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THE KARYOTYPES OF SIX SPECIES FROM THE GENUS *HYMENOLEPIS* (PLATYHELMINTHES, CESTOIDEA)

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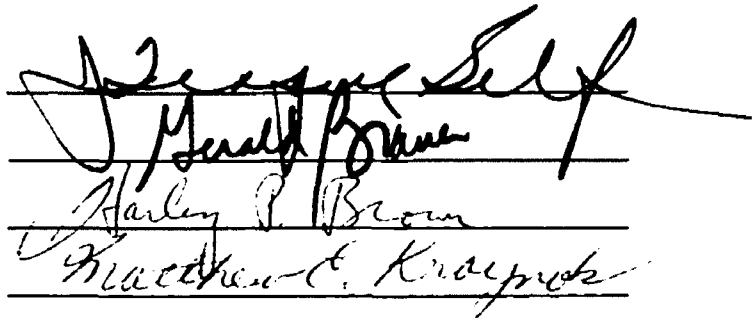
THE KARYOTYPES OF SIX SPECIES FROM THE GENUS

HYMENOLEPIS (PLATYHELMINTHES, CESTOIDEA)

APPROVED BY



DISSERTATION COMMITTEE

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THE KARYOTYPES OF SIX SPECIES FROM THE GENUS *HYMENOLEPIS* (PLATYHELMINTHES, CESTOIDEA)

INTRODUCTION

Karyotypes are often variant among related species. Karyotype evolution probably has been extensive and complex, involving structural chromosomal rearrangements and their consequences. Despite its being one of the most important aspects of the whole evolutionary process, the role of karyotype evolution, and the reasons it has taken different courses in different groups of organisms is still not clear.

Jones (1945) described the chromosomes of nine species from the family Hymenolepididae. He determined the number of chromosomes to be 10 and 12 diploid, and that the morphologies of the nine representative species from the family showed considerable uniformity, suggesting that the Hymenolepididae constitute a homogeneous group. The morphology of the hymenolepids is quite uniform, and the family has few genera which, however, contain many species. Ecological data support this uniformity hypothesis, most species of the family being parasites of shore or water birds. Since there is coincidence of uniformity from morphological and ecological data, one would

expect cytological uniformity to form a part of a natural system of classification for this family.

Walton (1959) stated that, from cytological records, the Cestoda do not demonstrate any definite chromosomal taxonomic patterns above the species level, and that studies of chromosomal numbers, structure, or behavior do not substantiate the belief that the Cyclophyllidea are probably the most specialized among the Cestoda.

Jones (1945) was the first to study the chromosomes of Hymenolepis diminuta. At that time he stated that the chromosome number was probably a diploid number of 12, but his results were not conclusive because of fixation problems.

Kisner (1962) prepared aceto-orcein squashes of selected regions of the strobila of H. diminuta containing developing embryos. The chromosomes, ranging in stage from late prophase to early anaphase were drawn by camera lucida. He concluded that the normal 2N number was 12, thus confirming what Jones reported in 1945. He also found from observations of the chromosome movement during anaphase that all the chromosomes were acrocentric. He constructed an idiogram based on the average lengths of the chromosomes from seventeen cells which were assumed to be in first cleavage. The average length in microns for each pair, was as follows: 7, 5.5, 5, 5, 4.5, and 3: in other words one pair of long chromosomes, four pair of medium length chromosomes, and one pair of short chromosomes. One of the medium length

chromosomes was shown to be slightly shorter than the others in this group. He also stated that the chromosomes seemed to decrease in length progressively with each succeeding division. Occasionally he observed a cell in which there appeared to be one less, or one more than the normal number.

Douglas (1961) wrote that during cleavage the number of separate chromosome units was reduced from 12 to 6 in H. diminuta. He hypothesized that somatic pairing of homologous chromosomes would explain this reduction. It is my opinion that extrusion of chromosomes during preparation is probably the explanation for the reduction in the number of discernable chromosomal units. Douglas negates the possibility of chromosomal extrusion by stating that he looked at a large number of cells and never saw extruded chromosomes. But it would be extremely unlikely that extruded chromosomes would remain close enough to their cell of origin to be observed in a smear or squash preparation. Douglas (1961) also states that "The importance of somatic pairing to survival of the species is evident provided it enhances somatic crossing over. Somatic crossing over in germinal tissue near the scolex could provide for phenotypic variation among proglottids."

Douglas (1962) idiogramed the chromosome pairs of H. diminuta in the following manner: three short chromosomes, two of medium length, and one longer than the others. He identified chromosome number 1 because of its long length

and small satellite, chromosome numbers 2 and 3 because of a secondary constriction near one end, chromosome number 4 because of the satellite which is considerably separated from the rest of the chromosome, chromosome number 5 because of its short length and tapered shape, and chromosome number 6 because it is short and slightly bent on its long axis. He idiogramed and studied the chromosome structure during prophase and prometaphase of the first cleavage division when the male and female chromosomes are still separate. He states that in no other stage in this organism's life history thus far examined is the chromosomal structure so clearly defined.

Douglas (1962) and Kisner (1962) probably differ in their results because neither of them standardized their length measurements and Douglas had a larger sample size than did Kisner.

Hossain and Jones (1963) studied the chromosomes of H. microstoma in Feulgen stained squash preparations of germ cells and early embryos. They found the centromeres to be terminal and the chromosomes to occur in a diploid number of 12. Four pairs were short, one pair of medium length, and one pair obviously longer than the others. They observed some lightly stained regions near one end of the chromosome in some metaphase figures. These regions were believed not to be subterminal centromeres because their studies of anaphase figures showed all chromosomes to be rod shaped.

Therefore, they suggested that these are heterochromatic regions which extend from the terminal centromeres.

H. nana also has a normal diploid number of 12 rod shaped chromosomes, there being a cytological variant with a diploid complement of 10 rod shaped chromosomes (Jones and Ciordia, 1955). In addition to their chromosomal similarities, H. nana and H. microstoma also resemble each other morphologically.

Hossain and Jones (1963) noted that the chromosomes of H. microstoma conform to the pattern of chromosome number and form observed by Jones (1945) in other species of Hymenolepis. The normal pattern has a diploid number of 12, the exceptions being the cytological variant of H. nana with a 2N number of 10 and H. fraterna which also has a 2N number of 10 including one metacentric chromosome. Terminal, subterminal and median centromeres have also been found in the chromosomes of Hymenolepis spp.

In addition to the species discussed above my work includes for the first time studies of Hymenolepis citelli from Citellus tridecemlineatus, H. farciminosa from Sturnus vulgaris, and H. sp. (possibly H. microcirrosa or H. planestici) from Turdus migratorius.

MATERIALS AND METHODS

Laboratory mice, Mus musculus, were used as the definitive hosts of Hymenolepis diminuta, H. nana, and H. microstoma. Tribolium confusum was the intermediate host.

H. citelli was collected from Citellus tridecemlineatus, the thirteen lined ground squirrel. Tribolium confusum was infected with the eggs from H. citelli, and when cysticercoids developed in the beetle they were used to infect Cricetus auratus, the golden hamster. Cricetus auratus were used as definitive hosts because of the ease with which they could be kept and handled in the laboratory.

Hymenolepis farciminosa and H. sp. were collected from the starling, Sturnus vulgaris, and from the robin, Turdus migratorius, respectively. Sections of tapeworms were studied for identification, and the eggs containing cleaving stages from mature proglottids were used for karyotyping. Plate VII shows photomicrographs of mitotic metaphase chromosomes from cleaving embryos. Plate I, figure f, is the karyotype of these chromosomes.

The cestodes were kept alive during the collection and pretreatment period. Pretreatment was by incubation for

two hours in a 0.1% colchicine saline solution at 37 C and then for ten minutes in a hypotonic solution of 1.0% sodium citrate. The colchicine arrested cell division at metaphase, thereby providing more than the normal number of metaphase figures for study. The hypotonic solution spread and swelled the chromosomes thus dispersing them for easy study. The tapeworms were fixed for fifteen minutes in a 3:1 solution of methyl alcohol to acetic acid. After fixation, the strobila was sliced with a razor blade in 60% acetic acid. Cells and eggs thus released in the acetic acid were kept in it for not more than five minutes. Within the five minutes the egg and cell solution was dropped with a pasteur pipette onto a slide which had been soaked in cold absolute ethyl alcohol. When the drop hit the cold slide, the eggs and cells dispersed outward and were flame fixed to the slides. They were stained with Giemsa. Microscopy and photography were performed with a Zeiss Standard RA Routine and Research Microscope equipped with an adapter holder and bellows for 4" x 5" plates. The film used was high contrast kodak ortho film (ASA-50). Pictures were taken at a magnification of 1,000X (oil immersion) and the negatives were enlarged 1.51X on the print paper. Thus the total enlargement of the original chromosomes was 1,510X.

The "Student-Neuman-Keuls-Test," Sokal and Rohlf (1969), was used to rank and demonstrate significant length differences between intraspecific chromosomes and also

between interspecific chromosomes. Measurements of the chromosomes were taken in mm. from the karyotypes. The measurements were made on the prints with a mm rule along the central axis of the chromosomes. Each curve was measured in increments of straight lines, which were summed up in order to know the lengths of the chromosomes, including their curves. The entire lengths of all the chromosomes of a karyotype were totaled. This result was then divided into each individual chromosomal length to get a corrected value for each chromosome. The corrected values were averaged and these averages were compared with the SNK Test.

DESCRIPTION OF PLATES

Plate I, figures* a - f

Karyotypes of Hymenolepis diminuta (1,510X)

Plate II, figures* a - f

Karyotypes of Hymenolepis microstoma (1,510X)

Plate III, figures* a - f

Karyotypes of Hymenolepis nana (1,510X)

Plate IV, figures* a - f

Karyotypes of Hymenolepis citelli (1,510X)

Plate V, figures* a - f

Karyotypes of Hymenolepis farciminosa (1,510X)

Plate VI, figures* a - f

Karyotypes of Hymenolepis sp. (1,510X)

Plate VII, Photomicrographs of Mitotic Metaphase Chromosomes from Cleaving Embryo of Hymenolepis diminuta (1,510X)

*Each figure represents the karyotype of the mitotic metaphase chromosomes from a cell of a cleaving embryo of a different parent.

PLATE I

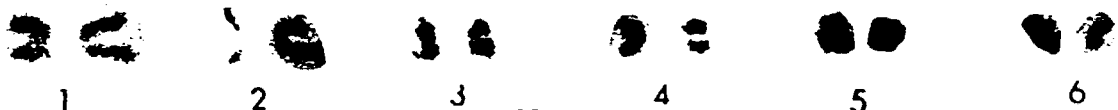

fig a

fig b

fig c

fig d

fig e

fig f

PLATE II

fig a

fig b

fig c

fig d

fig e

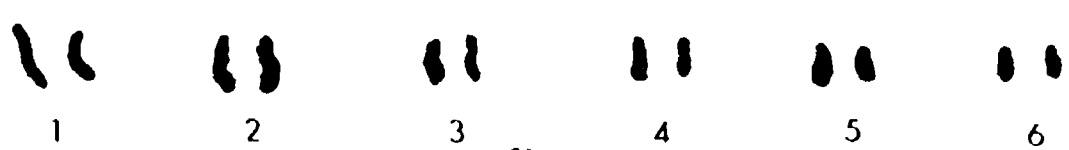

fig f

PLATE III

fig a

fig b

fig c

fig d

fig e

fig f

PLATE IV

fig a

fig b

fig c

fig d

fig e

fig f

PLATE V

fig a

fig b

fig c

fig d

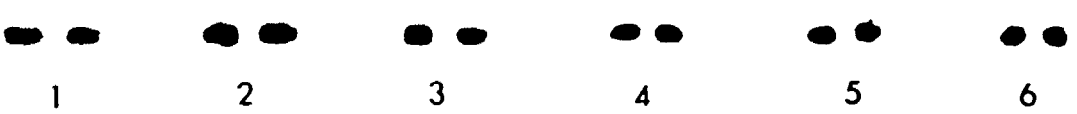

fig e

fig f

PLATE VI

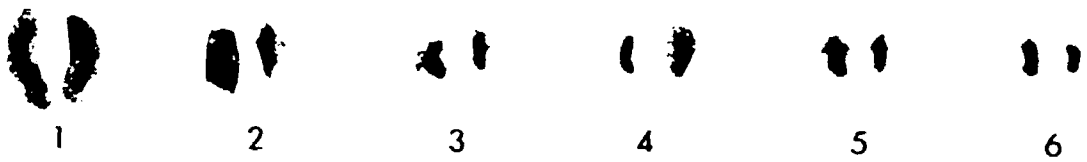

fig a

fig b

fig c

PLATE VII

TABLE I

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES
OF HYMENOLEPIS DIMINUTA

Chromosome Number	N	Corrected Mean	Variance
1	12	0.1202	0.0005
2	12	0.0958	0.0002
3	11	0.0851	0.0001
4	12	0.0803	0.0001
5	11	0.0676	0.0000
6	11	0.0542	0.0001

Maximum Nonsignificant Ranges

Subset	Chromosome Number	
1	2	3
2	3	4

Legend:

Chromosome Lengths Expressed As Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

TABLE II

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES
OF HYMENOLEPIS MICROSTOMA

Chromosome Number	N	Corrected Mean	Variance
1	12	0.1253	0.0001
2	12	0.1085	0.0001
3	12	0.0788	0.0001
4	12	0.0727	0.0000
5	12	0.0616	0.0001
6	12	0.0532	0.0000
All Means Significantly Different			

Legend:

Chromosome Lengths Expressed As Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

TABLE III

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES
OF HYMENOLEPIS NANA

Chromosome Number	N	Corrected Mean	Variance
1	12	0.1182	0.0004
2	12	0.0918	0.0001
3	12	0.0814	0.0001
4	12	0.0763	0.0001
5	12	0.0701	0.0000
6	12	0.0622	0.0000

Maximum Nonsignificant Ranges

Subset	Chromosome Number	
1	3	4
2	4	5
3	5	6

Legend:

Chromosome Lengths Expressed As Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

TABLE IV

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES
OF HYMENOLEPIS CITELLI

Chromosome Number	N	Corrected Mean	Variance
1	12	0.1311	0.0001
2	12	0.0888	0.0000
3	12	0.0782	0.0001
4	12	0.0703	0.0001
5	12	0.0703	0.0001
6	12	0.0582	0.0000

Maximum Nonsignificant Ranges

Subset	Chromosome Number	
1	3	4
2	4	5

Legend:

Chromosome Lengths Expressed As Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

TABLE V

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES
OF HYMENOLEPIS FARCIMINOSA

Chromosome Number	N	Corrected Mean	Variance
1	10	0.0970	0.0001
2	9	0.0964	0.0001
3	10	0.0913	0.0000
4	9	0.0772	0.0001
5	10	0.0730	0.0001
6	9	0.0625	0.0000

Maximum Nonsignificant Ranges

Subset	Chromosome Number	
1	1	3
2	4	5

Legend:

Chromosome Lengths Expressed As Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

TABLE VI

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES OF HYMENOLEPIS SP.

Chromosome Number	N	Corrected Mean	Variance
1	2	0.1562	0.0005
2	2	0.0859	0.0001
3	2	0.0781	0.0005
4	2	0.0703	0.0001
5	2	0.0586	0.0000
6	2	0.0508	0.0000

Maximum Nonsignificant Ranges

Subset	Chromosome Number	
1	2	6

Legend:

Chromosome Lengths Expressed As Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

TABLE VII

COMPARATIVE MEAN LENGTHS OF CHROMOSOME OF EACH SPECIES

Chromosomes	Species A	B	C	D	E	F
1	.1202	.1253	.1182	.1311	.0970	.1562
2	.0958	.1085	.0918	.0888	.0964	.0859
3	.0851	.0788	.0814	.0782	.0913	.0781
4	.0803	.0727	.0763	.0703	.0772	.0703
5	.0676	.0616	.0701	.0703	.0730	.0586
6	.0542	.0532	.0622	.0582	.0625	.0508

Legend:

Chromosome Lengths Expressed as Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

Vertical Lines Represent Maximum Nonsignificant Ranges

Underlined Numbers Designate Medium Length Range

A = Hymenolepis diminuta

B = Hymenolepis microstoma

C = Hymenolepis nana

D = Hymenolepis citelli

E = Hymenolepis farciminosa

F = Hymenolepis sp.

TABLE VIII

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES 1

Species Number	N	Corrected Mean	Variance
5	9	0.0970	0.0001
3	12	0.1182	0.0004
1	12	0.1202	0.0005
2	12	0.1253	0.0001
4	12	0.1311	0.0001

Maximum Nonsignificant Ranges

Subset	Species Number	
1	3	4

Legend:

Chromosome Lengths Expressed As Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

1 = *Hymenolepis diminuta*

2 = *Hymenolepis microstoma*

3 = *Hymenolepis nana*

4 = *Hymenolepis citelli*

5 = *Hymenolepis farciminosa*

TABLE IX

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES 2

Species Number	N	Corrected Mean	Variance
4	12	0.0888	0.0000
3	12	0.0918	0.0001
1	12	0.0958	0.0002
5	10	0.0964	0.0001
2	12	0.1085	0.0001

Maximum Nonsignificant Ranges

Subset	Species Number	
1	4	5

Legend:

Chromosome Lengths Expressed as Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

1 = Hymenolepis diminuta

2 = Hymenolepis microstoma

3 = Hymenolepis nana

4 = Hymenolepis citelli

5 = Hymenolepis farciminosa

TABLE X

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES 3

Species Number	N	Corrected Mean	Variance
4	12	0.0782	0.0001
2	12	0.0788	0.0001
3	12	0.0814	0.0001
1	11	0.0851	0.0001
5	10	0.0913	0.0000

Maximum Nonsignificant Ranges

Subset	Species Number	
1	4	1
2	1	5

Legend:

Chromosome Lengths Expressed as Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

1 = Hymenolepis diminuta

2 = Hymenolepis microstoma

3 = Hymenolepis nana

4 = Hymenolepis citelli

5 = Hymenolepis farciminosa

TABLE XI

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES 4

Species Number	N	Corrected Mean	Variance
2	12	0.0727	0.0000
4	12	0.0734	0.0000
3	12	0.0763	0.0001
5	9	0.0772	0.0001
1	12	0.0803	0.0001

Maximum Nonsignificant Ranges

Subset	Species Number	
1	2	1

Legend:

Chromosome Lengths Expressed as Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

1 = Hymenolepis diminuta

2 = Hymenolepis microstoma

3 = Hymenolepis nana

4 = Hymenolepis citelli

5 = Hymenolepis farciminosa

TABLE XII

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES 5

Species Number	N	Corrected Means	Variance
2	12	0.0616	0.0001
1	11	0.0676	0.0000
3	12	0.0701	0.0001
4	12	0.0703	0.0001
5	10	0.0730	0.0001

Maximum Nonsignificant Ranges

Subset	Species Number	
1	2	4
2	1	5

Legend:

Chromosome Lengths Expressed as Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

1 = Hymenolepis diminuta

2 = Hymenolepis microstoma

3 = Hymenolepis nana

4 = Hymenolepis citelli

5 = Hymenolepis farciminosa

TABLE XIII

COMPARATIVE MEAN LENGTHS OF CHROMOSOMES 6

Species Number	N	Corrected Mean	Variance
2	12	0.0532	0.0000
1	11	0.0542	0.0001
4	12	0.0582	0.0000
3	12	0.0622	0.0000
5	9	0.0625	0.0000

Maximum Nonsignificant Ranges

Subset	Species Number	
1	2	4
2	4	5

Legend:

Chromosome Lengths Expressed as Ratio =

$$\frac{\text{Length of Each Chromosome in mm}}{\text{Total Length of All Chromosomes in mm}}$$

1 = Hymenolepis diminuta

2 = Hymenolepis microstoma

3 = Hymenolepis nana

4 = Hymenolepis citelli

5 = Hymenolepis farciminosa

TABLE XIV

COMPARATIVE MEAN LENGTHS OF CHROMOSOME COUNTERPARTS* BETWEEN SPECIES

Species	Chromosomes					
	1	2	3	4	5	6
Hymenolepis farciminosa	<A,B,C,D	< B	>B,C,D		>B	>A,B
Hymenolepis microstoma	>E	>A,C,D,E	<E		<E	<C,E
Hymenolepis diminuta	>E	<B			<E	<C,E
Hymenolepis nana	>E	<B	<E		<E	>A,B
Hymenolepis citelli	>E	<B	<E		<E	<C,E

Legend:

* = chromosomes of same number

> = longer than

< = shorter than

A = Hymenolepis diminuta

B = Hymenolepis microstoma

C = Hymenolepis nana

D = Hymenolepis citelli

E = Hymenolepis farciminosa

OBSERVATIONS

The centromere on a telocentric chromosome is located at one end of the chromosome; whereas the centromere of an acrocentric chromosome is positioned slightly subterminally, leaving a minute arm on one end and a longer one on the opposite side of the centromere. The minute arm was very difficult to observe in this work because of the lack of adequate resolution at the magnification required. It is therefore often difficult to distinguish between telecentric and acrocentric chromosomes. From my observations and those in the literature, most if not all of the chromosomes studied here are acrocentric. Therefore, I am treating them all as acrocentric.

The metaphase chromosomes represented by the karyotypes in Plates I through VI are numbered from the longest to the shortest as 1 through 6. All the chromosomes are acrocentric as demonstrated by their rod shapes. The chromatid arms of most of the chromosomes are spread 180° apart. The normal diploid number is 12.

The chromosomes of all the species studied are partitioned into three main ranges according to their corrected

lengths. The limits of these ranges are arbitrarily set as follows: chromosomes with length ratios exceeding 0.100 are placed in the long length range, chromosomes with length ratios between 0.0999 and 0.0700 are placed in the medium length range, and chromosomes with length ratios shorter than 0.0700 are placed in the short length range.

Plate I, figures a through f, represents the karyotypes for individuals of Hymenolepis diminuta. Each chromosome for these karyotypes can be identified by its length, and chromosome 2 is also distinguished by a possible secondary constriction (figures c and d). These can not be positively identified as secondary constrictions because they were only infrequently observed.

Table I compares the mean chromosome lengths of H. diminuta. Chromosome 1 is alone in the long length range. Chromosomes 2, 3, and 4 are all in the medium length range where they are separated into two different length subranges which overlap each other by both 2 and 4 overlapping chromosome 3. Chromosome 2 is significantly longer than chromosome 4. Chromosomes 5 and 6 constitute two subranges within the short length range, 5 being significantly longer than 6.

Each figure on Plate II represents a karyotype of an individual of H. microstoma. The one characteristic differentiating these chromosomes from each other is their length.

The mean lengths of the chromosomes of H. microstoma are collated in Table II. There are two significantly different subranges within each of the three main length ranges. Chromosomes 1 and 2 are in the long length range, chromosome 1 being significantly longer than 2. In the medium length range chromosome 3 is significantly longer than 4. Chromosome 5 is significantly longer than 6 in the short length range. None of the ranges overlap.

Individual karyotypes of H. nana are represented in Plate III, figures a through f. They are distinguishable only by their lengths, except that in the case of chromosome 2, where a possible secondary constriction (figures b and f) also occurs.

Comparisons are made between the mean lengths of the chromosomes of H. nana in Table III. Only chromosome 1 falls in the long length range. The next four are in the medium length range. These are segregated into three subranges, two of which overlap each other. Chromosome 2 is alone in its subrange being the longest. Chromosome 3 is significantly longer than 5, but their respective medium subranges overlap that of 4. The short length range is occupied by chromosome 6 except that the latter is not always distinguishable from 5.

Plate IV, figures a through f, represents individual karyotypes of H. citelli. The chromosomes can be differentiated only by their lengths.

Table IV collates the mean chromosome lengths of this species. Only chromosome 1 is in the long length range. The medium length range includes chromosomes 2 through 5. These are divided into three subranges, 2 occupying the longest subrange, 3 the middle subrange, and 5 the shortest. Four is not always distinguishable from 3 or 5. Six is the only member of the short length range.

Individual karyotypes of H. farciminosa are illustrated on Plate V, figures a through f. The distinguishing structural features between the chromosomes of this species are their lengths, and possible secondary constrictions in chromosomes 1 (figure b) and 2 (figures b and c).

The mean chromosome lengths of H. farciminosa are collated in Table V. None of the chromosomes fall in the long length range. Chromosomes 1 through 5 are differentiated into two subranges within the medium length range. Chromosomes 1 through 3 constitute the longest subrange, and 4 and 5 the shortest subrange. The short length range includes only chromosome 6.

In Plate VI, figures a through c, are illustrated the karyotypes of H. sp. Only length differentiates them.

Comparisons among the mean lengths of the chromosomes of this species are presented in Table VI. Chromosome 1 alone belongs in the long length range. Two through 6 are all indistinguishable, occupying indeterminately both the

medium and short length ranges. This nonconformity in their lengths is considered due to an inadequate sample size.

Table VII will be mentioned in the discussion.

Table VIII collates the mean lengths of chromosomes 1 interspecifically. The collation shows that this chromosome is the same length in every species, except in H. farciminosa in which it is significantly shorter than its counterparts in the other species.

The mean lengths of chromosome 2 counterparts are compared among the five species studied in Table IX. It is the same length in every species except that in H. microstoma it is longer than in the other species.

Table X shows the collations of the mean lengths of chromosomes 3 from the species concerned in this study. This chromosome is longer in H. farciminosa than in H. nana, H. microstoma, and H. citelli. However, its length in H. diminuta is not discernible from that in all the other species.

The collations of chromosomes 4 in Table XI show no significant variation in length among all five species.

The interspecific collation of the mean lengths of chromosomes 5 are displayed in Table XII. This chromosome is significantly longer in H. farciminosa than it is in H. microstoma. Its length does not vary significantly among H. diminuta, H. nana, and H. citelli.

Table XIII compares the mean lengths of chromosomes 6 interspecifically. Those in H. farciminosa and H. nana are significantly longer than those in H. diminuta and H. microstoma. The length of this chromosome in H. citelli is not distinguishable from that of its counterparts in all the other species.

The chromosomes of H. sp. are not included because the sample size is too small.

Table XIV will be mentioned in the discussion.

DISCUSSION

My findings show that H. diminuta possesses one pair of long chromosomes, three pairs of medium length chromosomes, and two pairs of short chromosomes (Table I). This observation differs from that of Douglas (1962). He counted one pair of long chromosomes, two pairs of medium length chromosomes, and three pairs of short chromosomes. The discrepancy between my results and those of Douglas is probably due to our using different methods of measurements. He studied the chromosomes from idiograms constructed by drawings made with the aid of a camera lucida, and he also did not standardize his measurements. Drawings made with the aid of a camera lucida are not as accurate as photographs. It is necessary to standardize measurements of the lengths of these chromosomes because they are differentially condensed throughout metaphase. I measured all the chromosomes from karyotypes made from photographs, and their length measurements were all expressed as the length of each chromosome over the total length of all the chromosomes of the same cell.

Kisner (1962) observed that the chromosomes of H. diminuta were segregated into one that was long, four that

were of medium length, and one that was short. This observation also does not agree with mine. He did not standardize his chromosomal length measurements, instead he idiogramed them by camera lucida drawings.

I believe from the above comparisons of methodology that my measurements are more accurate than those of Douglas and Kisner.

Douglas (1961) reported observing a reduction in the number of chromosome units from 12 to 6 in H. diminuta. Kisner (1962) and I observed a small number of cells with both low and high chromosome counts. Douglas explained that this reduction was due to somatic pairing. However, it is possible for a ruptured cell to lose, or to gain chromosomes from a nearby ruptured cell. He identified chromosomes 2 and 3 by secondary constrictions near one end, while I also found a secondary constriction only on chromosome 2 (Plate I, figures c and d). He reported that chromosomes 1 and 4 had satellites at one end, and his idiograms show these satellites to be terminally located on one chromatid arm of each chromosome. The identification of a satellite body would be more convincing if it appeared on both chromatid arms, and I could not identify any satellite bodies.

Hossain and Jones (1963) reported that H. microstoma has one pair of long chromosomes, one pair of medium length chromosomes, and four pairs of short chromosomes. I am reporting two pairs of chromosomes in each of the

aforementioned length categories (Table II). My measurements seem more reliable because I corrected the length measurements for the continuously changing condensation state of the chromatin throughout metaphase.

H. diminuta, H. microstoma, H. nana, H. citelli, H. farciminosa, and H. sp. appear to have experienced moderate chromosomal changes in their evolution. They have acrocentric chromosomes, a diploid number of 12 and a uniformity of structure. Hossain and Jones (1963) stated that there have been telocentric, acrocentric, and metacentric chromosomes in H. sp. In 1945 Jones reported that H. fraterna has a 2N number of 10 including one pair of metacentric chromosomes. If this pair fragmented into two pairs of acrocentric chromosomes, a diploid number of 10 with one pair of metacentric chromosomes could increase to a diploid number of 12 with all acrocentric chromosomes. Thus a species like H. nana might have gradually evolved from an ancestor like H. fraterna. Evolution could also have involved a progression in the other direction if centric fusions occurred. The centric fusions of each of two pairs of acrocentric chromosomes would result in one pair of metacentric chromosomes and a 2N number of 12 would decrease to a 2N number of 10. This is a possible explanation of Jones's finding in H. fraterna.

Jones and Ciordia (1955) also described a cytological race of H. nana with a 2N number of 10 acrocentric chromosomes. It is possible that the 2N number could have

been reduced by a centric fusion followed by a pericentric inversion which resulted in 10 acrocentric chromosomes of about equal lengths, if the centric fusion involved two small chromosomes. Mather (1953) stated that low chromosome numbers represent an adaptation to a life cycle with several or many generations in a year, reasoning that if recombinations via independent assortment were not reduced to a low level in such organisms, the hereditary mechanism would suffer too much from the disruptive influences of natural selection acting in different directions in successive generations. If recombination is decreased by tying together genic material into fewer units of transmission, then the effects of a changing environment on selection will be decreased, and a well adapted species, like any of the species in this study, will not be changed drastically even though great changes in the environment might occur. Cestodes certainly are capable of completing several life cycles in a year and since, during their life cycles, they often encounter drastic environmental changes, reduced recombinations result from a decrease in their chromosome number and these have positive selective value. These factors could be the key to the evolution of the family Hymenolepididae, and the species of the genus Hymenolepis studied here, most of which have a normal 2N number of 12 acrocentric chromosomes according to studies made to date.

These cestodes are probably dependent upon recombination within chromosomes for their evolutionary advances because of their low chromosome number. Mather (1953) pointed out that similar factors are important in the evolutionary advances of Drosophila.

Tables I through VI are summarized in Table VII, which collates the mean lengths of the chromosomes for each species. Each of the species has a different number of chromosomes in each of the main length ranges. These differences suggest the possibility that reciprocal translocations occurred in the evolution of each species. These differences also support the validity of the present classification of these species.

Tables VIII through XIII are summarized in Table XIV, which reveals the interspecific length differences between each chromosome and its counterparts. These species can be distinguished from each other by comparing the lengths of each of their chromosomes with their counterparts interspecifically. In H. farciminosa chromosome 1 is shorter and 6 is longer than their respective counterparts in H. diminuta. Chromosomes 1 and 2 are shorter and 3, 5, and 6 are longer than their respective counterparts in H. microstoma. Chromosome 1 is shorter and 3 is longer than their counterparts in H. nana. Also chromosome 1 is shorter and 3 is longer than their specific counterparts in H. citelli. In H. microstoma chromosome 2 is longer than its counterpart in H.

diminuta, 2 is longer and 6 is shorter than their counterparts in H. nana. Chromosome 2 is longer than its counterpart in H. citelli. Five and 6 are shorter and 1 and 2 are longer than their counterparts in H. farciminosa. In H. diminuta chromosomes 5 and 6 are shorter and 1 is longer than their counterparts in H. farciminosa. Chromosome 2 is shorter than its counterpart in H. microstoma. Chromosome 6 is shorter than its counterpart in H. nana and 3 shows no significant differences in length from its counterparts in all of the other species. In H. nana chromosomes 3 and 5 are shorter and 1 is longer than their specific counterparts in H. farciminosa. Two is shorter and 6 is longer than their respective counterparts in H. microstoma and 6 is longer than its counterpart in H. diminuta. In H. citelli 3, 5, and 6 are shorter and 1 is longer than their counterparts in H. farciminosa, and 2 is shorter than its counterpart in H. microstoma. Six is shorter than its counterpart in H. nana. The length of chromosome 4 is interspecifically invariant.

The collation of the mean lengths of chromosome counterparts (Table XIV) supports the present classification of H. diminuta, H. microstoma, H. nana, H. citelli and H. farciminosa, while at the same time indicating a close relation between the species. H. farciminosa parasitizes avian hosts as opposed to the other species which parasitize mammalian hosts. It is expected therefore that H. farciminosa would be cytologically more variant from these species than

they would be among themselves. Comparison of the mean lengths of the interspecific chromosome counterparts in this work tends to verify this point. Chromosomes in H. farciminosa differ more in lengths from their respective counterparts in all the other species studied than is the case for the same chromosomes among the latter. H. microstoma is almost as distantly related to H. diminuta, H. nana, and H. citelli as is H. farciminosa. In H. microstoma there are, however, fewer chromosomes than in H. farciminosa which vary in their length from their respective interspecific counterparts. These two species are not closely related because in each it is different chromosomes which vary in length from their respective counterparts in H. diminuta, H. nana and H. citelli. That H. microstoma is so variant cytologically is not surprising because it also is a physiological deviant. A different physiology is required for the shift from a gut to bile duct habitat. H. diminuta, H. nana, and H. citelli appear to be closely related cytologically in that their respective chromosomes are similar as revealed by the observations employed. Differences are reflected in that chromosome 3 in H. diminuta is similar to rather than being shorter than its counterparts in H. farciminosa. Chromosome 6 in H. nana is longer than its respective counterpart in H. diminuta and H. microstoma and 6 is not shorter than its respective counterpart in H. farciminosa.

When the chromosomal mean lengths are collated intraspecifically (Tables I through VI and summarized in Table VII) and interspecifically (Tables VIII through XIII and summarized in Table XIV) H. diminuta, H. microstoma, H. nana, H. citelli and H. farciminosa are more thoroughly verified as distinct species. The morphology of the chromosomes from these species seems cytologically stable. They are all acrocentric and rod shaped. The only morphologically distinctive features are possible secondary constrictions on chromosome 2 in H. diminuta (Plate I, figures c and d) and in H. nana (Plate III, figure b). All of these species probably benefit by a low rate of recombination via independent assortment because their environment undergoes drastic changes at various times in their life cycle.

CONCLUSIONS

The karyotypes of H. diminuta, H. microstoma, H. nana, H. citelli, and H. farciminosa support their present classification. The chromosomes of each species fit into a specific group of length ranges, while certain chromosomes differ in length from their counterparts interspecifically. This indicates that in the evolution of these species a number of reciprocal translocations could have occurred. Reciprocal translocations probably occur in a random manner, but only those translocations which have survival value can be observed in karyotype evolution. Thus the trends of karyotype evolution are of a non-random nature.

The karyotypes of the species of the genus Hymenolepis studied indicate that they are cytologically quite uniform, as is the case with all the members of the family Hymenolepididae studied to date. However, Hymenolepis has many species, while the family Hymenolepididae contains few genera, indicating that genetic variation is primarily on the species level.

White (1957) stated that the analysis of cytotaxonomic differences is easier in groups where the differences

are not numerous. The processes of chromosomal evolution are best understood in organisms which show a large degree of evolutionary stability. As long as the number of individuals comprising a species is large, there is a greater probability for centric fusions to occur in species with more than one pair of acrocentric chromosomes. However, most of these centric fusions probably would not survive because they must be genetically adaptive. If the heterozygotes in their meiotic configurations are mis-oriented, aneuploid gametes will arise and the fusions could not establish themselves in the genome. There must not be any deleterious position effects or the relocated genes may not function, or may be influenced to function in a detrimental fashion. There must also be a normal and regular cycle through all the phases of mitosis so that the centric fusions can be duplicated in the somatic tissue.

H. farciminosa from Sturnus vulgaris, appears to be distantly related cytologically to H. diminuta, H. microstoma, H. nana, and H. citelli which are all from rodents. H. microstoma from the bile duct of Mus musculus is also remotely akin cytologically to the other aforementioned species. However H. diminuta, H. nana, and H. citelli seem to be cytologically close relatives.

Only a few species of the genus Hymenolepis have been karyotyped. For cytotaxonomy of this genus to be of much value in taxonomy most of its species should be compared as should also be done with genera in closely related families.

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