CHROMOSOMES OF HELMINTHOGLYPTIDAE

(PULMONATA: GASTROPODA)

by

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A Dissertation Submitted to the Faculty of the

DEPARTMENT OF BIOLOGICAL SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN ZOOLOGY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by Noorullah Babrakzai

entitled CHROMOSOMES OF HELMINTHOGLYPTIDAE

(PULMONATA: GASTROPODA)

be accepted as fulfilling the dissertation requirement of the degree of DOCTOR OF PHILOSOPHY

Walter L. Miller — 20 March 1975

Dissertation Director — Date

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

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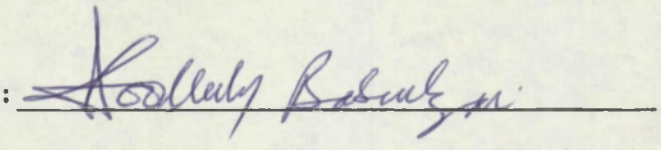
SIGNED: 

ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. Walter B. Miller, major professor and dissertation director, and to Drs. Peter E. Pickens and William B. Heed of the major committee for critically examining this manuscript. Appreciation is also extended to Drs. Wayne R. Ferris and Oscar G. Ward for their service on the minor committee.

I am greatly indebted to Dr. Walter B. Miller for enthusiastically encouraging and endorsing this study, taking me on numerous fieldtrips for collection of specimens, letting me use specimens from his own collection, and in identification of specimens collected from the field; to Dr. Oscar G. Ward for letting me use his research facilities; to Dr. Joseph T. Bagnara for his help in photomicrography and use of his darkroom; to Donald B. Sayner and Alison Habel for their help and assistance in printing thousands of photomicrographs; to Dr. Wayne R. Ferris for letting me use many chemicals; to my wife Sianoosh Samsam-Bakhtiary for her help in constructing karyotypes, assistance, useful suggestions, and understanding; to Carl Christensen, Pete D'Eliscu, Wendell O. Gregg, David Richman, Richard Russel, and Richard Reeder for assistance in arduous collecting and for their

companionship on several difficult expeditions in Arizona and California.

I wish to thank Margaret Vescovi for her hard work on the drawings and illustrations; Sally Viparina for typing and editing the manuscript; Drs. Joseph C. Bequaert and M. Robinson for their encouragement and suggestions; Drs. John B. Burch and Charlotte M. Patterson for sending me numerous rare reprints on snail chromosomes which were useful in this study.

Special thanks are reserved for Drs. Walter B. Miller and Oscar G. Ward for their daily encouragement, genuine interest, and readiness to discuss any and all aspects of malacology and cytogenetics during the past four years.

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ABSTRACT

This study reports, for the first time, chromosome morphology and numbers for ten species belonging to nine genera of the pulmonate family Helminthoglyptidae. The haploid and diploid chromosome numbers for Sonorella odorata, Sonorelix borregoensis, Greggelix indigena, and Monadenia infumata are 29 and 58, respectively. Helminthoglypta cf. lowei, H. micrometallioides, Xerarionta areolata and Humboldtiana högeana each have a haploid number of 30 and a diploid number of 60. The diploid chromosome number of Micrarionta rufocincta beatula and Eremarionta indioensis is 60. Karyotypes and idiograms of nine species belonging to nine genera of Helminthoglyptidae have been constructed, figured and compared. Indications of primary trisomy were found in one specimen each of M. infumata and G. indigena. Evidence of pericentric inversion was found in one specimen of M. r. beatula. Heteromorphism in homologous chromosome pairs was found in two species. Supernumerary chromosomes were detected in the mitotic metaphase of one species, and a chromosome fragment in meiosis of another.

Phylogenetic relationships within the subfamilies of Helminthoglyptidae and in Helicacea-Polygyracea are discussed in the light of chromosome numbers and morphology.

INTRODUCTION

In the past two decades chromosomes of molluscs have been studied by an increasing number of workers and interest in molluscan cytotaxonomy has been growing. According to Patterson's (1969) review and my estimate, chromosome numbers are now known for more than 590 species of molluscs. Of these cytologically-known species the majority are gastropods belonging to the subclass Pulmonata.

Earlier reports on the chromosomes of the land snail genus Helix have been reviewed by Naville (1923), Perrot (1930), Perrot and Perrot (1937), and Perrot (1938). Pennypacker (1930) counted chromosomes of the land snail Polygyra appressa, and Hickman (1931) determined the chromosome number of Succinea ovalis. The first major work on the chromosomes of pulmonate land snails was that of Husted and Burch (1946). These authors counted haploid and diploid chromosome numbers in 17 species of the Polygyridae.

One reason for the scarcity of successful cytological research on molluscs earlier in this century was that workers were using the paraffin sectioning technique. Burch, in 1959, was among the first to successfully use the chromosome squash technique on aquatic pulmonate snails. Once the switch from sectioning to the chromosome squash

technique was made, the study of molluscan chromosomes became easy and rewarding.

To the cytotaxonomist and cytogeneticist, number and morphology of chromosomes are two important aspects of the karyotype. Karyotype analysis can either strengthen the known taxonomic relationships in a group of related species or throw new light on their relationships. However, karyotypic studies on molluscs have been few, even though the chromosome number is known for a large number of species. Patterson (1971) estimated that the chromosome morphology of less than 25 species of molluscs is known.

The vast majority of reports on molluscan chromosomes deals with haploid numbers only, obtained from spermatogonial meiosis. The lack of information on karyotype analysis of molluscs has been attributed to the lack of adequate techniques to spread the chromosomes in mitotic metaphase. Patterson (1971) described a technique to obtain metaphase spreads utilizing freshwater snail embryos. However, this technique is difficult to apply to desert land snails because of the time required to rear them to maturity and the complexity of obtaining eggs and maintaining them to successful hatching. Therefore, a new technique was developed to obtain metaphase spreads of land snails by utilizing ovotestis tissue (Babrakzai and Miller, 1974a). With this technique the karyotypes of Helicina orbiculata (Babrakzai and Miller, n.d.), Oreohelix anchana,

O. concentrata, Radiocentrum clappi, R. chiricahuana (Babrakzai, Miller, and Ward, in press, 1975), and Sonorella virilis (Babrakzai, Miller, and Ward, n.d.), have been worked out.

The present research deals with the study of the chromosomes of the land snail family Helminthoglyptidae, a large family of many genera and numerous species. Pilsbry (1939) revised the taxonomy of this family for the North American species north of Mexico. Miller (1967) revised the systematics of the helminthoglyptid genus Sonorella in the light of new morphological and anatomical studies. More recently, Bequaert and Miller (1973) have discussed and reviewed the taxonomy and zoogeography of Southwestern Helminthoglyptidae.

Although the chromosome number of one helminthoglyptid species, Monadenia fidelis, has been reported by Ford (1962) and Burch (1965), Laws (1973) correctly points out that information for the superfamily Helicacea is incomplete since only one species of Helminthoglyptidae is known cytologically. This conclusion had been reached here earlier, so that the present work was started in 1971 in an attempt to provide this much needed information. It was decided that the best approach was to investigate the chromosomes of representative species of the genera of helminthoglyptids rather than to try to study all of the

species. As a consequence, this investigation provides the following:

1. Data on meiosis in 8 species belonging to 7 genera.
2. A report on the chromosome numbers, karyotypes, and idiograms of species from 9 genera belonging to this family.
3. Postulations about the phylogenetic relationships between various genera of Helminthoglyptidae based on chromosome morphology, karyotype analysis and the already-known anatomical features.
4. A discussion of the relationship between Helminthoglyptidae and other Helicacean families in the light of the results of these cytological studies.

MATERIALS AND METHODS

The snails used for this study were collected from their type localities whenever possible. In collecting and rearing specimens the methods of Miller (1967) were followed. The localities and number of specimens used for this study are as follows:

1. Sonorella odorata, Pilsbry and Ferriss, 1919. Head of Alder Canyon, Marshal Gulch, Santa Catalina Mountains, elevation 7600 ft., Pima County, Arizona, 30 specimens.
2. Xerarionta areolata (Pfeifer, 1845). Magdalena Plain, Baja California, 5 specimens (collected by W. B. Miller).
3. Sonorelix borregoensis (Berry, 1929). Borrego Palm Canyon, San Diego County, California, 3 specimens.
4. Humboldtiana högeana (Von Martens, 1892). 16.3 miles southwest of Buenaventura, elevation 7400 ft., Chihuahua, Mexico, 31 specimens (collected by R. L. Russell).
5. Greggelix indigena (Mabille, 1895). La Purisma Canyon, Baja California, 6 specimens (collected by W. B. Miller).

6. Helminthoglypta cf. lowei (Bartsch, 1918). Warner Springs, San Diego County, California, elevation ca. 5000 ft., 8 specimens.
7. Helminthoglypta micrometallioides, W. B. Miller, 1970. El Paso Mountains, Kern County, California, 1 specimen (collected by W. B. Miller).
8. Monadenia infumata (Gould, 1855). Green Hill Road, Sonoma County, California, 1 specimen (collected by W. B. Miller).
9. Micrarionta rufocincta beatula, Cockerell, 1929. Avalon, Catalina Island, California, 7 specimens (collected by W. B. Miller).
10. Eremarionta indioensis (Yates, 1890). Fish Traps, Indio Valley, Riverside County, California, 2 specimens.

Chromosome Spreads

The ovotestes of the snails were used for the chromosome squash preparations. The classical chromosome squash technique (La Cour, 1941), utilizing acetic orcein did not prove to be of much help due to the following reasons:

1. The preparation of the stain requires heating. After cooling the stain gradually precipitates in the stock solution, and it is necessary to filter the stain each time before use.

2. The stain is dissolved in 45% acetic acid and it evaporates relatively quickly from a slide containing a few drops of the stain. As a result, there is a possibility of precipitation of the stain on the cellular preparation, rendering it unsuitable for chromosomal study.
3. When the snail ovotestis tissue is left in stain for a long time (15 minutes or more), the acetic acid makes the tissue hard to squash (Darlington and La Cour, 1961).
4. The prepared slides, even if sealed (with nail polish, etc.), can be stored only for a week to ten days in the refrigerator. The study of chromosomes, including photomicroscopy and checking the photomicrographs against the actual spreads under the microscope, often takes more than a week. During that time, parts of a temporary slide would dry out and be lost.
5. At times, for unknown reasons, one-layered cellular smears could not be obtained.

Due to these difficulties I began to look into other methods of chromosome squash preparation. After experimenting with the known techniques, a newly modified technique suitable for land snails was developed with the help and guidance of Dr. W. B. Miller. In this technique

(Babrakzai and Miller, 1974a), the use of lactic-acetic-orcein (Cooperrider and Morrison, 1967) instead of acetic-orcein solved most of the problems encountered with the use of the latter. The stain can be prepared at room temperature and does not precipitate for a relatively long time (over a year in the refrigerator). Lactic acid is an oily liquid which does not evaporate easily; a tissue can be immersed in lactic-acetic-orcein for 1-24 hours without staining the cytoplasm. One difficulty encountered when using lactic acid is that it dissolves cytoplasm if the tissue is not well fixed; in such cases the nuclei are released. At times when many adjacent cells are dividing chromosome counts are difficult or impossible because the chromosomes from two or three dividing cells mix together. To overcome this difficulty, the ovotestis was fixed in Newcomer's fluid (Newcomer, 1953) for 36-48 hours prior to staining, making them sufficiently hard so that some cytoplasm remained undissolved by the stain.

The Use of Colchicine

In the technique that I have followed (Babrakzai and Miller, 1974a) precautions must be taken in using colchicine to arrest mitotic metaphase chromosomes. The synthetic analog of colchicine, colcemid, is known to cause hypo-diploidy (Fitzgerald and Brehaut, 1970) due to continued cytokinesis of some chromosomes in human blood lymphocyte

tissue cultures and to cause chromosomal non-disjunction of Chinese hamster cells *in vitro* (Cox and Puck, 1969). The main reason for this effect is that insufficient quantities of colchicine or colcemid were used. Furthermore, it has been shown that a 10^{-7}M colchicine solution can arrest metaphases in animal tissues (Levan, 1954). Therefore, I use a rather strong solution of colchicine (10^{-3}M) to prevent these problems. After injecting the colchicine into the snail, some hemolymph oozes out and with it possibly some colchicine. Since a 10^{-3}M solution of colchicine has to be diluted more than 10,000 times in order to cause hypodiploidy, the loss of this colchicine can be tolerated. An interesting fact is that the land snails used in this research can tolerate relatively strong doses of colchicine for at least 10 hours.

Micromanipulation

It is sometimes difficult to make a chromosome spread of a cell seen under the microscope. Resquashing the material either deos not spread the chromosomes, or the cell may be pushed out of the field of view. I have successfully used a fine dissection needle, placing it on the dividing cell and pressing it gently while looking under the low power of the microscope (10X objective). This technique worked about 80% of the time and was useful in making

chromosome squashes when there were few snail specimens or a scarcity of cells undergoing meiosis and mitosis.

Slides and Coverslips

In all cases No. 1 coverslips were used. The quality of slides and coverslips is important in chromosomal studies. I found that even the so-called "precleaned" microscope slides and coverslips were of little use because commercial slides and coverslips have microscopic granules of glass strongly adhering to them. By trial and error I found that the following method of cleaning slides and coverslips is effective:

1. Submerge the slides and coverslips in a mixture of 70% ethanol, 23% glacial acetic, and 2% hydrochloric acid for at least 24 hours.
2. Wipe the slides and coverslips with tissue paper such as "Kimwipe," etc., to remove the cleaning solution and dry them.
3. Use the slides and coverslips immediately to avoid subsequent contamination. Any unused slides can be stored in the cleaning solution.
4. Use a fresh cleaning solution for each batch of 72 slides and for each ounce of coverslips.

Permanent Mounts

Permanent mounts from squash preparations were made by first heating the temporary mount on an alcohol lamp for

about 5-7 seconds. The mount was then placed in a Coplin jar containing 75% acetic acid and 25% ethanol until the coverslip dropped from the slide. Then the coverslip and the slide were processed separately by placing them successively for one to two minutes in solutions of 50% acetic acid and 50% ethanol, 25% acetic acid and 75% ethanol, and 100% ethanol. The slide with its dehydrated squash was mounted in Euparol using a new coverslip; similarly, a new slide was used to go with the original coverslip of the preparation. Thus, two permanent mounts were obtained from one temporary slide. Placing the original coverslip and slide together would make the observation of the material under the microscope difficult since the chromosome spreads on the coverslip and on the slide are separated by the mounting medium. Therefore, one would need to focus separately on the cells under the coverslip and on the slide. There is only a 70% chance that temporary slides containing favorable chromosome spreads can be made permanent with the spreads intact. I therefore make permanent mounts only for record-keeping purposes and to double check the results. All the observations and photomicrographs were taken from freshly squashed material.

Photomicrographs

Observations and photomicrographs were made with a Wild microscope using both brightfield and phase contrast objectives. Photomicrographs were taken on Kodak High Contrast Copy film with an ASA rating of 10-20.

Drawings

Some chromosome spreads, especially in the pachytene stage of meiosis, have considerable overlap, and a photomicrograph of such a spread is hard to interpret. In order to overcome this difficulty camera lucida drawings of chromosome spreads were used.

Endopolyploidy

Endopolyploidy is a common and most probably an adaptive phenomenon in the ovotestis of pulmonate gastropods (Babrakzai and Miller, 1974b). However, in the construction of karyotypes, polyploid cells in endometaphase were not included.

Karyotype Analysis

The standard nomenclature for chromosomes proposed by Levan, Fredga, and Sandberg (1964) has been followed, and symbols have been used in describing individual chromosomes as follows:

p = short arm of chromosome.

q = long arm of chromosome.

q/p = arm ratio (r).

p/p+q = centromeric index (C.I.) expressed as percentage of the short arm to the total length of the chromosome.

p+q = length of chromosome.

RL = relative length of a chromosome expressed as percentage of total length of all chromosomes.

m chromosome = a chromosome with arm ratio (r) between 1.01 and 1.69; a centromeric index (C.I.) between 49.9 and 37.5.

sm chromosome = a chromosome with (r) between 1.7 and 2.9, and C.I. between 37.4 and 25.1.

st chromosome = a chromosome with r value more than 3.0 and C.I. below 25.0.

Photographs were printed on 8" x 10" Kodabromide printing paper with maximum magnification for chromosome measurements. The original photographic negatives were also used in determining the arm ratio by placing them in a slide projector and projecting them on a screen and measuring the magnified chromosomes (3,000 X or more). The arm ratios, chromosome lengths, and centromeric indices were used to arrange the mitotic metaphase chromosomes in homologous pairs. The haploid chromosome lengths of a genome were added together and each chromosome was expressed as a percentage of the entire genome. Since the largest

chromosome in each species studied was less than 10% of the entire genome, the percentage relative lengths were multiplied by a factor of 10 to describe relative chromosome lengths in "units." The use of relative lengths rather than absolute lengths is of special importance for comparative purposes because metaphase chromosomes from different specimens seldom undergo the same degree of contraction. Thus, it is possible for two chromosomes having the same relative length to be classified differently if one chromosome belonging to one cell has undergone greater contraction than one from another cell. In comparing karyotypes from different species with different degrees of contraction this problem becomes evident.

It would have been ideal if enough data could have been obtained for each species to determine the range of length of each chromosome of the genome, its mean, standard deviation, etc., over a large sample. Unfortunately, the excessive amount of time required for such an operation, as well as the scarcity of specimens of certain species, precluded this level of perfection.

In constructing karyotypes, I noticed that arranging chromosomes according to decreasing length produces confusion rather than clarity when comparing karyotypes from two different species, because the chromosomes in each genome tend to have overlapping lengths, and comparison becomes difficult. This problem has been observed by other

cytotaxonomists, notably Bogart (1970) and Raicu, Taisescu, and Banarescu (1973). Consequently, I have followed the procedure of Bogart (1970) and Raicu et al. (1973) in constructing the karyotypes and idiograms of the haploid set of chromosomes. The method essentially consists of separating m, sm, and st chromosomes into three groups. Thus, differences in lengths of chromosomes, centromeric position and the number of chromosomes in a group become more clearly established, and because of this it is easier to compare karyotypes. The three main groups can be subdivided into subgroups for convenience, if desired.

MEIOSIS IN HELMINTHOGLYPTIDAE

Meiotic chromosomes were studied in diplotene, Diakinesis and metaphase I. However, attempts were also made to study them in the pachytene stage but they were not successful except in two cases (Figs. 11 [p. 30], 15 [p. 35]). No meiosis in the specimens of Micrarionta rufocincta beatula Cockerell, 1929 and Eremarionta indioensis Yates, 1890 was observed.

Sonorella odorata Pilsbry and Ferriss, 1919

Specimens of this species are found in relative abundance in their type locality because it is apparently well adapted to a forest floor habitat, living on the underside of dead logs and in detritus. Consequently, 30 specimens of S. odorata were obtained for cytological studies. On subsequent examination I found that 16 specimens of S. odorata had "aborted ovotestes," i.e., the tissue of the ovotestis had no germinal cells. The acini of the ovotestes were filled with a brown, rather hard material resembling the color of the digestive gland. Since my work did not involve a pathological study, I discarded these specimens.

Five specimens were found to be undergoing meiosis and mitosis. Twenty-nine bivalents were observed in

diakinesis (Fig. 1) and in metaphase I (Fig. 2). There appeared to be various chromosomal aberrations in the meiosis of some individuals in the form of translocations and evidence of isochromosomes. Therefore, the haploid number of 29 recorded for this species should be considered tentative since other species of Sonorella have a haploid number of 30 (Babrakzai, Reeder, and Miller, in press, 1975).

Xerarionta areolata (Pfeiffer, 1845)

All five specimens used in this study were undergoing meiosis. Six chromosome spreads in late diakinesis from one specimen had 30 bivalents each (Fig. 3a). Similarly, 48 spreads of metaphase I from 4 other specimens were studied with 30 bivalents occurring in each case (Fig. 3b).

Sonorelix borregoensis (Berry, 1929)

Meiosis was seen in only one specimen. Eight chromosome spreads at diakinesis were obtained. In each case 29 bivalents were encountered (Fig. 4). In three cases an additional chromosomal element that did not seem to be paired was also found (Fig. 4). The haploid chromosome number of S. borregoensis is therefore 29. The individual specimen in question (with an extra chromosomal element) probably had a supernumerary chromosome or a fragment.

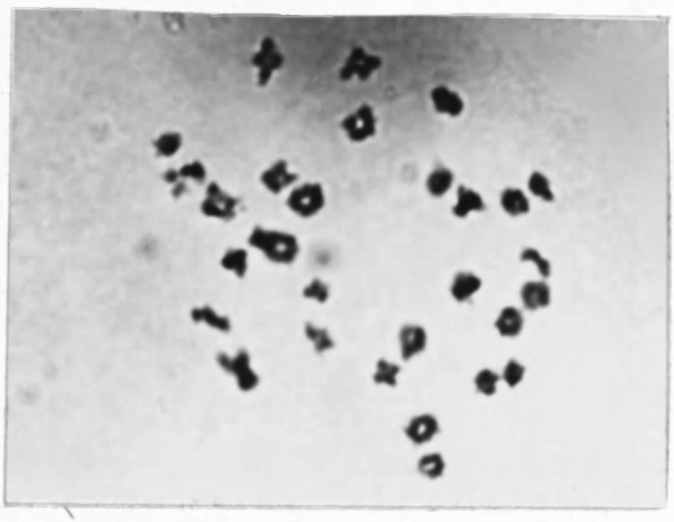


Fig. 1. Meiotic diakinesis of Sonorella odorata.

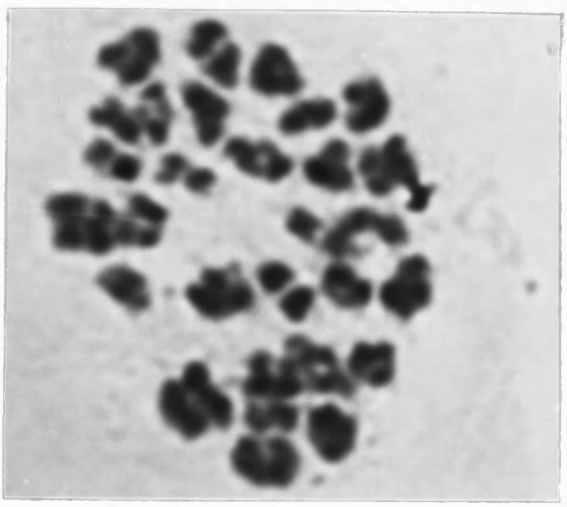
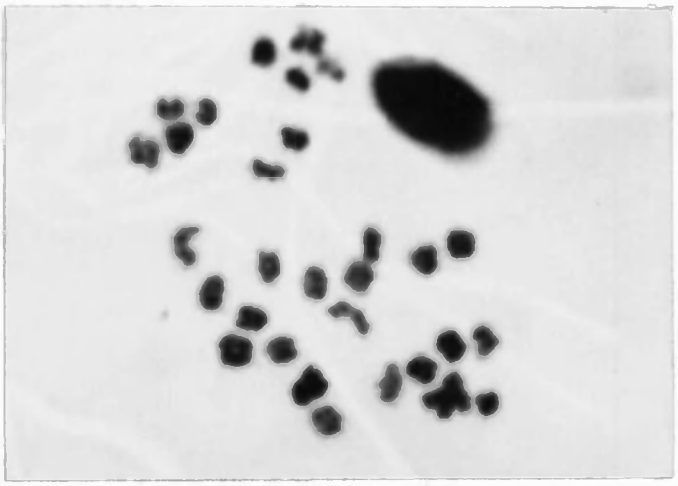
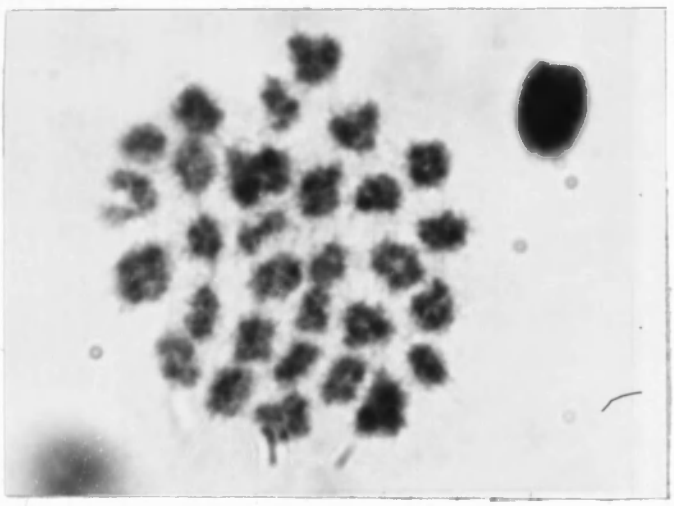


Fig. 2. Meiotic metaphase I of S. odorata.



(a) Meiotic diakinesis



(b) Meiotic metaphase I

Fig. 3. Meiosis in Xerarionta areolata.

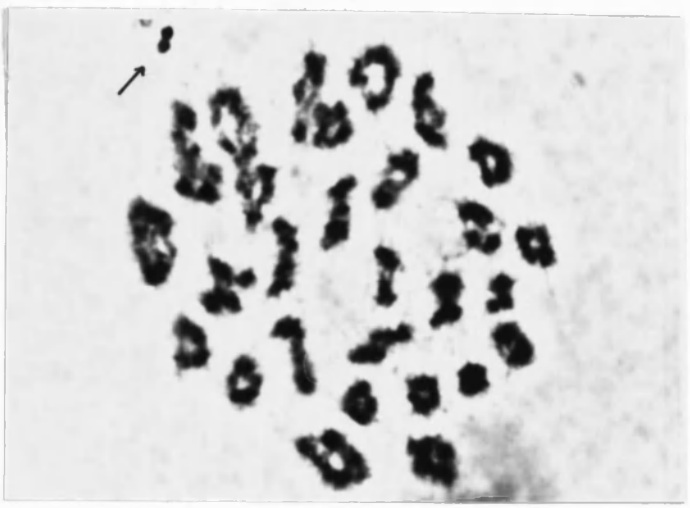


Fig. 4. Meiotic diakinesis of Sonorelix borregoensis -- A small chromosome fragment is also visible (arrow).

Humboldtiana högeana (Von Martens, 1892)

I examined 31 specimens of this species. Only 4 specimens had normal gonads; in the other 27, the ovotestis of each specimen was without germinal cells and reduced to a dark-brown, scarlike tissue. Meiotic chromosomes were found in only three specimens. Twenty-six spermatogonial spreads at diakinesis (Fig. 5) and 54 at metaphase I (Fig. 6) were obtained. In each case there were 30 bivalents. Meiosis was "normal," i.e., there were no chromosomal aberrations. The haploid chromosome number of H. högeana is therefore 30.

Greggelix indigena (Mabille, 1895)

Three specimens of G. indigena were studied. In view of the fact that I found numerical chromosomal aberrations, the results for each specimen are listed separately.

Specimen #1

Meiotic bivalents were counted from 85 spreads of spermatogonial tissue at metaphase I. In each case the count was 29 (Fig. 7). Furthermore, 45 favorable chromosome spreads at late diakinesis had 29 bivalents in each case (Fig. 8).

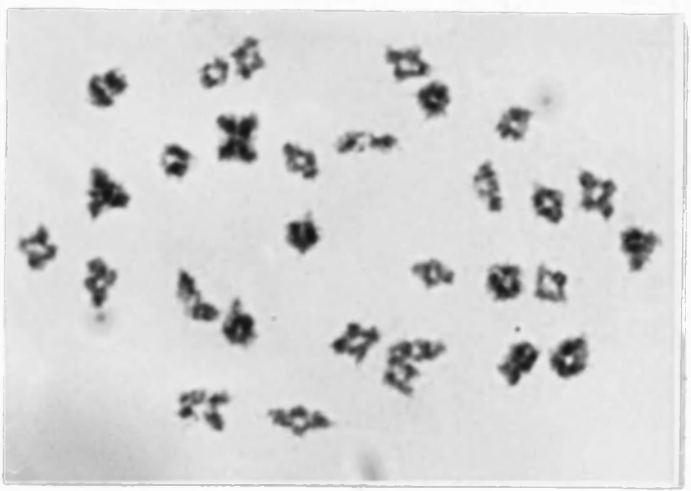


Fig. 5. Meiotic diakinesis of Humboldtiana högeana.

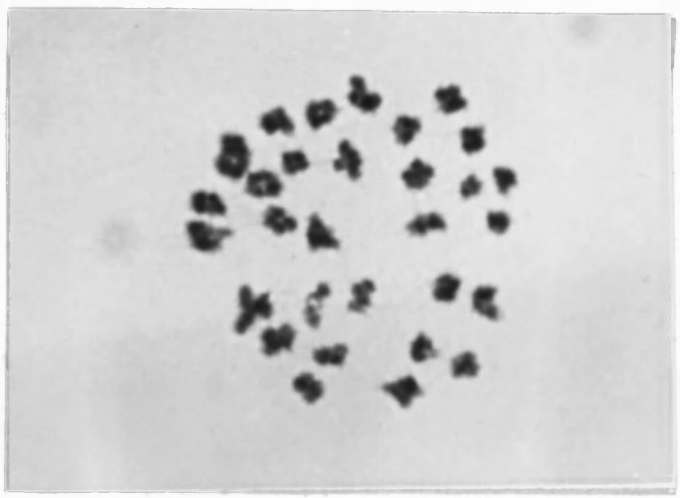


Fig. 6. Meiotic metaphase I of H. högeana.

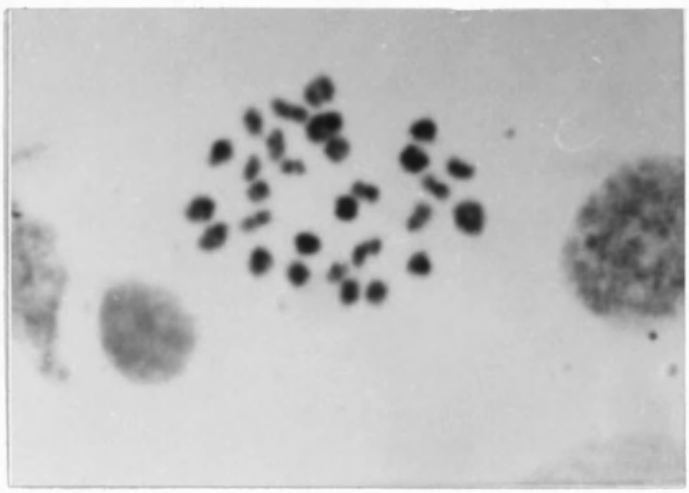


Fig. 7. Meiotic metaphase of Greggelix indigena with 29 bivalents.

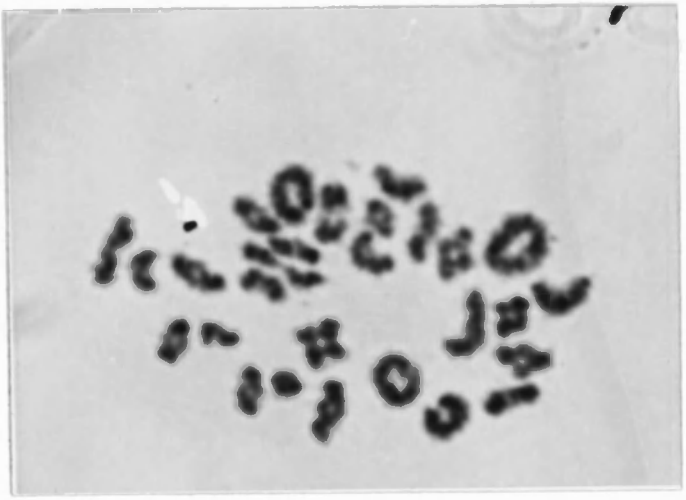


Fig. 8. Meiotic diakinesis of Greggelix indigena with 29 bivalents.

Specimen #2

Meiotic chromosomes at metaphase I were found in two primary spermatocytes with 29 bivalents in each cell.

Specimen #3

Meiotic chromosomes at diakinesis (five cells) and metaphase I (7 cells) were observed in this specimen. In each case, 28 bivalents and one trivalent were found. The trivalent was either "Y"-shaped (Fig. 9) or a chain of three chromosomes (Fig. 10). This specimen was apparently trisomic for one chromosome pair. The haploid number of G. indigena is 29.

Helminthoglypta

I studied two species of Helminthoglypta cytologically. The meiotic account of each species is given separately below.

Helminthoglypta cf. lowei (Bartsch, 1918)

Eight specimens of this species were examined cytologically. Five chromosome spreads in pachytene stage of meiosis yielded 30 bivalents (Fig. 11) in each case. Forty-one chromosome spreads in diplotene (Fig. 12) had 30 bivalents each. Similarly, 25 spreads in metaphase I showed 30 bivalents (Fig. 13). The nucleolus in the primary spermatocytes is clearly visible under the microscope with the nucleolar organizer chromosome. The nucleolar organizer

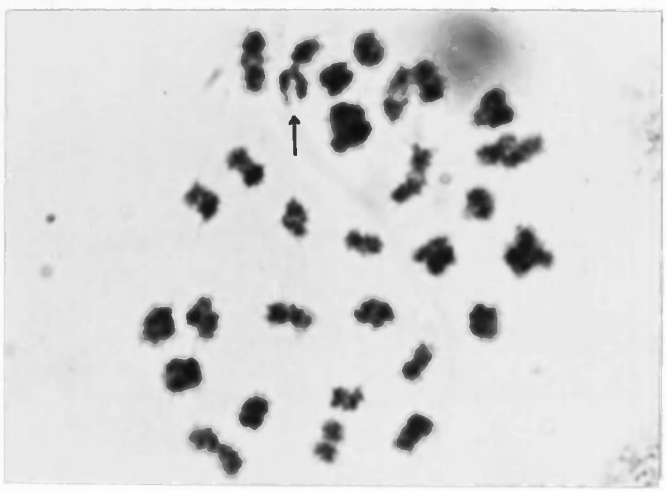


Fig. 9. Meiotic metaphase I of Greggelix indigena with 28 bivalents and a "Y"-shaped trivalent (arrow).

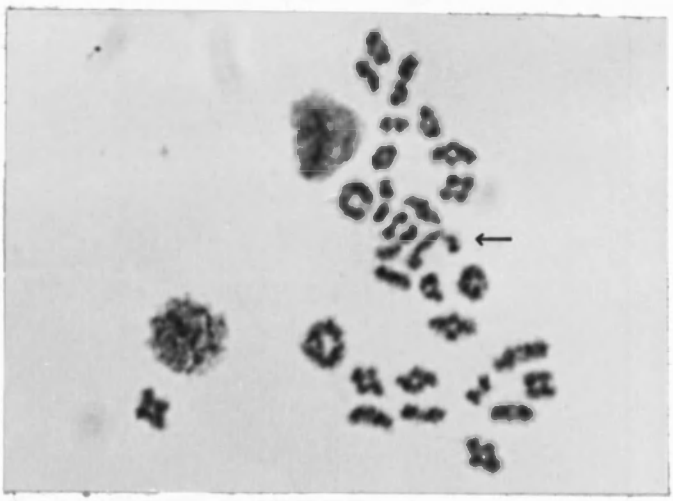


Fig. 10. Meiotic diakinesis of Greggelix indigena with 28 bivalents and a chain of three chromosomes (arrow).

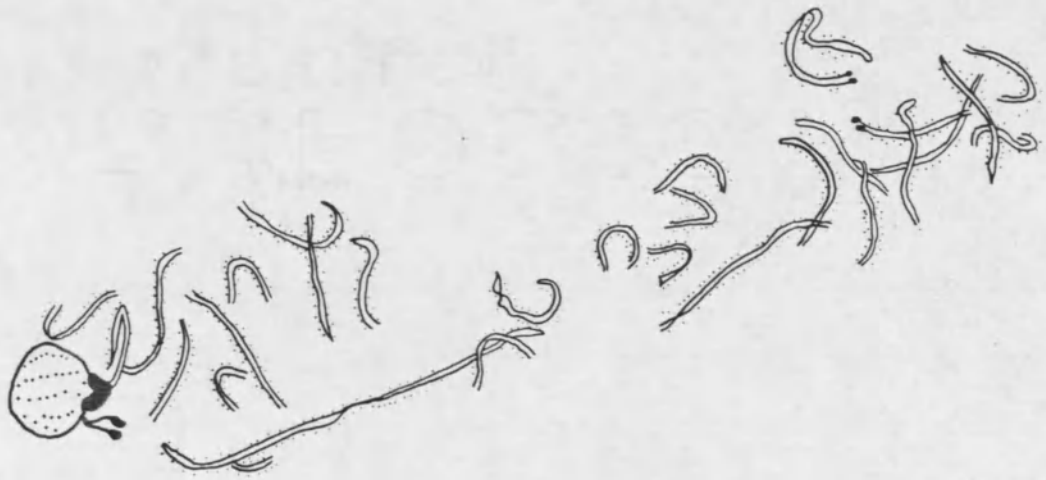


Fig. 11. Camera lucida drawing of pachytene stage of Helminthoglypta cf. lowei.

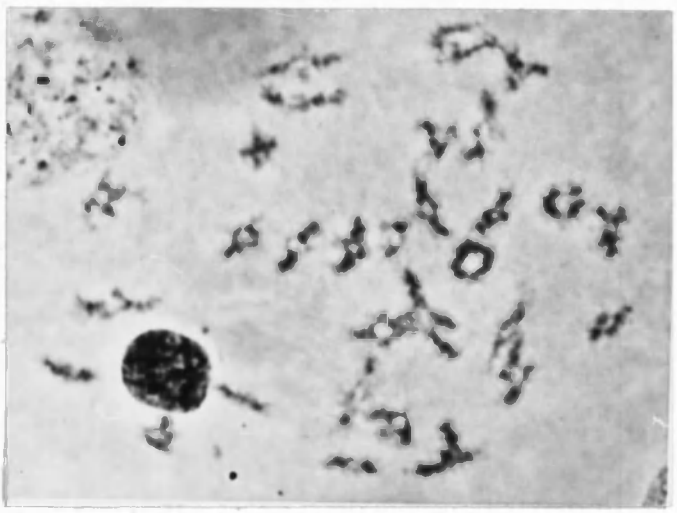


Fig. 12. Meiotic diplotene stage of H. cf. lowei.

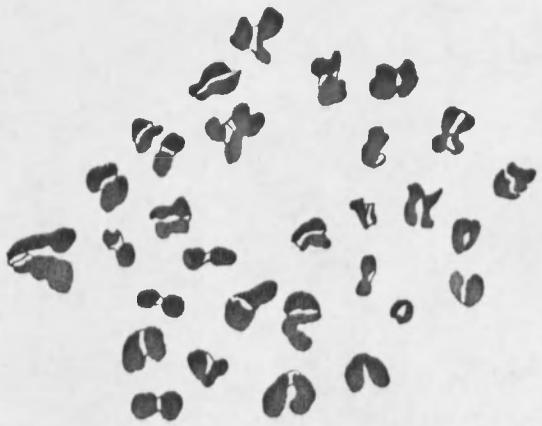


Fig. 13. Camera lucida drawing of meiotic metaphase I of H. cf. lowei.

bivalent has a heterochromatic short arm in pachytene (Fig. 11). The long arm of this bivalent also has heterochromatin near the nucleolus. The nucleolar heterochromatin is clearly seen in interphase nuclei (Fig. 14). The haploid chromosome number of H. cf. lowei is 30. There were no chromosomal aberrations observed in this species.

Helminthoglypta micrometallioides W. B. Miller, 1970

Only one specimen of this rare species was obtained and examined cytologically. The specimen in question had been estivating for more than one year in a terrarium in our laboratory. I found only one chromosome spread in pachytene (Fig. 15) with 30 bivalents. Observations of the nucleolar organizer and nucleolus under phase contrast microscope revealed that the second arm of the nucleolar organizer chromosome seen in pachytene of H. cf. lowei is missing (Fig. 16).

Monadenia infumata (Gould, 1855)

Only one specimen of M. infumata was available for study. I found three primary spermatocytes at diakinesis with 29 bivalents (Fig. 17). The haploid chromosome number of M. infumata is 29.

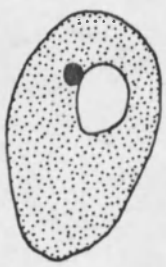


Fig. 14. Camera lucida drawing of interphase nucleus from a squash of ovotestis of H. cf. lowei -- Heterochromatin is clearly seen associated with the nucleolus.

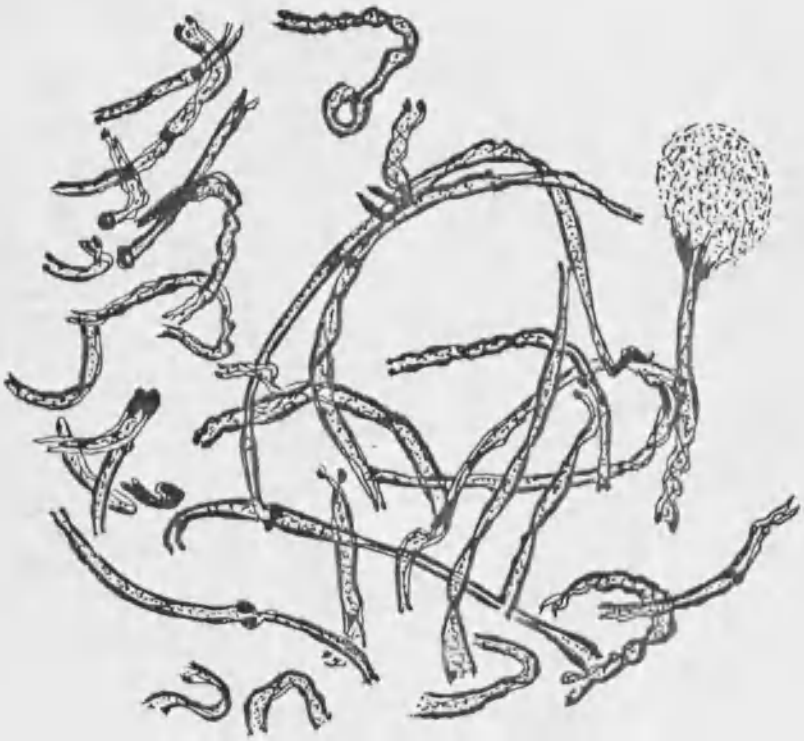
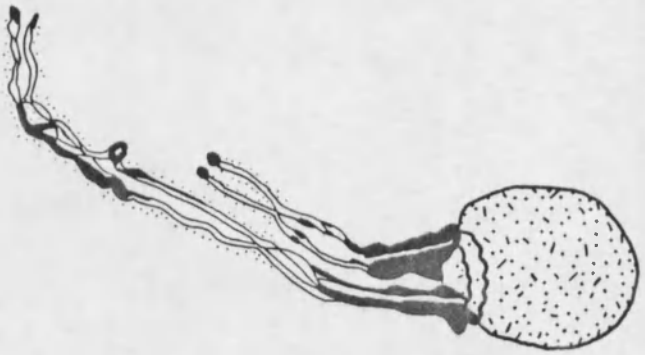
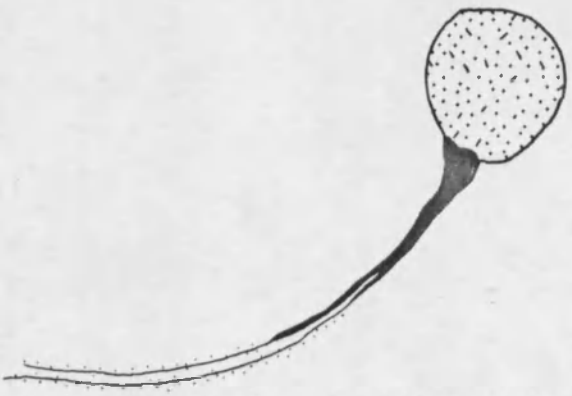


Fig. 15. Camera lucida drawing of pachytene stage of H. micrometallioides.



(a) Drawing of nucleolus, and nucleolar organizer of H. cf. lowei.



(b) Drawing of nucleolus and nucleolar organizer of H. micrometallioides. A heterochromatic short arm found in H. cf. lowei is missing in H. micrometallioides.

Fig. 16. Morphology of nucleolar organizer in H. cf. lowei and H. micrometallioides.

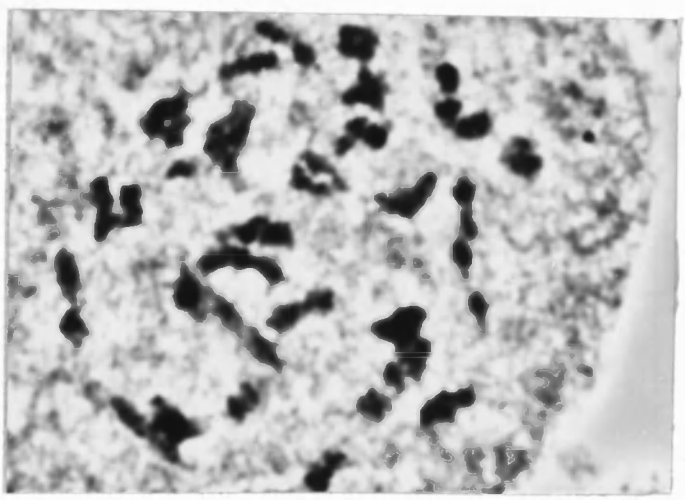


Fig. 17. Meiotic diakinesis of Monadenia infumata.

KARYOTYPES OF HELMINTHOGLYPTID GENERA

The mitotic metaphase chromosomes of the ten helminthoglyptid species described here are mostly metacentric (m), and submetacentric (sm), with one to three subtelocentric (st) chromosomes per karyotype. In all cases the chromosomes are monocentric. No attempt was made to locate secondary constrictions on chromosomes due to scarcity of enough specimens and adequate techniques suitable for pulmonate land snails.

Sonorella odorata Pilsbry and Ferriss, 1919

Mitotic metaphase spreads were obtained from only three specimens. In all, chromosomes from 42 spreads were counted. The haploid number was consistently 58. The karyotype of S. odorata (Fig. 18) has 15 pairs of metacentric (m), 13 pairs of submetacentric (sm), and one pair of subtelocentric (st) chromosomes. The relative lengths (RL), arm ratios, and centromeric indices (C.I.) for the haploid set are tabulated in Table 1. Figure 19 is the idiogram of the haploid set of S. odorata based on the data of Table 1. The largest chromosome is 6.7% and the smallest one is 1.9% of the haploid genome.

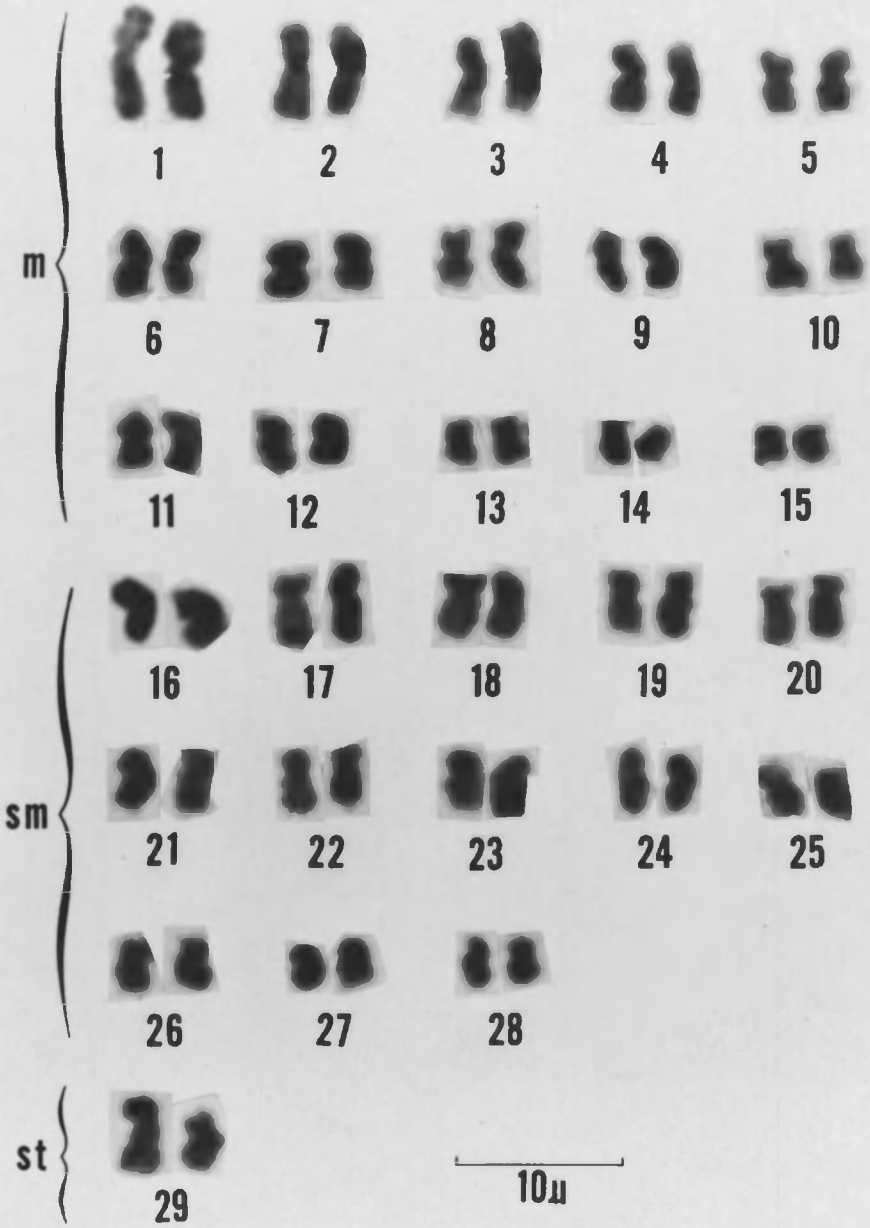


Fig. 18. Karyotype of Sonorella odorata.

Table 1. Relative lengths (RL), arm ratios (q/p), centromeric indices (C.I.), and chromosome types of the haploid set of Sonorella odorata.

Chromosome Number	RL	q/p	C.I.	Chromosome Type
1	67.3	1.2	46.5	m
2	58.3	1.4	40.9	m
3	50.7	1.3	44.4	m
4	45.4	1.4	41.4	m
5	38.2	1.4	41.0	m
6	35.1	1.2	46.4	m
7	34.5	1.2	45.5	m
8	32.9	1.3	42.9	m
9	32.9	1.1	47.6	m
10	32.3	1.6	38.8	m
11	27.2	1.4	40.2	m
12	26.3	1.4	40.5	m
13	24.0	1.4	41.6	m
14	21.9	1.3	42.9	m
15	18.8	1.4	41.7	m
16	39.5	2.5	28.6	sm
17	38.2	2.3	30.3	sm
18	37.5	2.0	33.3	sm
19	37.5	2.4	29.2	sm
20	36.0	2.8	26.1	sm
21	34.5	2.7	27.3	sm
22	31.9	2.4	29.4	sm
23	29.8	2.2	31.6	sm
24	28.2	2.0	33.3	sm
25	27.6	1.9	34.1	sm
26	24.0	2.1	32.5	sm
27	22.5	2.0	33.3	sm
28	20.4	2.2	30.8	sm
29	46.4	3.2	23.6	st

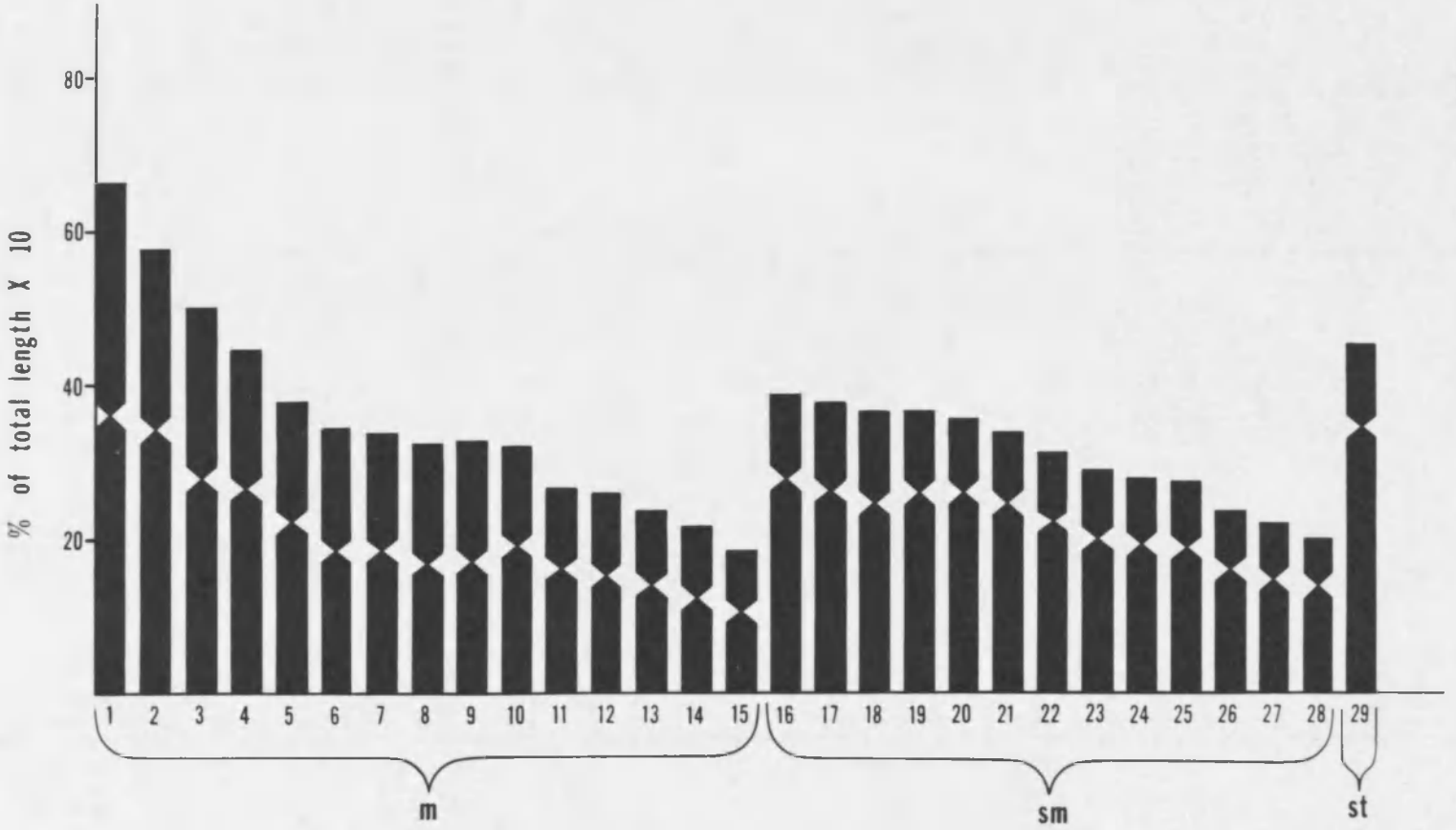


Fig. 19. Idiogram of the haploid set of S. odorata based on data in Table 1.

Xerarionta areolata (Pfeiffer, 1845)

Chromosome counts were made on 20 spermatogonial cells undergoing mitosis from 4 specimens. The diploid number in each case was 60. The karyotype of X. areolata (Fig. 20) has 19 pairs of metacentric (m), 10 pairs of submetacentric (sm), and one pair of subtelocentric (st) chromosomes. The chromosomal data are shown in Table 2. Figure 21 is the idiogram of X. areolata based on the data of Table 2. The largest chromosome is 8.7%, and the smallest one is 1.8% of the haploid genome.

Sonorelix borregoensis (Berry, 1929)

Spermatogonial mitoses were found in only one specimen. Chromosomes were counted from 15 metaphase plates. Based on these counts, the diploid number of S. borregoensis is 58. The karyotype (Fig. 22) consists of 13 pairs of metacentric (m), 15 pairs of submetacentric (sm), and one pair of subtelocentric (st) chromosomes. The chromosomal data are tabulated in Table 3. Figure 23 is the idiogram of the haploid set of S. borregoensis based on data of Table 3. The largest chromosome is 7.5% and the smallest one is 1.7% of the haploid genome.

Humboldtiana högeana (Von Martens, 1892)

Mitotic chromosome spreads were obtained from 4 specimens. In all, favorable spreads from 57 spermatogonial

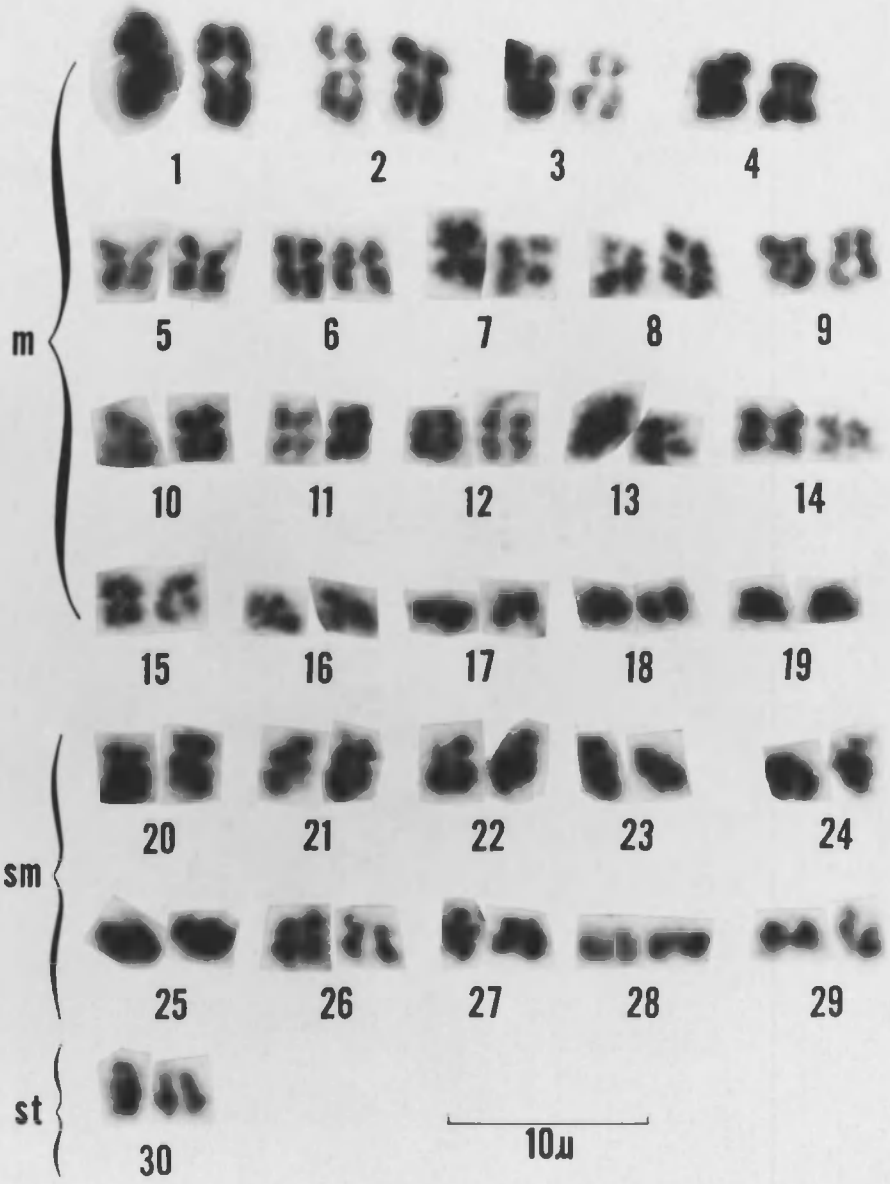


Fig. 20. Karyotype of Xerarionta areolata.

Table 2. Relative lengths (RL), arm ratios (q/p), centromeric indices (C.I.), and chromosome types of the haploid set of Xerarionta areolata.

Chromosome Number	RL	q/p	C.I.	Chromosome Type
1	86.5	1.2	45.8	m
2	67.4	1.6	39.0	m
3	47.6	1.4	42.4	m
4	45.0	1.3	44.0	m
5	43.2	1.4	41.6	m
6	37.1	1.3	43.6	m
7	36.0	1.2	45.0	m
8	34.2	1.2	45.3	m
9	32.8	1.3	42.9	m
10	30.3	1.5	40.5	m
11	29.5	1.1	48.8	m
12	28.8	1.5	40.0	m
13	28.8	1.4	41.3	m
14	27.7	1.2	45.5	m
15	27.0	1.4	44.0	m
16	26.3	1.2	45.2	m
17	22.7	1.3	42.9	m
18	19.9	1.4	41.9	m
19	19.8	1.2	45.5	m
20	39.6	2.1	31.8	sm
21	32.4	2.0	33.3	sm
22	31.0	2.1	32.6	sm
23	30.6	2.6	28.2	sm
24	29.2	2.7	27.2	sm
25	28.1	2.4	29.5	sm
26	26.3	2.3	30.1	sm
27	24.5	2.4	29.4	sm
28	19.1	2.3	30.2	sm
29	18.0	2.3	30.0	sm
30	30.3	4.9	16.7	st

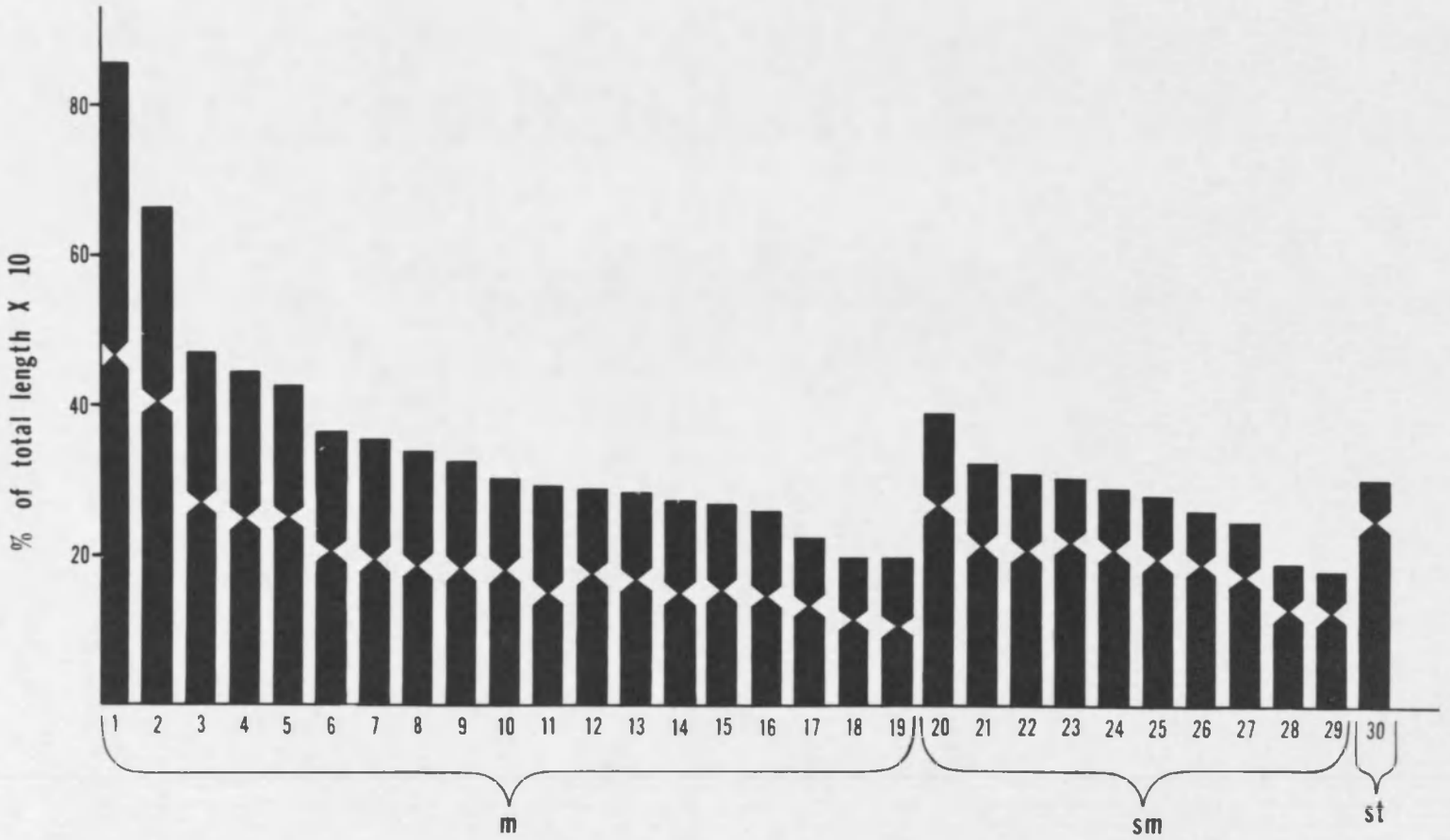


Fig. 21. Idiogram of the haploid set of X. areolata based on data in Table 2.

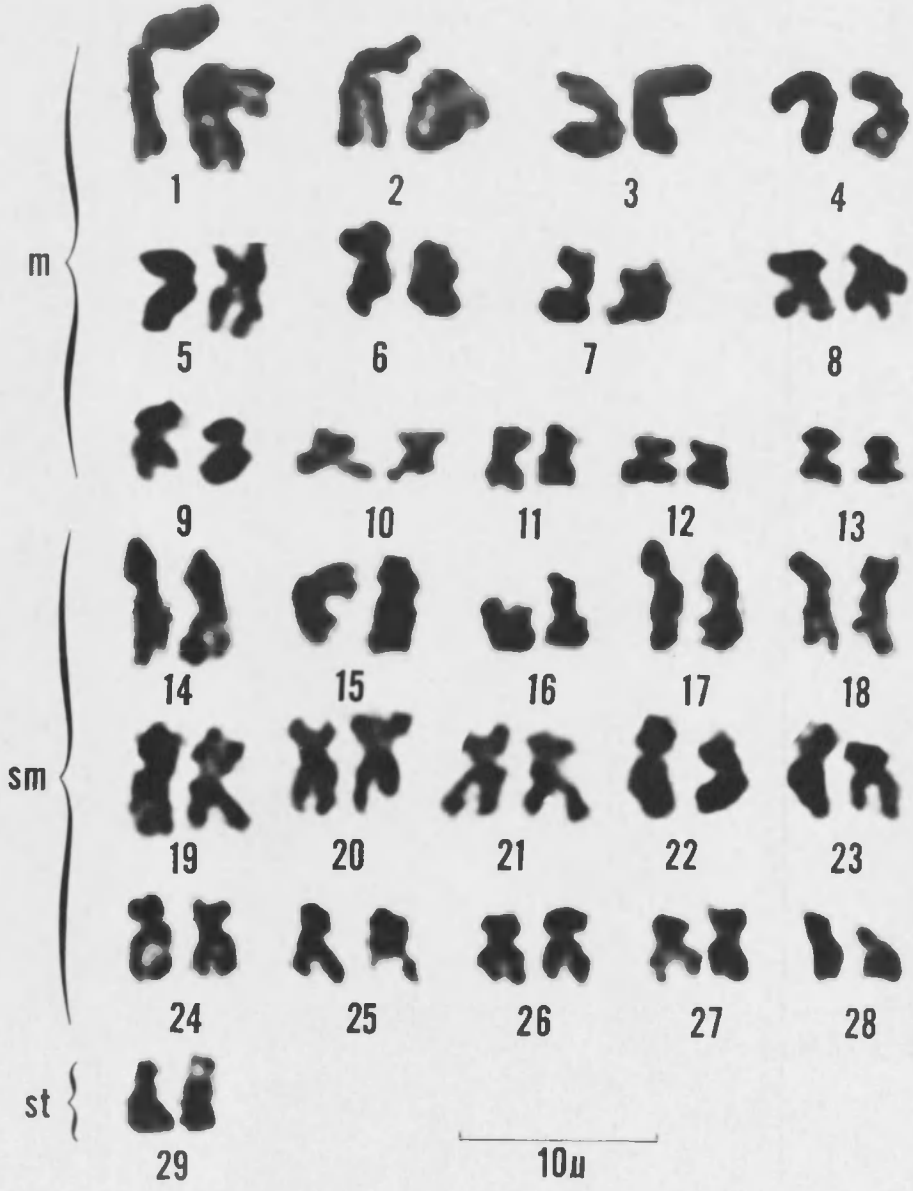


Fig. 22. Karyotype of Sonorelix borregoensis.

Table 3. Relative lengths (RL), arm ratios (q/p), centromeric indices (C.I.), and chromosome types of the haploid set of *Sonorelix borregoensis*.

Chromosome Number	RL	q/p	C.I.	Chromosome Type
1	75.0	1.3	43.0	m
2	62.6	1.1	47.0	m
3	57.0	1.1	46.7	m
4	52.2	1.1	47.3	m
5	38.2	1.3	42.8	m
6	30.4	1.3	43.8	m
7	29.2	1.2	45.5	m
8	28.9	1.2	46.1	m
9	24.7	1.4	42.3	m
10	22.4	1.5	40.7	m
11	20.9	1.2	45.5	m
12	19.9	1.6	38.1	m
13	17.1	1.3	44.4	m
14	47.5	1.9	34.0	sm
15	42.7	2.0	33.3	sm
16	41.8	2.7	27.3	sm
17	39.9	2.8	26.2	sm
18	39.9	2.2	31.0	sm
19	39.9	2.0	33.3	sm
20	35.7	2.4	29.8	sm
21	31.3	1.7	36.4	sm
22	30.0	1.7	36.7	sm
23	28.5	2.0	33.3	sm
24	27.5	2.2	31.0	sm
25	24.6	2.2	30.7	sm
26	23.3	2.7	26.8	sm
27	21.5	2.4	29.2	sm
28	19.9	2.5	29.6	sm
29	25.6	4.5	18.5	st

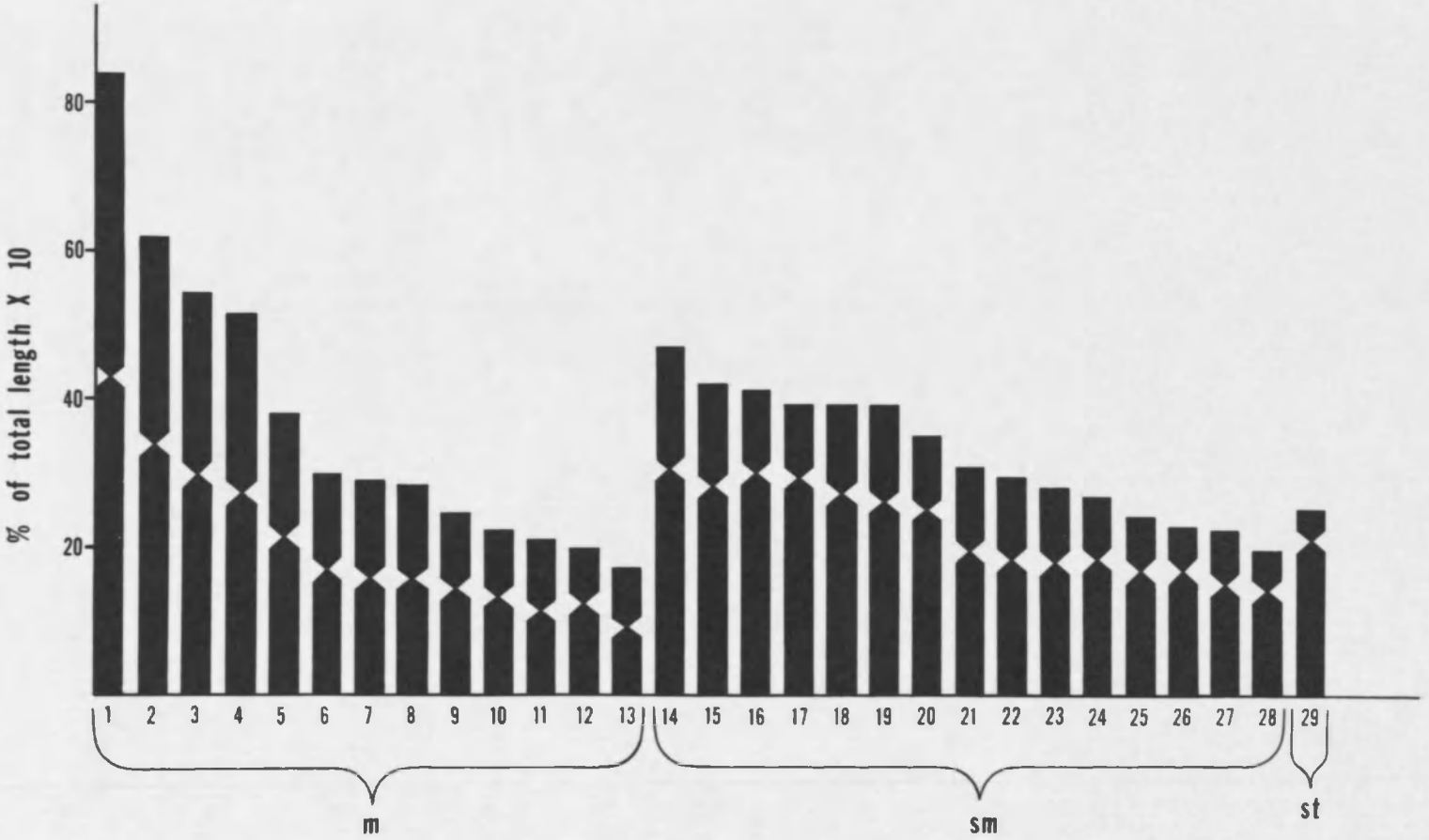


Fig. 23. Idiogram of the haploid set of S. borregoensis based on data in Table 3.

cells were counted. The diploid number in each case was 60. The karyotype of H. högeana (Fig. 24) has 16 pairs of metacentric (m), 11 pairs of submetacentric (sm), and 3 pairs of subtelocentric (st) chromosomes. The chromosomal data are tabulated in Table 4. Figure 25 is the idiogram of H. högeana based on the data of Table 4. The largest chromosome is 7.4% and the smallest one is 2.0% of the haploid genome.

Greggelix indigena (Mabille, 1895)

The first attempt to obtain mitotic chromosomes from three specimens of G. indigena failed and instead only meiotic chromosomes were obtained. Therefore, the rest of three specimens were reared in terraria for one year before a second attempt was made to obtain mitotic chromosome spreads. This time spermatogonial metaphase chromosomes were obtained from all three specimens of G. indigena. In all, chromosomes were counted from 22 chromosome spreads. Based on these counts, the diploid number was determined to be 58. The karyotype of G. indigena (Fig. 26) has 16 pairs of metacentric (m), 10 pairs of submetacentric (sm), and three pairs of subtelocentric (st) chromosomes. Chromosomal data are tabulated in Table 5 from which the idiogram of G. indigena has been constructed (Fig. 27). The largest chromosome is 7.0% and the smallest one is 1.7% of the haploid genome.

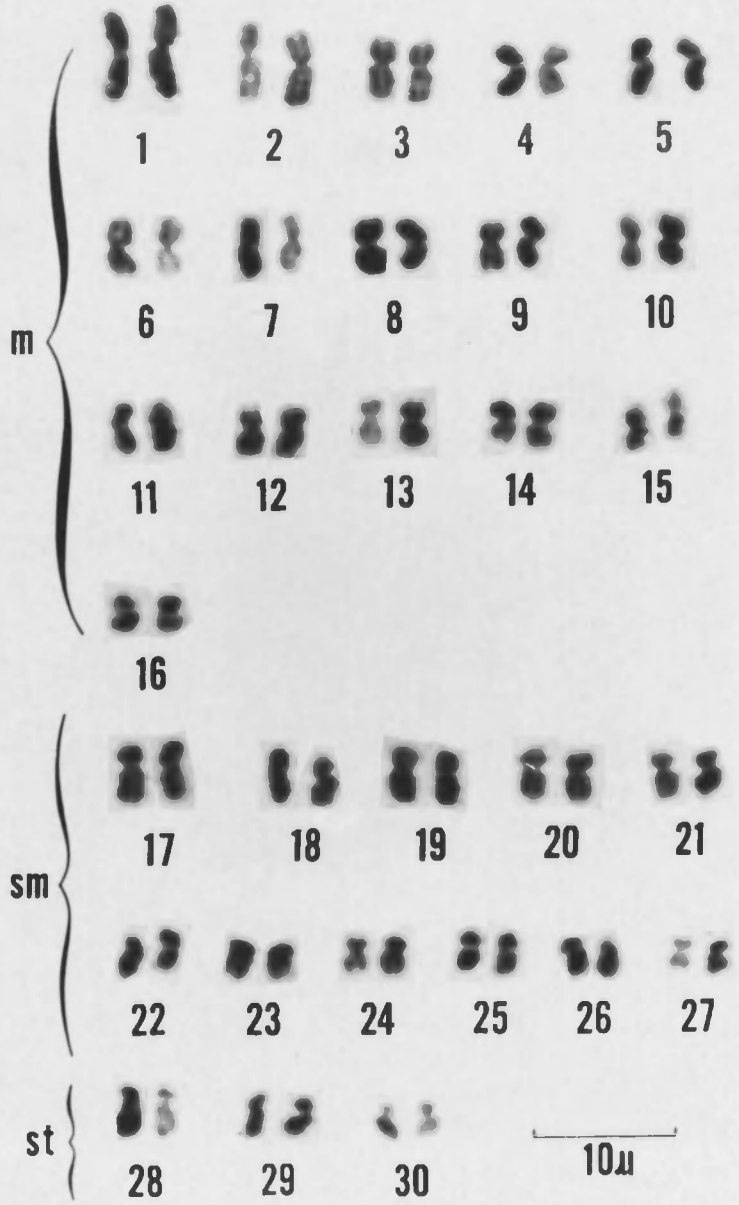


Fig. 24. Karyotype of Humboldtiana högeana.

Table 4. Relative lengths (RL), arm ratios (q/p), centromeric indices (C.I.), and chromosome types of the haploid set of Humboldtiana högeana.

Chromosome Number	RL	q/p	C.I.	Chromosome Type
1	73.8	1.2	45.1	m
2	57.2	1.3	44.3	m
3	43.4	1.3	42.6	m
4	41.6	1.4	40.9	m
5	41.6	1.3	43.4	m
6	41.6	1.2	45.2	m
7	36.9	1.4	41.2	m
8	36.2	1.2	45.0	m
9	34.7	1.6	39.6	m
10	34.5	1.4	42.1	m
11	33.3	1.3	43.5	m
12	30.4	1.5	40.5	m
13	30.0	1.4	42.2	m
14	29.0	1.4	42.5	m
15	26.1	1.3	44.4	m
16	23.5	1.2	46.2	m
17	39.8	2.7	27.3	sm
18	36.6	2.1	32.7	sm
19	34.4	1.7	36.8	sm
20	32.6	2.0	33.3	sm
21	27.0	2.8	26.6	sm
22	25.3	2.5	28.6	sm
23	23.5	2.3	30.8	sm
24	22.4	2.1	32.3	sm
25	21.7	2.0	33.3	sm
26	21.0	2.2	31.0	sm
27	19.9	1.8	36.4	sm
28	31.5	3.8	20.7	st
29	27.5	3.2	23.7	st
30	22.1	3.1	24.6	st

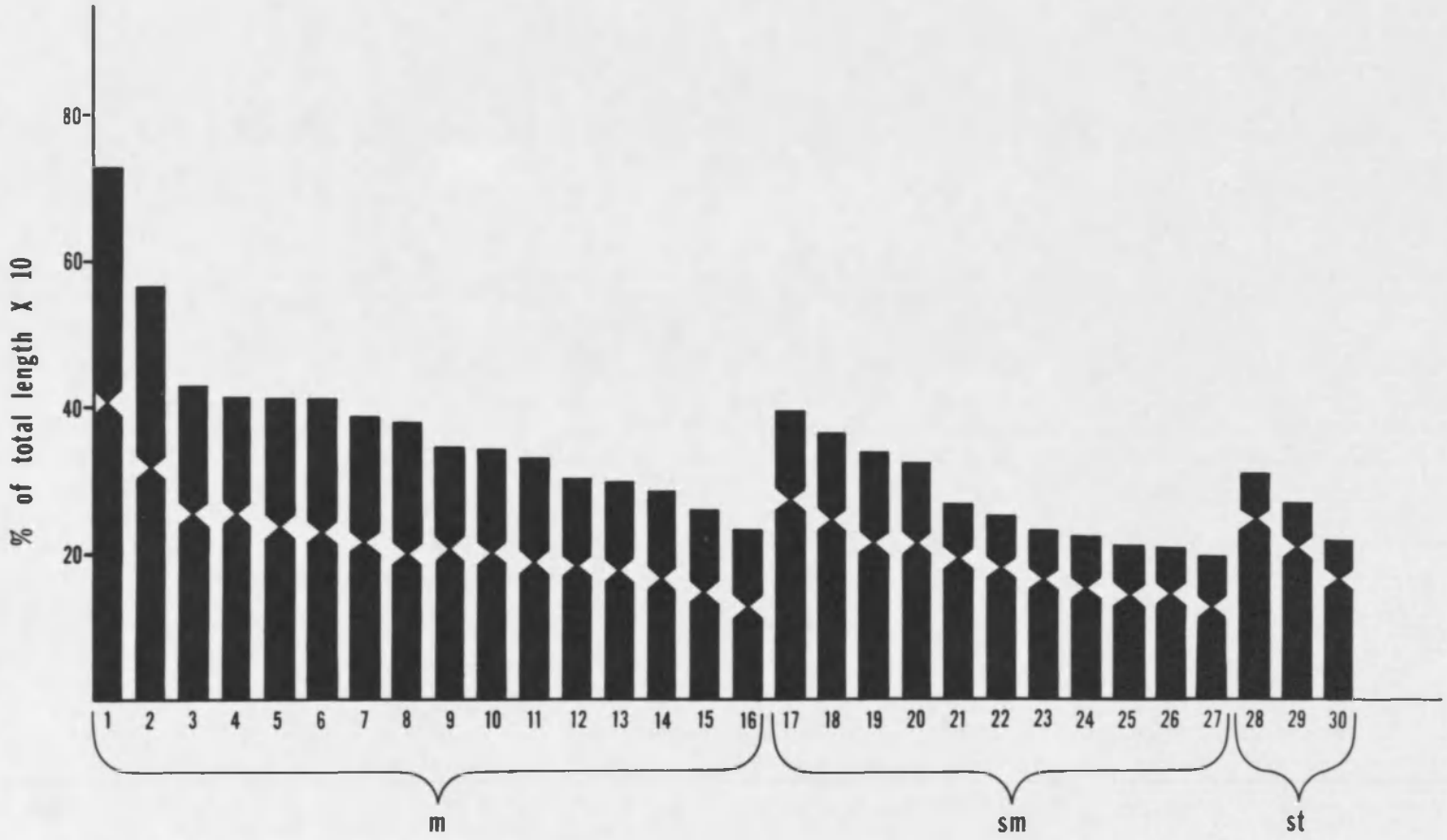


Fig. 25. Idiogram of the haploid set of H. högeana based on data in Table 4.

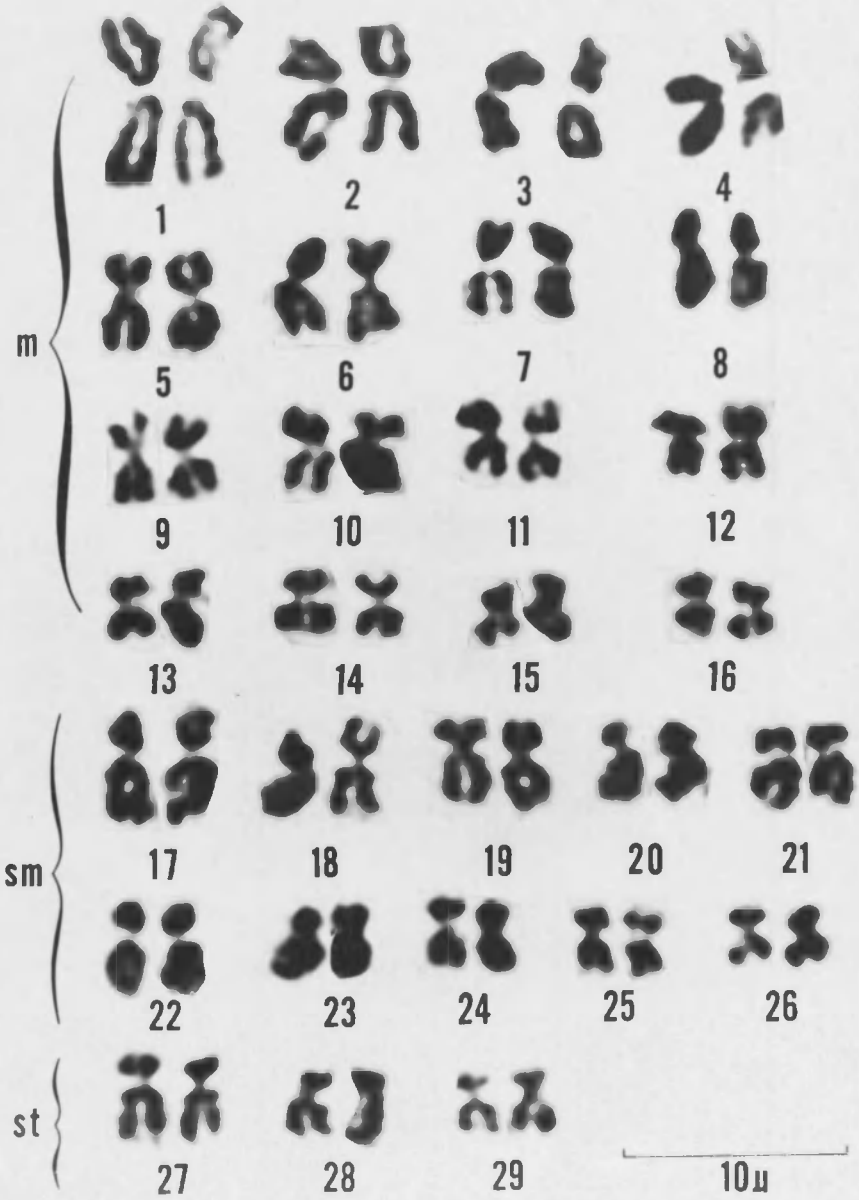


Fig. 26. Karyotype of Greggelix indigena.

Table 5. Relative lengths (RL), arm ratios (q/p), centromeric indices (C.I.), and chromosome types of the haploid set of *Greggelix indigena*.

Chromosome Number	RL	q/p	C.I.	Chromosome Type
1	69.7	1.2	45.8	m
2	61.0	1.2	46.0	m
3	51.3	1.03	49.1	m
4	50.3	1.2	46.2	m
5	46.5	1.4	41.7	m
6	43.6	1.3	44.4	m
7	39.7	1.2	46.3	m
8	36.8	1.1	47.4	m
9	35.8	1.1	48.6	m
10	34.8	1.6	38.9	m
11	31.0	1.1	46.9	m
12	30.6	1.03	49.4	m
13	24.2	1.3	44.0	m
14	21.3	1.2	45.5	m
15	17.4	1.3	44.4	m
16	16.8	1.1	48.3	m
17	46.5	2.1	32.6	sm
18	41.6	1.9	34.9	sm
19	34.8	2.0	33.3	sm
20	32.9	1.8	35.3	sm
21	31.1	1.9	34.8	sm
22	30.8	2.5	28.3	sm
23	28.0	1.9	34.5	sm
24	24.2	1.8	36.0	sm
25	20.3	2.0	33.3	sm
26	18.2	1.8	36.2	sm
27	32.9	3.3	23.5	st
28	25.2	3.3	23.1	st
29	22.6	3.04	24.8	st

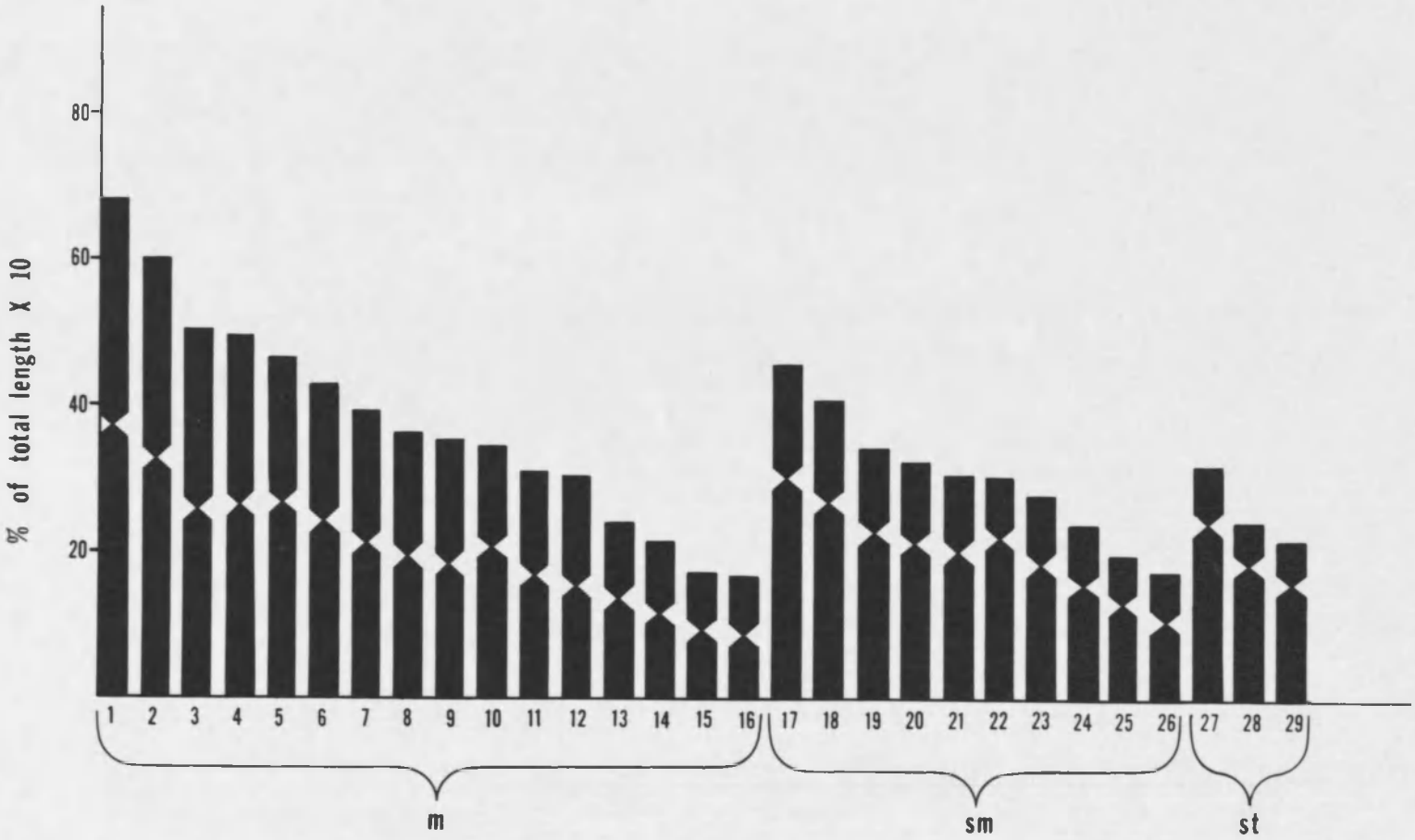


Fig. 27. Idiogram of the haploid set of G. indigena based on data in Table 5.

Helminthoglypta cf. lowei (Bartsch, 1918)

Spermatogonial mitotic chromosomes were obtained from 5 specimens. In all, 39 favorable mitotic metaphase spreads were counted to determine the diploid number. Based on these counts, the diploid number of H. cf. lowei is 60. The karyotype of H. cf. lowei (Fig. 28) has 17 pairs of metacentric (m), 10 pairs of submetacentric (sm), and three pairs of subtelocentric (st) chromosomes. The chromosomal data are tabulated in Table 6, from which the idiogram of H. cf. lowei (Fig. 29) has been constructed. The largest chromosome is 5.7% and the smallest one is 2.0% of the haploid genome.

Helminthoglypta micrometallioides W. B. Miller, 1970

Only one spermatogonial metaphase chromosome spread was obtained from the single available specimen. Due to lack of chromosome spreads and considerable overlap in chromosomes of the single available one, no attempt was made to construct the karyotype and idiogram of H. micrometallioides. Instead, a camera lucida drawing of the chromosome spread under the microscope was made and is shown in Fig. 30. The diploid number of H. micrometallioides, based on this spread, is 60.

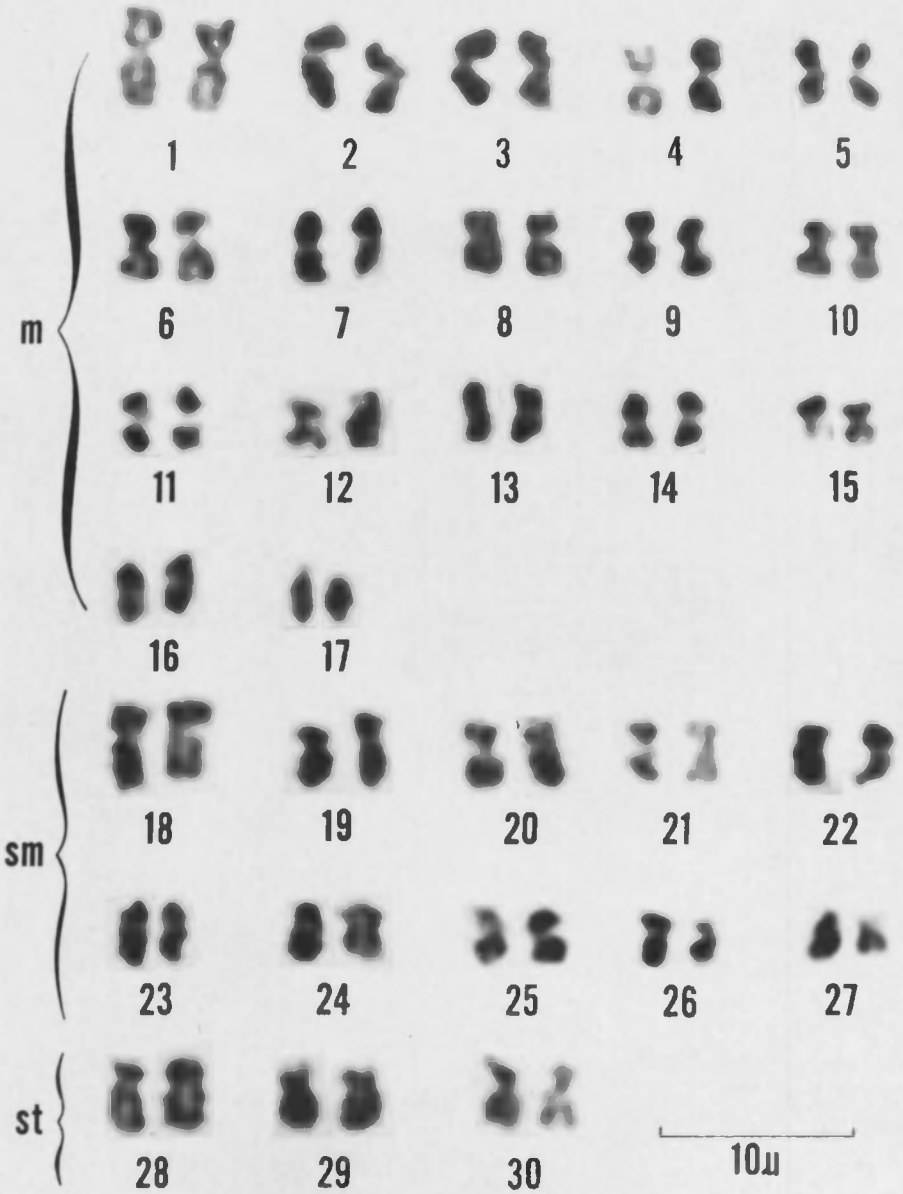


Fig. 28. Karyotype of Helminthoglypta cf. lowei.

Table 6. Relative lengths (RL), arm ratios (q/p), centromeric indices (C.I.), and chromosome types of the haploid set of *Helminthoglypta cf. lowei*.

Chromosome Number	RL	q/p	C.I.	Chromosome Type
1	56.7	1.2	45.0	m
2	54.6	1.1	47.9	m
3	50.7	1.01	49.7	m
4	40.1	1.01	49.6	m
5	38.3	1.5	40.7	m
6	36.1	1.6	39.3	m
7	34.8	1.2	44.7	m
8	32.7	1.5	39.1	m
9	31.2	1.2	45.4	m
10	31.2	1.6	38.0	m
11	29.8	1.1	47.6	m
12	28.8	1.02	49.3	m
13	28.2	1.01	49.6	m
14	26.8	1.01	49.5	m
15	25.5	1.3	44.4	m
16	22.7	1.6	37.5	m
17	19.9	1.1	46.7	m
18	48.2	1.8	35.2	sm
19	41.1	2.2	31.0	sm
20	35.9	2.0	33.3	sm
21	32.1	2.0	36.5	sm
22	29.8	2.0	33.3	sm
23	29.1	1.7	36.5	sm
24	25.5	2.0	33.3	sm
25	24.0	1.8	35.2	sm
26	20.5	2.3	30.5	sm
27	20.0	2.0	33.3	sm
28	36.9	3.3	23.0	st
29	36.9	3.3	23.0	st
30	29.0	4.8	17.2	st

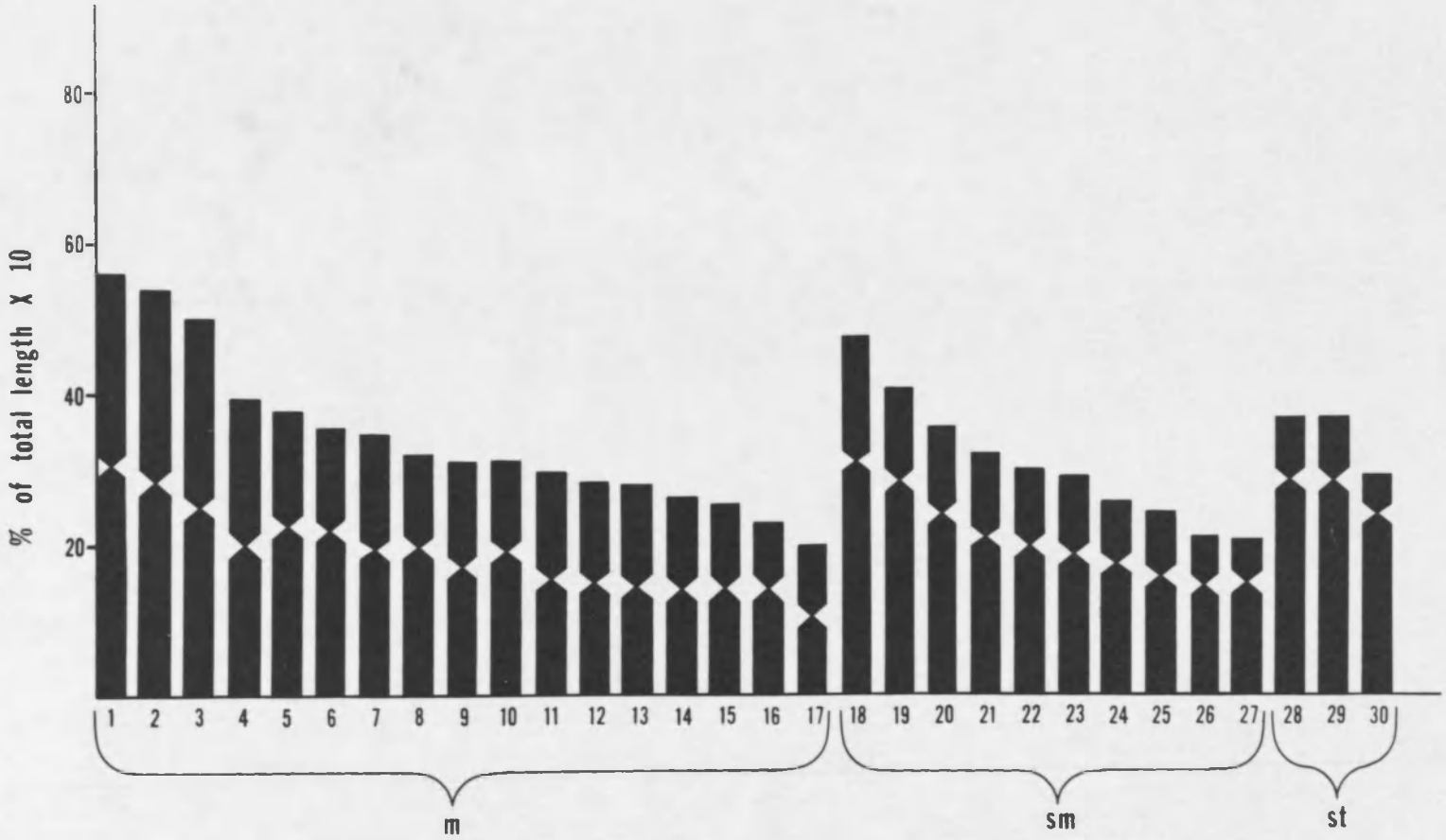


Fig. 29. Idiogram of the haploid set of H. cf. lowei based on data in Table 6.

Fig. 30. Camera lucida drawing of the mitotic metaphase of Helminthoglypta micrometallioides.

Monadenia infumata (Gould, 1855)

Only one early mitotic metaphase spread was obtained from the single available specimen of M. infumata. The data presented here should be regarded as tentative. The karyotype of M. infumata has 16 pairs of metacentric (m), 12 pairs of submetacentric (sm), and one pair of subtelocentric (st) chromosomes (Fig. 31). There are two additional chromosomes labeled B_1 and B_2 in Fig. 31. Although both are subtelocentric (st), their lengths are unequal and it is most likely that they are nonhomologous in nature. They could be supernumerary or B chromosomes. Further cytological investigation will probably resolve this question. However, there is not sufficient data to accept or reject the possibility of supernumerary chromosomes in M. infumata. The specimen of M. infumata was probably also a primary trisomic as can be seen in the three chromosomes with identical morphology (No. 28) in Fig. 31. Based on this scanty information it can be assumed that the diploid number of M. infumata is 58+1 (to reflect to 2n+1 condition). This diploid number can be modified to accommodate the supernumerary chromosomes as 2n=58+1+2B's.

The largest chromosome is 6.6% and the smallest chromosome is 1.5% of the haploid genome, without considering the supernumerary chromosomes. The chromosomal data are tabulated in Table 7. Figure 32 is the idiogram of M. infumata constructed from the data of Table 7.

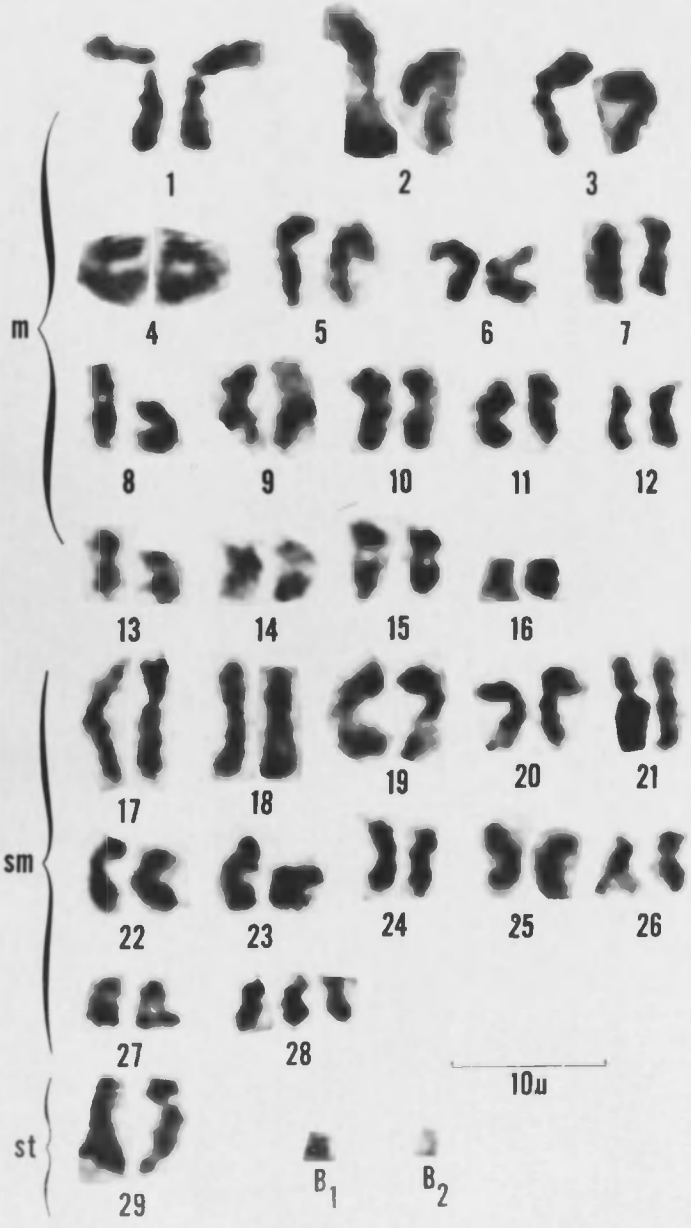


Fig. 31. Karyotype of Monadenia infumata -- B_1 and B_2 are two supernumerary chromosomes. There are apparently 3 homologous chromosomes in No. 28.

Table 7. Relative lengths (RL), arm ratios (q/p), centromeric indices (C.I.), and chromosome types of the haploid set of Monadenia infumata.

Chromosome Number	RL	q/p	C.I.	Chromosome Type
1	65.7	1.2	45.7	m
2	60.0	1.5	38.8	m
3	51.1	1.2	45.6	m
4	45.5	1.4	42.2	m
5	37.1	1.6	38.4	m
6	36.6	1.1	49.4	m
7	30.9	1.4	41.4	m
8	30.7	1.5	39.1	m
9	30.4	1.5	39.5	m
10	27.9	1.6	37.7	m
11	27.0	1.4	41.7	m
12	25.0	1.4	39.0	m
13	24.0	1.1	46.9	m
14	22.8	1.2	46.2	m
15	21.3	1.02	49.3	m
16	14.9	1.5	40.2	m
17	47.8	1.7	37.1	sm
18	43.5	1.8	36.2	sm
19	43.5	1.8	36.8	sm
20	38.3	2.4	29.4	sm
21	37.2	1.8	35.5	sm
22	34.7	1.9	34.8	sm
23	34.6	2.2	30.0	sm
24	28.6	1.7	36.8	sm
25	25.6	2.4	29.3	sm
26	23.6	2.0	33.3	sm
27	16.5	1.8	36.4	sm
28	15.5	1.7	36.9	sm
29	37.6	3.1	24.0	st

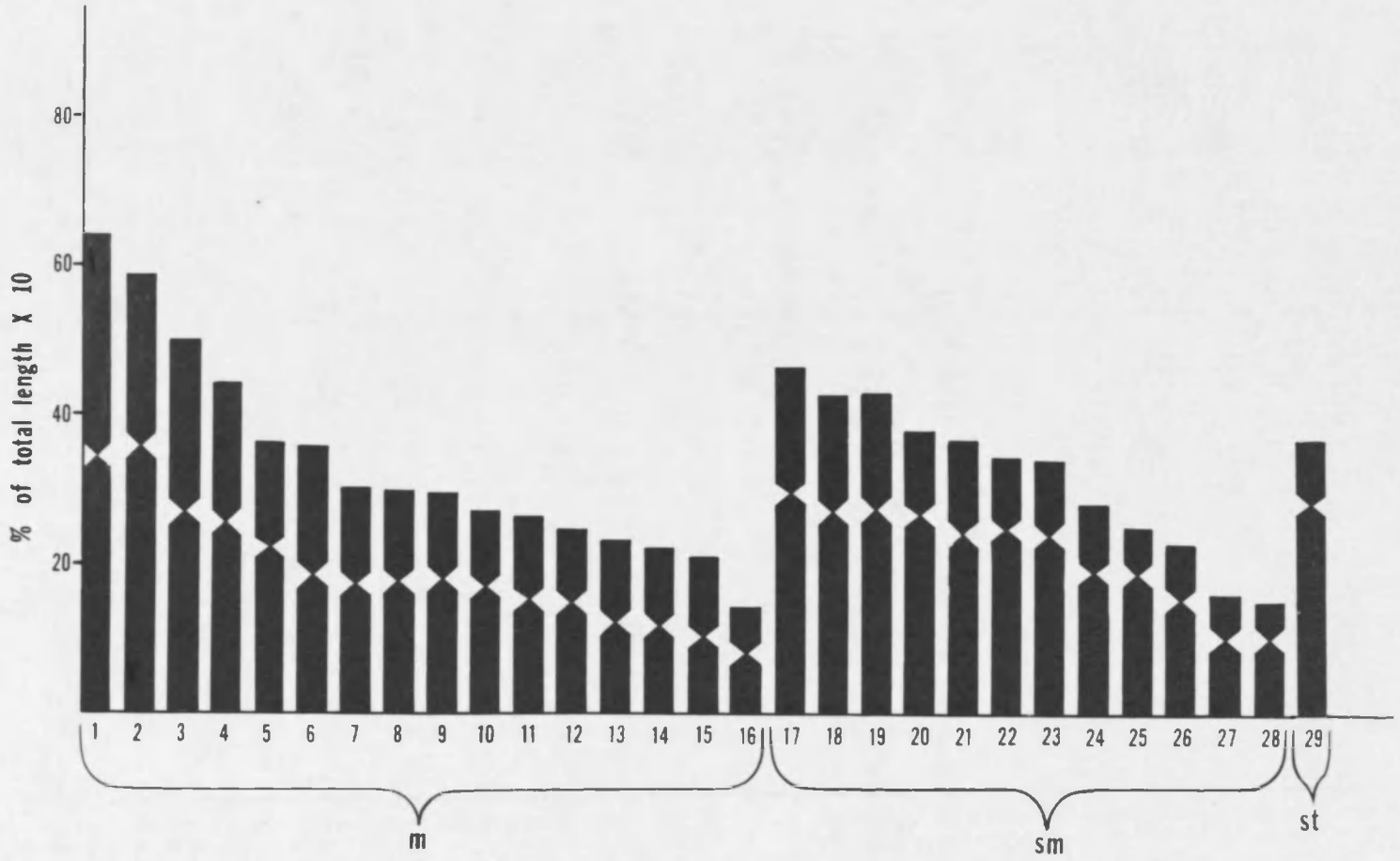


Fig. 32. Idiogram of the haploid set of M. infumata based on data in Table 7 -- B_1 and B_2 are omitted from this idiogram.

Micrarionta rufocincta beatula Cockerell, 1929

Mitotic metaphase chromosomes were obtained from only one out of the seven specimens studied cytologically. In all, chromosome spreads from seven cells were obtained. Chromosome counts revealed a diploid number of 60. The karyotype of *M. r. beatula* (Fig. 33) has 18 pairs of metacentric (*m*), 10 pairs of submetacentric (*sm*), and 2 pairs of subtelocentric (*st*) chromosomes. The chromosomal data are tabulated in Table 8. An idiogram of the haploid set of *M. r. beatula* (Fig. 34) has been constructed from the data of Table 8. The largest chromosome is 7.6% and the smallest one is 1.7% of the haploid genome.

The first two metacentric pairs of chromosomes (Nos. 1 and 2 in Fig. 33) are heteromorphic. In chromosome pair "1" the chromosome on the left side is metacentric, with a relatively large, uncoiled portion near the centromere. The second chromosome of pair "1" is subtelocentric. The uncoiled nature of the "left" chromosome is unique since it is in metaphase when all the chromosomes are usually contracted. The heteromorphism in the first chromosome pair could best be explained by assuming a pericentric inversion in the left chromosome. The second pair of heteromorphic chromosomes is No. 2 in Fig. 33. Here the left chromosome appears to be longer in size than the right one. One reason for this heteromorphism in two

Fig. 33. Karyotype of Micrarionta rufocincta beatula -- Heteromorphism in chromosome pairs Nos. 1 and 2 is evident.

Table 8. Relative lengths (RL), arm ratios (q/p), centromeric indices (C.I.), and chromosome types of the haploid set of Micrarionta rufocincta beatula.

Chromosome Number	RL	q/p	C.I.	Chromosome Type
1	75.5	1.6	38.0	m
2	58.9	1.3	43.5	m
3	49.2	1.3	42.9	m
4	41.1	1.1	48.5	m
5	39.8	1.1	47.0	m
6	37.1	1.1	47.2	m
7	34.7	1.3	43.5	m
8	32.0	1.2	45.3	m
9	27.2	1.2	44.4	m
10	26.3	1.02	49.4	m
11	24.4	1.2	44.4	m
12	24.0	1.5	40.0	m
13	22.9	1.1	47.4	m
14	21.7	1.2	44.4	m
15	21.1	1.3	42.9	m
16	20.5	1.4	41.2	m
17	19.6	1.6	38.5	m
18	17.2	1.4	42.1	m
19	69.8	2.5	28.3	sm
20	43.8	2.2	31.0	sm
21	39.2	2.3	30.7	sm
22	33.8	2.5	28.6	sm
23	32.0	1.9	33.7	sm
24	30.2	1.8	35.0	sm
25	28.7	1.9	33.7	sm
26	26.3	2.5	28.7	sm
27	20.5	1.9	33.8	sm
28	19.0	2.2	31.7	sm
29	35.3	3.4	23.0	st
30	28.4	3.7	21.3	st

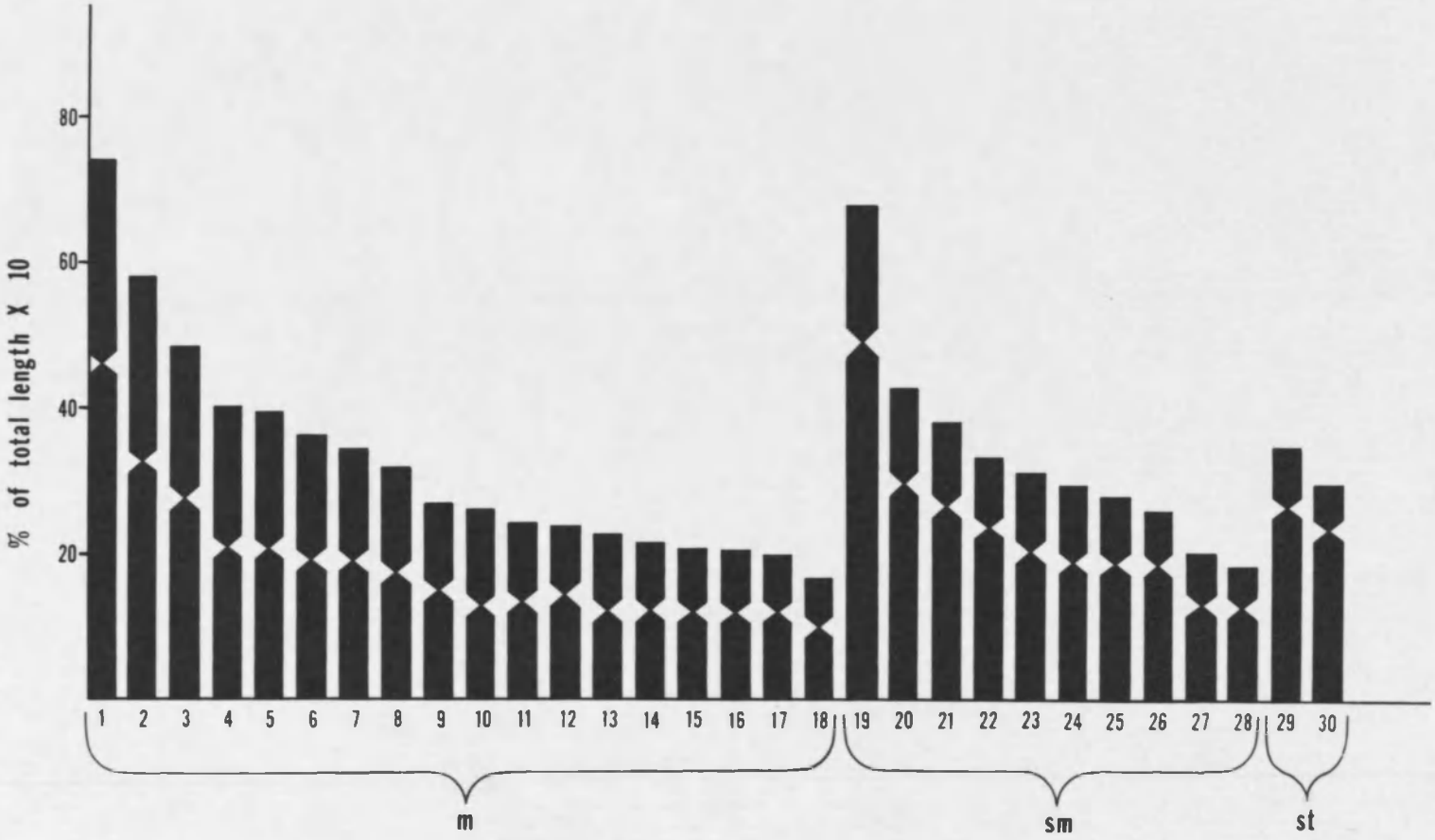


Fig. 34. Idiogram of the haploid set of M. r. beatula based on data in Table 8.

otherwise homologous chromosomes from the same cell could be a differential rate of contraction in each homolog. It is hard to postulate any causitive agents or forces that could allow chromosomes to contract differentially.

Eremarionta indioensis (Yates, 1890)

Nineteen mitotic metaphase chromosome spreads from the two available specimens of E. indioensis were obtained. The diploid chromosome number from these counts was determined to be 60 in all cases. The karyotype (Fig. 35) of E. indioensis has 18 pairs of metacentric (m), 11 pairs of submetacentric (sm), and one pair of subtelocentric (st) chromosomes. Heteromorphism in length of the first pair (No. 1 in Fig. 35) is evident. The largest chromosome is 8.0% and the smallest one is 1.5% of the haploid genome. Chromosomal data are tabulated in Table 9, from which the idiogram of E. indioensis (Fig. 36) has been constructed.

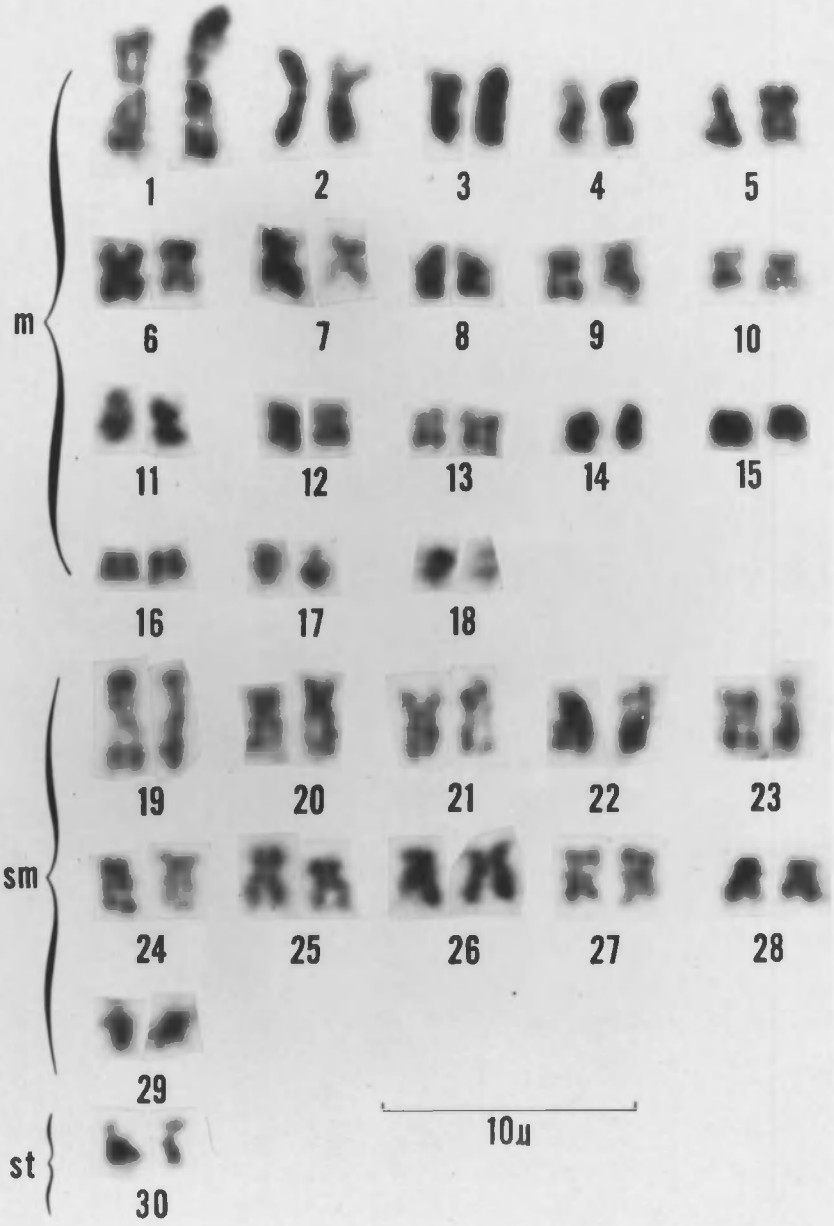


Fig. 35. Karyotype of Eremarionta indioensis -- Heteromorphism in length of the first pair of chromosomes is evident.

Table 9. Relative lengths (RL), arm ratios (q/p), centromeric indices (C.I.), and chromosome types of the haploid set of Eremarionta indioensis.

Chromosome Number	RL	q/p	C.I.	Chromosome Type
1	79.5	1.2	46.4	m
2	61.0	1.4	42.2	m
3	42.2	1.4	41.6	m
4	40.8	1.3	43.1	m
5	35.8	1.5	39.3	m
6	35.2	1.01	49.5	m
7	33.9	1.5	41.5	m
8	31.2	1.6	39.1	m
9	29.8	1.4	40.9	m
10	25.2	1.4	40.9	m
11	24.4	1.3	44.4	m
12	22.0	1.4	41.1	m
13	23.0	1.6	38.9	m
14	19.6	1.4	42.1	m
15	16.5	1.3	43.6	m
16	19.4	1.3	44.0	m
17	18.4	1.1	47.8	m
18	15.4	1.3	44.4	m
19	60.1	1.8	36.0	sm
20	45.7	2.6	27.7	sm
21	40.7	2.0	33.3	sm
22	36.8	2.8	26.2	sm
23	35.8	2.2	31.7	sm
24	35.2	2.3	30.7	sm
25	34.6	2.2	31.3	sm
26	32.9	2.5	28.8	sm
27	30.3	1.7	37.4	sm
28	27.8	1.9	34.5	sm
29	19.0	1.8	35.7	sm
30	27.1	3.0	25.0	st

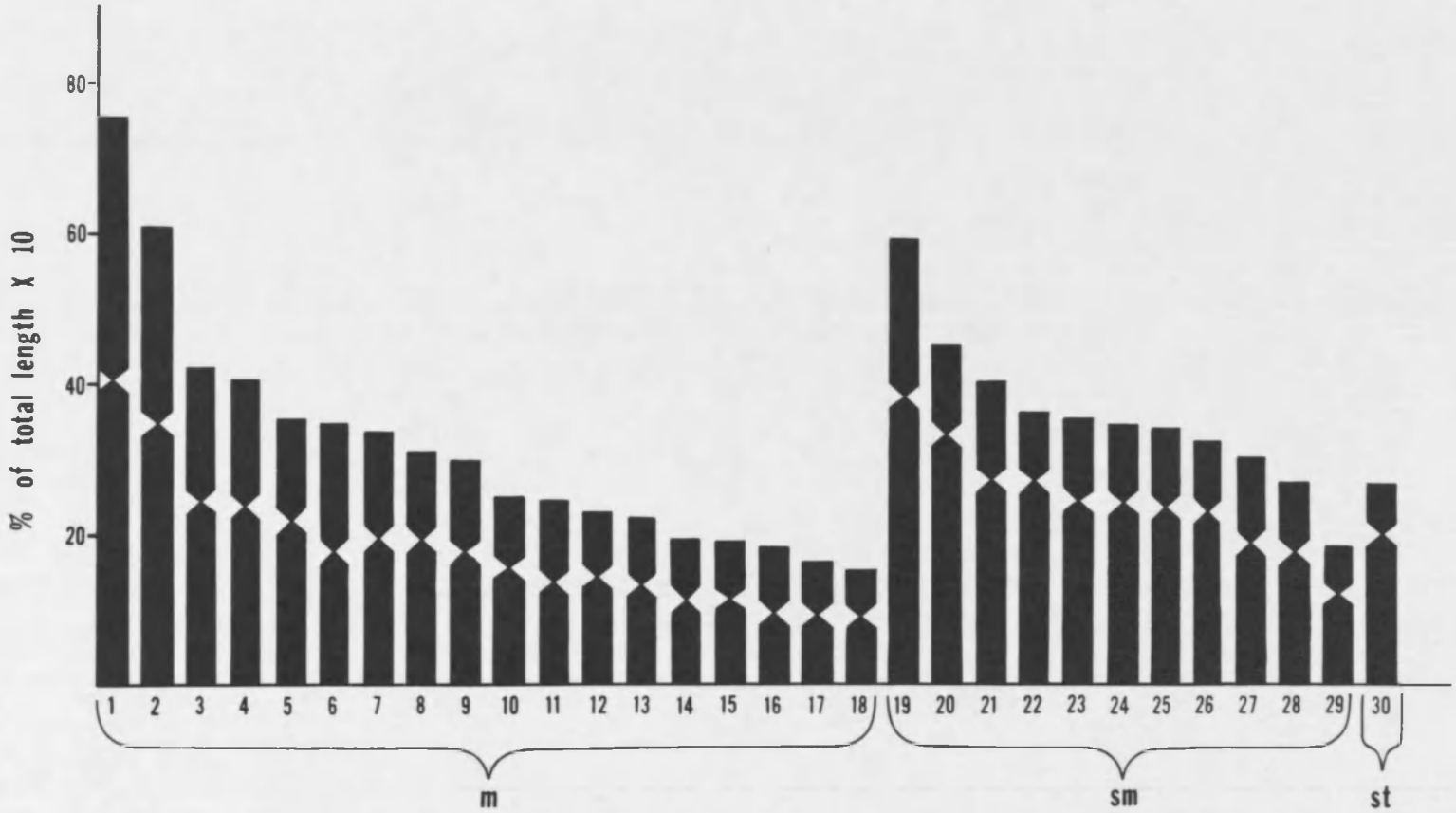


Fig. 36. Idiogram of the haploid set of E. indioensis based on data in Table 9.

PHYLOGENETIC RELATIONSHIPS IN HELMINTHOGLYPTIDAE

Pilsbry (1939) revised the taxonomy of the Helminthoglyptidae. According to that classification, the nine genera of Helminthoglyptidae examined cytologically in this study, would fall into three subfamilies as follows:

1. Subfamily Sonorellinae
   a. *Sonorelix*
   b. *Sonorella*
   c. *Greggelix*
2. Subfamily Helminthoglyptinae
   a. *Monadenia*
   b. *Helminthoglypta*
   c. *Eremarionta*
   d. *Micrarionta*
   e. *Xerarionta*
3. Subfamily Humboldtianinae
   a. *Humboldtiana*

Species belonging to the subfamily Helminthoglyptinae have one dart sac in their reproductive system, while the Humboldtianinae have four, and the dart apparatus is lacking in Sonorellinae. Originally, only the genus *Sonorella* was placed in the subfamily Sonorellinae. Investigations after Pilsbry's have shown that *Sonorelix*, *Greggelix*, *Mohavelix*

and Tryonigens are dartless. Miller (1973) suggested two possibilities for the evolution of dartless genera in Helminthoglyptidae. One possibility is that all the dartless genera are monophyletic, and the second one is that they are polyphyletic, with the dartless condition arising de novo in some or all genera.

Table 10 summarizes the cytological features of the 9 representative species belonging to 9 genera of Helminthoglyptidae. The subfamilial classification, haploid and diploid chromosome numbers, description of karyotype in terms of number of chromosomes in each group (m, sm, st) and the sums of the relative lengths for that group are shown. The taxonomically important characters of the reproductive anatomy for the nine genera of Helminthoglyptidae are summarized in Table 11. Pilsbry's three subfamilies are considered separately in the light of cytological and anatomical data.

Sonorellinae

The common character in Sonorellinae is the absence of the dart apparatus. Cytologically, the absence of the dart apparatus cannot be correlated with chromosome numbers alone. Sonorelix and Greggelix have a haploid number of 29. While one species of Sonorella, S. odorata, apparently has a haploid number of 29, subsequent chromosomal studies of S. sabinoensis tucsonica, S. virilis and S. simmonsi have

Table 10. Summary of chromosome morphology in Helminthoglyptidae.

Subfamily	Genus	No. of (m)	% of Entire Genome	No. of (sm)	% of Entire Genome	No. of (st)	% of Entire Genome	n	2n
Sonorel-linae	Sonorelix	13	47.9	15	49.5	1	2.6	29	58
	Sonorella	15	54.6	13	40.8	1	4.6	29	58
	Greggelix	16	61.1	10	30.8	3	8.1	29	58
Helmintho-glyptinae	Monadenia	16	57.3	12	38.9	1	3.8	29	58+1
	Helmintho-glypta	17	59.1	10	30.6	3	10.3	30	60
	Eremarionta	18	57.6	11	39.9	1	2.5	--	60
	Micrarionta	18	66.2	10	27.4	2	6.4	--	60
	Xerarionta	19	69.1	10	27.9	1	3.0	30	60
Humboldt-tianinae	Humboldtiana	16	61.5	11	30.4	3	8.1	30	60

Explanation of symbols:

No. of: total number of chromosomes in a group
(m): metacentric group
(sm): submetacentric group
(st): subtelocentric group

n and 2n refer to haploid and diploid numbers of chromosomes, respectively.

Table 11. Summary of the taxonomically important characters of reproductive anatomy in genera of Helminthoglyptidae.

Name of Genus	Dart Sac	Epiphallic Caecum	Spermathecal Diverticulum	Verge
Sonorella	none	vestigial	no	yes
Sonorelix	none	yes	yes	yes
Greggelix	none	yes	yes	yes
Helminthoglypta cf. lowei	one	yes	yes	no
Helminthoglypta micrometallioides	one	yes	yes	no
Monadenia	one	yes	yes	no
Micrarionta	one	yes	no	yes
Xerarionta	one	yes	yes	no
Eremarionta	one	yes	yes	no
Humboldtiana	four	yes	yes	no

revealed that the haploid number in these thre species of Sonorella is 30 (Babrakzai et al., in press, 1975).

Karyotypes of the genera from representative species do reveal similarities and differences. By placing Sonorella, n = 29-30, at the base of a phylogenetic tree (Fig. 37A) it is easy to derive the karyotype of Sonorelix by assuming that two of the metacentric (m) chromosomes of Sonorella were transformed into the submetacentric (sm) chromosomes of Sonorelix, and, also, that there was loss of a chromosome pair. Similarly, the karyotype of Greggelix can be derived from that of Sonorella if we assume that two of the submetacentric (sm) chromosomes of Sonorella were transformed into two of the three subtelocentric (st) chromosomes of Greggelix and that, possibly, a chromosome pair was lost. The third sm chromosome of Sonorella may have given rise to a metacentric chromosome of Greggelix. Such transformations probably involved reciprocal translocations and pericentric inversions in their evolution.

Other alternative phylogenetic schemes can also be considered (Figs. 37B and C). In any case one has to assume quite a few changes in the karyotypes to derive Greggelix from Sonorelix. Nevertheless, these changes in karyotype are not very drastic in the light of the "Hypothesis of Saltational Chromosomal Speciation" (Lewis, 1962, 1966; Miller, 1973). Such changes could probably have occurred

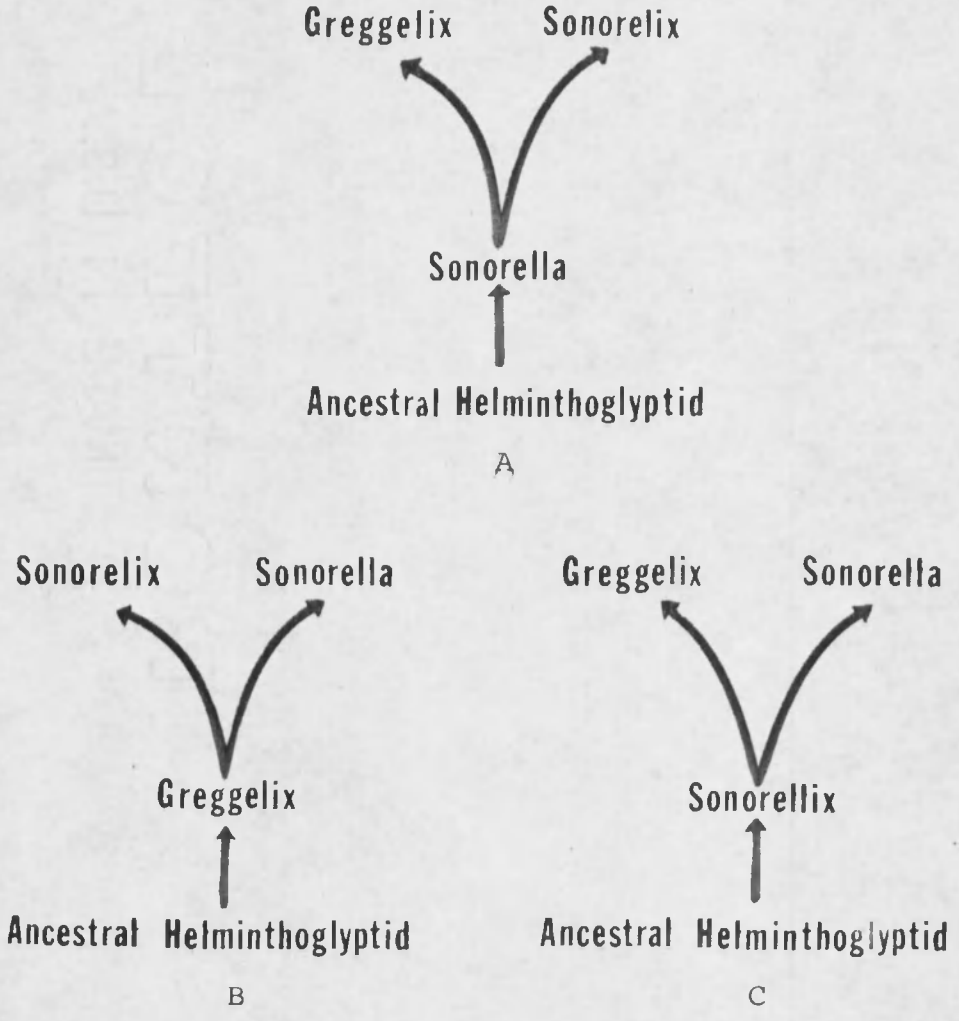


Fig. 37. Phylogenetic schemes within Sonorellinae -- A, B, and C are three different hypothetical phylogenetic schemes for the evolution of the three Sonorelline genera.

in the ancestral Sonorella population from which Sonorelix and Greggelix subsequently evolved.

If geographical considerations are also taken into account, the most probable course of evolution in Sonorellinae is the one shown in Fig. 37A. Bequaert and Miller (1973) have discussed the zoogeography of the Southwestern molluscs. They do not mention any overlapping region between species of Sonorella and Greggelix. Sonorella occurs mostly in Arizona while Greggelix is confined to central Baja California, and Sonorelix is distributed from southern California to about the middle of the Baja peninsula. It is therefore rather tempting to hypothesize that Greggelix and Sonorelix evolved from an ancestral Sonorella population, with Greggelix speciating at the southern end of the population and Sonorelix at the western end while Sonorella remained at the northeastern end. This process probably involved chromosomal changes in the karyotypes of the ancestral Sonorelix and Greggelix populations, followed by subsequent adaptive radiation.

The ancestral Sonorella population evolved either directly from the base of the Helminthoglyptid stick or somewhere near the base of Eremarionta lines. After the separation of Sonorella from the Helminthoglyptid stock the Helminthoglyptinae and Sonorellinae evolved in different directions. Loss of the dart apparatus may have occurred in the ancestral Sonorella population as an adaptive

character to survive in the more arid desert environment (see below).

A second hypothesis regarding the evolution of Greggelix has been suggested by Miller (1972, 1973). According to Miller's hypothesis, Greggelix probably evolved from an ancestral Xerarionta population with loss of the dart apparatus through random genetic drift in an increasingly drier climate. Other anatomical features listed by Miller (1972) that would relate Greggelix to Xerarionta are the unusually long epiphallic caecum and spermathecal diverticulum in both genera. These two structures are much shorter in Sonorelix.

If we accept the hypothesis of random genetic drift as the causative agent for the evolution of Greggelix from Xerarionta, then we have to derive Sonorella and Sonorelix in the same manner from dart-bearing ancestral population of Eremarionta, as proposed by Bequaert and Miller (1973). In this case, besides the loss of the dart apparatus, one also has to assume the loss of a pair of chromosomes during the evolution of Sonorelix and Greggelix, while in the case of Sonorella the chromosome number probably did not change. Again in this case it would be easy to derive Sonorella from Eremarionta and Greggelix and Sonorelix from Sonorella. In the first case only the loss of the dart apparatus is involved, while in the second case loss of a pair of chromosomes is also involved.

In comparing the karyotypes of the three sonorelline genera, Sonorelix appears to be quite different from both Sonorella and Greggelix. Sonorelix has 15 pairs of submetacentric (sm) chromosomes and 13 pairs of metacentric (m) chromosomes. Sonorella and Greggelix, on the other hand, have 13 and 10 submetacentric (sm), and 15 and 16 metacentric (m) pairs of chromosomes, respectively. The karyotypes of Sonorella and Greggelix are more similar to each other than to Sonorelix. The apparent resemblance between the karyotypes of Greggelix and Helminthoglypta is probably superficial since the chromosome number and anatomy of the reproductive organs differ markedly between the two genera.

As far as the difference in lengths of spermathecal diverticulum and epiphallic caecum between Sonorelix and Greggelix are concerned, they can be equally explained by assuming that these characters are controlled by quantitative genes. Thus, the occurrence of varying lengths of spermathecal diverticula and epiphallic caeca could be due to selection pressures on the genes controlling their lengths. It is therefore possible that the lengths of spermathecal diverticula and epiphallic caeca could be different in closely related species while similar lengths could be found in relatively unrelated ones due to convergent evolution.

It is also possible that random genetic drift did occur in an ancestral Sonorella population, accompanied by loss of the dart apparatus.

The loss of the dart apparatus is not unique to the sonorelline genera of Helminthoglyptidae. It has also occurred in Oreohelicidae, Camaenidae and Ammonitellidae to cite a few. It seems that the loss of the dart apparatus is apparently not a random process. It has an adaptive significance not completely understood at present. It is worth mentioning that the Sonorellinae are mostly inhabitants of xeric environments. The functions of the dart apparatus have been postulated to be stimulation of the "courting" snails by shooting the dart at the partner. Elimination of such an elaborate courtship behavior and the dart apparatus is probably an adaptive act. Thus, it would allow the desert snails to take maximum advantage of a relatively very short favorable time to reproduce and feed themselves. From such sonorelline stock that became adapted to the desert environment, the three genera probably evolved. Their subsequent evolution probably involved "chromosomal saltation" (Miller, 1967), and geographic isolation. Most of the species are dwellers of the arid Southwest.

Helminthoglyptinae

The chromosome number in all genera except Monadenia of this subfamily is 30. In Monadenia it is 29. The karyotypes of the representative species of the five genera are quite comparable. The number of metacentric (m) chromosome pairs is 16 in Monadenia, 18 in Eremarionta and Micrarionta, 19 in Xerarionta, and 17 in Helminthoglypta. Similarly, the number of submetacentric (sm) chromosome pairs is as follows: 12 in Monadenia; 11 in Eremarionta; 10 in Micrarionta, Xerarionta, and Helminthoglypta. Furthermore, Monadenia, Eremarionta and Xerarionta have one subtelocentric (st) chromosome pair, while Micrarionta and Helminthoglypta have two and three pairs respectively. The phylogenetic relationships among the Helminthoglyptinae are drawn in two schemes (Figs. 38 and 39). Three genera, Eremarionta, Micrarionta and Xerarionta, have almost identical karyotypes, with a difference of only one chromosome between Eremarionta and Xerarionta. Originally, these three genera were placed under one genus, Micrarionta, and each was considered a subgenus. At present it can only be said that these are very closely related genera with similar karyotypes. The same can be said about Helminthoglypta. The similarity in the karyotypes of all the genera of Helminthoglyptinae does justify placing them in a subfamily of their own. All the species of the subfamily Helminthoglyptinae have a dart apparatus.

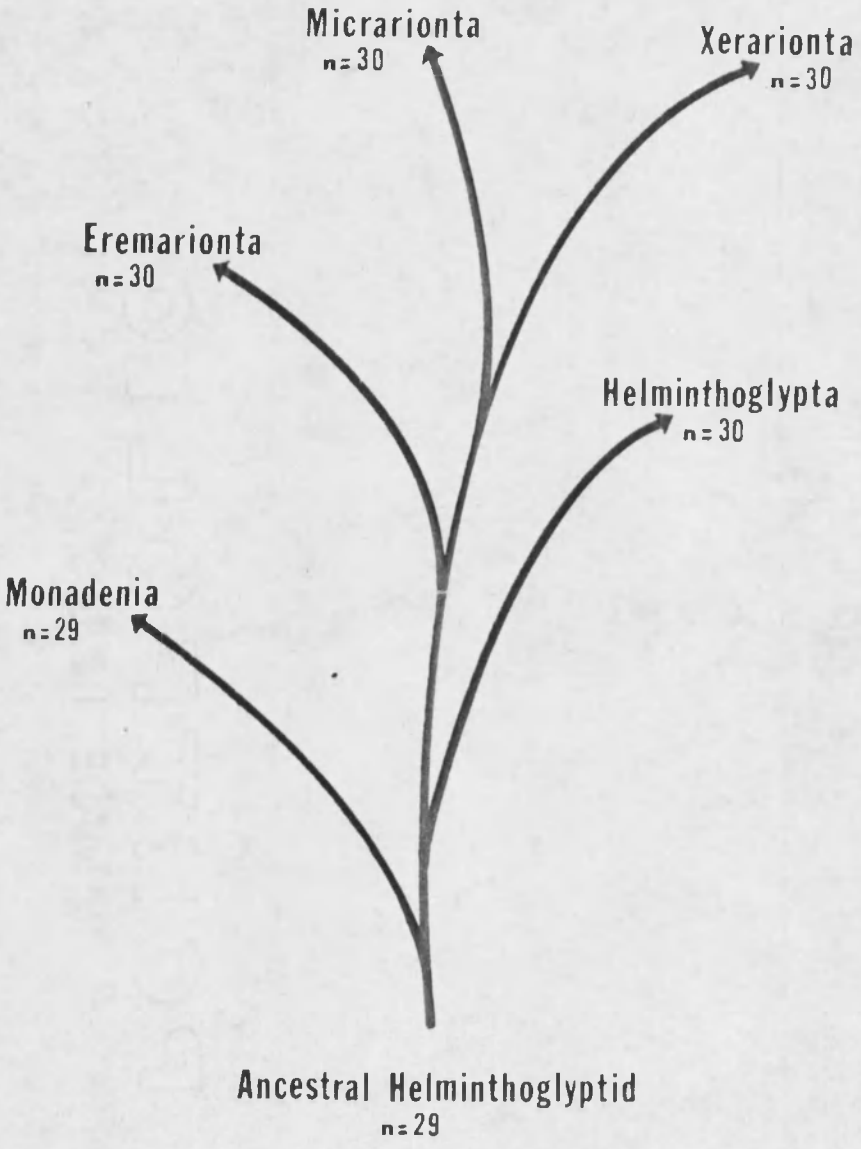


Fig. 38. Phylogenetic tree of the Helminthoglyptinae considering Helminthoglypta primitive and ancestral to Eremarionta.

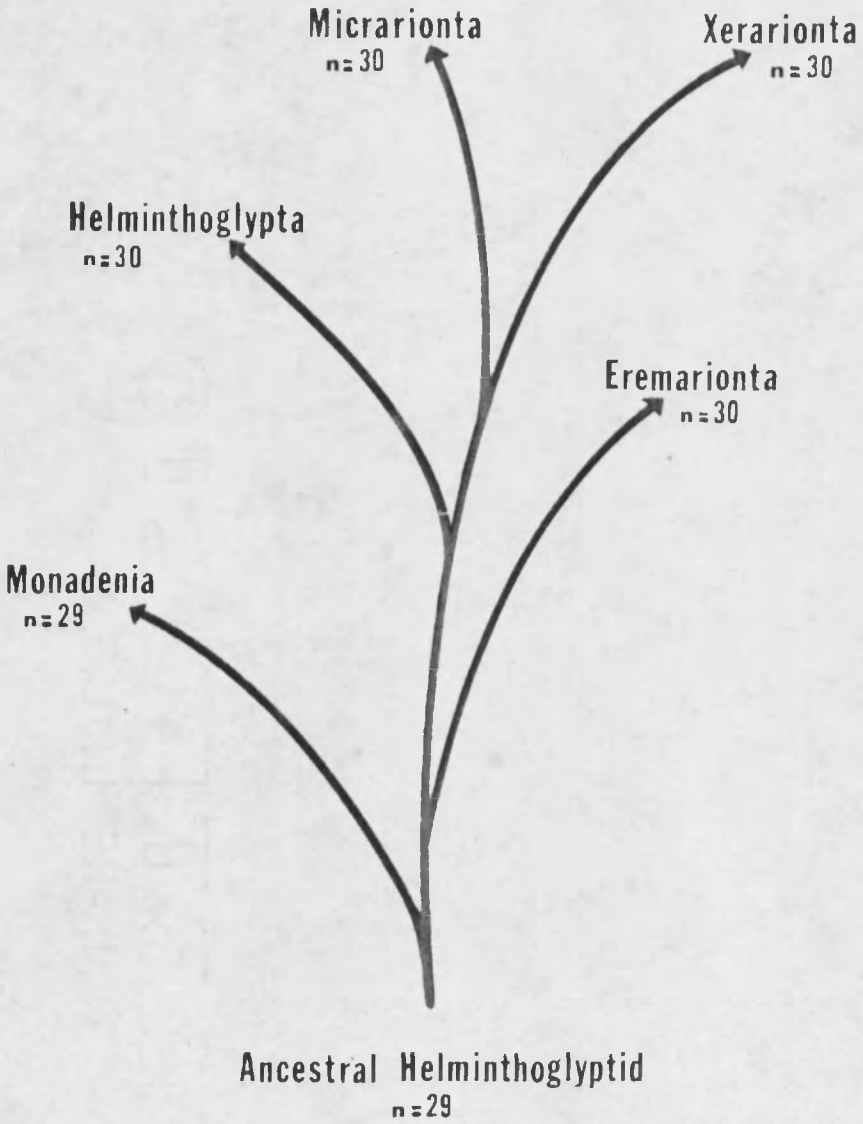


Fig. 39. Phylogenetic tree of Helminthoglyptinae considering Eremarionta more primitive than Helminthoglypta.

The Old World Bradybaenidae have been considered as the closest group to Helminthoglyptidae. The haploid chromosome number of Bradybaenidae is 28-29 (Inaba, 1959; Kawabe, 1947; Perrot, 1938; Burch, cited by Patterson, 1969; Babrakzai and Miller, in press, 1975). It is therefore possible that the haploid chromosome number of 29 is a primitive condition in Monadenia and that the subsequent karyotypic evolution of the Helminthoglyptinae involved the addition of a pair of chromosomes.

The haploid number of 29 has been reported by Ford (1962) and Burch (1965) in another species of Monadenia, M. fidalis. Therefore, Monadenia could be one of the more primitive genera of Helminthoglyptinae as well as Helminthoglyptidae.

The genus Helminthoglypta is considered to be the primitive one from which the other genera evolved (Fig. 38). Although the haploid chromosome number in Helminthoglypta cf. lowei is 30, it is considered to be primitive and it would not be surprising if a haploid number of 29 is found in other species of this genus. Another possibility is that Helminthoglypta evolved from an Eremarionta-like ancestor as shown in Fig. 39.

The distribution of Eremarionta, however, supports its probable evolution from Helminthoglypta. According to Bequaert and Miller (1973), Eremarionta is in an active

evolutionary phase, judging from the fact that it has several subspecies or incipient species.

Humboldtianinae

This subfamily is represented by the genus Humboltiana which has many species. The karyotype of H. högeana has 16 pairs of metacentric (m), 11 pairs of submetacentric (sm), and three pairs of subtelocentric (st) chromosomes. This is quite comparable to Helminthoglypta with 17 pairs of metacentric (m), 10 pairs of submetacentric (sm), and three pairs of subtelocentric (st) chromosomes. The ancestral Humboldtiana stock probably evolved from a Helminthoglypta population. The chromosome number in Helminthoglypta and Humboldtiana is the same (n=30). Anatomically, Helminthoglypta has one dart sac while Humboldtiana has four.

Figure 40 shows a possible phylogenetic tree of the Helminthoglyptidae for the genera used in this study. It should be noted that only one species belonging to each genus has been considered as representing the genus. Further cytological study might reveal cytological variation within a genus.

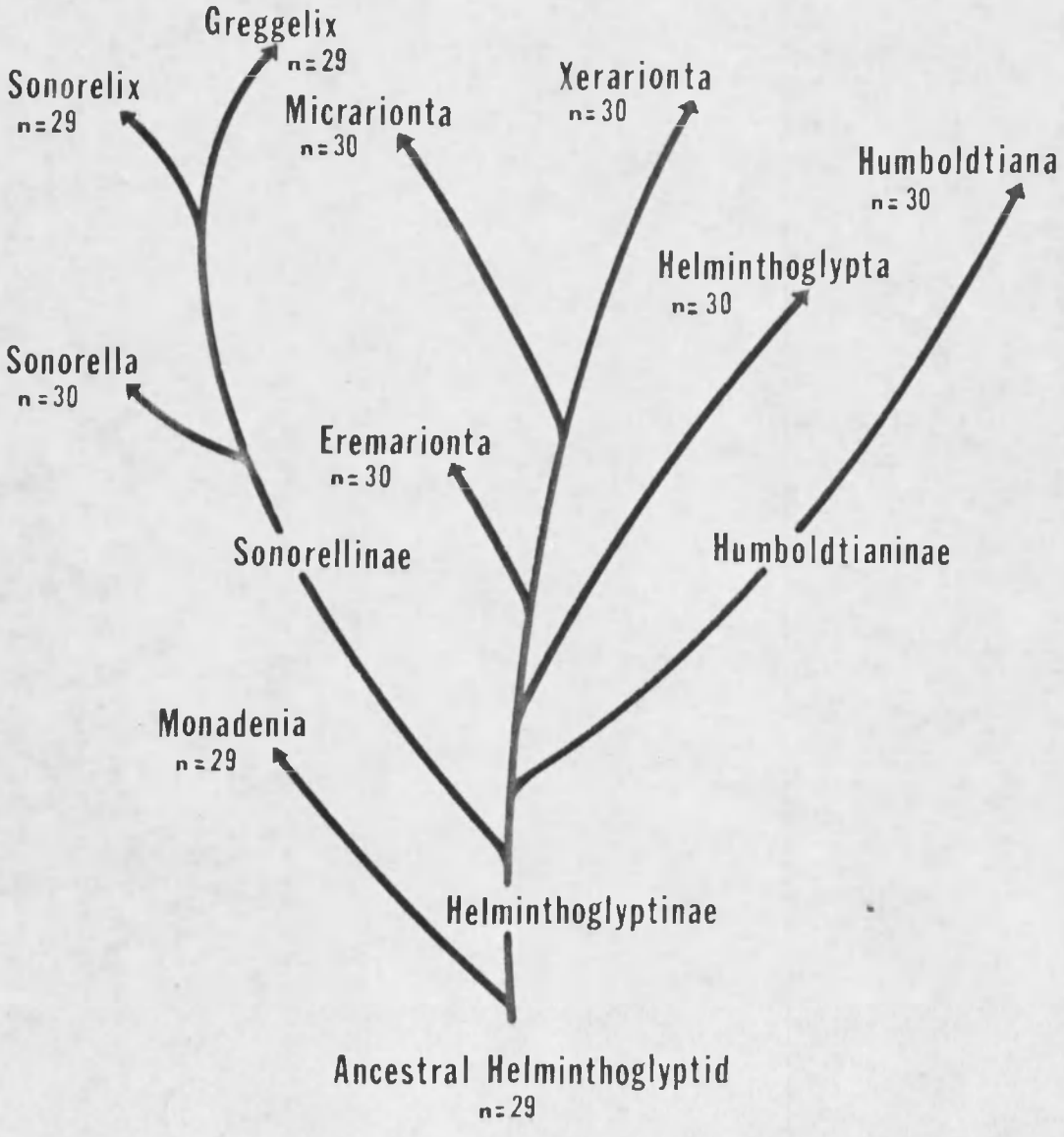


Fig. 40. Tentative phylogeny of Helminthoglyptidae.

PHYLOGENETIC RELATIONSHIPS BETWEEN HELICACEA AND POLYGYRACEA

The chromosome numbers in Helicacea and Polygyracea are summarized in Table 12. The family Helicidae shows a wide range of chromosome numbers. Several different numbers are recorded for the Polygyridae, but the majority have a haploid number of 29. Similarly, the Camaenidae have a haploid number of 29 in all species studied so far. It is interesting to note that the Bradybaenidae have haploid numbers of 28-29. This contrasts with n=29-30 for the Helminthoglyptidae. Although the Helminthoglyptidae and the Bradybaenidae are morphologically similar and sometimes indistinguishable, there is a finite cytological difference between the two families. All the species of Helminthoglyptidae studied so far have a majority of chromosomes in the metacentric-submetacentric (m-sm) group (Table 10). The karyotype of one species of Bradybaenidae, Bradybaena similaris, is quite different from any helminthoglyptid species. It has a haploid number of 28. The karyotype of B. similaris has one pair of metacentric (m), 2-3 pairs of submetacentric (sm), and the rest of its chromosomes are telocentric (terminal centromere) (Babrakzai and Miller, in press, 1975).

Table 12. Chromosome numbers of Polygyracea and Helicacea: number of species known for each family.[a]

Family (number of genera known cytologically)	Haploid Chromosome Numbers											
	21	22	23	24	25	26	27	28	29	30	31	32
Polygyridae (7)						2			18	1 (1)	1	
Camaenidae (15)									26			
Bradybaenidae (5)								9	8			
Helminthoglyptidae (9)									2	12		
Haplotrematidae (2)									1	1		
Oreohelicidae (2)											1	6
Ammonitellidae (6)							1	1	4			
Helicidae (20)	1	3	8	3	2	8	10		3	6 (1)		

[a]From Burch (1965, 1967); Rainer (1967); Inaba (1959); Perrot (1938); Kawabe (1947); Husted and Burch (1946, 1953); Laws (1965, 1973); Ford (1962); Babrakzai and Miller (1974a); Babrakzai, Miller, and Ward (in press); Babrakzai, Miller, and Ward (n.d.); Natarajan (cited by Patterson, 1969); Stern and Metcalf (1974); Stern (1975); and this study.

It will be of great interest if other members of the Bradybaenidae have karyotypes comparable to B. similaris. Further study of the karyotypes of Bradybaenidae is needed. If B. similaris is an "average" representative of the family, then, despite morphological resemblances between Helminthoglyptidae and Bradybaenidae, the two families are very far apart cytologically.

According to Burch (1965, 1967), there is a progressive increase in chromosome number from the more primitive families to the advanced ones. This trend has also been shown within families. It is likely that some genera of Helminthoglyptidae with n=30 are more specialized.

Solem (1959) proposed that the Camaenidae and probably the Polygyridae were derived from an ancestral helicacean stock. The higher families of Helicacea may have, in turn, evolved from a camaenid ancestor. An opposing view has been advanced by Laws (1973). According to Laws (p. 234),

> The high (chromosome) numbers of the Camaenidae, Bradybaenidae and Helminthoglyptidae and especially the single known number of Camaenidae suggest that modern families diverged from a stock which already showed a range of chromosome number. The Camaenidae probably diverged early since they are morphologically primitive members of the Helicacea and the only family of Helicacean-Polygyracean complex to occur in both the Old and New Worlds. It is extremely unlikely, however, that the family Helicidae in the higher Helicacea could have arisen as Solem suggested, as an offshoot of early Camaenids . . . .

Laws (1973) also suggested that in the light of Burch's (1965, 1967) hypothesis that there is an increase in chromosome number with increasing specialization, one would not expect low chromosome number helicids to have evolved from an ancestral stock with 29 chromosome pairs. Laws (1973) favors the hypothesis that the Helminthoglyptidae and Bradybaenidae could have been derived from a Camaenid stock.

There is a danger in relying too heavily on chromosome numbers alone. While Burch's hypothesis may hold true for many families of pulmonate land snails, it is possible that it will not always prove to be correct. One wonders if exceptions do exist, then where do they occur and why? It is worth mentioning that the karyotypes of all the helicid species have not been worked out. Could the variable chromosome number of Helicidae be due to "dysploid decrease and dysploid increase" (Togby, 1943; Sherman, 1946; Jackson, 1964; Grant, 1966a, 1966b) caused by translocations or by centric fusion and centric fission of the chromosomes, since chromosome numbers vary in some closely related species of Helicidae. For example, Cepea nemoralis has a haploid number of 22, C. hortensis has n=22, 30, and C. silvatica has n=25 (Perrot, 1938; Rainer, 1967).

Some interesting examples of dysploid decrease in chromosome numbers are found among other animals such as the species of pulmonate snails Catinella (n=5-6) (Burch, 1964; White, 1973), the Indian muntjac deer, Muntiacus muntjak

(n=3-4) (Wurster and Benirschke, 1970), and the chocolate Gourami fish, Sphaerichthys osphromonoides (n=8) (Calton and Denton, 1974).

The majority of the Succinidae to which Catinella belongs have a haploid chromosome number of 12-25. Their systematic relationships have been discussed by Patterson (1969). White (1973) considers the low chromosome numbers in species of Catinella to be secondarily derived. The Indian muntjac deer (n=3-4) probably arose from an ancestor with 2n=46. One of the proposed mechanisms for this chromosome reduction is the rearrangement of acrocentric chromosomes of an ancestor through centric fusion. Brown (1972) has proposed that the primary mechanism for dysploid decrease is the translocation of active genes between non-homologous chromosomes, resulting in one chromosome that is genetically active and one that is eventually eliminated owing to gene loss. Should the newly formed chromosome become homologous, the result is the elimination of a chromosome pair. Similar mechanisms have been proposed by Calton and Denton (1974) for the reduction in chromosome number in the chocolate Gourami. Members of related species have 2n=42-47 compared to n=8 in the chocolate Gourami.

Whether the Helicidae evolved from Camaenidae as proposed by Solem (1959), or Camaenidae evolved from Helicidae as proposed by Laws (1973), cannot be resolved. At present, with insufficient cytological information, the

evidence at best is inconclusive. The Helminthoglyptidae and Bradybaenidae, as well as the Oreohelicidae, Haplotrematidae, and Ammonitellidae, could have evolved from Camaenidae as suggested by Laws (1973).

Based on cytological evidence, the hypothesis of Solem (1959) is considered the more plausible one (Fig. 41). The Oreohelicidae and Ammonitellidae probably evolved from a camaenid ancestor. These two families were considered as subfamilies of Camaenidae by Pilsbry (1939). The highest haploid chromosome number in the Helicacea is recorded for the Oreohelicidae with 31 in one species and 32 in six species (Babrakzai et al., in press, 1975; Stern, 1975). Haploid chromosome numbers of 29-30 in Haplotrematidae show an intermediate situation between Camaenidae (n=29) and Oreohelicidae (n=31-32). Morphologically, Haplotrematidae resemble the Oreohelicidae. They differ from Oreohelicidae in being omnivorous. Haplotrematidae is placed between Camaenidae and Oreohelicidae.

Based on cytological evidence, it is probable that Bradybaenidae evolved from a Camaenid-Helicid ancestral stock. Helminthoglyptidae evolved from Bradybaenidae, while the Helicidae differentiated early from the ancestral group. Ammonitellidae and Oreohelicidae evolved from a later Camaenid stock. It is also possible that the Polygyracean stock also separated from a Camaenid ancestor. One genus,

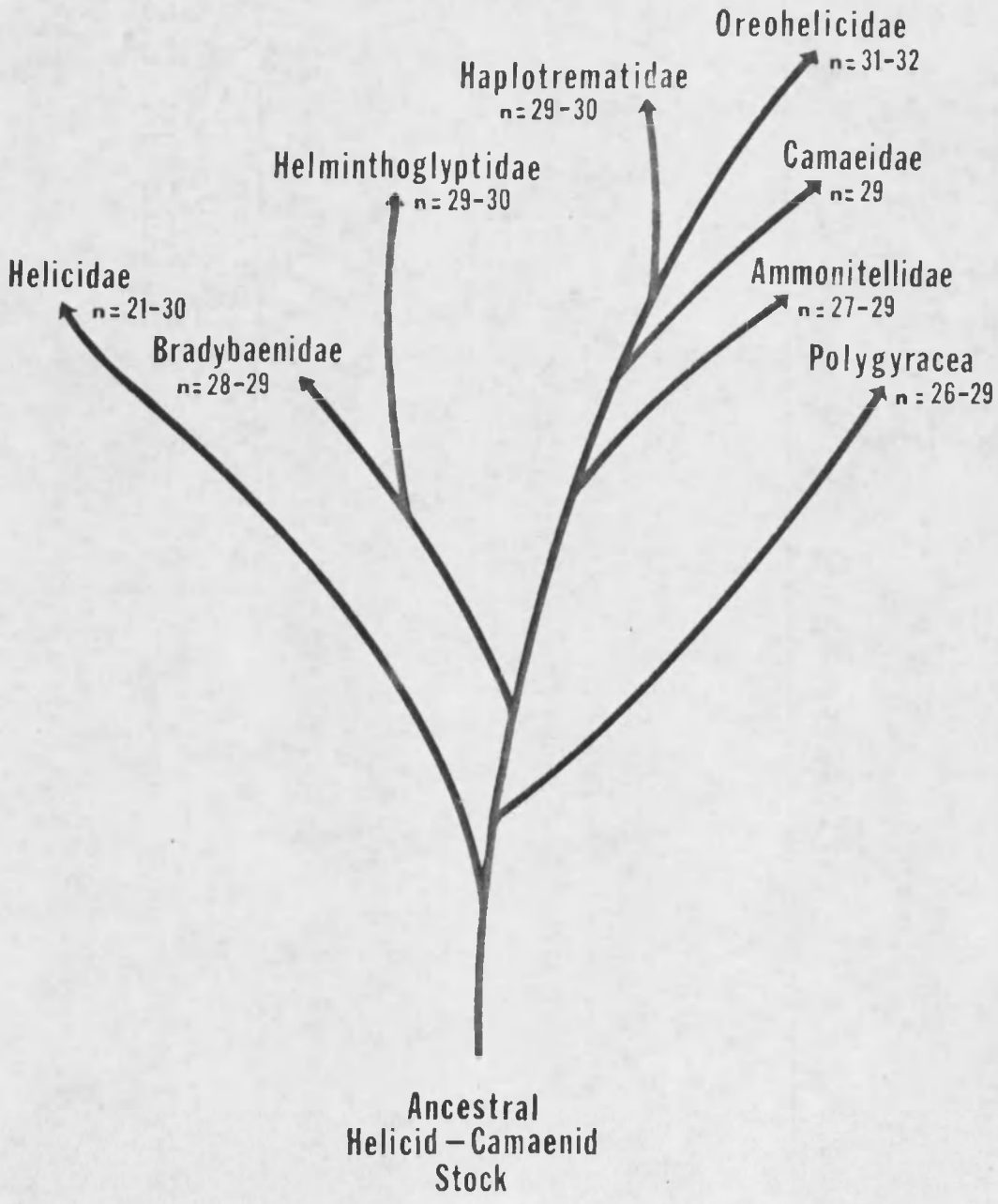


Fig. 41. Phylogenetic tree of Helicacean and Polygyracean families.

Allogona, in Polygyridae has a haploid number of 26 (Husted and Burch, 1953). Allogona does show primitive characters, e.g., retention of epiphallus and flagellum. The majority of Polygyridae have n=29. It is therefore tempting to think that reduction in chromosome number could be a secondary phenomenon in Allogona.

Another Polygyrid species, Triodopsis fraudulenta, was reported to have n=29-31 by Husted and Burch (1946). However, White (1954, 1973) and Patterson (1969) consider the variable number of chromosome in T. fraudulenta to be due to supernumerary chromosomes. Husted and Burch (1946), however, postulated that the increase was due to duplication of some chromosomes. Nevertheless, they failed to note that if there was any such duplication, i.e., primary trisomy or double trisomy (2n+1 or 2n+2), it would involve multivalent association in the multiple, homologous chromosomes which would have been easy to observe in meiosis. Postulating supernumerary chromosomes would also explain the variable chromosome number in a single individual of T. fraudulenta. The suggested phylogenetic relationships are shown in Fig. 41.

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