4 molar in Medium 199) was added to each culture 3 to 4 hours prior to the metaphase harvest time. Photomicrographs of metaphase spreads and cells were taken by means of a microscope camera attachment, utilizing a 12.5X ocular and a 100X oil-immersion objective. The film used was 4 x 5 inch Kodak contrast process ortho. Karyotypes were prepared from 8 x 10 inch enlargements by cutting out the individual chromosomes, placing apparent homologous pairs together and pasting them on white cardboard in an appropriate order.

Two hundred metaphase figures, suitable for karyotype analysis, were selected, photographed and karyotypes prepared from each print. The 200 included: 10 karyotypes from each of 5 female and 5 male domestic swine (2n=38); 5 from each of 5 female and 5 male European wild swine (2n=36); 5 from each of 5 female and 5 male European wild-domestic hybrids (2n=37). Each arm and each chromatid of the metaphase chromosomes were measured with a digital printing-measuring device located at the Oak Ridge National Laboratory (courtesy of Dr. M. A. Bender).

RESULTS AND DISCUSSION

No standard system for the arrangement of chromosomes within karyotypes of domestic animals has been set forth. A system was therefore needed to facilitate the identification of chromosomes for preparation of karyotypes to be utilized in the measurement and quantitative analysis of individual chromosomes. The following system was utilized for preparation of the domestic swine (2n=38) karyotypes. The chromosomes making up the karyotype were placed in four groups excluding the sex chromosomes. These groups were arranged visually by centromere location with no reference to quantitative data. Within groups the chromosomes were arranged according to decreasing length, from left to right. Figure 1 illustrates the groups of chromosomes as arranged within a typical karyotype.

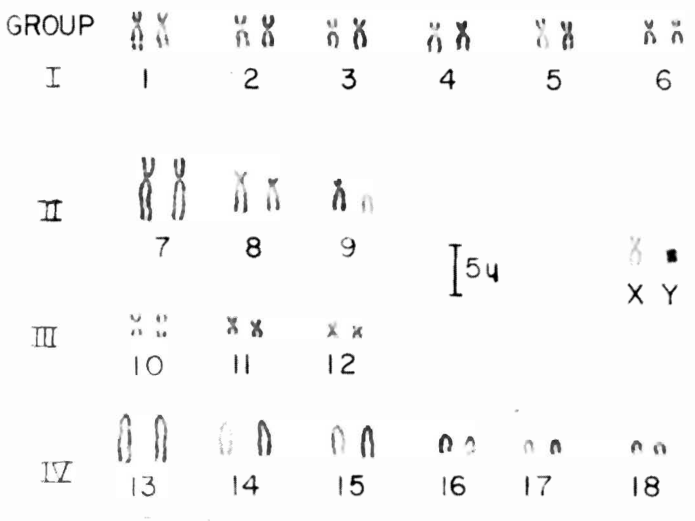

Figure 1. Karyotype of a domestic swine (2n=38) showing numbers assigned to homologous pairs of chromosomes.

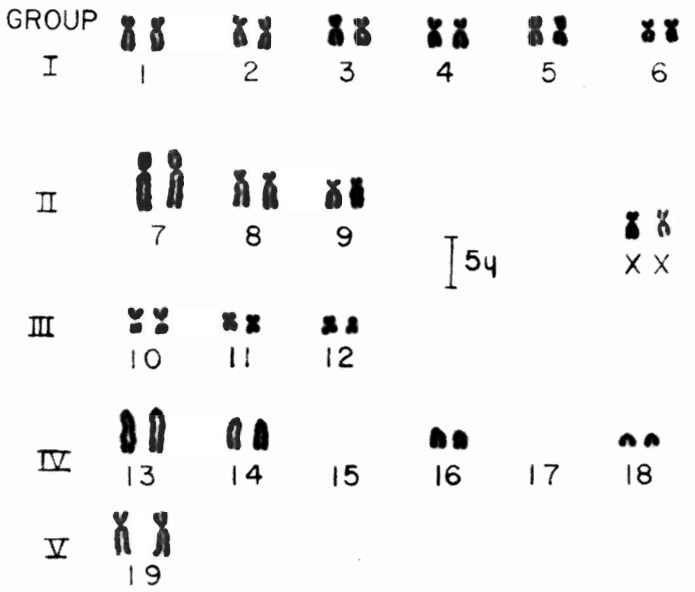

Figure 2. Karyotype of a European wild swine (2n=36) showing numbers assigned to homologous pairs of chromosomes.

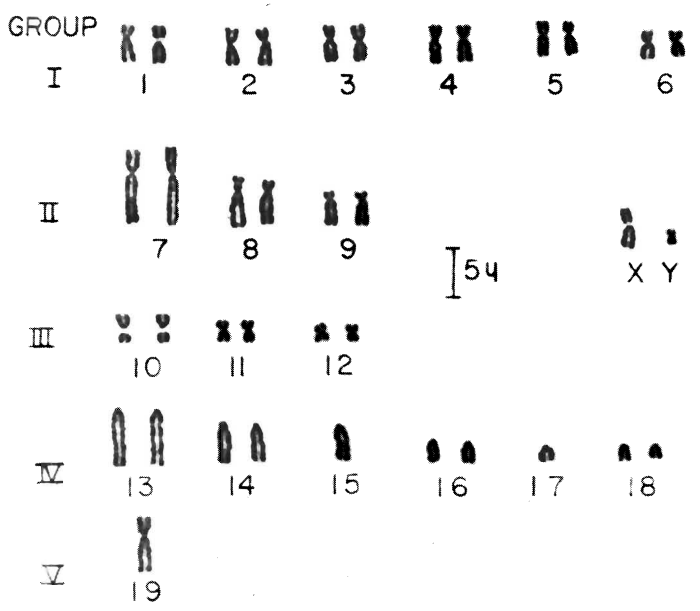

Figure 3. Karyotype of a domestic-European wild hybrid swine (2n=37) showing numbers assigned to homologous pairs of chromosomes.

The four basic groups of chromosomes utilized in the preparation of the domestic swine karyotypes were also utilized in preparing karyotypes of the European wild (2n=36) and the European wild-domestic hybrid (2n=37). The European wild and the European wild-domestic hybrid both however have one more group (Group V) than does the domestic karyotype. (Figures 2 & 3). The extra group contains only one chromosome which is not present in the domestic karyotype.

Numbers were assigned to homologous pairs of chromosomes for the purpose of quantitative analysis of chromosomes within a group, between groups and also for comparison of quantitative data from the three different swine karyotypes. The homologous pairs of chromosomes of the domestic swine were numbered 1 through 18 with X or Y designating the apparent sex chromosomes (Figure 1). The homologous pairs within the complements of the 36 and 37 chromosome swine were numbered 1 through 19 with either X or Y designating the sex chromosomes. By utilizing this system the homologous pair numbers 1 through 18 represent chromosomes which are apparently homologous within the karyotypes of 36, 37, and 38 chromosome swine.

TABLE I

THE RELATIVE LENGTHS OF THE DOMESTIC SWINE (2n=38), THE EUROPEAN WILD (2n=36) AND THE EUROPEAN WILD-DOMESTIC HYBRID (2n=37)

	2n=38			2n=37			2n=36		
Chromosome Number	Relative Length	S.E.	Coefficient of Variation	Relative Length	S.E.	Coefficient of Variation	Relative Length	S.E.	Coefficient of Variation
1	63.94	0.344	7.58	57.60	0.50	8.59	62.66	0.51	8.15
2	57.37	0.271	6.74	47.57	0.42	8.83	54.75	0.50	9.06
3	54.27	0.247	6.46	44.50	0.44	9.95	50.21	0.52	10.32
4	52.01	0.264	7.17	42.33	0.46	10.96	47.71	0.48	10.02
5	49.80	0.261	7.36	38.70	0.44	11.44	44.01	0.62	14.13
6	42.36	0.178	6.00	27.39	0.55	11.67	34.35	0.72	21.19
7	120.06	0.599	7.03	129.46	1.08	8.38	135.64	1.70	12.50
8	69.55	0.359	7.33	64.92	0.52	7.93	71.16	0.64	9.00
9	52.07	0.300	8.31	43.13	0.51	11.85	49.11	0.58	11.83
10	41.84	0.308	8.13	25.37	0.56	22.11	30.08	0.82	27.23
11	30.24	0.245	11.50	13.43	0.42	31.20	18.92	0.75	39.75
12	24.97	0.217	12.24	8.69	0.31	35.02	15.32	0.72	47.13
13	89.64	0.399	6.24	109.70	1.06	9.65	110.30	1.49	13.52
14	64.69	0.266	5.77	72.88	0.77	10.62	74.49	0.97	12.99
15	57.78	0.260	6.33	65.39	0.83	11.08	-----		
16	36.49	0.258	10.11	35.19	0.52	14.89	36.74	0.46	12.49
17	28.12	0.187	9.42	23.08	0.19	5.80	-----		
18	23.49	0.199	12.17	18.65	0.32	17.10	20.72	0.46	22.01
19	-----			87.09	0.84	6.78	92.51	1.17	12.66
X	54.84	0.372	8.29	44.63	0.60	11.67	50.83	0.80	13.70

The average relative length, the standard error and the coefficient of variation for each of the homologous pairs of chromosomes of the domestic swine karyotype are shown in Table I. A relatively large standard error or coefficient of variation for a particular pair of chromosomes indicates a chromosome which contracts out of phase with the remainder of the complement (Moore, 1965). As can be seen in Table I, all of the standard errors and coefficients of variation are relatively small and also have a rather narrow range. If indeed there is a chromosome of the domestic swine karyotype which is contracting out of phase, it is apparently not out of phase enough to be detected in the way just described.

11

TABLE II

LONG ARM/SHORT ARM RATIOS OF THE CHROMOSOMES OF THE EUROPEAN WILD-DOMESTIC HYBRID (2n=37), THE EUROPEAN WILD SWINE (2n=36), AND THE DOMESTIC SWINE (2n=38), WITH THE STANDARD ERROR OF EACH

	2n=38		2n=37		2n=36	
Chromosome Number	L/S	S.E.	L/S	S.E.	L/S	S.E.
1	1.84	0.023	2.55	0.104	2.42	0.094
2	1.65	0.020	2.22	0.029	1.98	0.055
3	1.56	0.021	1.57	0.048	1.82	0.067
4	1.52	0.022	1.94	0.088	1.82	0.067
5	1.53	0.022	2.05	0.086	1.93	0.045
6	1.45	0.017	1.81	0.023	1.71	0.057
7	2.11	0.014	2.59	0.012	2.44	0.038
8	3.29	0.035	6.91	0.250	6.09	0.088
9	3.22	0.044	8.03	0.335	6.13	0.250
10	1.12	0.013	1.53	0.020	1.17	0.034
11	1.22	0.015	1.45	0.022	1.35	0.043
12	1.19	0.015	1.33	0.020	1.30	0.046
19			2.66	0.029	2.55	0.052
X	1.58	0.026	2.11	0.033	1.72	0.060

The t-test at the 5 percent level of probability was used to determine significant differences between the mean relative lengths of the chromosomes within each of the respective groups of the domestic swine karyotype. Significant differences were found between the mean relative lengths of all the chromosomes within each of the four groups. The mean relative length of the X chromosome fell into the range of the mean relative lengths of the Chromosomes of Group I. Other criteria should, however, be used before a quantitative separation of the chromosomes within individual groups is formulated. For example, the L/S, the centric index and differential contraction of chromosomes should also be considered.

The long arm/short arm ratios of the chromosomes of the domestic swine are shown in Table II. The long arm/short arm ratios are very useful in the

TABLE III

CENTRIC INDICES AND STANDARD ERRORS OF THE CHROMOSOMES OF THE DOMESTIC, EUROPEAN WILD, AND THE EUROPEAN WILD-DOMESTIC HYBRID

	2n=38		2n=37		2n=36	
Chromosome Number	Centric Index	S.E.	Centric Index	S.E.	Centric Index	S.E.
1	39.67	0.303	29.72	0.620	30.95	0.652
2	38.23	0.311	32.82	0.703	34.95	0.598
3	38.51	0.368	40.12	0.643	37.26	0.930
4	40.32	0.338	36.18	0.790	37.04	0.720
5	39.81	0.345	34.98	0.805	35.52	0.685
6	41.20	0.277	37.52	0.770	38.46	0.710
7	32.32	0.162	28.27	0.314	29.37	0.300
8	23.62	0.194	13.82	0.396	16.07	0.556
9	24.19	0.266	12.45	0.426	15.95	0.590
10	46.20	0.250	41.57	0.847	46.54	0.620
11	45.29	0.297	43.27	0.908	43.77	0.670
12	45.99	0.302	45.12	0.758	45.10	0.790
19	47.40	0.098	28.01	0.600	28.60	0.380
X	39.31	0.429	34.40	0.973	38.09	2.067

determination of nomenclature for chromosomes in respect to centromeric position. Chromosome number 1 of group I of the domestic swine is a submetacentric (sm) while the remainder of the chromosomes of group I are metacentrics (m). Chromosome number 7 of group II with a L/S ratio of 2:1. would be termed a submetacentric (sm), while chromosomes 8 and 9 with ratios of 3.29 and 3.22 respectively would be subtelocentrics (st). All chromosomes of group III were determined to be metacentrics (m).

The mean centric indices and the S.E. of each of the chromosomes of the domestic swine are shown in Table III.

The relative lengths with standard errors and coefficients of variation for each of the chromosomes of the European wild swine karyotype are shown in Table I. The coefficients of variation of the relative lengths of chromosome numbers 6, 10, 11, 12, and 18 are considerably larger than the remainder of the chromosomes of the complements.

It is possible that one or more of these chromosomes is contracting out of phase in relation to the other chromosomes. In this case, however, the comparatively large coefficients of variation probably result from the small relative lengths of the chromosomes. The smaller chromosomes would necessarily entail a proportionally greater error which was introduced during measurement of the chromosomes. All of the mean relative lengths of the chromosomes within each of the groups were found to differ at the 5 percent level of probability. The L/S ratios were again used to provide a basis for determination of appropriate nomenclature for the chromosomes of the European wild swine. All the chromosomes of group I were determined to be submetacentric (sm). Chromosome number 7 of group II was determined to be a submetacentric while 8 and 9 of the same group are subtelocentrics. Chromosomes 10, 11, and 12 were metacentrics and chromosome number 19 (Group V) was a submetacentric.

The centric indices of the chromosomes of the European wild swine are shown in Table III. The relative lengths with standard errors and coefficients of variation of the European wild-domestic hybrid are shown in Table I. Each of the relative lengths was determined to be significantly different from that of each of the other chromosomes within their respective group.

The coefficients of variation for chromosomes of group III are considerably larger than the coefficients of variation for the other chromosomes of the complement. The relatively short lengths of the chromosomes of group III are probably responsible for the larger coefficients of variation.

The long arm/short arm ratios of the chromosomes of the European wild-domestic hybrid are presented in Table II. The chromosomes of group I are all submetacentrics, with the exception of number 3 which is a metacentric. Chromosome number 7 of group II is a submetacentric while

numbers 8 and 9 of the same group are subtelocentrics. Group III chromosomes were determined to be metacentrics. Chromosome number 19 with a long arm/short arm ratio of 2.66, therefore would be termed a submetacentric.

The centric indices of the chromosomes of the European wild-domestic hybrid are shown in Table III.

One of the major questions to be considered now is how many of the chromosomes within each of the groups can be positively identified either by the quantitative data just presented and/or by distinct morphological features? What criteria, as far as the quantitative data is concerned, should be met before a chromosome of group I, for example, can be considered as separate and distinct from others of the same group? One must consider, first of all, the basis by which the chromosomes of group I were arranged, information of the group within each of the original karyotypes. In the case of group I, the chromosomes were arranged in order of decreasing length from left to right. Using the system just described it would be entirely possible, in fact highly probable, that many of the chromosomes were placed in the wrong position. The type of error just described results from the fact that there exist only very slight differences in the total length of each of the chromosomes of group I. The error might actually be compounded by differential contraction of some chromosomes and also by some of the cells being in slightly different stages of mitosis. From the information just considered one may not be correct in distinctly separating all or even some of the chromosomes of group I. Should one accept then, as true, the statistics presented earlier which indicated each of the relative lengths of group I to be different at the 5 percent level of probability? On the other hand, consideration should be given to the fact that a distinct system was used for arranging the chromosomes within group I. From this standpoint the most logical quantitative approach would be to accept the data as setting forth quantitative criteria for the identification of group I and not individual chromosomes within that group.

Similarities and differences in the 36, 37 and 38 chromosome karyotypes. The chromosomes of group I of each of the three swine karyotypes appear to be similar, visually and quantitatively. Chromosomes of group I in each case show a gradual decrease in relative length from longest to shortest. There appears to be a decrease in the relative lengths of the chromosomes of group I in respect to the 36, 37, and 38 chromosome karyotypes. The chromosomes of group I of the 38 chromosome karyotypes have the longest relative lengths, the 37 chromosome karyotype the shortest with 36 being intermediate. Group II chromosomes (7, 8, and 9) visually appear to be the same in each of the different swine karyotypes. Quantitatively however, the relative lengths of the chromosomes of group II

of the 36 chromosome karyotypes are the greatest, with the 37 karyotype being the shortest and 38 intermediate. No visible difference was detected between the chromosomes of group III between the 36, 37, and 38 chromosome karyotypes. Quantitatively there was a decrease similar to group I in that the relative lengths of the 38 chromosome karyotypes were the greatest, 36 the least and 37 intermediate. The relative lengths of group IV of the 36 chromosome karyotype are larger, with 37 intermediate and 38 the smallest.

The relative lengths indicate that chromosomes 15 and 17 are the chromosomes involved in either the origin or disappearance of chromosome number 19. The best evidence which supports the idea that 15 and 17 are the chromosomes involved in the origin or disappearance of number 19 lies in the 37 chromosome karyotype (the 37 chromosome karyotype is the only swine karyotype in which chromosomes 15, 17, and 19 are all present). If one adds the average relative lengths of chromosomes 15 and 17, a sum of 88.47 is obtained. The sum of 88.47 is very close to the relative length of chromosome number 19 which has a relative length of 87.09 (see Table I). The most striking difference in the long arm/short arm ratios of the chromosomes of the 36, 37, and 38 chromosome karyotypes was found in group III, more specifically chromosomes 8 and 9 of group III. The long arm/short arm ratios of chromosomes 8 and 9 of the 38 chromosome karyotype are 3.29 and 3.22 (Table II). The long arm/short arm ratios of chromosomes number 8 and 9 of the 36 and 37 chromosome karyotypes were much higher, all above 6 (see Table II).

A very pronounced secondary constriction was present in chromosome number 10 (see Figure I) in all karyotypes prepared from the 36, 37, and 38 chromosome swine. Chromosome number 16 (see Figure I) was found to have a small secondary constriction near the end of the arms. More than 90 percent of the number 16 chromosomes of the karyotypes prepared from 36, 37, and 38 chromosome animals possessed this secondary constriction. Secondary constrictions were observed in other chromosomes in 36, 37, and 38 chromosome karyotypes, their appearance, however, was very inconsistent and at a very low frequency.

SUMMARY

The relative lengths, long arm/short arm ratios and centric indices for the chromosomes of the 36, 37, and 38 chromosome swine were presented. Considerations for the quantitative identification of particular chromosomes of each of the different karyotypes were discussed. The 36, 37, and 38 chromosome karyotypes were compared in respect to visual differences and also quantitative differences as indicated by the relative lengths centric indices, and long arm/short arm ratios.

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