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CHROMOSOMES OF AQUATIC PULMONATE SNAILS (BASOMMATOPHORA)

by
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of the requirements for the degree of
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INTRODUCTION

Freshwater pulmonate snails (Basommatophora) are of considerable interest not only because of their variation and distribution, but also because many are important intermediate hosts of human parasites. The North American basommatophoran fauna consists of two major groups, the freshwater "higher limnic Basommatophora" and the more primitive marine and brackish-water forms belonging to the families Siphonariidae and Melampidae (=Ellobiidae). The freshwater group comprises the families Lymnaeidae, Physidae, Planorbidae, and Ancylidae, each with a world-wide distribution. Although the literature dealing with the taxonomy and biology of these snails is voluminous, the systematics of the lower taxonomic categories in almost every group still remain an enigma, both from the practical viewpoint of identifying species and in understanding the mechanisms of speciation. Hubendick (1951) indicated the need for cytogenetic studies of freshwater pulmonates; White (1956) suggested that perhaps polyploidy occurs among them. Nevertheless, chromosome studies have been made in only nineteen species and two subspecies. This is less than one per cent of the total number of described species. The meager amount of information in so large a group led to this study of the chromosomes of North American and European Basommatophora.

The first chromosome studies of basommatophoran snails

were all on various lymnaeid species and reported by Linville (1900), Crabb (1927), and Larambergue (1929) (Table I). As indicated by the work of J.-L. and M. Perrot (1930, 1934, 1938) the chromosome numbers reported in these earlier investigations were unreliable. The latter authors studied six species and subspecies of lymnaeids and suggested that cytological studies would help clarify systematics in this group. In effect, on basis of their results, they advocated separating Radix as a distinct taxon (subgenus) and combining Stagnicola with Lymnaea. Following the Perrots were the reports of Koslowsky (1933), Mahoney (1940), Archie (1942), Bretschneider (1948), Inaba (1950, 1953), Inaba and Tanaka (1953), Le Calvez and Certain (1950), and Meyer (1955). The most significant of these, because of the nature of the work and the number of snails studied, are the investigations of Inaba (1953), Inaba and Tanaka, and Le Calvez and Certain. Chromosome numbers as determined by the above authors are listed in Table I.

The haploid numbers reported for species of Basommatophora range from six to eighteen; within this range are found the numbers eight, ten, twelve, sixteen, and seventeen. The sequence of numbers such as six, twelve, and eighteen, or eight and sixteen, all suggest that the evolution of chromosome numbers in this group was perhaps not a gradual alternation by duplication or elimination of existing chromosomes (aneuploidy) but rather by duplication of entire complements (polyploidy). According to Muller

TABLE I

Chromosome Numbers Reported for Basommatophora

Species	Chromosome Number n	2n	Source	Author
MELAMPIDAE				
Melampus boholensis (= Pira angiostoma)	8(♂I)	16s	Haberadove, Ceylon	Koslowsky, 1933
Ovatella (= Phytia) myosotis	18(♂I)		Wilhelmshaven, Germany	Meyer, 1955
AMPHIBOLIDAE				
Salinator takii	18(♂I, II)		Ariake Bay, Japan	Inaba, 1950, 1953
SIPHONARIIDAE				
Siphonaria japonica	16(♂I, II)	32s	Hiroshima, Japan	Inaba, 1933
LYMNAEIDAE				
Lymnaea sp.	16(♀ II)		Cambridge, Mass., U. S. A.	Linville, 1900
Lymnaea stagnalis	16(♀ I)		France?	Larambergue, 1929
Lymnaea stagnalis (ssp. jugularis?)	12(♂I)	24s	Wisconsin, U. S. A.	Archie, 1942
Lymnaea stagnalis	16(♀ I)		Netherlands?	Bretschneider, 1948
Lymnaea stagnalis appressa (= jugularis)	10(♂I,♀ I, II)		Michigan, U. S. A.	Crabb, 1927
Lymnaea stagnalis lacustris	18(♂I, II)	36s	Geneva, Switzerland	Perrot, J.-L., 1930
Lymnaea stagnalis rhodani	18(♂I, II, ♀ I)	36s	Geneva, Switzerland	Perrot, J.-L., 1930, 1934
Lymnaea (= Stagnicola) palustris	16(♀ I)		France?	Larambergue, 1929
Lymnaea (= Stagnicola) palustris	18(♂I)		Geneva, Switzerland	Perrot and Perrot, 1938
Lymnaea (= Stagnicola) elodes (= palustris elodes)	16(♀ I, II)		Cambridge, Mass., U. S. A.	Linville, 1900
Lymnaea (= Radix) auricularia	16(♀ I)	32mi	France?	Larambergue, 1929
Lymnaea (= Radix) auricularia	17(♂I)		Versoix, Switzerland	Perrot and Perrot, 1938
Lymnaea (= Radix) ovata	17(♂I)		L. of Bourget, France	Perrot and Perrot, 1938
Lymnaea (= Radix) pereger	17(♂I)		Ferney, Ain, France	Perrot and Perrot, 1938
Lymnaea (= Radix) limosa	18(♂I)		Brittany, France	Le Calvez and Certain, 1950
Lymnaea (= Radix) japonica	18(♂I, II)	36s	Hiroshima and Osaka, Japan	Inaba, 1950; Inaba and Tanaka, 1953
Lymnaea (= Fossaria) ollula pervia	17(♂I)	34s	Osaka, Japan	Inaba and Tanaka, 1953
PHYSIDAE				
Physa gyrina	6(♂)		Boulder, Colorado, U. S. A.	Mahoney, 1940
Physa acuta (integra?)	18(♂I)	36s	Yokohama and Han-Shin, Japan	Inaba, 1953
Physa acuta	18(♂I)		Brittany, France	Le Calvez and Certain, 1950
Physa fontinalis	18(♂I)		Brittany, France	Le Calvez and Certain, 1950
PLANORBIDAE				
Gyraulus hiemantium	16(♂I)	32s	Hiroshima, Japan	Inaba and Tanaka, 1953
Planorbis cornuta (= Planorbarius corneus)	18(♂I)		Brittany, France	Le Calvez and Certain, 1950
Planorbis (= Anisus) vortex	18(♂I)		Brittany, France	Le Calvez and Certain, 1950
ANCYLIDAE				
Ancylastrum costulatum	ca. 59(♂I)		Brittany, France	Le Calvez and Certain, 1950

(♀ I) refers to observations made on the first meiotic division in the oöcyte.
(♂I, II) refers to observations made on the first and second meiotic divisions during spermatogenesis.
s refers to observations made on spermatogonial cells.
mi refers to observations made on somatic cells.

(1925), polyploidy in animals should be confined to groups in which reproduction is hermaphroditic (as in pulmonate snails), parthenogenetic, or clonal. In such animals polyploidy would not upset the sex-chromosome mechanism. Since self-fertilization has been reported for a number of species of freshwater pulmonates, ample opportunity exists for the production of polyploid strains (White, 1956), and it is possible that polyploidy has been as important in snail evolution as in plant evolution. Inaba (1953) suggested that the physid snail[1] he studied in Japan is a polyploid[2] of the American Physa gyrina since its chromosome number is an exact multiple by three of that reported by Mahoney (1940) for the latter species.

A description of the shapes of mitotic chromosomes, which is essential for caryotype comparisons involving individual chromosomes, was given by Crabb (1928) for Lymnaea (=Stagnicola) palustris (ssp. elodes?); J.-L. Perrot (1930) for Lymnaea stagnalis; Inaba (1953) for Siphonaria japonica and Physa acuta (?); and Inaba and

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[1] Inaba (1953) stated that the species he studied, Physa acuta, was imported from North America to Japan in about 1940. However, regardless of statements to the contrary by authors (e.g., Hannibal, 1912), this species (or subspecies) does not occur in North America. If Inaba's specimens were identified by shell characters they were probably P. integra or a closely related species.

[2] Inaba used the term triploid; however, he must have been referring to a hexaploid. He reported eighteen bivalents at meiosis and thirty-six chromosomes in spermatogonial cells. Mahoney stated that Physa gyrina had a haploid number of six.

Tanaka (1953) for Lymnaea (Radix) japonica, L. (Fossaria) ollula pervia, and Gyraulus hiemantium. Although Crabb did not establish a definite chromosome number for S. palustris, he stated that he "frequently found V-shaped chromosomes arranged in an indifferent equatorial plate suggesting monocentric mitosis." The investigations of chromosome numbers of J.-L. and M. Perrot and Inaba and Tanaka appear to be much more reliable than those by Crabb, but the former authors figure and describe the mitotic chromosomes as "en forme de bâtonnet ou de boule" and dot- or short rod-like." From descriptions and figures given by the former authors it appears that caryotype studies comparing the individual chromosomes of related species are unrewarding since the centromere, a valuable morphological character in caryotype analysis, is diffuse, terminal, or the chromosomes are too small for it to be visible.

The chromosome number and the shape of the mitotic chromosomes as reported in these snails, (i.e., less than twenty haploid, rod and dot-like in mitotic divisions) has been used to characterize the Basommatophora as distinct from the land-dwelling pulmonates, the Stylommatophora (Inaba, 1953). In the latter group the chromosomes are V- or J-shaped (i.e., monocentric and medianly or submedianly constricted) during metaphase and anaphase of mitosis, and all reliable reports give haploid numbers greater than twenty (Perrot and Perrot, 1937; M. Perrot, 1937; Inaba, 1945, 1950a, b, 1953; Husted and Burch, 1946, 1953).

The objectives of this study were 1) to determine the chromosome numbers of as many species of freshwater pulmonates as possible, thereby obtaining an indication of the extent to which the numbers vary, and to see if any variance found would prove useful in determining systematic divisions and relationships, and 2) to recheck previous descriptions of the gross morphology of mitotic metaphase chromosomes and determine if these might be useful in caryotype studies to clarify systematics in the lower taxonomic categories.

SNAILS STUDIED

Thirty-eight species and subspecies of fresh- and brackish-water pulmonate snails were used in this investigation. These animals came from ten different regions: Ontario, Canada; Minnesota, Michigan, Ohio, Maryland, Virginia, and Colorado, U. S. A.; Puerto Rico (?); Sweden; and Turkey (Table II). Most of the material was from Michigan. A systematic list, with authors, follows to indicate the species used in this study as well as to give exact localities for obtaining the animals. Catalogue numbers of shells in my collections and the numbers of duplicate shells in the Museum of Zoology, University of Michigan (UMMZ), the United States National Museum (USNM), and the University of Colorado Museum (UCM) follow each locality. Such information has been found necessary if one wishes to make a check on this material using the same or other methods.

MELAMPIDAE Stimpson 1851 (=Ellobiidae H. and A. Adams 1855)

Melampus bidentatus lineatus Say 1822.

Salt marsh near Waterside Drive, Huggins Point, Breton Bay, Potomac River, near Leonardtown, St. Marys County, Maryland. 2190.

Hurry's Boat Dock, Breton Bay, Potomac River, St. Marys County, Maryland. 2192; UMMZ 197468; USNM 628900.

Detracia floridana (Pfeiffer 1856) Morrison 1951a.

Salt marsh near Waterside Drive, Huggins Point, Breton Bay, Potomac River, near Leonardtown, St. Marys County, Maryland. 2188.

Hurry's Boat Dock, Breton Bay, Potomac River, St. Marys County, Maryland. 2191; UMMZ 197469; USNM 628901.

Phytia myosotis marylandica (Pilsbry 1900) Morrison 1951b.

Rappahannock River, Mollusk, Lancaster County, Virginia. Collected by Joseph P. E. Morrison.

LYMNAEIDAE Rafinesque 1815 emend

Lymnaea stagnalis jugularis Say 1817a.

Small tributary of St. Marys River in Ontario, on Canadian shore, across Lake George from Duck Island, Chippewa County, Michigan. Collected by Henry van der Schalie. 2010.

Au Sable River, one-half mile southwest of Grayling, highway M-72, Crawford County, Michigan. 2167, 2194; UMMZ 197470, 197471; USNM 628902, 628903.

Stagnicola (Stagnicola) exilis (Lea 1837) F.C. Baker 1928.

West Lake, Island Lake Road, near Dexter, Washtenaw County, Michigan. 2129.

Small pond at junction of Dancer and Trinkle Roads, near Dexter, Section 11, Lima Township, Washtenaw

County, Michigan. 2072; UMMZ 197473; USNM 628905.

Stagnicola (Stagnicola) lanceata (Gould 1848) F. C. Baker 1928.

Vicinity of Lake Itasca, Minnesota. Collected by Dorothea Franzen. 693; UMMZ 197710.

Stagnicola (Stagnicola) reflexa (Say 1821) F.C. Baker 1928.

Grassy swale bordering extensive Typha marsh, north edge of highway O-2, four miles west of Port Clinton, Ottawa County, Ohio. Collected by Harold J. Walter. 2065; UMMZ 197475; USNM 628906.

Stagnicola (Stagnicola) umbrosa (Say 1842) F.C. Baker

Roadside pond, three miles northeast of East Tawas on U. S. 23, Iosco County, Michigan. 2091; UMMZ 197475; USNM 628907.

Stagnicola (Stagnicola) palustris (Müller 1774) Draparnaud 1801.

Lake Orlangen, Södermanland, Sweden (about twenty Km. south of Stockholm). Collected by Bengt Hubendick. 2213.

Stagnicola (Stagnicola) palustris elodes (Say 1821) F. C. Baker 1928.

Woods pool at junction of Liberty and Zeeb Roads, Scio Township, Washtenaw County, Michigan 2058; UMMZ 197476; USNM 628908.

Mill pond on highway M-132, Section 5, Scio Town-

ship, Dexter, Washtenaw County, Michigan. 2080; UMMZ 197477; USNM 628909.

Drainage ditch at Maple Road and Huron River Drive, Section 12, Scio Township, Washtenaw County, Michigan. 2075; UMMZ 197478; USNM 628910.

Stagnicola (Stagnicola) palustris desidiosa (Say 1821) F. C. Baker 1911, 1928.

Tire ruts, southside of Reedsburg Dam empoundment one and one-fourth miles northeast of Reedsburg Dam, Roscommon County, Michigan. 2085; UMMZ 197479; USNM 628911.

Stagnicola (Stagnicola) catascopium (Say 1817a) F. C. Baker 1928.

Au Sable River, public fishing site, just off highway M-72, four miles east of U. S. 27, near Grayling, Crawford County, Michigan. 2169; UMMZ 197480; USNM 628912.

Stagnicola (Stagnicola) emarginata angulata (Sowerby 1872) F. C. Baker 1928.

North beach of Long Point, North Bay, Houghton Lake, Roscommon County, Michigan. 2193; UMMZ 197481; USNM 628913.

Stagnicola (Stagnicola) emarginata serrata (Haldeman 1841) F. C. Baker 1936.

Silver Strand, northwest beach of Douglas Lake, Cheboygan County, Michigan. 2008; UMMZ 197482; USNM 628914.

Stagnicola (Hinkleyia) caperata (Say 1829) F.C. Baker 1928.

Flooded wooded bottomlands on south side of Stillwater River, one-half mile upstream from Siebenthaler Avenue Bridge, north edge of Dayton, Montgomery County, Ohio. Collected by Harold J. Walter. 2029; UMMZ 197483; USNM 628915.

Radix pereger (Müller 1774).

Swampy ground and small stream below a spring, about eighteen Km. from Ankara, Turkey. Collected by B. T. Simms.

Acella haldemani (Binney 1867) F. C. Baker 1911.

Public fishing site, southeast end of Sages Lake, Ogemaw County, Michigan. 2173.

Pseudosuccinea columella (Say 1817b) F.C. Baker 1911.

Greenhouse, University of Virginia Campus, Charlottesville, Albemarle County, Virginia. 2198; USNM 628923.

Bulimnea megasoma (Say 1824) Haldeman 1841.

Small creek draining into St. Marys River near Indian Point, southeast shore of Sugar Island, Chippeawa County, Michigan. Collected by Henry van der Schalie. 2011.

Roadside drainage ditch, highway M-55, about two miles west of Houghton Lake, Roscommon County, Michigan. 2195; UMMZ 197472; USNM 628904.

Fossaria parva (Lea 1841) F. C. Baker 1928.

Mill pond on highway M-132, Section 5, Scio Township, Dexter, Washtenaw County, Michigan. 2144: UMMZ 197486; USNM 628921.

Fossaria modicella (Say 1825) F. C. Baker 1928.

Grassy swale bordering extensive Typha marsh, north edge of highway O-2, four miles west of Port Clinton, Ottawa County, Ohio. Collected by Harold J. Walter. 2062; UMMZ 197485; USNM 628920.

Fossaria modicella rustica (Lea 1841) F. C. Baker 1928.

Mill pond on highway M-132, Section 5, Scio Township, Dexter, Washtenaw County, Michigan. 2206; UMMZ 197484; USNM 628917.

PHYSIDAE Haldeman 1842 emend

Physa (Physella) sayii crassa (Walker 1901) Winslow 1926.

North shore of Lake Margrethe, about three and one-half miles west of Grayling, Crawford County, Michigan. 2050; USNM 628886.

Physa (Physella) heterostropha (Say 1817a) Say 1821.

South shore of James River, Riverside Drive, highway VA-679, about two miles west of Richmond, Chesterfield County, Virginia. 2196; UMMZ 197459; USNM 628885.

Physa (Physella) gyrina Say 1821.

Au Sable River, one-half mile southwest of Grayling, highway M-72, Crawford County, Michigan.

2186; USNM 628883.

Roadside sloughs along Baseline Road just east of Boulder, Colorado. Collected by Hugo G. Rodeck. 2181; UMMZ 197456; USNM 628880; UCM 26230.

Varsity Lake on the campus of the University of Colorado, Boulder, Colorado. Collected by Hugo G. Rodeck. 2183, 2186; UMMZ 197457; USNM 628882, 628883; UCM 26228.

Physa (Physodon) anatina Lea 1864.

Roadside sloughs along Baseline Road just east of Boulder, Colorado. Collected by Hugo G. Rodeck. 2184; UMMZ 197458; USNM 628879; UCM 26231.

Varsity Lake on the campus of the University of Colorado, Boulder, Colorado. Collected by Hugo G. Rodeck. 2185; USNM 628881; UCM 26229.

Aplexa hypnorum (Linné 1758) Fleming 1820.

Swampy area near north shore of Long Point, North Bay, Houghton Lake, Roscommon County, Michigan. 2032; USNM 628887.

Small pond at junction of Dancer and Trinkle Roads, near Dexter, Section 11, Lima Township, Washtenaw County, Michigan. 2199; UMMZ 197460; USNM 628888.

PLANORNIDAE Rafinesque 1815

Gyraulus (Gyraulus) deflectus (Say 1824) F.C. Baker 1928.

Mill pond on highway M-132, Section 5, Scio Township, Dexter, Washtenaw County, Michigan. 2202;

UMMZ 197463; USNM 628894.

Drainage ditch at Maple Road and Huron River Drive, Section 12, Scio Township, Washtenaw County, Michigan. 2201; UMMZ 197462; USNM 628893.

Gyraulus (Torquis) circumstriatus (Tryon 1866) F. C. Baker 1928.

Mill pond on highway M-132, Section 5, Scio Township, Dexter, Washtenaw County, Michigan. 2203; UMMZ 197464; USNM 628895.

Armiger crista (Linné 1758) Hartmann 1844.

Mill pond on highway M-132, Section 5, Scio Township, Dexter, Washtenaw County, Michigan. 2208; UMMZ 197488.

Australorbis glabratus (Say 1818) Pilsbry 1934 (ssp.? olivaceus Spix and Wagner 1827[3]).

UMMZ stock from Parke-Davis Pharmaceutical Company, Detroit, Michigan. Perhaps originally from Puerto Rico. 2016; UMMZ 197487; USNM 628922.

Helisoma (Helisoma) anceps (Menke 1830) F.C. Baker 1945.

Small pond near Charlottesville, Albemarle County, Virginia. 2020.

South Branch, Au Sable River, highway M-72, Crawford County, Michigan. 2052; USNM 628891.

Helisoma (Pierosoma) trivolvis (Say 1817a) F.C. Baker 1928.

Hardwood Lake, about twelve miles east of West

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[3] Personal communication from Joseph P. E. Morrison.

Branch, Ogemaw County, Michigan. 2088; USNM 628890.

Huron River, Dexter-Huron Municipal Park, Section 5, Scio Township, Washtenaw County, Michigan. 2200; UMMZ 197461; USNM 628889.

Planorbula crassilabris (Walker 1907) F.C. Baker 1928.

Swampy area near north shore of Long Point, North Bay, Houghton Lake, Roscommon County, Michigan. 2030; USNM 628892.

Mill pond on highway M-132, Section 5, Scio Township, Dexter, Washtenaw County, Michigan. 2149.

Promenetus exacuous (Say 1821) F. C. Baker 1935.

Small pond at junction of Dancer and Trinkle Roads, near Dexter, Section 11, Lima Township, Washtenaw County, Michigan. 2204; UMMZ 197465; USNM 628896.

Mill pond on highway M-132, Section 5, Scio Township, Dexter, Washtenaw County, Michigan. 2146.

ANCYLIDAE Rafinesque 1815 emend

Ferrissia (Ferrissia) parallela (Haldeman 1841) F. C. Baker 1924.

Roadside drainage ditch, highway M-55, about two miles west of Houghton Lake, Roscommon County, Michigan. 2155; UMMZ 197467; USNM 628899.

Ferrissia (Ferrissia) tarda (Say 1831) F. C. Baker 1922.

Huron River, Geddes Bridge, Section 26, Ann Arbor Township, Washtenaw County, Michigan. Collected by Paul F. Basch. 2205; UMMZ 197466; USNM 628898.

Ferrissia (*Laevapex*) *fusca* (C. B. Adams 1840) Walker 1917.

Pendleton's pond, Forest Lodge, Old Lynchburg Road, Charlottesville, Albemarle County, Virginia. 2021; USNM 628897.

METHODS OF PREPARATION

Snails, collected from the middle of March to the first of October, 1957 and from March to September, 1958, were cleaned, wiped dry, and the upper several whorls of each shell were removed with a sharp bone scapel in a manner similar to that used by Husted and Burch (1946) with the Polygyridae. The ovotestis, together with the surrounding digestive gland tissue, was removed and immediately submerged into fixative, or in the case of water-pretreatment (Makino and Nishimura, 1952) into distilled water. Pieces of tissue removed were no larger than four cubic millimeters and the procedure took only a few seconds. Since it is difficult to remove the ovotestis from the small planorbid snails in the above manner, their shells were simply crushed and the entire animal dropped into the fixative, the ovotestis was removed later prior to staining. The tissues were fixed in the fluid of Sanfelice (1918) (cf. White, 1940; Ross, 1953) for sectioning, Carnoy's (1887) (acetic-ethanol-chloroform, 1:6:3) or Newcomer's (1953) fluids for aceto-carmine and acetic-orcein squashes (Belling, 1926; Painter, 1939; La Cour, 1941), and in twenty-five per cent glacial acetic acid for water-pretreated squashes.

The tissues fixed in Sanfelice's solution were stained by means of the Feulgen reaction (Feulgen and Rossenbeck, 1924; De Tomasi, 1936; Coleman, 1938) or with one per cent aqueous crystal violet (Newton, 1926; Huskins, 1927; Conn,

1936). Hydrolysis for the Feulgen reaction was carried out in normal hydrochloric acid at fifty-eight degrees Centigrade for twenty minutes (Bauer, 1932; Hillary, 1939). Light green (yellowish), alone and in combination with Orange G, was used as a counter stain for tissues stained with the Feulgen reaction, and Orange G was used following crystal violet for histological studies of the reproductive glands. Sections used to study the chromosomes, however, were not counter-stained. Tissues were dehydrated in ethanol, cleared in chloroform, embedded in paraffin, and sectioned at fifteen micra. The tissues fixed in Carnoy's and Newcomer's fluids were stored immediately at four degrees Centigrade. Prior to squashing the ovotestes were rapidly separated from the surrounding tissues under a dissecting microscope. Parasitized snails were unsuitable for these investigations. Temporary slides were made permanent by the method of McClintock (1929) and Darlington and La Cour (1950).

Observations were made with a Tiyoda microscope using a 90X (n.a. 1.25) oil immersion objective and 10-30X oculars. The chromosomes were drawn with the aid of a camera lucida and reproduced at a magnification of 4650X.

STRUCTURE AND HISTOLOGY OF THE OVOTESTIS

The ovotestis (or hermaphroditic gland) in the spired lymnaeid snails and in the physids is an elongate gland situated in the upper whorls and embedded in the columellar surface of the hepatic gland. It is irregularly lobulated, each lobe formed of numbers of acini which communicate with the hermaphroditic duct on the central columellar surface of the ovotestis. In the planorbs used in this study the ovotestis is similar, but coiled in the same plane, and occupies most or all of the last several whorls. There is very little hepatic gland covering it. In the sinistral, helmet-shaped Ancylids the ovotestis is situated in the right posterior quadrant of the shell and is embedded to varying degrees in the hepatic gland. In the melampids, which have a spired shell with no internal whorls, the ovotestis is located in the apex of the shell and covers the hepatic gland.

Gametogensis occurs in the acini and appears to be strongly cyclic during the early reproductive period in the spring, abundant spermatogensis followed by extensive egg-laying. Gametogensis continues sporadically throughout the summer and early fall, and during this period both spermatogensis and oogonial development may be observed to occur simultaneously in the same acinus.

The histological structure of the ovotestis and the details of spermatogenesis are almost identical in all of

the species studied. By way of description, therefore, Stagnicola emarginata angulata (Sowerby) was chosen. It is a snail common to the sandy shoals of lakes in northern United States and southern Canada. The ovotestis in this species is separated from the hepatic gland lobules by loose connective tissue and blood vessels and sinuses. The connective tissue covering the acini is one-cell-layer thick and very often heavily pigmented, particularly in older individuals. Beneath this layer, and with the latter forming the wall of the acinus, is a single cell layer of germinative epithelium, which, especially in regions of spermatogonia, consists of small, rather cuboidal cells about 5 micra (in sectioned material) in diameter. It is from this tissue that the spermatogonia arise.

Often one can find all stages of spermatogensis as well as developing egg cells in the same acinus. Under such conditions the acinus appears to be quite tightly packed, the bundles of long sperm tails occupying the center. Acini which are not so active have a definite lumen which leads indirectly into the hermaphroditic duct.

The male germ cells are attached to nutritive cells ("Sertoli cells") during most of their development. These large cells lie along the wall of the acinus and contain large nuclei and conspicuous nucleoli. Adjacent to each spermatogonial cluster several cells larger than the other cells of the germinal epithelium always appear. Apparently one of these cells will act as the nurse cell which is so

conspicuous in later divisions. Spermatocytes, spermatids, and mature sperm are quite obviously clustered around a large nurse cell, each cluster having a single nurse cell.

Once a cell in the germinative epithelium begins dividing to produce male sex cells, a very precise synchrony is established which continues until the sperm are mature. Although J.-L. Perrot (1930) speaks of primary, secondary, and tertiary spermatogonial cells in Lymnaea stagnalis, my studies indicate that there are actually six spermatogonial divisions in all the lymnaeids, (which includes L. stagnalis jugularis). I have counted spermatogonial clusters of four, eight, sixteen, and thirty-two cells. All cells of any particular cluster are always in exactly the same stage of division. The primary spermatocytes are always in clusters of sixty-four, the secondary spermatocytes in clusters of one hundred and twenty-eight, and developing and mature sperm in clusters of two hundred and fifty-six.

THE CHROMOSOME CYCLE DURING SPERMATOGENSIS

In this investigation cells of the male germ line were used exclusively because their meiotic divisions were much more numerous and they occurred in the ovotestis. The eggs were not used because they go through meiosis after they are fertilized and laid, and many collections were not made during the active egg-laying season. J.-L. Perrot (1930, 1934) has shown that the chromosome number is the same in both spermatocytes and oocytes of the same species (Lymnaea stagnalis rhodani), proving, therefore, that there is no difference in the number of chromosomes between the two germinal lines.

In the discussions that follow observations were made from both sectioned and squashed preparations. Since the paraffin embedding technique shrinks the chromosomes from one-third to one-half the size of those prepared by squash preparations (Figs. 40-43, Plate IV) and does not lend as clear a definition, the following descriptions and measurements, unless otherwise stated, are all from squash preparations.

The nuclei of interphase spermatogonial cells vary in diameter, depending on whether the cells have just divided or just ready to divide. These nuclei range from 6 to 11 micra, the latter or larger measurement representing about that of the prophase nuclei. Thus, the interphase exhibits considerable growth as the cell prepares for the

succeeding cell division. Once cell division begins, it is apparently very rapid since spermatogonial division stages are rather rare.

The chromosomes of spermatogonial cells vary in length depending on the degree of spindle formation and congression. Late prophase and prometaphase chromosomes measure from a little over 1 to about 4 micra in length in Stagnicola emarginata angulata. At the completion of spindle formation and when the chromosomes are orientated in the late metaphase position, the larger chromosomes have decreased 30 to 50 per cent in length and the position of the chromosomes is difficult to alter by pressure on the coverglass. The chromosomes now measure 0.7 to 2 micra in length.

In contrast to descriptions of the mitotic chromosomes given by J.-L. Perrot (1930), Inaba (1953), and Inaba and Tanaka (1953), for various species of freshwater pulmonates, spermatogonial late prophase and metaphase chromosomes in Stagnicola emarginata angulata and all other basommatophorans studied are elongate and medianly or submedianly constricted (Figs. 62-65, Plate VIII: Fig. 85, Plate XI; Figs. 104, 105, Plate XIV). These constrictions, often referred to as "primary constrictions", indicate the points of attachment of the chromosomes to the spindle. In the late prophase chromosome the constriction is seen as a lightly staining or nonstaining band (the centromere) which divides the chromosome into either two equal or unequal arms. In sectioned material spermatogonial metaphase chromosomes measure only 0.4 to 1.4 micra in length, and the primary

constrictions are not visible (Figs. 38, 39, Plate IV). This accounts for previous descriptions of these chromosomes as dot-or small rod-shaped. Equatorial plates with J- or V-shaped chromosomes were not found in Stagnicola emarginata angulata, but they were observed by me in other lymnaeids and in all the other basommatophorans studied. (Fig. 75, Plate IX; Figs. 87, 88, Plate XII; Fig. 102, Plate XIV; Fig. 108, Plate XV).

Following the last spermatogonial interphase the cells begin an extended prophase in preparation for the first meiotic division. The relatively long period of time taken for this phase is evidenced by the great number of cells always found in the various prophase stages. The early prophase cells of the first meiotic division may be distinguished from the spermatogonial prophases by the greater number of cells (i.e., sixty-four) and their large size. The chromatin of the earliest prophase I stage (leptonema) appears in a bundle of fine threads with very obvious chromomeres, the threads completely filling the nuclear membrane. Thus, the "bouquet" arrangement of the chromosomes at this stage is difficult to discern, but is easily seen later, particularly during pachynema. It is difficult to distinguish the next stage, zygonema, because of the nature of the material, but pachynema is easily detected and is one of the most abundant stages present. The chromatin threads, now bivalents due to homologue pairing, are twice as thick as the leptonema threads. These bi-

valent chromatin strands appear to be paired but, at least during late pachytene, must actually be made up of four chromatids. During diplonema these chromatids separate in pairs, i.e., "open out", but are kept from separating completely by one or more chiasmata. During late diplonema and early diakinesis there are usually twelve bivalents in Stagnicola emarginata angulata which appear as crosses (held together by one chiasma), five as loops (held by two chiasmata), and one as a series of loops (having three or more chiasmata).

During diplonema and diakinesis the chromosomes shorten considerably by coiling. Usually the coils cannot be distinguished as such because of the small size of the chromosomes, but occasionally the major coils may very clearly be seen. The chromosomes continue to shorten until late metaphase, at which time they are rather closely crowded on the spindle. Accurate counts are often difficult during this stage because there is a tendency for smaller chromosomes to separate before larger chromosomes, and often separation of the smaller elements begins before all the chromosomes have been brought to lie in one plane. (Fig. 44, Plate IV). It is during late diakinesis and prometaphase that the bivalents may be counted most readily and accurately. Side views of metaphase-anaphase I clearly confirm that the chromosomes are monocentric with medianly or submedianly placed centromeres (Fig. 44, Plate IV; Figs. 80, 81, Plate X).

Following metaphase, the chromosomes separate and pass

to opposite poles with terminalization of the chiasmata. In nearly all of the lymnaeids and many of the planorbids studied one set of chromosomes tends to lag behind at anaphase, apparently having difficulty terminalizing. This delayed separation is often accompanied by a bridge between the separating chromosomes at late anaphase but need not be confused with the "true bridges" of inversion heterozygosity, because acentric fragments are not found. However, in one of three individuals of Gyraulus deflectus from Ann Arbor, bridges and fragments were found indicating that in this population there are snails heterozygous for a paracentric inversion. In most of the dividing meiotic cells of this specimen studied there was only one fragment, indicating a single crossover in the inversion loop produced at synapsis (Figs. 82-84, Plate XI). In one cell two acentric fragments were found possibly indicating a four-strand double crossover in the loop (Fig. 86, Plate XII). Some sperm resulting from such cells will undoubtedly be inviable.

Following the first meiotic anaphase each cell divides forming two secondary spermatocytes. The chromosomes do not become diffuse during interkinesis but are immediately orientated at the equatorial plates of the secondary spermatocytes and pass directly through metaphase into anaphase II.

CHROMOSOME NUMBERS AND CARYOTYPE ANALYSIS

Thirty-one species and subspecies were found to have eighteen pairs of chromosomes. These include three species of melampids, seventeen species and subspecies of lymnaeids, five species of physids, and six species of planorbids (Table II). Figures of the chromosomes of these species are shown in the plates. One individual of Gyraulus deflectus from Dexter had three extra elements present in its nuclei. A spermatogonial metaphase with thirty-nine chromosomes is shown in Fig. 88, Plate XII. These elements appear as three univalents, one univalent and a bivalent, or one trivalent during diakinesis (Figs. 91-95, Plate XIII) where they are readily recognized because they are much more deeply stained than the bivalents of the normal complement. The pairing of these supernumeraries is not normal and there is probably little homology between their segments. Their fate during cell division was not determined.

Finding Physa gyrina from the Au Sable River in Michigan with eighteen pairs of chromosomes raised some doubt concerning Mahoney's (1940) report of Physa gyrina in Colorado with a haploid number of six. To settle this point Dr. Hugo G. Rodeck, Director of the University of Colorado Museum, kindly sent live specimens of this species from several of the same localities from which Dr. Mahoney obtained his material. Six of these individuals all clearly has eighteen bivalents at meiosis and thirty-six chromosomes

TABLE II

Chromosome Numbers of Species Used in This Investigation

Species	Chromosome Number x	Chromosome Number 2x	Number of Individuals Examined	Source	Collection Number
MELAMPIDAE					
Melampus bidentatus lineatus	18	36	10	Breton Bay, Potomac R., Md.	637, 643
Detracia floridana	18	36	10	Breton Bay, Potomac R., Md.	635, 639
Phytia myosotis marylandica	18	36	7	Mollusk, Va.	638
LYMNAEIDAE					
Lymnaea stagnalis jugularis	18	36	3	Sugar Island, Mich.	150, 151
	18	36	10	Au Sable R., Grayling, Mich.	252, 295
Stagnicola exilis	18	36	9	West Lake, Dexter, Mich.	519, 531
	18	36	10	Dancer Rd., Dexter, Mich.	317, 325
Stagnicola lanceata	18	36	5	Lake Itasca, Minn.	640
Stagnicola reflexa	18	36	10	Port Clinton, Ohio	303, 316
Stagnicola umbrosa	18	36	10	East Tawas, Mich.	374, 594
Stagnicola palustris	18	36	10	Södermanland, Sweden	656
Stagnicola palustris elodes	18	36	10	Maple Rd., Ann Arbor, Mich.	324, 538
	18	36	10	Liberty Rd., Ann Arbor, Mich.	283, 284
	18	36	10	Huron R., Dexter, Mich.	319, 338
Stagnicola palustris desidiosa	18	36	10	Roscommon Co., Mich.	359, 362
Stagnicola catascopium	18	36	10	Au Sable R., Grayling, Mich.	258, 580
Stagnicola emarginata angulata	18	36	10	Houghton Lake, Mich.	248, 601
Stagnicola emarginata serrata	18	36	10	Douglas Lake, Mich.	138, 504
Stagnicola caperata	18	36	10	Dayton, Ohio	216, 244
Radix pereger	17	34	7	Ankara, Turkey	657, 658
Acella haldemani	18	36	10	Sages Lake, Ogemaw Co., Mich.	495, 589
Pseudosuccinea columella	18	36	3	Charlottesville, Va.	171, 175
Bulimnea megasoma	18	36	5	Sugar Island, Mich.	152, 153
	18	36	10	Houghton Lake, Mich.	263, 566
Fossaria parva	18	36	10	Huron R., Dexter, Mich.	542, 562
Fossaria modicella	18	36	10	Port Clinton, Ohio	297, 298
Fossaria modicella rustica	19	38	10	Huron R., Dexter, Mich.	333, 334
PHYSIDAE					
Physa sayii crassa	18	36	10	Lake Margrethe, Mich.	267, 595
Physa heterostropha	18	36	10	Richmond, Va.	653
Physa gyrina	18	36	8	Au Sable R., Grayling, Mich.	255, 257
	18	36	6	Boulder, Colo.	632, 644
Physa anatina	18	36	4	Boulder, Colo.	631, 642
Aplexa hypnorum	18	36	9	Houghton Lake, Mich.	222, 223
	18	36	2	Dancer Rd., Dexter, Mich.	343, 344
PLANORBIDAE					
Gyraulus deflectus	18	36	3	Maple Rd., Ann Arbor, Mich.	536, 539
	18*	36*	4	Huron R., Dexter, Mich.	543a, 559
Gyraulus circumstriatus	36	72	10	Huron R., Dexter, Mich.	543, 560
Armiger crista	18	36	2	Huron R., Dexter, Mich.	545, 558
Australorbis glabratus	18	36	10	Parke-Davis Pharmaceutical Co.	130, 203
Helisoma anceps	18	36	6	Charlottesville, Va.	176, 186
	18	36	7	Au Sable R., Grayling, Mich.	271, 272
Helisoma trivolvis	18	36	4	Hardwood Lake, Mich.	368, 369
	18	36	2	Huron R. Dr., Dexter, Mich.	329, 330
Planorbula crassilabris	19	38	7	Houghton Lake, Mich.	219, 220
	19	38	2	Huron R., Dexter, Mich.	547
Promenetus exacuous	18	36	10	Dancer Rd., Dexter, Mich.	340, 341
	18	36	10	Huron R., Dexter, Mich.	544, 561
ANCYLIDAE					
Ferrissia parallela	30	60	3	Houghton Lake, Mich.	551, 641
Ferrissia tarda	17	34	3	Huron R., Ann Arbor, Mich.	557
Ferrissia fusca	17	34	3	Charlottesville, Va.	181

in spermatogonial cells (Figs. 106 and 108, Plate XV).

Seven species were found to have haploid numbers other than eighteen. Three species, a lymnaeid and two ancylids, had seventeen pairs of chromosomes; two species, a lymnaeid and a planorbid, had nineteen pairs; one ancylid had thirty pairs; and a planorbid had thirty-six.

In addition to obtaining chromosome numbers, the individual chromosomes of any species may be identified, their shapes described, and comparisons made with other species. Such studies usually use the chromosomes as found in somatic metaphase and anaphase because in these stages the chromosomes have reached their maximum contraction. The relative lengths, volumes, and shapes of such chromosomes under ordinary conditions are remarkably constant for any cell in any individual of a particular species. The chromosomes of different species are usually sufficiently characteristic to provide criteria that can be used for systematic purposes.

As compared with chromosomes of many plants, numerous insects, and prosobranch snails, pulmonate mitotic chromosomes are difficult to study. In previous work the classical method of cutting sections of ovotestis tissue or eggs has been used almost entirely. By this method one can study dividing cells in normal relation to other cells in the ovotestis and obtain a clear histological picture of this organ, but the individual chromosomes are difficult to analyze. Inaba (1953) is the only author to attempt a

caryotype analysis, but he realized the difficulties presented by mollusk material and did not try to compare the mitotic chromosomes of the species he investigated. He did, however, attempt to compare the metaphase chromosomes of the primary spermatocytes. His results were described as follows (pp. 226, 227):

> "The chromosomes of Lymnaeidae are a little larger than the others. And the chromosomes of 2 families living in salt water distinctly smaller than those of living in freshwater... The karyotypes of Limnaeid snails are very similar to each other...Except the chromosome numbers, the karyotypes of Limnaea stagnalis is very similar to 2 Japanese species and especially shows the same appearance with that of L. japonica."

Such information, while worthwhile, does not serve to clarify systematics at the level of the lower taxonomic categories.

In order to overcome the difficulties indicated above, several of the squash techniques were employed since they have proven so useful in plant and insect cytogenetics and cytotaxonomy. These techniques permit better observation of the individual chromosomes for several reasons: (1) the chromosomes are not subjected to the shrinkage of paraffin methods and, accordingly, are larger; (2) the flattening brought about by the pressure applied to the coverglass brings all the chromosomes nearly into a single optical plane; and (3) the chromosomes are spread out and thereby separated. In addition, the squash techniques are simpler and less laborious than paraffin techniques.

As mentioned previously, dividing spermatogonial cells are rather rare in basommatophoran material. Also, when congression is complete the chromosomes are so tightly attached to the spindle that they cannot be sufficiently spread for adequate observation. These conditions, of course, add considerable difficulty to the already inherent disadvantage presented by the small size of the chromosomes. However, spermatogonial cells at early metaphase, before congression is complete, have been found in individuals of six species -- four lymnaeids and two ancylids. One set of chromosomes of each of these species are arranged in order of decreasing length on Plate VIII and Plate XIV, Figs. 104, 105. Another cell is shown on Plate XIV, Fig. 103, with the chromosomes reproduced in their original spread positions.

There is no sharp division of the chromosomes of these six species (Stagnicola emarginata angulata, S. palustris elodes, Pseudosuccinea columella, Fossaria modicella, Ferrissia fusca, and F. tarda) into well-defined size groups. The sizes in each species decreases gradually from the largest pair to the smallest. All of the chromosomes are monocentric, i.e., have one primary constriction, and in each species this constriction divides some of the chromosomes into equal arms. In none of the chromosomes is the centromere terminal. Most of the chromosomes which are not medianly constricted present a problem in terminology. Here the centromeres are sufficiently far from the

median position to be as easily considered subterminal as submedian. Therefore, in this investigation chromosomes with short arms one-third or more of their total lengths are considered submedianly constricted, and those with short arms less than one-third their total lengths subterminal. However, since this distinction is relative to the overall lengths of the individual chromosomes, the short arms of large subterminally constricted chromosomes may be longer than the long arms of small submedianly constricted chromosomes (cf. sets 1 and 16 of Fig. 104). A comparison of the caryotypes of the above six species is given in Table III.

TABLE III

Comparison of Caryotypes of Four Freshwater Snails and Two Limpets

Species	Number of Medianly Constricted Chromosomes	Number of Submedianly Constricted Chromosomes	Number of Subterminally Constricted Chromosomes	Chromosome Number (2n)
LYMNAEIDAE				
Stagnicola palustris elodes	8	3	7	36
Stagnicola emarginata angulata	13	1	4	36
Pseudosuccinea columella	9	1	8	36
Fossaria modicella	10	5	3	36
ANCYLIDAE				
Ferrissia tarda	4	2	11	34
Ferrissia fusca	4	6	7	34

DISCUSSION

Fifty-four species and subspecies of the pulmonate order Basommatophora have now been examined critically enough to determine their chromosome numbers. This assemblage includes those reported in the investigations of the Perrots' (1930, 1934, 1938), Le Calvez and Certain (1950), Inaba (1953), Inaba and Tanaka (1953), and Meyer (1955), whose reports are regarded as reliable. Of these fifty-four species and subspecies, forty-two, or over seventy per cent, have the single haploid number of eighteen. Thus, it appears that the basic number of chromosomes for the order is eighteen pairs. This statement is strengthened by the observations that all but one of the more primitive members critically studied, i.e., the Melampidae, and the Amphibolidae, have eighteen pairs of chromosomes. The only family (other than the one species of siphonarid investigated) in which the haploid number eighteen is not the dominant one, and indeed, has not been found at all, is the Ancylidae, which is considered to be the most highly evolved of the Basommatophora.[4] Reports of haploid numbers other than eighteen for Lymnaea stagnalis and its American variety jugularis, Stagnicola palustris and its American variety elodes, and Physa gyrina are erroneous. The number

[4]This family is usually considered to have been derived from the Planorbidae.

reported for Pira angiostoma is not only doubtful, but should be considered invalid, especially when one considers the technique employed. Therefore, the numbers six, eight, ten, twelve, and most of the sixteens are eliminated.

Because of this constancy in the chromosome number, those species with haploid numbers other than eighteen are very interesting. Those with nineteen are a lymnaeid and a planorbid, and since they are only very remotely related, each obviously gained an extra bivalent independently. On the other hand, four lymnaeida have seventeen pairs of chromosomes and three of these are very closely related, belonging to the same genus (Radix) in the same general geographic area. J.-L. and M. Perrot (1938) have pointed out the systematic significance of these facts, but their suggestion of putting Lymnaea stagnalis and Stagnicola palustris into the same taxonomic group because they both have eighteen pairs of chromosomes was, of course, premature. This last point has been discussed by Inaba and Tanaka (1953). The fact that the three species of European Radix all have seventeen pairs of chromosomes is perhaps quite significant. It suggests that a corollary, either direct or indirect, in the evolution of this group was a loss of one bivalent. This loss may have been either directly correlated with the genetic separation of the early Radix ancestors or resulted independently. However, it became fixed very early in the history of the group. Radix japonica of Japan with eighteen as the haploid number per-

haps regained a bivalent during its separation from the European stock; or, its Radix-like morphology may be the result of parallel evolution. However, it should be stated here that taxonomic characters of infraspecific, specific, and subgeneric rank are at present too ill-defined for many lymnaeids to definitely place them in nomenclatural groups. Fossaria ollula pervia with seventeen pairs of chromosomes may actually belong to the Radix group, or again, may have lost a bivalent independently.

The haploid numbers of sixteen reported for Siphonaria japonica and Gyraulus hiemantium are interesting but until more investigations are made on their respective groups little can be said about them. The only other anomalous numbers reported in the Basommatophora are in the ancylids, which have haploid numbers of seventeen, thirty, and fifty-nine, and Gyraulus circumstriatus with thirty-six. Both Ferrissia tarda and F. fusca have seventeen pairs of chromosomes, but they belong to different subgenera (i.e., Ferrissia s.s. and Laevapex). On the other hand, F. parallela has a haploid number of thirty but is placed, on the basis of shell characters, with the subgenus Ferrissia. Ancylastrum costulatum has been reported to have about fifty-nine bivalents during the first meiotic division (Le Calvez and Certain, 1950). The latter two species may possibly be polyploids. However, Le Calvez and Certain are very doubtful about the polyploid nature of the last named species. They suggest that the difference in its chromosome number and the usual eighteen is due to diphylogeny in the Basommatophora.

Indeed, recent evidence suggests that the ancylid group itself is polyphyletic (Bengt Hubendick, personal communication; Basch, 1958). It will be interesting to determine chromosome numbers of the other ancylids.

A much more probable case of polyploidy, i.e., tetraploidy, is that of Gyraulus (Torquis) circumstriatus with thirty-six pairs of chromosomes. The only other North American gyraulid investigated has eighteen pairs (exactly one-half), as do all but two of the other planorbids studied. Since this case is apparently the only bona fide one of polyploidy in the Basommatophora, this hermaphroditic, self-fertilizing group has not measured up to previous expectations. Polyploidy has undoubtedly been of little importance in its speciation and evolution.

The stability of the chromosome number in the Basommatophora has interesting implications with regard to the origin of that group. A common theory is that the Basommatophora arose from the land-dwelling Stylommatophora (cf. Pilsbry and Bequaert, 1927). Since the Stylommatophora differ from all other molluscan groups in that landshell species are characterized by a greater number of chromosomes, i.e., more than twenty pairs (Perrot and Perrot, 1937; M. Perrot, 1938; Inaba, 1945, 1950a, b, 1953; Husted and Burch, 1946, 1953) it is difficult to understand how land pulmonates can be ancestral to the freshwater lung-breathers when the transition would involve a gain of chromosomes with land specialization and the subsequent loss of chromosomes with adaptation to freshwater. The Basommatophora may have been derived from either the prosobranchs or opisthobranchs since

both groups are characterized by haploid numbers less than twenty.

Since the chromosomes of all the snails studied in this investigation, and presumably also in all other freshwater pulmonates, are not as previously described for this group, but are monocentric with well-defined primary constrictions, the individual chromosomes of each species may be described and corresponding chromosomes identified in other individuals of each species. Thus, caryotype analysis may prove very useful in future cytotaxonomic studies. However, because the caryotypes are now known in only a few species, none of which are very closely related, little can be said concerning systematic implications, except that although the chromosome numbers of each group are the same, the caryotypes of the various species are different. It will be interesting to compare caryotypes of other species, especially closely related ones.

SUMMARY AND CONCLUSIONS

The basic number of chromosomes for the Basommatophora appear to be eighteen pairs. This is the number found in thirty-one species and subspecies used in this study, and has been reported by other authors for eleven other species of the group. Seven species were found to have haploid numbers other than eighteen , and two of these may be polyploid. The haploid number (n=36) of Gyraulus circumstriatus indicates a good possibility of its being tetraploid; Ferrissia parallela (n=30) may also be polyploid, but this case is more doubtful since no ancylid with fifteen pairs of chromosomes has been found. Polyploidy and anauploidy appear rare in freshwater snails and are probably of little importance in their speciation and evolution.

The chromosome numbers reported by J.-L. Perrot and M. Perrot for Stagnicola palustris and Radix pereger have been confirmed. The numbers reported by various authors for Lymnaea stagnalis jugularis, Stagnicola palustris elodes, and Physa gyrina are erroneous. The number reported for Pira angiostoma also is not reliable.

Any hypothesis concerning the origin of the Basommatophora must take into account its stability in chromosome numbers. Since the Stylommatophora differ from all other pulmonate groups in that its species are characterized by greater number of chromosomes, it is difficult to visualize land pulmonates as ancestral to the freshwater lung-breathers,

since the transition involves gain of chromosomes with land specialization and subsequent loss of chromosomes with adaptation to freshwater.

Supernumerary chromosomes were found in one population of Gyraulus deflectus, and chromosomal bridges and fragments indicating structural heterzygosity were found during the first meiotic anaphase in another population.

The mitotic chromosomes of species of Basommatophora are not oval or dot-shaped as previously described, but are elongate and monocentric. Therefore the shapes of mitotic chromosomes cannot be used to distinguish this group from the Stylommatophora, but may prove useful in discerning taxonomic relationships at lower systematic levels.

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PLATE I (continued)

Fig. 11: Stagnicola emarginata serrata (Haldeman). Douglas Lake, Cheboygan County, Michigan.

Fig. 12: Stagnicola (Hinkleyia) caperata (Say). Dayton, Montgomery County, Ohio.

Fig. 13: Stagnicola (Hinkleyia) caperata (Say). Baseline Road, Boulder, Colorado.

PLATE I

SHELLS OF STAGNICOLA

Fig. 1: Stagnicola exilis (Lea). Dancer and Trinkle Roads, near Dexter, Washtenaw County, Michigan.

Fig. 2: Stagnicola palustris elodes (Say). Maple Road and Huron River Drive, Ann Arbor, Washtenaw County, Michigan.

Fig. 3: Stagnicola palustris elodes (Say). Mill pond, Dexter, Washtenaw County, Michigan.

Fig. 4: Stagnicola palustris desidiosa (Say). Reedsburg Dam, Roscommon County, Michigan.

Fig. 5: Stagnicola palustris elodes (Say). Liberty and Zeeb Roads, Scio Township, Washtenaw County, Michigan.

Fig. 6: Stagnicola palustris elodes (Say). Honey Creek, Huron River Drive, Washtenaw County, Michigan.

Fig. 7: Stagnicola umbrosa (Say). Near East Tawas, Iosco County, Michigan.

Fig. 8: Stagnicola reflexa (Say). Near Port Clinton, Ottawa County, Ohio.

Fig. 9: Stagnicola catascopium (Say). Au Sable River, near Grayling, Crawford County, Michigan.

Fig. 10: Stagnicola emarginata angulata (Sowerby). Houghton Lake, Roscommon County, Michigan.

PLATE I

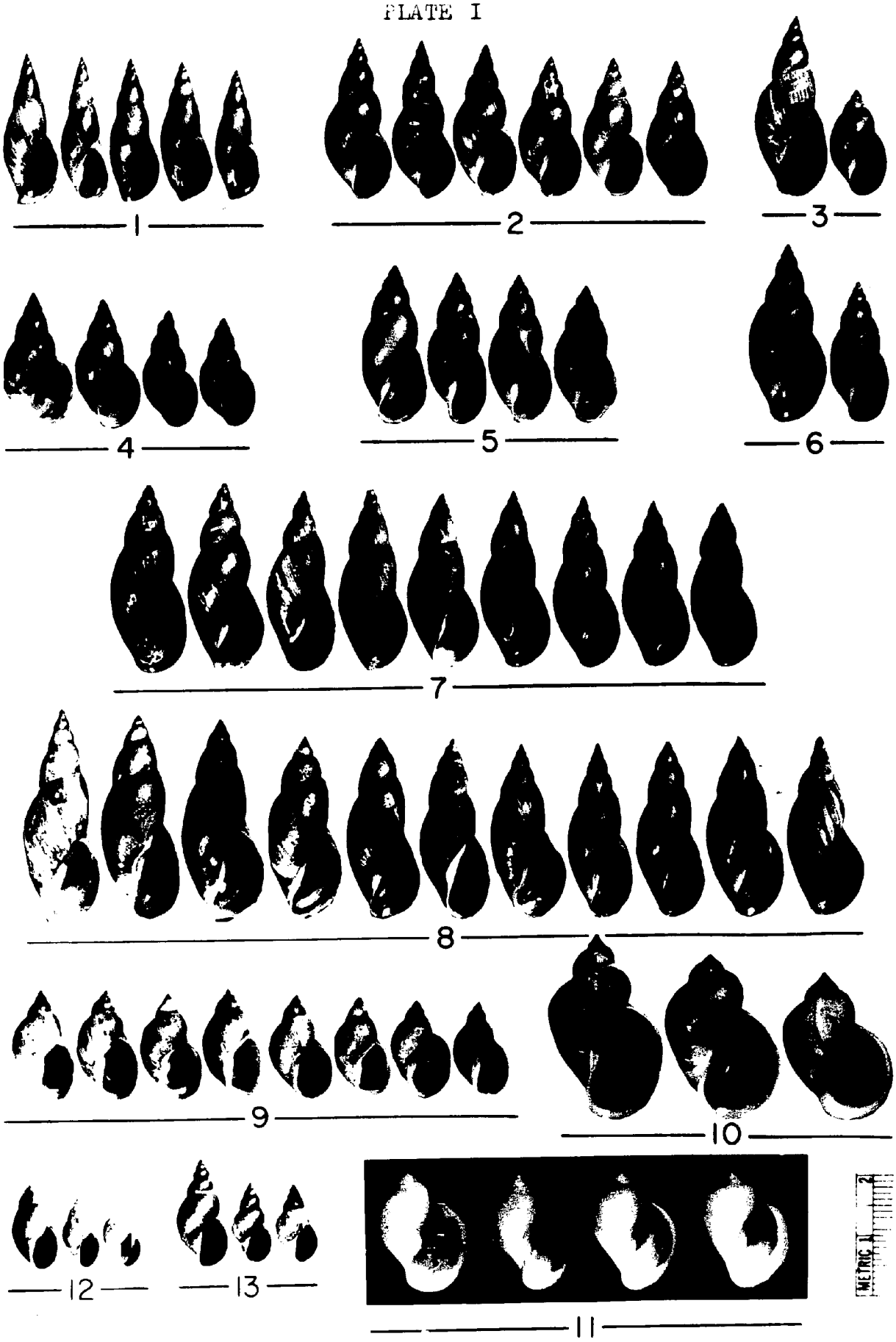


PLATE II (continued)

Fig. 26: Helisoma anceps (Menke). Au Sable River, near Grayling, Crawford County, Michigan.

PLATE II

SHELLS OF FRESHWATER PULMONATES

Fig. 14: Bulimnea megasoma (Say). Near Houghton Lake, Roscommon County, Michigan.

Fig. 15: Lymnaea stagnalis jugularis (Say). Au Sable River, near Grayling, Crawford County, Michigan.

Fig. 16: Pseudosuccinea columella (Say). Greenhouse, Charlottesville, Albemarle County, Virginia.

Fig. 17: Acella haldemani (Binney). Sages Lake, Ogemaw County, Michigan.

Fig. 18: Helisoma (Pierosoma) trivolvis (Say). Hardwood Lake, Ogemaw County, Michigan.

Fig. 19: Helisoma (Pierosoma) trivolvis (Say). Huron River, Scio Township, Washtenaw County, Michigan

Fig. 20: Aplexa hypnorum (Linne). Houghton Lake, Roscommon County, Michigan.

Fig. 21: Physa (Physella) sayii crassa (Walker). Lake Margrethe, Crawford County, Michigan.

Fig. 22: Physa (Physodon) anatina Lea. Baseline Road, Boulder, Colorado.

Fig. 23: Physa (Physella) gyrina Say. Au Sable River, near Grayling, Crawford County, Michigan.

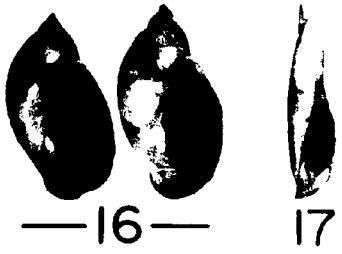
Fig. 24: Physa (Physella) gyrina Say. Varsity Lake, Boulder, Colorado.

Fig. 25: Physa (Physella) gyrina Say. Baseline Road, Boulder, Colorado.

PLATE II



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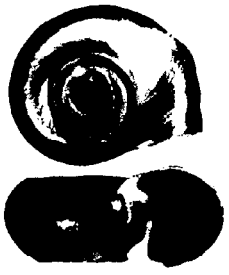
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METRIC 1 2

PLATE III

SHELLS OF SMALL FRESHWATER PULMONATES

Fig. 27: Fossaria parva (Lea). Mill pond, Dexter, Michigan.

Fig. 28: Fossaria modicella (Say). Near Port Clinton, Ottawa County, Ohio.

Fig. 29: Fossaria modicella rustica (Lea). Mill pond, Dexter, Michigan.

Fig. 30: Gyraulus deflectus (Say). Mill pond, Dexter, Michigan.

Fig. 31: Gyraulus deflectus (Say). Maple Road and Huron River Drive, Ann Arbor, Washtenaw County, Michigan.

Fig. 32: Gyraulus (Torquis) circumstriatus (Tryon). Mill pond, Dexter, Michigan.

Fig. 33: Promenetus exacuous (Say). Dancer and Trinkle Roads, near Dexter, Michigan.

Fig. 34: Planorbula crassilabris (Walker). Houghton Lake, Roscommon County, Michigan.

Fig. 35: Ferrissia (Laevapex) fusca (Adams). Charlottesville, Albemarle County, Virginia.

Fig. 36: Ferrissia tarda (Say). Huron River, Ann Arbor, Washtenaw County, Michigan.

Fig. 37: Ferrissia parallela (Haldeman). Near Houghton Lake, Roscommon County, Michigan.

PLATE III

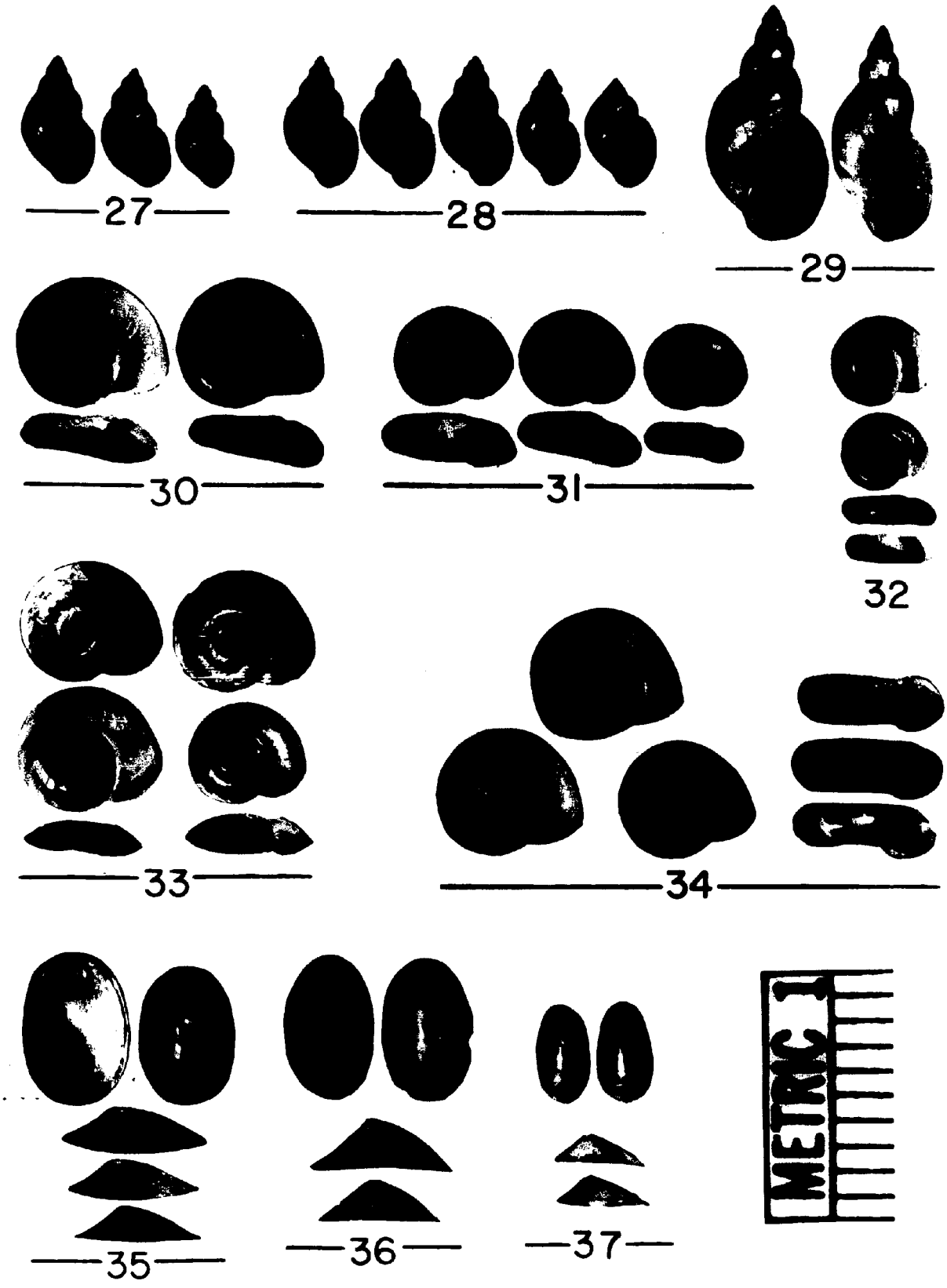


PLATE IV

CHROMOSOMES OF FRESHWATER SNAILS

Fig. 38: Lymnaea stagnalis jugularis (Say). Polar view, spermatogonial metaphase.

Fig. 39: Fossaria modicella rustica (Lea). Polar view, spermatogonial metaphase.

Fig. 40: Fossaria modicella rustica (Lea). Polar view, meiotic metaphase I.

Fig. 41: Fossaria modicella rustica (Lea). Polar view, meiotic metaphase II.

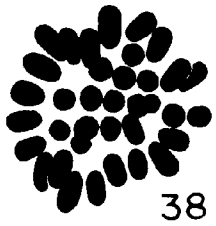
Fig. 42: Fossaria modicella rustica (Lea). Polar view, meiotic metaphase I.

Fig. 43: Fossaria modicella rustica (Lea). Polar view, meiotic metaphase II.

Fig. 44: Lymnaea stagnalis jugularis (Say). Side view, meiotic metaphase-anaphase I. Two elements have completely separated.

Drawings on this and all subsequent plates were made with the aid of a camera lucida and reproduced at a magnification of 4650X. Figs. 38-41 are from sectioned material stained with crystal violet; Figs. 42-44 are from aceto-carmine squashes. Note the differences in sizes of Figs. 40 and 42, 41 and 43.

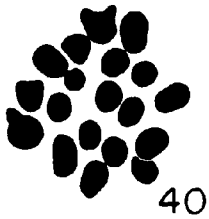
PLATE IV



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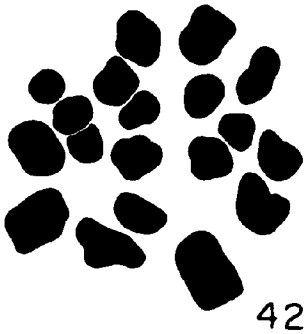
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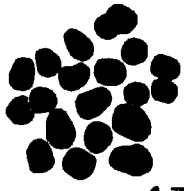
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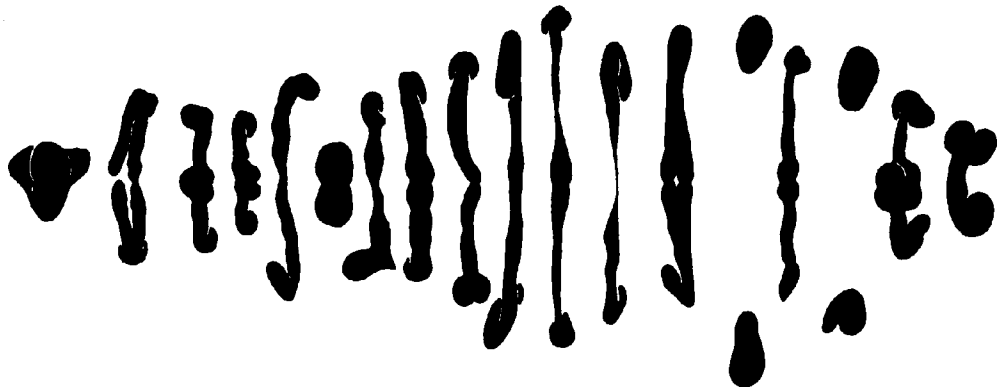
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PLATE V

CHROMOSOMES OF FRESHWATER SNAILS

Fig. 45: Lymnaea stagnalis jugularis (Say). Late diakinesis.

Figs. 46, 47: Stagnicola palustris elodes (Say). Polar views, meiotic metaphase I.

Fig. 48: Stagnicola palustris desidiosa (Say). Late diakinesis.

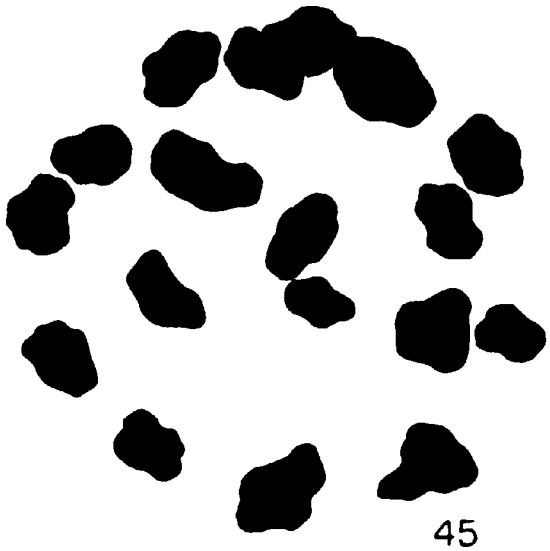
Fig. 49: Stagnicola umbrosa (Say). Late diakinesis.

Fig. 50: Stagnicola exilis (Lea). Polar view, meiotic metaphase I.

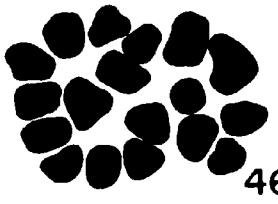
Fig. 51: Stagnicola reflexa (Say). Meiotic prometaphase I.

Figs. 45-47 from acetocarmine squashes; Figs. 48-51 from acetic-orcein squashes. Fig. 50 from material pretreated with distilled water and killed and fixed in 25 per cent acetic acid.

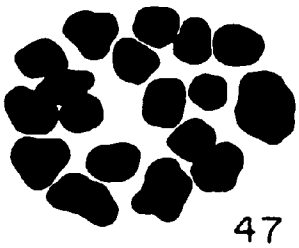
PLATE V



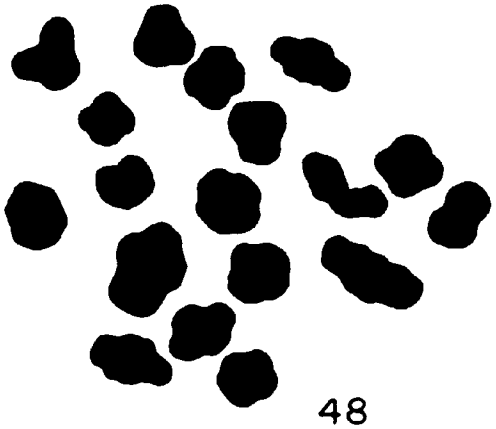
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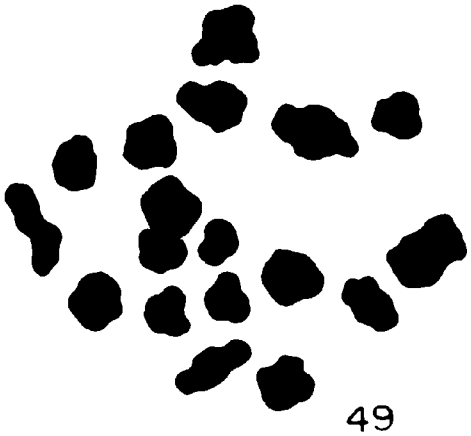
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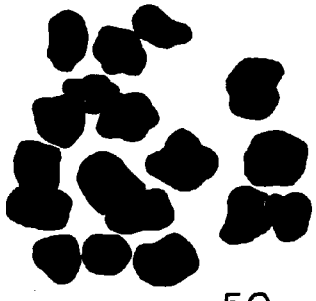
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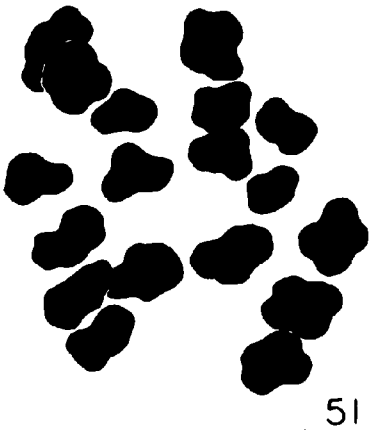
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PLATE VI

CHROMOSOMES OF FRESHWATER SNAILS

Fig. 52: Bulimnea megasoma (Say). Late diakinesis.

Fig. 53: Bulimnea megasoma (Say). Polar view, meiotic metaphase I.

Fig. 54: Pseudosuccinea columella (Say). Late diakinesis.

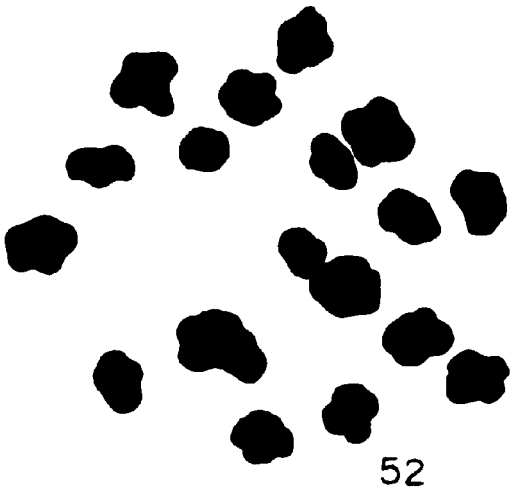
Fig. 55: Stagnicola (Hinkleyia) caperata (Say). Late diakinesis.

Fig. 56: Stagnicola catascopium (Say). Meiotic prometaphase I.

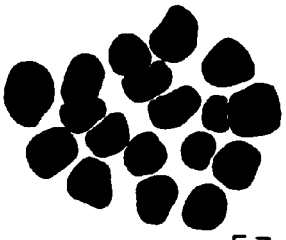
Fig. 57: Stagnicola emarginata angulata (Sowerby). Meiotic prometaphase I.

Figs. 53, 54 from acetocarmine squashes; Figs. 52, 55-57 from acetic-orcein squashes.

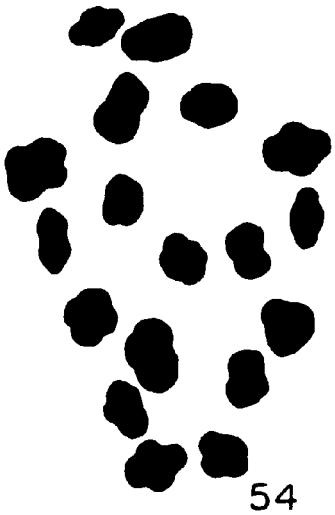
PLATE VI



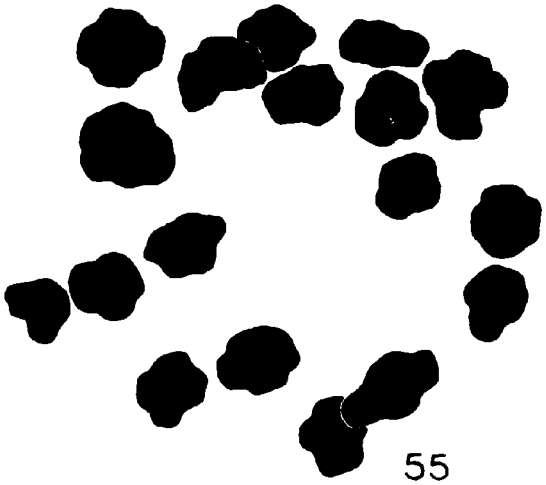
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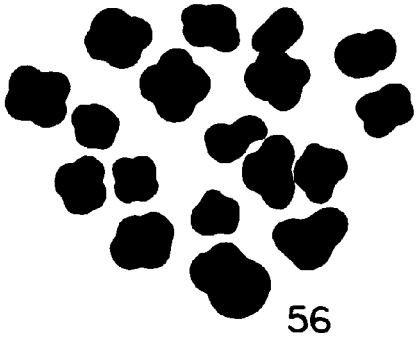
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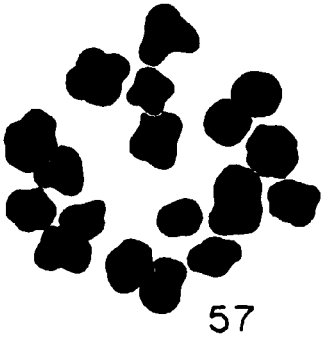
54



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PLATE VII

CHROMOSOMES OF FRESHWATER SNAILS

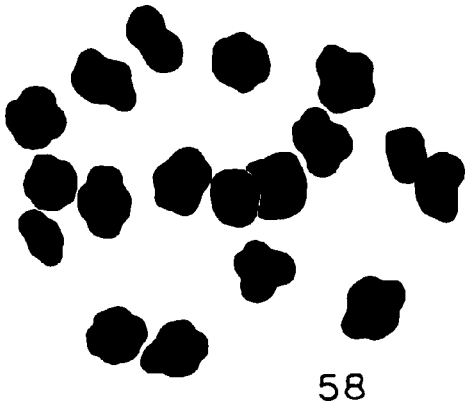
Fig. 58: *Fossaria parva* (Lea). Late diakinesis.

Fig. 59: *Fossaria modicella* (Say). Polar view, meiotic metaphase I.

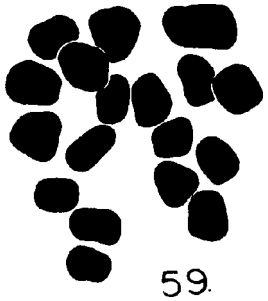
Figs. 60, 61: *Fossaria modicella rustica* (Lea). Polar views, meiotic metaphase I.

Fig. 58 from acetocarmine squash; Figs. 59-61 from acetic-orcein squashes.

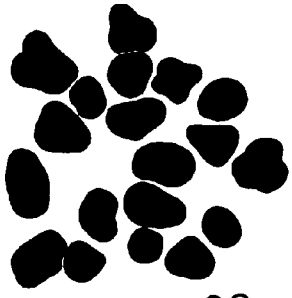
PLATE VII



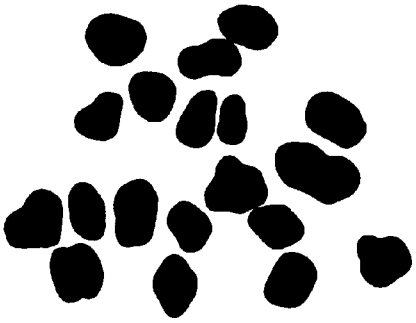
58



59.



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61

PLATE VIII

SPERMATOGONIAL CHROMOSOMES OF FOUR LYMNAEIDS

ARRANGED IN ORDER OF DECREASING LENGTH

Fig. 62: *Stagnicola palustris elodes* (Say).

Fig. 63: *Stagnicola emarginata angulata* (Sowerby).

Fig. 64: *Pseudosuccinea columella* (Say).

Fig. 65: *Fossaria modicella* (Say).

All figures drawn from acetocarmine squashes.

PLATE VIII

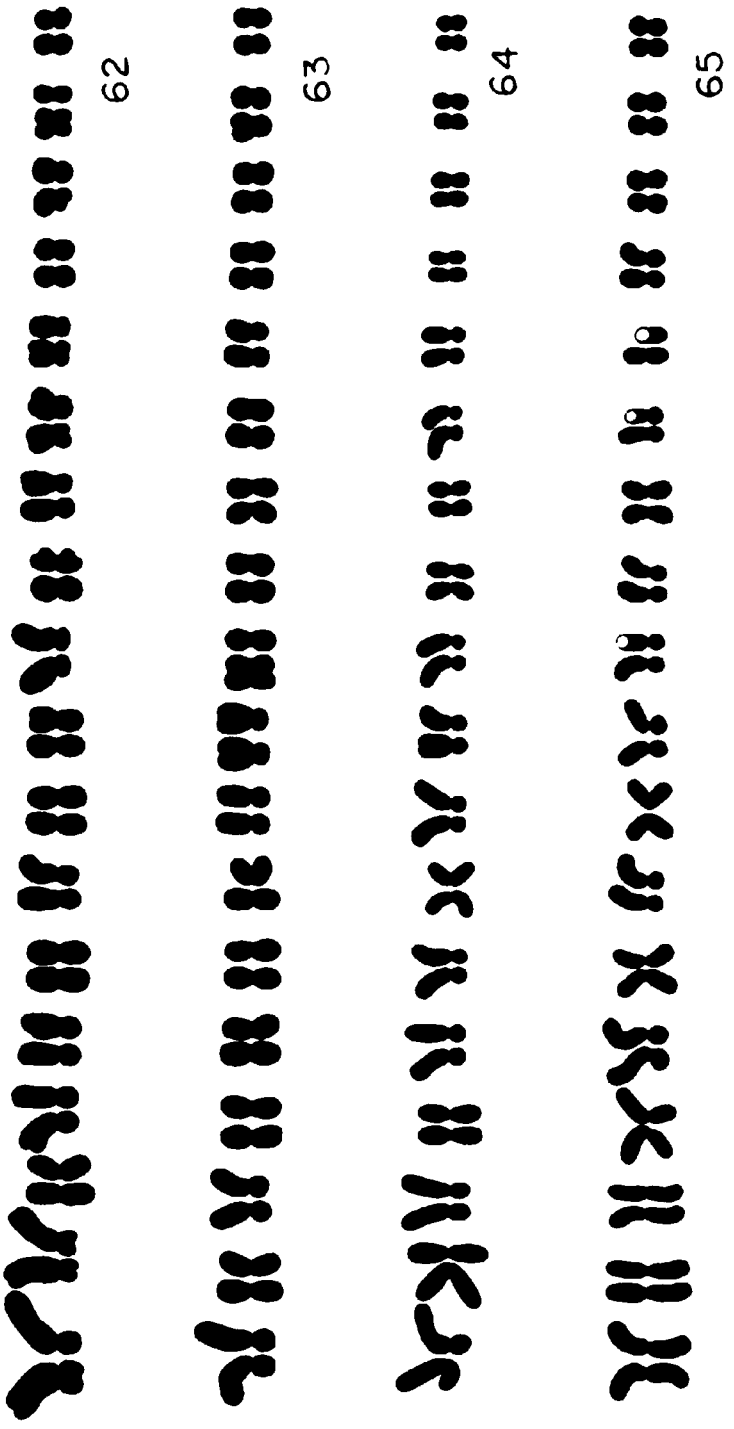


PLATE IX

CHROMOSOMES OF FRESHWATER SNAILS

Fig. 66: Physa (Physella) gyrina (Say). Michigan. Polar view, meiotic metaphase I.

Fig. 67: Physa (Physella) sayii crassa (Walker). Polar view, meiotic metaphase I.

Fig. 68: Aplexa hypnorum (Linné) Polar view, meiotic metaphase I.

Fig. 69: Armiger crista (Linné). Meiotic prometaphase I.

Figs. 70, 73: Planorbula crassilabris (Walker). Polar view, meiotic metaphase I.

Fig. 71: Promenetus exacuous (Say). Polar view, meiotic metaphase I.

Fig. 72: Helisoma (Pierosoma) trivolvis (Say). Polar view, meiotic metaphase I.

Fig. 74: Australorbis glabratus (Say). Polar view, meiotic metaphase I.

Fig. 75: Helisoma (Pierosoma) trivolvis (Say). Polar view, spermatogonial metaphase. Notice the J- and V-shaped chromosomes.

Fig. 76: Helisoma anceps (Menke). Virginia. Polar view, meiotic metaphase I.

Figs. 66, 68, 70, 71, 74, 76 from acetocarmine squashes; Figs 67, 69, 72, 73, 75 from acetic-orcein squashes.

PLATE IX

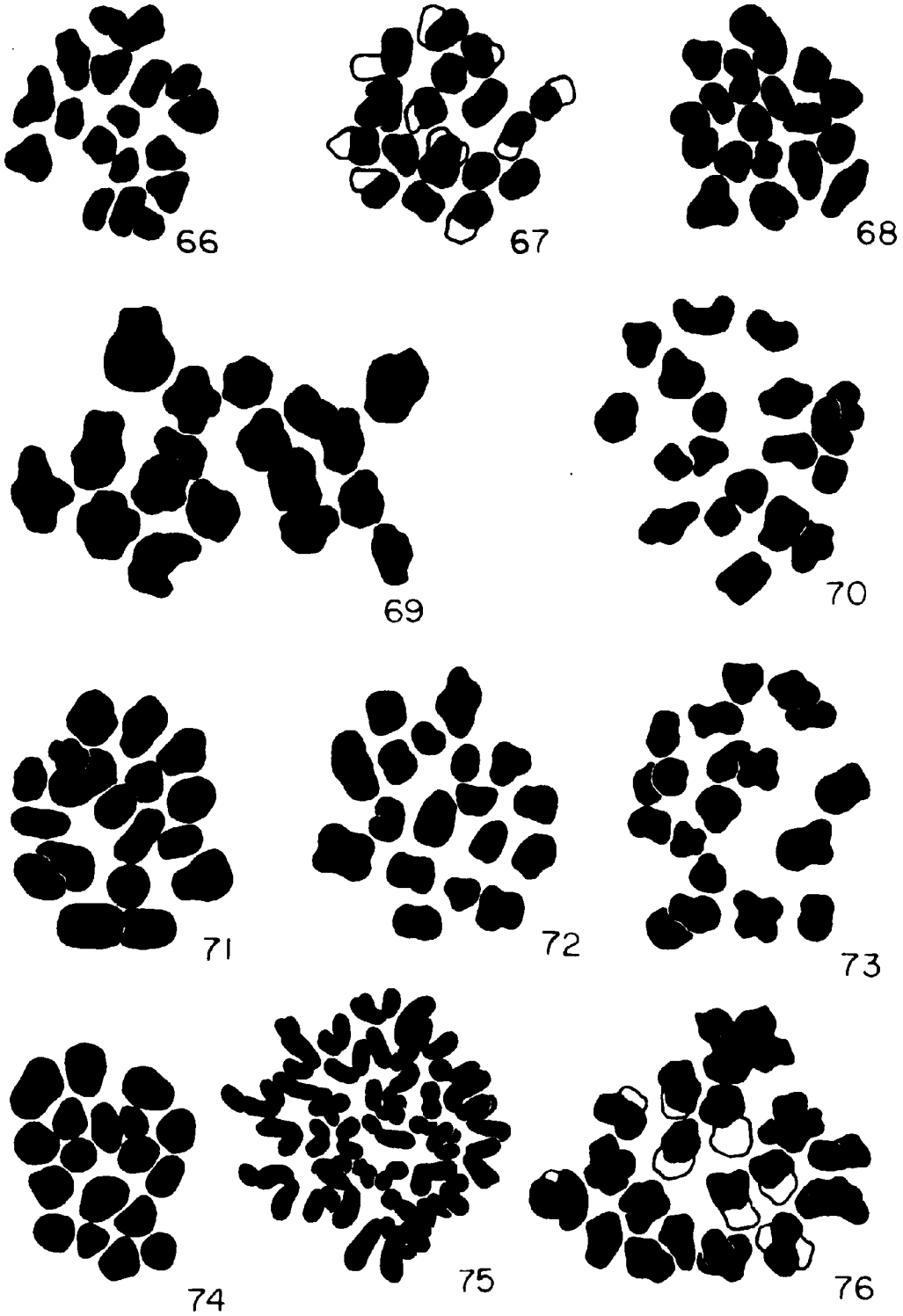


PLATE X

CHROMOSOMES OF FRESHWATER SNAILS

Fig. 77: Gyraulus deflectus (Say). Ann Arbor. Polar view, meiotic metaphase I.

Fig. 78: Gyraulus deflectus (Say). Dexter. Meiotic prometaphase I.

Fig. 79: Helisoma anceps (Menke). Michigan. Polar view, meiotic metaphase I.

Figs. 80, 81: Helisoma anceps (Menke). Virginia. Side views, meiotic metaphase I.

Figs. 77, 78 from acetic-orcein squashes; figs. 79-81 from acetocarmine squashes.

PLATE X

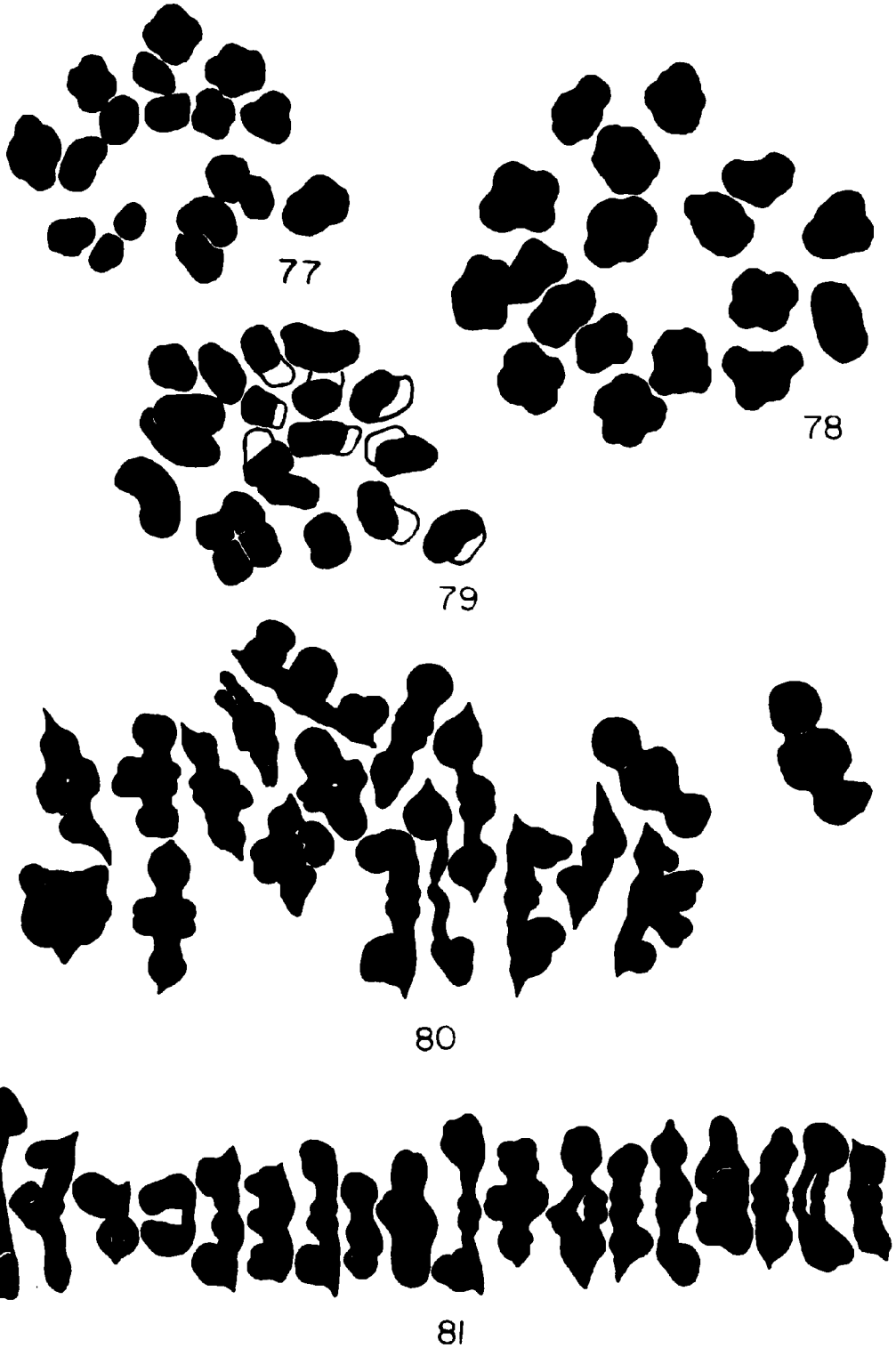


PLATE XI

CHROMOSOMES OF FRESHWATER SNAILS

Figs. 82-84: Gyraulus deflectus (Say). Side view, meiotic anaphase I showing bridges and fragments indicative of inversion heterozygosity.

Fig. 85: Gyraulus deflectus (Say). Spermatogonial prometaphase.

All figures from acetic-orcein squashes from an individual of the Ann Arbor population.

PLATE XI

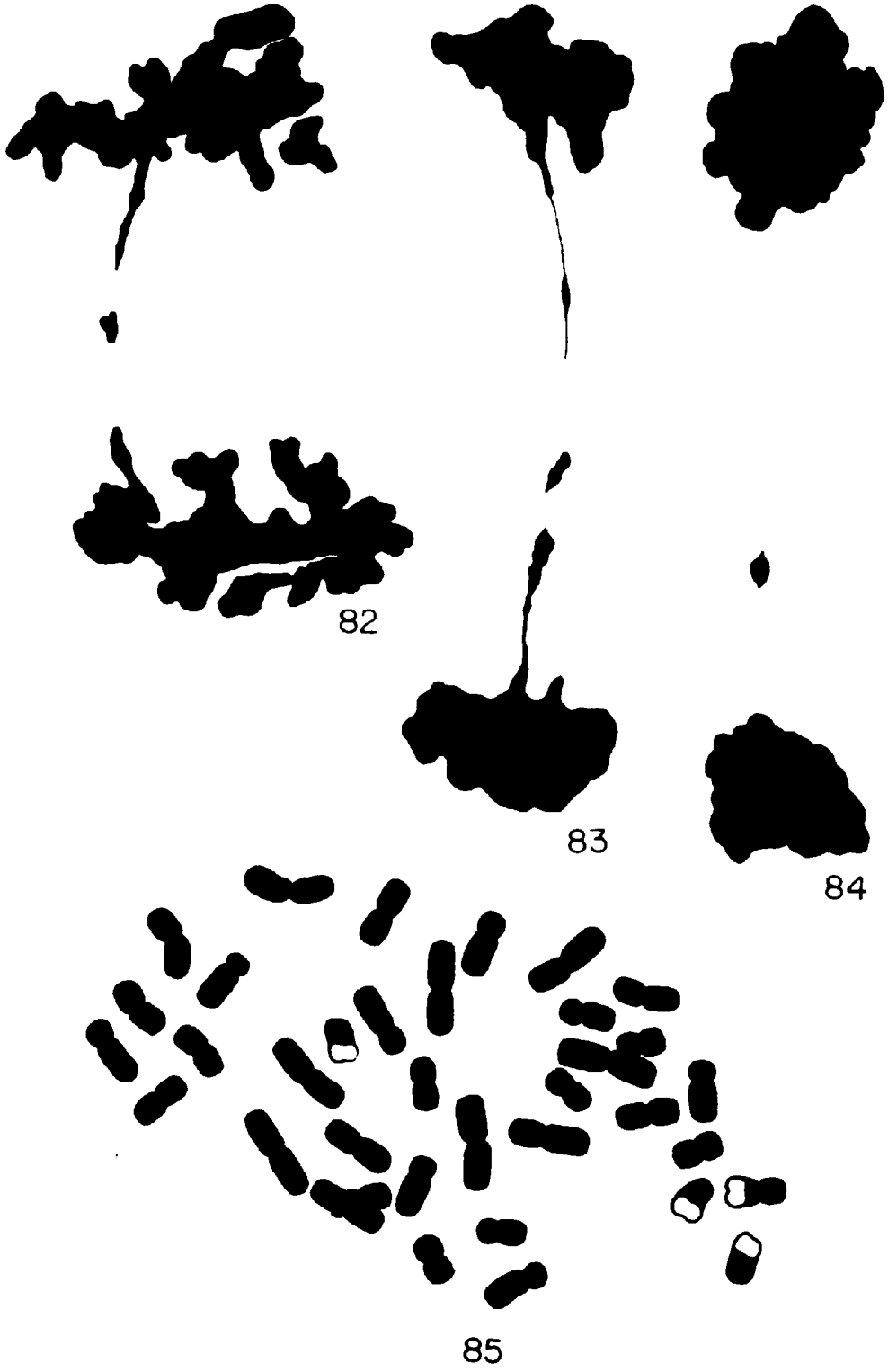


PLATE XII

CHROMOSOMES OF FRESHWATER SNAILS

Fig. 86: Gyraulus deflectus (Say). Ann Arbor. Side view of meiotic anaphase I showing two acentric fragments indicating a four-strand double cross-over in the inversion loop.

Fig. 87: Gyraulus deflectus (Say). Dexter. Polar view of spermatogonial metaphase with thirty-six chromosomes.

Fig. 88: Gyraulus deflectus (Say). Dexter. Polar view of spermatogonial metaphase with thirty-nine elements on the equatorial plate.

Fig. 89: Gyraulus deflectus (Say). Dexter. Diakinesis.

Fig. 90: Gyraulus deflectus (Say). Ann Arbor. Diakinesis.

All figures from acetic-orcein squashes.

PLATE XII

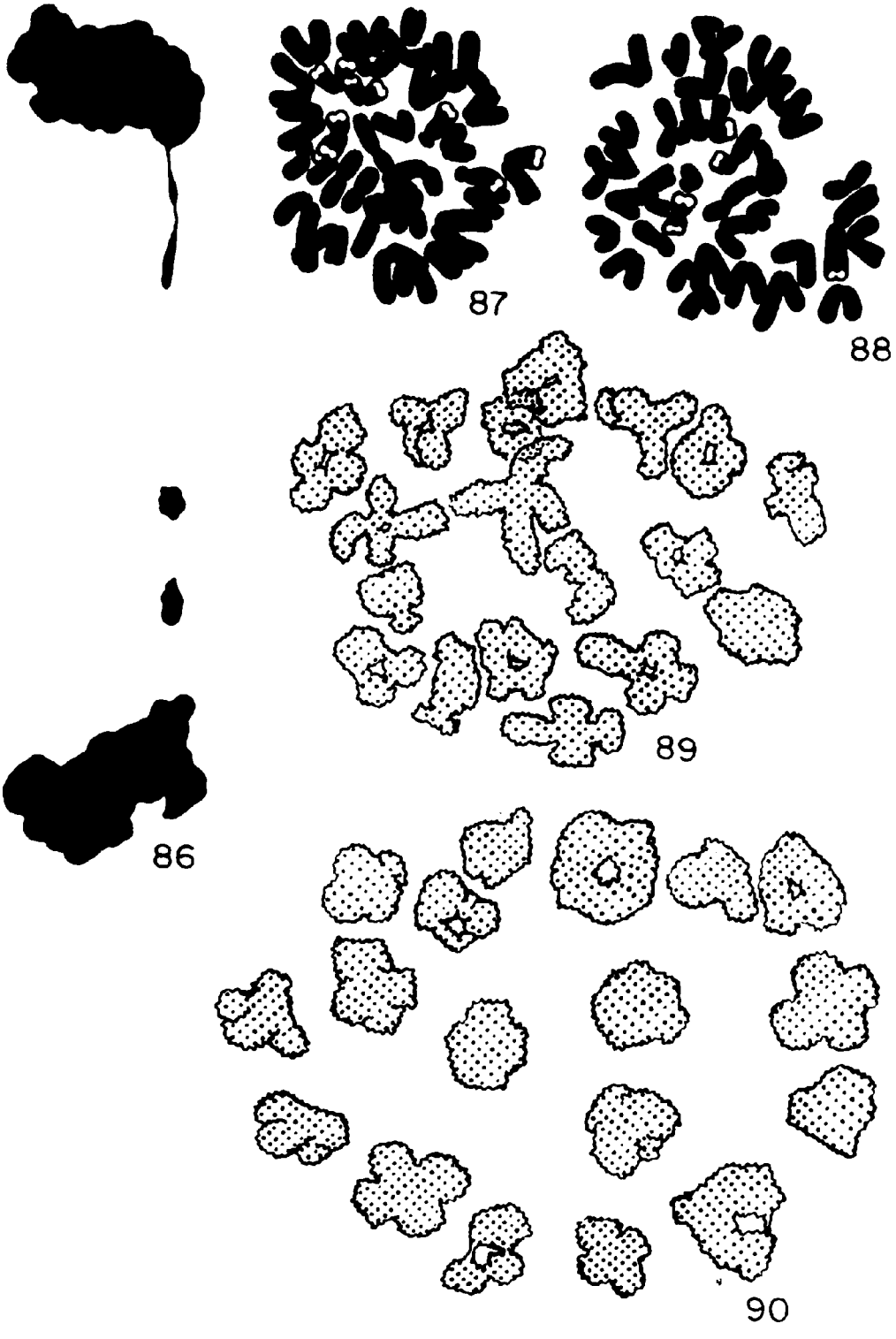


PLATE XIII

CHROMOSOMES OF AQUATIC SNAILS

Fig. 91: Gyraulus deflectus (Say). Dexter. Diakinesis with deeply-staining supernumeraries.

Figs. 92-95: Gyraulus deflectus (Say). Dexter. Nine sets of supernumeraries from nine meiotic prophase I cells.

Figs.96, 97: Gyraulus (Torquis) circumstriatus (Tryon). Meiotic prometaphase I.

Fig. 98: Ferrissia tarda (Say). Spermatogonial prometaphase.

All figures from acetic-orcein squashes.

PLATE XIII

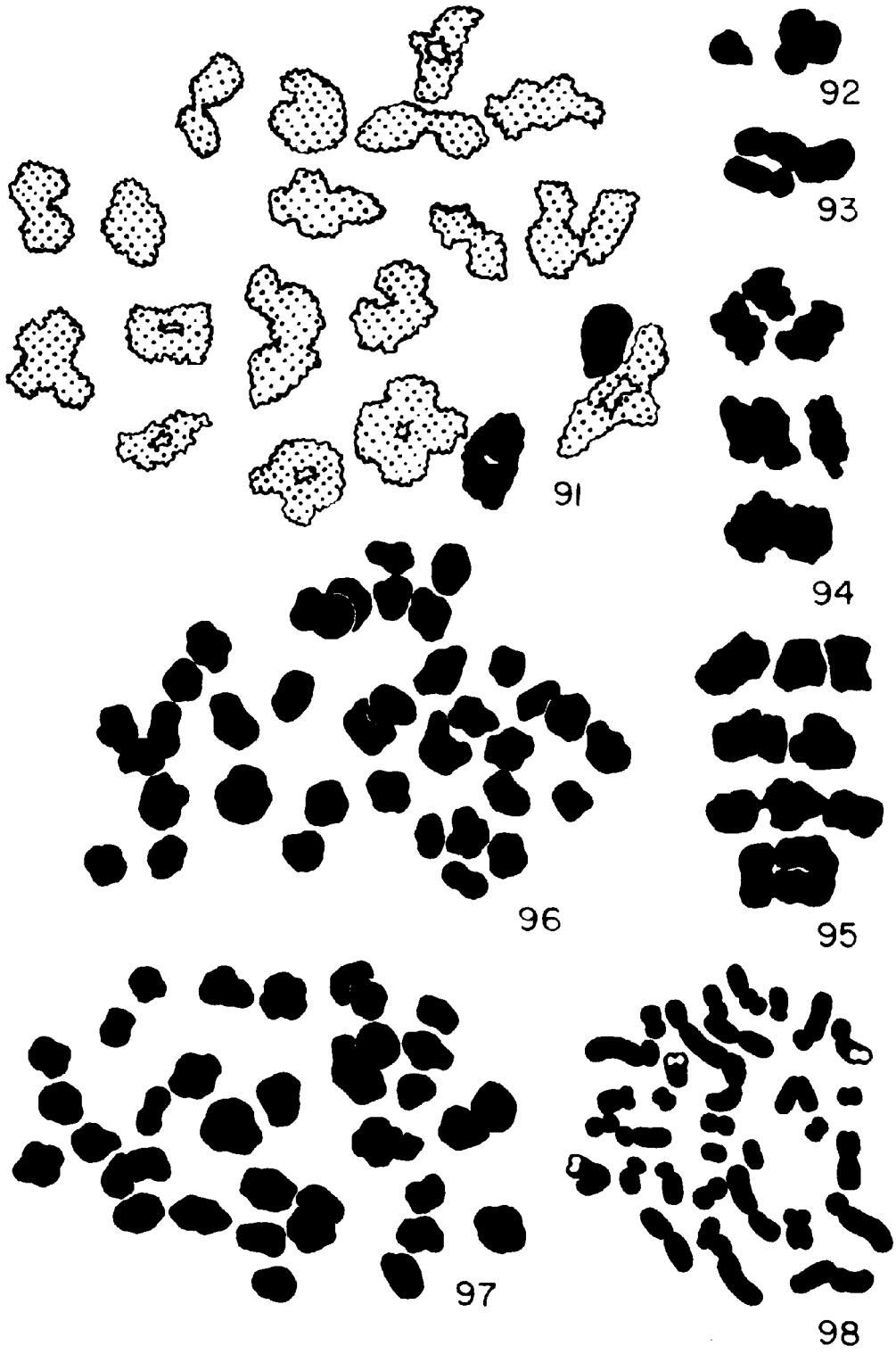


PLATE XIV

CHROMOSOMES OF AQUATIC SNAILS

Fig. 99: Ferrissia tarda (Say). Polar view, meiotic metaphase I.

Fig. 100: Ferrissia fusca (Adams). Polar view, meiotic metaphase I.

Fig. 101: Ferrissia parallela (Haldeman). Polar view, meiotic metaphase I.

Fig. 102: Ferrissia parallela (Haldeman). Spermatogonial prometaphase.

Figs. 103, 105: Ferrissia fusca (Adams). Spermatogonial prometaphase chromosomes.

Fig. 104: Ferrissia tarda (Say). Spermatogonial prometaphase chromosomes.

All figures from acetic-orcein squashes.

PLATE XIV

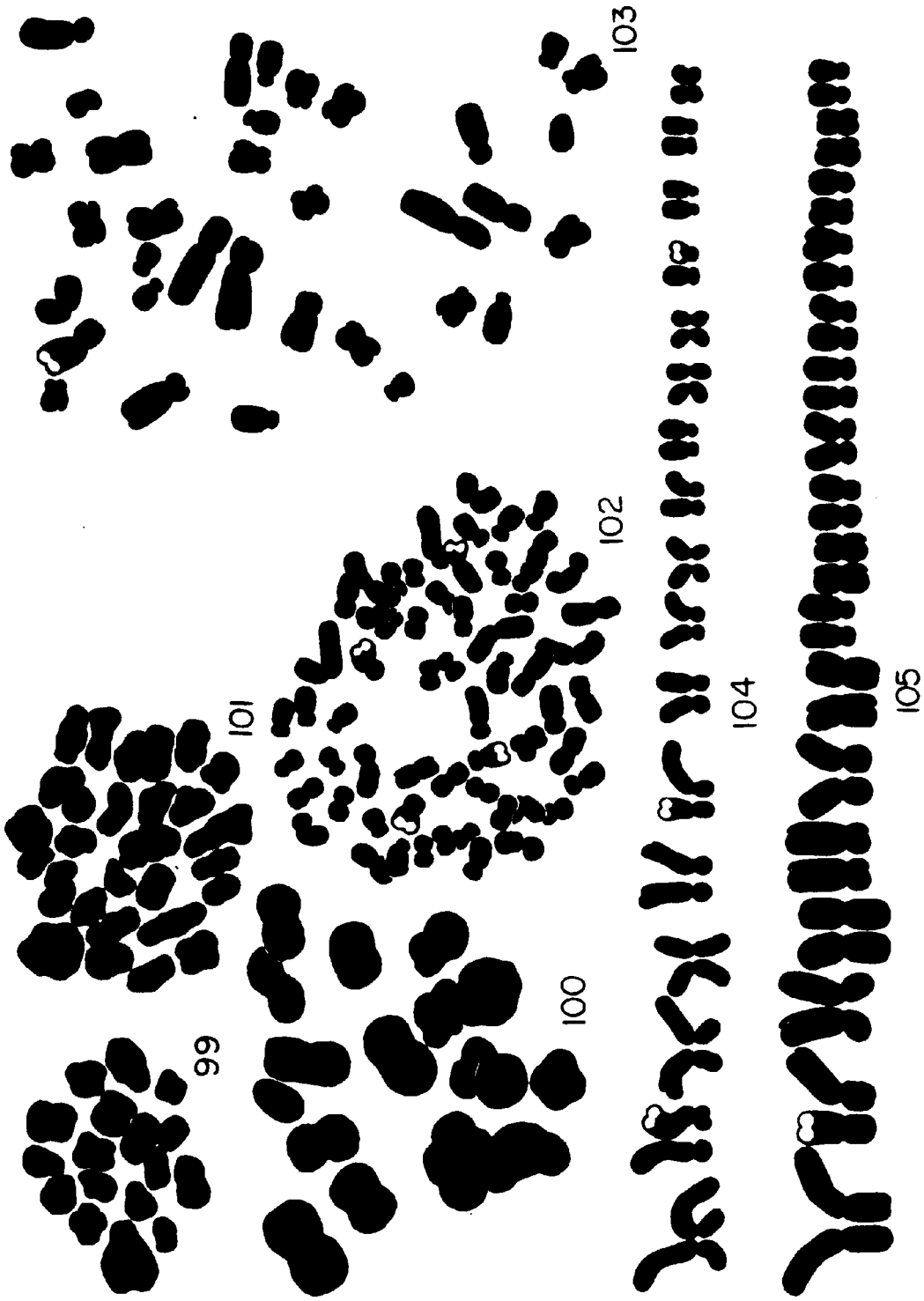


PLATE XV

CHROMOSOMES OF AQUATIC SNAILS

Fig. 106: Physa gyrina (Say). Colorado. Polar view, meiotic metaphase I.

Fig. 107: Physa anatina Lea. Polar view, meiotic metaphase I.

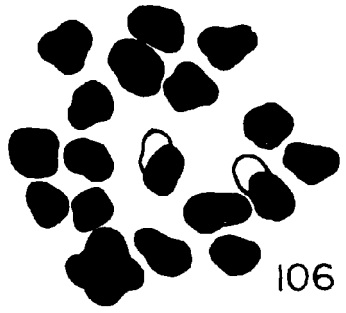
Fig. 108: Physa gyrina (Say). Polar view, spermatogonial metaphase.

Fig. 109: Melampus bidentatus lineatus Say. Polar view, meiotic metaphase I.

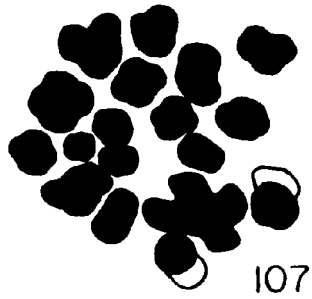
Fig. 110: Detracia floridana (Pfeiffer). Late diakinesis.

All figures from acetic-orcein squashes.

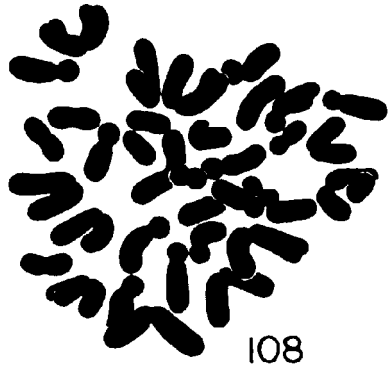
PLATE XV



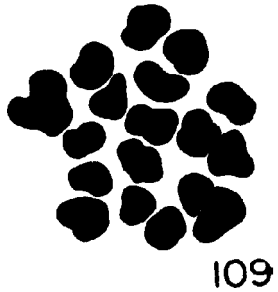
106



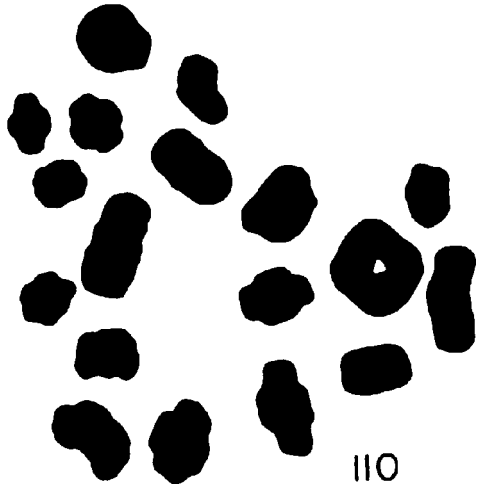
107



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109



110

PLATE XVI

PHOTOMICROGRAPHS OF CHROMOSOMES OF FRESHWATER SNAILS

Fig. 111: Bulimnea megasoma (Say). Spermatogonial cells in early prophase (top) and metaphase (bottom). The metaphase cells are in a cluster of eight; three cells are not in the picture.

Fig. 112: Bulimnea megasoma (Say). Spermatogonial cells in metaphase. There are sixteen cells in this cluster.

Fig. 113: Bulimnea megasoma (Say). Photographs taken at a lower power; cluster of thirty-two spermatogonial cells in metaphase.

Fig. 114: Bulimnea megasoma (Say). Spermatogonial metaphase. One cell in polar view and one in side view.

Fig. 115: Physa gyrina Say. Colorado. Spermatogonial metaphase.

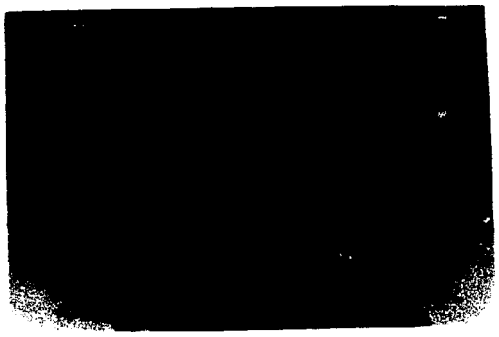
Fig. 116: Helisoma trivolvis (Say). Spermatogonial metaphase.

Fig. 117: Gyraulus deflectus (Say). Spermatogonial prometaphase.

Fig. 118: Gyraulus circumstriatus (Tryon). Spermatogonial metaphase (2n= 72).

All photomicrographs on this plate and Plate XVII are from acetic-orcein squash preparations. Fig. 113 was taken through a 10X ocular and a 20X objective. All other photomicrographs on this plate and Plate XVII were taken with a 10X ocular and an oil immersion objective.

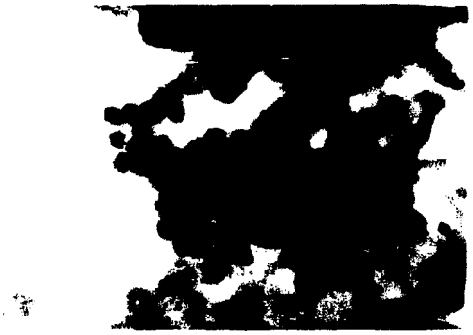
PLATE XVI



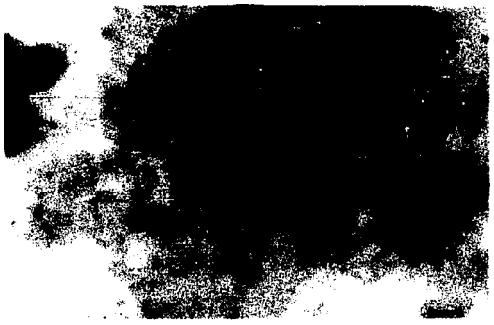
111



112



113



114



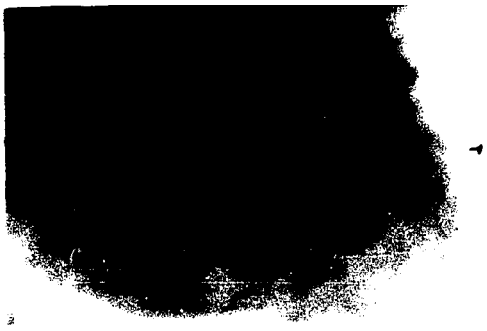
115



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PLATE XVII

PHOTOMICROGRAPHS OF CHROMOSOMES AND SPERM OF FRESHWATER AND BRACKISH-WATER SNAILS

Fig. 119: Melampus bidentatus lineatus Say. Pachynema chromosomes.

Fig. 120: Stagnicola emarginata angulata (Sowerby). Diakinesis. All the bivalents of the two cells in the center are in one plane and eighteen bivalents may easily be counted in each.

Fig. 121: Gyraulus deflectus (Say). Diakinesis; supernumerary chromosomes are more darkly stained.

Fig. 122: Physa gyrina Say. Colorado. Meiotic metaphase I. Eighteen bivalents can be seen.

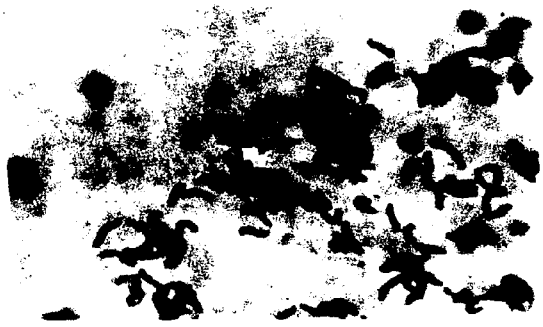
Fig. 123: Australorbis glabratus (Say). Meiotic metaphase I.

Fig. 124: Gyraulus circumstriatus (Tryon). Meiotic prometaphase I (n=36).

Fig. 125: Melampus bidentatus lineatus Say. Meiotic metaphase I. Side view of bivalents.

Fig. 126: Stagnicola palustris (Müller). Group of sperm clustered around a nurse cell (out of focus). Two hundred and fifty-one sperm heads can be counted in this photograph; five are out of focus.

PLATE XVII



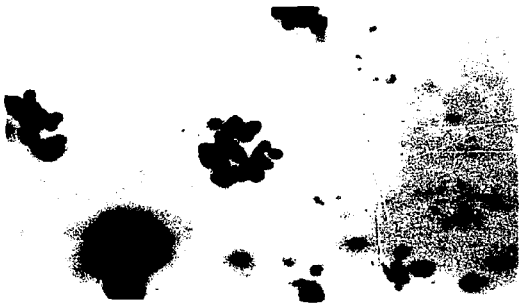
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120



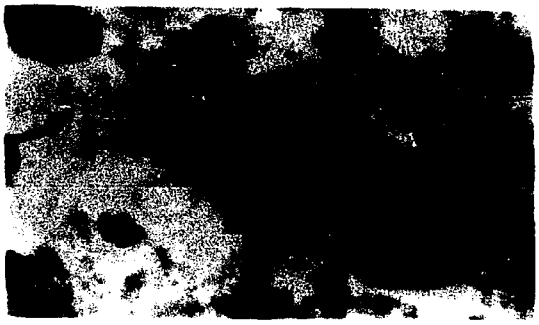
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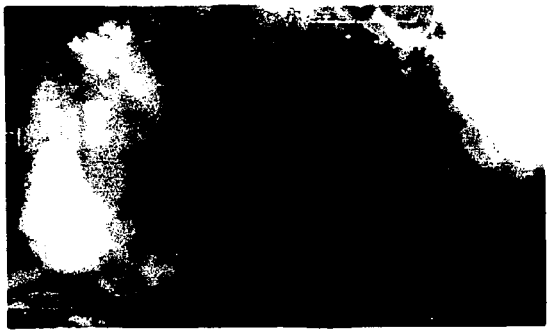
122



123



124



125



126