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Cytogenetics of *Paramecium putrinum* C. et L., 1858 [2]

Цитогенетика *Paramecium putrinum* C. et L., 1858

Contents

I. Introduction

At present, due to widering of taxonomic and genetic investigations on ciliates, a study of their nuclear apparatus, nuclear reorganization patterns and kinetome dynamics acquires larger significance.

A series of ciliate species exhibit differentiation into morphological groups (forms, varieties, subspecies) — a differentiation that is not specific for ciliates and occurs in other groups of animals also. Some ciliates possess, at the same time, their own patterns of intraspecific differentiation (see reviews of Sonneborn 1957, Poljan-

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[2] Abbreviated and modified sketch of a part of cand. biol. sci. dissertation presented in 1966 in the Institute of Cytology Acad. Sci. USSR. The research was carried under guidance of Prof. G. I. Poljansky in 1959–1960 years on the Chair of Invertebrate Zoology, Leningrad University, and in 1962–1965 years in the Laboratory of the Cytology of Unicellular Organisms, Institute of Cytology Acad. Sci. USSR. The entire work is planned to be published in three parts; this article will be supplemented by two more papers, on abnormal nuclear reconstruction patterns and on kinetome dynamics at division and conjugation.

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sky 1957, Dogiel et al. 1962); the systems of mating types and syngens, most carefully studied in many species, may be the best examples. Syngens, discovered in *Paramecium aurelia* by Sonneborn, are known still in a relatively small number of ciliates belonging to several orders; each species exhibits its own peculiarities in syngen structure, and therefore the study of syngens must not be limited by several "model" objects. One of the tasks of my research is the study of mating types and syngens in *P. putrinum* C. et L., 1858 (*P. trichium* Stokes, 1885) — a species that I regard to be the most primitive paramecium, presumed founder of the genus.

Since *P. putrinum* is poorly studied in both morphological and taxonomic aspects, I include short sketch of its structure, synonymics, phylogeny and taxonomic position within the genus.

The study of several hundreds of lines of *P. putrinum* yielded an evidence of micronuclear heteroploidy; chromosome counts were performed on some races. Due to different reasons, in 1965, at completion of this research, I came to conclusion that the "wild type" massive micronucleus of *P. putrinum*, *P. caudatum* and *P. bursaria* is normally polyploid and not diploid nucleus and that micronuclear polypolyploidy is specific character and not simply an individual or clonal aberration in paramecia with massive micronuclei.

The study of conjugation of *P. putrinum* is the central aspect of my research. Good knowledge of conjugation is significant for both taxonomic and genetic studies: all species of *Paramecium* differ in their nuclear reorganization patterns (behaviour of macro- and micronuclei, occurrence of autogamy), that facilitates taxonomic differentiation of species and correct determination of material. On the other hand, genetists need the correct "cytogenetic passport" of the lines under study, that is preparations showing the normal course of nuclear processes, frequency and kinds of aberrations, possibility of pathological conjugation, of not-amphimictic reorganization, etc. Cytogenetics of ciliates lags still behind the genetics: hundreds of papers, for example, deal with the genetics of *P. aurelia*, while a detailed analysis of the nuclear performance in conjugants was made by Dippel, Sonneborn and Kościuszko only in latest time. There was a case when authors, studying *Tetrahymena*, only after a series of genetic works with unusual results made preparations, showing the disturbance of the nuclear processes in all mates. Amicronuclearity of some lines was ascertained only after years of cultivation. Cytological studies must precede genetic ones, and I hope this will be the case with *P. putrinum*.

Conjugation in *P. putrinum* deserves, no doubt, an especially careful study: highly contradictory data have been accumulated in the literature; large number of anomalies in the nuclear behaviour were reported; strange "abbreviated" conjugation, atypical for ciliates, was described by Diller; doubtful copulation was found by Doflein.

Conjugating pairs of *P. putrinum* were reported by several early authors (Stokes 1885, 1888, Bütschli 1873, as "*P. aurelia*", 1876, 1889, Joukowsky 1898, Doflein 1907); Plate's 1888 "*P. putrinum*" is a typical *P. caudatum*. Diller 1934 and Wich-

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terman 1937 described, in abstracts, a typical amphimictic process in *P. putrinum*. Doflein 1916 (also in textbooks: Doflein und Reichenow 1953: 249–252; Raabe 1964: 167–168) illustrates on *P. putrinum* the general scheme of amphimixis in ciliates, but I show below that this is a kind of automictic conjugation (automixis I). Wenrich 1926 states, without details, that nuclear reorganization in his race coincides with that of Doflein's one.

Diller 1948, 1949, 1959 described a surprising "chaotic" variability of the nuclear behaviour in conjugating *P. putrinum*, but is seems that he had not tried to separate normally inherited cycles from occasional aberrations. May be, Diller tried to reflect all main aberrations noted on preparations, confusing these with stages, normal for his lines. His works leave an impression that reorganization processes in conjugants of *P. putrinum* may go in different ways, but possibly are not clonally inherited. "*P. putrinum*", says Diller 1948: 3, "is one of the most interesting and remarkable species of *Paramecium* on account of the extreme variability and lability of its nuclear performance at conjugation; this involves both micro- and macronucleus".

Sonneborn 1949 and Wichterman 1953 regard these cycles only as occasional aberrations, display of the individual variability of the nuclear processes, peculiar for ciliates — an "occasional lost of III division of micronucleus", "occasional inability to exchange of gametic nuclei" etc. May be, this resulted in the lack of interest to conjugating *P. putrinum* in subsequent years. Sonneborn 1949: 66 indicates that Diller's data were verified by R. V. Dippel on one race (line?) of *P. putrinum* and were not confirmed: a typical amphimictic cycle was found, by occasion only: there were equal chances to isolate an aberrant race.

My study was started in 1958 with an occasional isolation of a selfing strain of *P. putrinum* with repeatedly atypical conjugation. Diller's 1949: 342 words that "the versatility, lability and variability of micronuclear activity in *P. trichium* should be susceptible for experimental attack and analysis" encouraged me to start wide collectioning of this species. Beginning with 1958, near 400 lines from 26 races ("race" means population in one pond or river) were isolated, of which near 300 lines were conserved for analysis; syngen and nuclear reorganization patterns were determined in nearly 75% of these lines, and mating type in nearly 50%. In this way a multiple mating type system was discovered, and unexpected system of nuclear reorganization types was demonstrated. Clonally inherited nuclear cycles were separated from occasional ones (Jankowski 1960–1972 a–f). Unlike all other ciliates, *P. putrinum* is subdivided into several groups of lines and races, named "mixotypes" — groups inheriting a definite kind of the nuclear reorganization or several such kinds in a definite combinations. I distinguished five inherited ways of nuclear reorganization in *P. putrinum*: amphimixis, automixis I and II, apomixis I and II. Mixotype system is not correlated with mating type system within syngens, that makes possible intermixotype mating and complicates the scheme of reorganization patterns.

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To facilitate comparative studies on mixotypes, careful cytological study of amphimixis was undertaken; I present here the data on meiosis, nuclear exchange, caryogamy, development of macronuclear "anlagen", caryorrhexy and other processes that are not still well studied in *Paramecium*.

The following abbreviations are used throughout the text: Ma — macronucleus, Mi — micronucleus, mt — mating type, MT — mixotype, Nc — nucleole, PC — paroral cone, Sg — syngen, Sk — synkaryon. A new term, "pycnocaryon", means degenerated nucleus (derivate of Mi). The stages of Mi divisions, for the brevity, are designated below as "prophase Mi I", "anaphase Mi II" etc., those of synkaryon — as "prophase Sk I" etc.

II. Material and methods

Lines of *Paramecium putrinum*

Isolations were made in 1958–1960 years in Leningrad and its suburbs, in limits of 35 km outside of the town, and partly also in 1960–1961 years in Kirishi region, 160 km south-east of Leningrad. The species was found in 28 (of 65 examined) water reservoirs of different kinds — lakes, ponds, rivers, ditches, temporary spring pools. 309 lines of 22 races were conserved for analysis. Races ZN, SB, H, CR, LR and T were isolated in park water bodies in Leningrad, 13 other races in its suburbs — Duderhoff (LC), Lake Mozhajskoje (KS), Krasnoje Selo (YR), Staryj Petershoff (OM, MR), Novyj Petershoff (A), Sestroreck (M), Kurortnoje (LJ), Lake Heppo-Jaarvi near Kavgolovo (HR), Pushkin (PR), Lomonosov (LH), Gatchina (QS), Park Aeronavtov at Kolpino railway branch (NT). Collections from Kirishi region, in Volchov river basin, include races SZ (Budogosh), VK (village Belaja) and WH (Gorchakovo).

Isolation and cultivation of lines

Water and detritus samples were taken every several meters along the shore lines; points with domination of *P. putrinum* were sometimes found. *P. putrinum* is especially common in winter, under ice, in early spring and, less frequently, in autumn; it is a cold-water species. Specimens from the samples were isolated by micro-pipette into the test-tubes with a culture medium, prepared a day before sampling. Two types of cultures were supported, constant and temporary ones; first ones — on small boiled pieces of dry cow's manure (with re-culturing each 3–6 months), second ones — on Losina-Losinsky's mineral solution with constant addition of food (yeasts) and re-culturing every 7–10 days. To mate lines, I used temporary mass cultures or stable (constant) ones during first 1–2 weeks of their life, when both reproduction rate and culture density become stabilized. The crosses were made in depression slides (for determination of mt) or in Petri dishes (for obtaining abundant material and for making mass preparations). Selfing-lines

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and multiclones (progeny of several isolated cells, cultured together) were cultured mainly in Petri dishes on rice grains, for an easy establishement of the beginning of conjugation and isolation of pairs. To avoid an occasional lost of cultures, each line was multiplied in 3–20 test tubes. The majority of lines used in this research is not maintained now, but new isolations were made in Leningrad region, in fresh waters of the Baltic sea and near Vladivostok.

Observations in vivo

For in vivo studies of the nuclear processes in conjugants, pairs in late prophase or anaphase Mi I were put into Commandon–Fonbrune's oil chambers. Small drop on the cover glass, with ciliates, was laid down on a thin layer of the chemically inactive non-toxic vaseline oil spread on the object glass; in other cases, the edges of cover glass were simply sealed with vaseline oil. The ciliates were not compressed; they exhibited an increased thigmotaxis and remained motionless during the long period of time. Their cytoplasm was clear and free of food; mineral crystals gathered at the posterior body end; the nuclei, spindles and even chromosomes might be seen without phase contrast. Bismarck-braun dye was sometimes used for better contrasting of the nuclei and the cytoplasm. The use of Neutral red helped observations on food vacuole excretion in conjugants and on the beginning of feeding in exconjugants.

Fixation, impregnation and staining

The material (usually available in abundance) destined for nuclear staining was fixed in depression slides by Bouin's fluid and sticked to object glasses by a remarkable Chen's technique (alcohol–ether–celloidine) that gives guarantee of a firm adhesion and equal distribution of unlimited number of cells. Most preparations were stained after Feulgen and Lamateur (DNA) with counterstaining by light green or picric acid. Many preparations were stained by Böhmer's or Heidenhain's hematoxylins, on RNA by Unna-Pappenheim's methyl green–pyronine mixture and on proteins by Mazia's bromphenol blue–sublimate. Vegetative cells, conjugants and autogamonts were fixed by Champy's fluid and impregnated with $AgNO_3$ after Chatton and Lwoff, with development in diluted hydroquinone. The films of dried specimens were impregnated by Klein's technique.

III. Morphology of *Paramecium putrinum*

Correct species name

Claparede et Lachmann 1858–1861 perfectly described and figured a new species *P. putrinum* C. et L. 1858 from Switzerland; Stokes 1885, 1888, without reference on *P. putrinum*, gives less correct description of *P. trichium* Stokes 1885 from USA. It is widely indicated that swiss authors regarded the absence of tri-

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chocysts and presence of only one (anterior) contractile vacuole as distinctive characters of their species, but they figure both vacuoles and contours of trichocyst layer. It is clear that main characters of *P. putrinum*, which was compared by Claparede et Lachmann with *P. bursaria* and *P. aurelia*, are small dimensions, narrow body, curved Ma with large ovoid Mi, absence of zoochlorelles etc. The species is easily determinable according to these characters and should be considered as valid. Even now, when near 15 species of *Paramecium* are known, *P. putrinum* remains clearly distinct from all other species including its nearest relatives *P. bursaria* and *P. polycaryum*. The specific identity of races from Europe and USA is doubtless due to comparison of their morphology, morphogenesis, nuclei, conjugation processes. Diller 1948 has not found differences between races from France and USA. Although the identity of named species is evident for all who studied them (Wenrich 1927, 1928, Wichterman 1953), it is still maintained that swiss species is not still "determinable with confidence", and its name *P. putrinum* is not acceptable. This name was widely used by european authors, and *P. trichium* — by american ones (thus the rule of "50 years" of a taxonomic code is not applicable). After Wenrich's 1926, 1928 and especially after Wichterman's 1953 works the name *P. trichium* became widely distributed, regretfully, in Europe also (Danielova, Roque, Moravcova, Šramek-Hušek, Ammermann, and others). Since I recognize the first description of the studied species as giving good criteria for its correct determination, it remains to reject the name "*P. trichium*" as a synonym of *P. putrinum*. Other synonyms are: *P. ficarium* Kahl, 1926; *P. pseudoputrinum* Baumeister, 1931. *P. pseudotrichium* Dragesco, 1970 (from Cameroun) is not related to *P. putrinum* (*P. trichium*) and must be compared with *P. calkinsi*.

The main distinctive characters of *P. putrinum*

P. putrinum seems to be the most simple (primitive) species of the genus. It is a small form with typical size near 70–90×35–40 μ, with elongated cylindrical body more or less equal in width. The buccal complex lies in the anterior body half; vestibulum is not deep, prevestibular zone is weakly developed. The number of kineties in all kinetome zones is small. Two contractile vacuoles lie deeply in the cytoplasm and open outside by long curved channels; small secondary vacuoles instead of long radiating channels make the primary vacuole at their coalescence. Ma and Mi are of *P. bursaria* type, but smaller: Ma is elongated, ovoid or curved, dense, with numerous large nucleoles; Mi is ovoid, large, without polar cup. Trichocyst layer is typical, but it looks like well developed due to small body size. The green symbionts (zoochlorelles) are absent. Endoparasitic suctoria and bacteria of *Holospora* type, recorded in some other paramecia, are unknown in *P. putrinum*; infection of *Leptomonas* in Ma may be specific for *P. putrinum*. Deckart and Löfflath wrongly determined their paramecia, infected by bacteria in Ma, as *P. putrinum* (this was a green ciliate, *P. bursaria*).

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P. putrinum possesses a multiple mt system (an evidence of primitiveness in paramecia!). Natural autogramy in singles in unknown. Selfing is a common and peculiar phenomenon. The nuclear reorganization at conjugation may follow one of five established ways, and it is determined by mixotype of the line; amphimixis and various modifications of auto- and apomixis were described. The general scheme of nuclear behaviour at conjugation differs in many respects from that in *P. bursaria* and all other paramecia: unlike *P. bursaria*, e.g., Ma of *P. putrinum* become fragmented after branching, 4 Ma anlagen are formed, 3 nuclei degenerate after meiosis.

P. putrinum is a cold-water species, widely distributed in fresh waters of cold- and moderate-climate countries and possibly absent in tropics: it was not reported from hot countries (Egypt, Uganda, Cameroun, India) where the fauna of paramecia was specially studied and where *P. wichtermani*, *P. jenningsi*, *P. aurelia* and *P. africanum* were dominating. The species occurs commonly in pure and putrid waters (lakes, rivers, ponds, ditches), mainly near the bottom; it is most common in the spring, when it may be found even in many temporary small water bodies made by melting of the snow. *P. putrinum* is easily cultivated in the laboratory on media used for growing *P. caudatum*.

Taxonomic position and phylogeny

Two species groups are distinguished by authors within the genus *Paramecium:* "*aurelia*" and "*bursaria*" ones; I recognized three such groups — "*putrinum*", "*woodruffi*" and "*aurelia*" (Jankowski 1962 b, 1969 a). They are regarded as taxonomic subgenera. Subgenus *Helianter* includes most simple paramecia, *P. putrinum*, *P. bursaria* and new tropical forms found by Dragesco in Africa; an intermediate subgenus *Cypreostoma* includes *P. woodruffi*, *P. calkinsi*, *P. polycaryum*, *P. arcticum* and *P. pseudotrichium;* higher paramecia (*Paramecium* s. str.) include *P. aurelia*, *P. caudatum*, *P, jenningsi*, *P. africanum*, *P. multimicronucleatum* and *P. wichtermani*. The subgenera differ in many characters: in body size and shape, position of the cytosome, degree of deeping of the buccal complex, degree of development of vestibulum, of prevestibular zone, in number of kineties, in stomatogenetic patterns, in the nuclear characteristics, in syngen structure (multiple in *Helianter*, binary in *Cypreostoma* and *Paramecium*) etc. Miyake's data indicate that interspecific mating is possible within subgenera, at least in higher paramecia.

In almost all aspects *P. putrinum* is simpler than *P. bursaria*, but the way of the nuclear processes at conjugation is simpler in the last species: it follows *Colpidium* and *Tetrahymena* patterns (no branching and fragmentation of Ma, two anlagen of Ma). May be, it is a case of a heterochronous evolution of characters: the nuclear reorganization scheme has been secondarily modified in a primitive paramecium. Morphologically *P. putrinum* very closely resembles small species of *Frontonia* and not higher paramecia with their cigarette-like body. It has subanterior mouth, small vestibulum; body part between the mouth and anterior pole is only slightly

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elongated. On dry impregnated smears, when prevestibular cavity is not seen, the kinetome of *P. putrinum* and peniculines of *F. depressa* type look remarkably similar.

Further evidence of primitiveness of *P. putrinum* and its affinities with frontoniids comes from the study of its division process. Different groups of ciliates, including peniculines, recapitulate, with different degree of completeness, the structure of their predecessors at division (Jankowski 1972 b). In paramecia, the anterior daughter cell (proter) at the moment of fission resembles more or less the maternal cell, while opisthe recapitulates its ancestor. The proter of dividing *P. putrinum* resembles more an adult than opisthe, looking like a small frontoniid. Dividing *P. woodruffi* recapitulates "*P. putrinum*" stage, *P. aurelia* — "*P. woodruffi*" stage. All this makes evident the existence of an orthogenetic line *Frontonia* — *Helianter* — *Cypreostoma* — *Paramecium* s. str. Possibly *P. putrinum* gave rise to *P. polycaryum* — *P. woodruffi* — *P. aurelia* line, and *P. woodruffi* and *P. aurelia* were ancestors of other species within their subgenera.

Morphological subspecies

Body size and shape differ in various syngens of *P. putrinum*, and measurements on a single line are not typical for the entire species. My material yielded 3 morphotypes to be regarded as subspecies (Jankowski 1962 b, 1965 d). *P. p. vernalis* includes Sg 1, 2 and 3; representative line OM 11 includes middle-sized cells (69–97 × × 33–41 μ, mean 82 × 38 μ). *P. p. cheni* (Sg 4) includes large specimens; cells of a representative line M 6 reach 81–119 × 32–42 μ, mean 96 × 37 μ. *P. p. apomictum* (Sg 5) includes small ciliates: representative line CRn reaches only 64–83 × 27–32 μ, mean 72 × 30 μ. Since the subspecies include definite syngens, they differ in nuclear reorganization patterns also; *P. p. cheni* possibly had not elaborated the aberrant ways of reorganization, only amphimicts were isolated. Like in *P. aurelia* differences in the body shape in *P. putrinum* are not well expressed, and examination of large material is need for detection of a tendency to make a definite cell outlines. *P. p. apomictum* has thin tubular body, and *P. p. vernalis* more wide and flat one. No kinetome differences were found on impregnated mounts. From time to time I have found giant forms in natural populations (large series of such giants was isolated recently from Baltic sea); these cells resemble externally *P. woodruffi*. Gigantism is lost after several weeks of laboratory cultivation. Giants occur mainly in *P. p. vernalis*.

The kinetome

Details of infraciliature and argyrome of *P. putrinum* are shown on Plate I. The species has kinetome of *Frontonia*-type, especially immediately after division, when prevestibular zone is not developed. The body is curved, asymmetrical. Kinetome of our races coincides well with that of french and american ones (Roque 1961, Gillies and Hanson 1968). Ventral body side bears near 30 kineties, dorsal one near 25. Infraciliature includes 2 types of kineties — primary ones (longitudinal,

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united by kinetodesms) and secondary ones (diagonal or transversal, without kinetodesms), or "structural" and "physiological" kineties. The last ones appear at coordinated disposition of kinetosomes in a definite body area: kinetosomes of neighbouring kineties are located on single level, thus giving rise to diagonal rows. Gelei designated these as "falsche Cilienreichen". Since they are very widely distributed in ciliates of all orders, from prostomes to heterotrichs, I introduced a special term "adesmokineties" for their designation (Jankowski 1965 d, e). It corresponds possibly to the notion of "paratenes" of Ehret 1967.

The preoral (buccal) opening lies above the equatorial line, in the anterior body half. Buccal cavity bears two closely shifted peniculi, quadrulus and undulating ("endoral") membrane near the upper right border. Vestibular cavity includes several short radiating adesmokineties. Wide "peristomal" zone, that I propose to call "prevestibulum", extends between vestibulum and the anterior body end. Prevestibulum is wide cavity with near 35 transversal adesmokineties at left and four longitudinal ones at right. According to "the rule of stomodexy" (Jankowski 1971 c), prevestibular ciliature is especially developed at the left side of an "effective sector" and the entire prevestibular ciliature is a remarkable copy of spirotrich AZM. Longitudinal adesmokineties serve as undulating membrane (barrier to food current), and transversal ones drive the food particles from the anterior pole to vestibulum, like membranelles.

In the lower half of a right vestibular wall a distinct cilia-free area may be commonly seen; it bears several small kinetosomes not arranged in rows and triplets. I regard this group as anlage of a new buccal ciliary complex, that becomes activated before cell fission.

Macronucleus

The nuclear apparatus of *P. putrinum* (Plate II) resembles that in *Disematostoma* and small species of *Frontonia* and not that of higher paramecia. Ma is elongated, ovoid or curved, relatively large (25–30 × 12–16 μ). The extreme development of nucleoli causes a peculiar vacuolated or foamy appearance of Ma. Feulgen reaction is very intensive, the nucleus (outside nucleoles) looks dense, homogenous, chromatine grains are very closely shifted one to another. Ring like chromatine structures, 1–1.5 μ in diameter, typical for Ma of *P. caudatum*, were found only in Ma anlagen of *P. putrinum*. Elongated Ma in dividers or branching Ma in conjugants possesses numerous chromatine filaments, possibly chromosomes; they are rounded after fission or fragmentation of the nucleus.

Nucleoli reach 2–20 μ; they are semi-solid bodies, mostly spherical or pyriform. Single Ma contains variable number of nucleoles, sometimes more than one hundred. The total volume of nucleoli in some nuclei may reach, surely, that of a chromatine part of Ma (Fig. 1 I–N). Nucleoles contain none Feulgen-positive particles ("nucleolar organizers"); they are well stained by bromphenol blue, light green, acid fuchsin, pyronine (RNA), and weakly — by hematoxylins of Böhmer and Heiden-

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Fig. 1. The nuclei of *Paramecium*. Böhmer, Feulgen and Unna. A–C, G–U — *P. putrinum*; D — *P. woodruffi*; E — *P. caudatum*; F — micronuclei of paramecia, scheme. *P. putrinum*: A, B — line M 6; G–I — KS 14; J–N, P — OM 13; O — CRt; Q, R — M 3; S–U — monsters in races CR, QS and M

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hain. Fusion of neighbouring nucleoles is common. Nucleolar extrusion from the Ma is constant and easily detectable phenomenon (Fig. 1 A, K, N); nucleoles of different dimensions after extrusion are located at first near the nucleus, but are replaced then into the cytoplasm and become dispersed here.

The species of *Helianter* inherited such Ma from frontoniids. *Cypreostoma* has dense ovoid Ma with small nucleoles, and *Paramecium* s. str. — voluminous, amorphous, relatively faintly stainable Ma with discrete chromatine granules (Fig. 1 C–E).

Hemixis is very rare in *P. putrinum*. Diller noted three categories of hemixis in *P. aurelia* (extrusion of chromatine, Ma breakdown into 2–3 pieces and the entire fragmentation); of these, only Ma breakdown into large pieces was recorded in *P. putrinum*. Elimination of chromatine at cell division occurs only as exception. Several lines of Sg 2 (KS 14, 29, T 5) possess abnormally folded and rolled nuclei; this is a cause of their ruptures (Fig. 1 G–I). Hemixis and chromatine elimination are regarded presently as means of preventing the hyperploidization of Ma, and this may explain their occurrence in higher frontonians and paramecia (e.g. *P. aurelia*, *P. multimicronucleatum*) and rarity in lower forms (*P. bursaria*, *P. putrinum*) with Ma ploidy correspondingly near 900 n and 20 n.

Sg 1 and 2 include lines with abnormally clear, faintly stainable Ma; the differences become especially evident at mating in normal and aberrant lines (Fig. 3 N–S). It is a good marker for recognizing clones on preparations. The dimensions of chromatine granules coincide in both types of Ma, but they differ in their concentration. Mi of these lines quite resembles normal Mi, typical for the species, and heteroploidy of Ma is not explainable by that of Mi; it may be due to difference in the number of endomitotic cycles in developing Ma anlagen. The occurrence of "clear" Ma is not correlated with a definite mt of the line. The common occurrence of "clear" Ma in Sg 1 and 2 allows to suppose that this is not an occasional anomaly, but differentiation of lines into two groups in respect to Ma ploidy. Heteroploidy of Ma in different specimens, lines and races was reported in paramecia, tetrahymenas, trichodines etc. in the literature (Zeuthen, Blanc, Raikov, Cheissin, Stein et al.), and it was usually explained by different ploidy of Mi.

Micronucleus

At least five types of Mi may be distinguished in species of *Paramecium* — those of *P. putrinum* (large compact), *P. calkinsi* (small compact), *P. aurelia* (vesicular), *P. caudatum* (large, with polar cup, Fig. 1 F) and *P. jenningsi* (=*P. wichtermani*?) (large vesicular). Mi of *P. putrinum* is longitudinally striped, contains long parallel closely shifted chromosomes. "Light green zone", that becomes clearly visible on wide Mi pole in conjugants and dividers of *P. putrinum*, corresponds to the polar cup of *P. caudatum* and achromatine "endosome" of *P. aurelia*. As a rule, Mi of *P. putrinum* is located near the Ma or in its depression (Fig. 1 A, B, G–O). Similar, but somewhat smaller Mi may be found in *Frontonia atra*, *F. acuminata*, *F. elliptica*, *F. depressa* and other small frontonians, and also in species of *Disema-*

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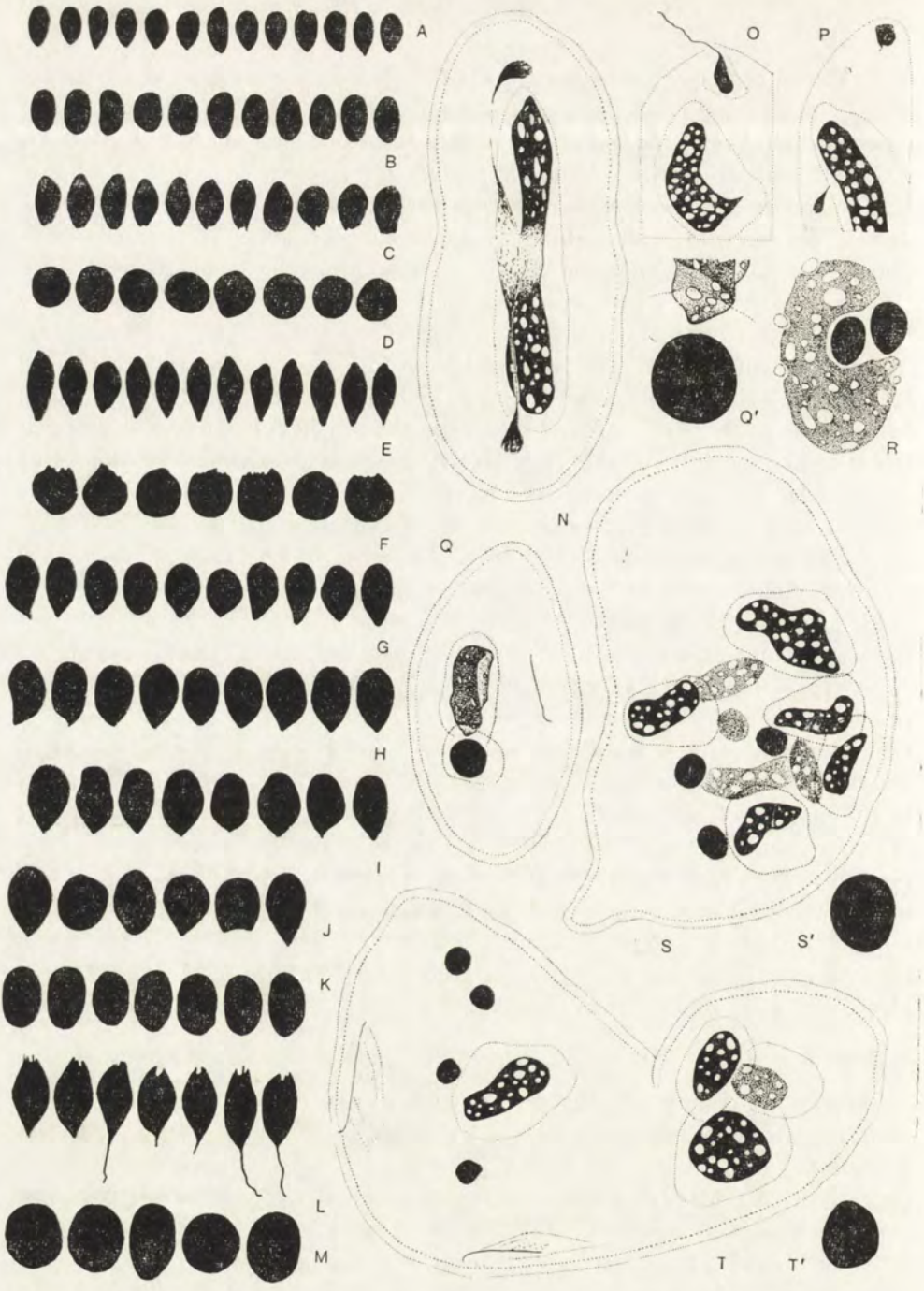

Fig. 2. Micronuclear heteroploidy in *P. putrinum*. Böhmer and Feulgen. A — OM 23; B — HR 23; C — VK 16; D — SZ 5; E — MR 11; F — T 3; G — KS 12; H — M 3; I — LH 24; J — M 15; K — CRn; L — SZ 14; M — CRt; N–P — abnormal mitosis, M 6; Q, R — aberrant cell with giant Mi, CRn; S, T — monsters, CRn

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tostoma. Vesicular Mi of *P. aurelia* type are not peculiar for peniculines, with possible exception of *Stokesia* and *Faurella;* they appeared in "intermediate" subgenus *Cypreostoma* and were conserved in higher paramecia, where they give rise to massive Mi of *P. caudatum*, *P. jenningsi*, *P. wichtermani*. Massive Mi appeared thus independently in two unrelated *Paramecium* subgenera. This indicates the existence of an interspecific polyploidization series of Mi in *Paramecium* and possibility of considering Mi polyploidy in *Paramecium* as a specific character and not simply clonal aberration.

Bimicronucleate specimens, noted by Wenrich 1926 and Diller 1948, are very rare in our races of *P. putrinum*. Amicronuclearity is a similar rarity: only two lines from nearly 300 studied possessed no Mi.

Diller 1940, 1948, 1949, 1959 noted difference in the dimensions of the nuclei at different stages of conjugation in *P. putrinum*, admitting the existence of a heteroploid Mi series. This hypothesis was not proved on clonal material. Diller's statements that "Mi is of a remarkably different size" and that it may be "aneuploid, normally euploid and hypoploid" are not based on comparisons of vegetative Mi and chromosome counts in *P. putrinum;* they are stated under impression of an extreme variability of the nuclear processes in conjugants. As it was customary, Diller considered the typical Mi as diploid, and larger or smaller ones as hyper- or hypoploid.

According to our data, *P. putrinum* possesses a tendency to formation of a definite type of Mi, but variation of Mi is striking. Analysis of 309 lines of 22 races and 4 syngens yielded 14 structural types of Mi, differing in their size, shape, DNA content (judging from the intensity of staining) and chromosome numbers, determined for several lines (Fig. 1–3, Plate II, Table 1). A "wild type" Mi, typical for the species, is massive ovoid body near 6–6.5×4–4.5 μ in size (e.g., Mi of M 3, M 15 or KS 12 lines). Mi are intensively stainable in most lines irrespective of their dimensions, and only several lines possess small clear Mi (SZ 18, OM 23, VK 16). To sum up, we see the gradual rise in Mi dimensions from 4.3 to 9.6 μ; as it might be expected, different types of Mi are not correlated with definite mt or Sg and seem to appear occasionaly. Figures 1–2 show the monsters encountered in cultures; many of these, and also some normal specimens (Fig. 2 Q) possess giant Mi, up to 10×9.3 μ in size. In monster, shown at Fig. 2 S, Ma has divided three times, and Mi — only two times, becoming hyperploid.

Chromosomes of *P. putrinum* are perfectly seen in meiosis. They reach maximal size (6.5–7 μ) in the late prophase Mi I, and become smaller and smaller at each subsequent division; they are only 4 μ long at the last synkaryon fission or in vegetative mitosis. All chromosomes in a set look alike.

The difficulties of chromosome counting in *Paramecium* were overstated. In paramecia with massive Mi chromosomes are large, numerous, tangled in prophase or metaphase meiotic nuclei, do not form distinct anaphase groups and equatorial plate. The technique of "pressed preparations" is not applicable for such nuclei.

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Table 1

Micronuclear heteroploidy in *Paramecium putrinum*

Line	Syngen	Shape of Mi	Number of measurements	Micronuclear size (μ)			
				length	*m*	width	*m*
SZ 18	1	oval	4	3.9–4.5	4.3	3.1–3.3	3.2
OM 23	1	lacrymoid	20	4.5–5.2	4.7	2.3–2.9	2.6
H 23	1	oval	17	4.6–5.4	5.0	2.9–3.4	3.2
VK 16	5	leaf-like	18	5.3–6.5	5.6	2.9–3.4	3.2
SZ 5	1	spherical	13	4.6–5.3	5.1	4.5–5.2	4.7
MR 11	4	spindleform	19	6.0–8.4	7.5	2.6–3.0	2.9
T 3	1	spherical	12	5.3–6.0	5.6	4.9–5.3	5.1
KS 12	2	lacrymoid	15	5.8–7.2	6.6	3.5–4.2	3.9
M 3	1	lacrymoid	14	6.6–7.5	7.1	4.0–4.8	4.5
LH 24	1	leaf-like	13	6.7–8.2	7.7	4.2–5.0	4.5
M 15	1	lacrymoid	12	6.5–8.5	8.0	4.7–5.6	5.0
CRn	5	ovoid	13	6.2–7.6	7.1	4.3–5.2	4.4
SZ 14	1	spindleform	14	9.3–10.5	9.6	3.6–4.4	4.3
CRt	5	ovoid	10	7.0–9.1	8.0	5.2–6.5	6.0

m – the arithmethic mean from all measurements.

Dippel counted chromosomes of *P. aurelia* in prometaphase — late metaphase Mi I; Chen consequently figured all chromosomes of a ball in prophase Mi I. I used much simpler technique: chromosomes are counted on photomicrographs or figures of optical (transversal) sections of nuclei in anaphase Mi II or Mi III (n), in pronuclei (n) or in anaphase Sk I (2 n). Chromosomes are not tangled in a ball, and are strictly parallel one to another in elongated nuclei. The error in counts is relatively low, ±10%.

"Diploid" phase Mi of M 6 line possesses 85–95 chromosomes, M 15 — 90–95, CRn — 109–124, LJ 2 — 116–134 (and 62–67 in pronuclei), CRt — near 180 ones. Small clear Mi of SZ 18 line possesses near 15 chromosomes in prometaphase Mi I, while its "normal" partner of WH 3 line — near 90 ones (Fig. 3 A–K). The difference in the size of pycnocaryons in partners (Fig. 3 L–M) deserves especial attention: it indicates that the "haploid" quantity of DNA may be determined at cytophotometric study of pycnocaryons.

Mi with 90–130 chromosomes is typical for *P. putrinum*, while the extremes (15 and 180) occur in aberrant strains. All studied lines were isolated in limits of Leningrad district (a city and suburbs), and heteroploidy is not explainable by their geographical isolation. The most significant sources of variation are aberrations in vegetative mitosis (unequal Mi fission), an extreme variability of the nuclear reorganization processes, abundance of aberrations in the nuclear behaviour and wide distribution in nature of non-amphimictic (auto- and apogamic) lines of *P. putrinum*.

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Fig. 3. Nuclear heteroploidy in *P. putrinum*. Böhmer and Feulgen. A, B — vegetative Mi, SZ 18; C–K — lines SZ 18×WH 3; L. M — pycnocarya of WH 3 and SZ 18; N–Q — macronuclear heteroploidy, OM 4×OM 19; R, S — difference in chromatine concentration in Ma fragments; T — normal mitosis, CRn; U — chromatine threads in fragmenting Ma

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The massive Mi, peculiar for *P. putrinum*, *P. bursaria*, *P. caudatum*, *P. jenningsi* and *P. wichtermani*, I consider polyploid, and not diploid nucleus; polyploidy of the Mi in these forms is a species character and not an individual or clonal aberration. In other words, diploidy of the Mi is anomaly here, and polyploidy — normal condition. The study of macronuclear fragmentation and regeneration yielded direct evidences for Mi polyploidy in *P. putrinum* and *P. caudatum*.

Macronuclear ploidy is determined from the relation of DNA quantity in Ma and Mi, where Mi is regarded as diploid nucleus. I found another way of determination of a minimal ploidy of Ma: counting of macronuclear fragments in exconjugants. It is well known that they are capable for regeneration into a new normal Ma in the absence of synkaryon derivates. Such regeneration was induced in *P. putrinum* at destroying Mi derivates by X-rays (Kovaleva and Jankowski 1965, 1966 a, 1966 b). Fragmentation of the Ma in conjugants is not the evidence of its degeneration (breakdown), unlike many authors suggest, and may be regarded as mechanism for providing exconjugants and their nearest progeny with RNA source (Jankowski 1966 c).

Table 2 summarizes the data on the number of Ma fragments immediately after fragmentation in five species of *Paramecium* (based on data from my preparations). *P. bursaria* and *P. polycaryum* have small numbers of rapidly growing anlagen, and Ma is dissolved entirely since there is no need of its fragmentation. Let us compare now the number of fragments and the data on Ma ploidy calculated in relation to a "conventionally diploid" Mi. Fragments are highly polyploid in first four species, and, really may develop into a normal Ma after conjugation (Table 3). Fragments of *P. caudatum* are low polyploid in groups A and B and still may yield normal Ma, but they are hypoploid in group C. The ploidy of fragment in *P. putrinum* equals to 0.20–0.37 n, what is improbable: fragment must be at least haploid for normal regeneration. Thus, Ma ploidy in *P. putrinum* must be equal to 65–120 n (according to the number of fragments) and not to 24 n (from relation of DNA quantity in Ma and Mi). Bearing in mind the last relation (12 : 1), we determine the minimal ploidy of Ma equal from 65/12 to 120/12, that is 5–10 n. Even the lowest limits of these values (65 n for Ma, 5 n for Mi) are much higher than those admitted previously (24 n and 2 n).

Analogous counts for C-group lines of *P. caudatum* allow to establish the minimal Ma ploidy as 40–70 n (instead of 25–30 n) and that of Mi from 40/12–15 to 70/12–15, that is 3.3–4.7 n, roughly 3–5 n (instead of 2 n). The data on the ploidy of Ma of *P. putrinum* and *P. caudatum*, obtained by cytophotometric study, appear to be understated 2–3 times, since massive Mi is polyploid.

The ploidy of Ma in *P. bursaria* was determined previously as 16 n only; this species has giant Mi, obviously polyploid. Mi ploidy in *P. bursaria* may be determined by minimalization of chromosome number at mating of normal and amicronucleate clones. The minimal quantity of DNA must be detected, that pro-

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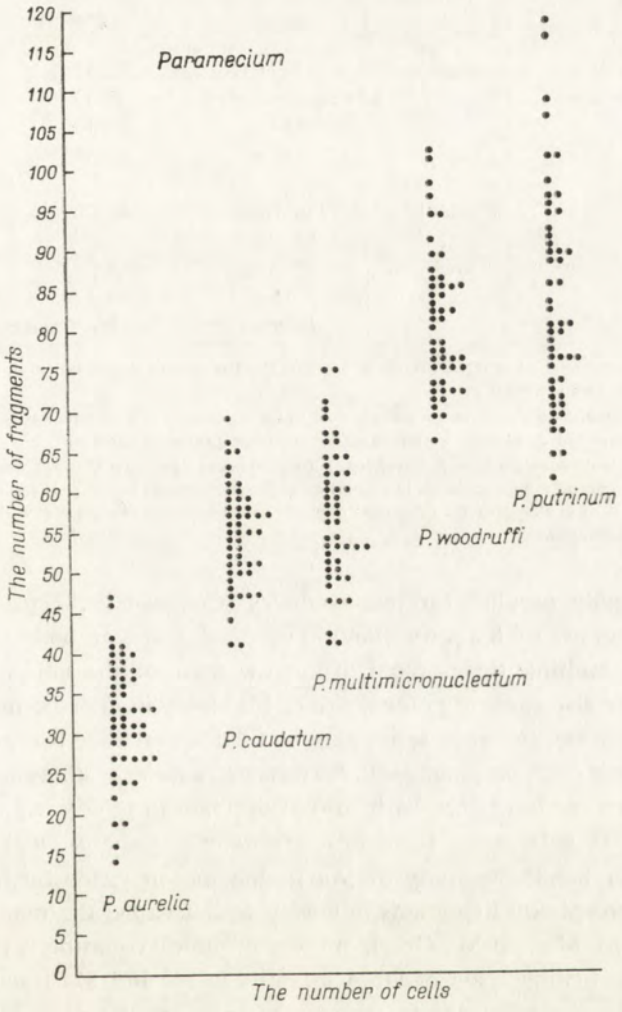
Table 2

The number of macronuclear fragments in paramecia (non-selective counts in early exconjugants)



vides the development of hemizygote (hemiploid) pronuclei into a new Ma. It seems that this way of research is appliable to all ciliates generally.

In the light of calculations, presented above, the hypothesis of the specific character of Mi polyploidy seems to be very probable. The existence of heteroploid series of Mi in paramecia with massive nuclei suggests the possibility of an extreme minimalization of chromosome number without injury to the line, what is possible only in case of the polyploidy of an initial "wild type" Mi. Unlike *Metazoa*, where the entire families may possess coinciding number of chromosomes, instability

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Table 3

The ploidy of the macronucleus and its fragments in *Paramecium*

Species	Macronuclear ploidy*		The number of fragments	Ploidy of fragments
	degree	value		
P. aurelia	high	860 n	15–45	19–57 n
P. multimicronucleatum	,,	minimum 860 n	40–75	12–25 n
*P. woodruffi***	,,	880 n	65–100	9–13 n
*P. calkinsi***	,,	560 n	12–15	37–43 n
*P. caudatum****				
Group A	middle	136–160 n	40–70	2–4 n
Group B	,,	84–100 n	,,	1–2 n
Group C	low	25–30 n	,,	0.43–0.75 n
*P. putrinum***	,,	24 n	65–120	0.20–0.37 n
P. bursaria	,,	16–60 n	No fragmentation	

* Precisely double relation of DNA content in Ma and Mi. The data of Woodard et al. 1961 Raikov et al. 1963, Cheissin et al. 1964 a, 1964 b.

** Photometric studies were made on the same lines in which the number of fragments was determined. *P. calkinsi*, *P. woodruffi*, *P. putrinum* and *P. bursaria* were isolated by author in Leningrad suburbs.

*** Other data: 50–60 n (Walker and Mitchison) 80 n (Moses) 128–150 n (Blanc) 160 n (Raikov et al.). According to Blanc different syngens coincide in proportion of DNA content in Ma and Mi and differ in the absolute quantity of DNA. Raikov et al. explain the discretness of ploidy values by clonal differences in both macro- and micronuclear ploidy in *P. caudatum*.

of ploidy is highly peculiar for many ciliates (*Chilodonella*, *Tetrahymena*, *Paramecium* — see review of Raikov 1969). There are cases of both multiple (polyploid) and non-multiple (heteroploid in narrow sense of the notion) variation of ploidy. There are also cases of gradual rise of Mi ploidy in interspecific evolutionary lines (e.g. in astomes), and such series may be for the first time formulated in *Paramecium* (*Frontonia — P. putrinum — P. bursaria; P. aurelia — P. jenningsi — P. caudatum*). There are, at last, lines with abrupt decrease in ploidy, e.g. *P. putrinum*— *P. woodruffi* or *P. polycaryum* (generally, *Helianter — Cyperostoma*).

On the other hand, the study of Ma fragmentation yields further support in favour of the polyploidy hypothesis, allowing to determine the minimal degree of the ploidy of both Ma and Mi. One more way of ploidy counting is possible: direct cytophotometrical comparison of DNA quantity in Mi and Ma fragments. It must be underlined that, visually, Ma fragments are much smaller, than Mi, and contain less stainable material in *P. putrinum*. Even the equality of their DNA content favors my hypothesis since each or most of 65–120 fragments would be "diploid" in this case. This comparison may open a new area of research.

Authors, who studied recently *P. caudatum* (Borchsenius et al. 1968) and *P. bursaria* (Golikova 1969), are inclined to recognize the massive Mi of these species as polyploid one, judging simply on the existence of heteroploidy and possibility of decrease of DNA content in Mi. These studies began after completion of the present work and formulation of my hypothesis in 1965.

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Diller discovered possibility of minimalization of chromosome number (at double hemizygotization) in a small vesicular Mi of *P. multimicronucleatum;* this may indicate polyploidy (near 8 n) of small Mi also. Heteroploidy found in *P. aurelia* by Dippel may support this unexpected conclusion.

The scheme of the categories of the nuclear apparatus in *Ciliophora* is more complicated now. Apart from two widely accepted combinations "diploid Mi+ +diploid Ma" and "diploid Mi+polyploid Ma" (Poljansky and Raikov 1960), the third kind of the nuclear set exists — "polyploid Mi+polyploid Ma". In respect to its ploidy, Mi of the last forms is just the same polygenomic system as Ma, but with lower degree of polyploidy. It may be noted also that nuclear isoploidy and not diploidy was proved for the first type of the nuclear set.

The number of cases of the arrest in nuclear development occurs in ciliates; e.g., "septimeric" phase of the nuclear division is inherited in higher hypostomes, inversion of the nuclear layers — in endogemmine chonotrichs, macronuclear branching before fragmentation — in *Plagiotoma*, Ma anlagen (in proportion Ma to Mi, 7 : 1, typical for thigmotrichs) — in *Myxophyllum steenstrupi* etc. The review of these cases will be presented in another paper. There is possibility that "diploid" Ma of *Loxodes*, which is not member of lower gymnostomes, are also anlagen arrested in their development and thus uncapable to vegetative fission.

Although micronuclear diploidy is proved for some species (judging from the cytology of meiosis in *Colpidium*, *Tetrahymena* etc.), it seems that the third type of the nuclear set is widespread among *Ciliophora*. Many genera include aberrant species with unusually large, atypical Mi: e.g., *Cyclidium citrullus* (*Pleuronematina*), *Tetrahymena chironomi* (*Tetrahymenina*), *Frontonia elliptica* (*Peniculina*), *Heterochona trifaria* (*Chonotricha*), *Scyphidia arctica* (*Peritricha*) etc. Giant Mi are typical for many thigmotrichs, hymenostomes, astomes, peritrichs, hypotrichs etc.

Multiple replication of genomes in Ma and Mi has a definite biological sense. Among numerous aberrations in the nuclear behaviour in *P. putrinum* I noted interesting cases of the loss of chromosomes by dividing nuclei (falling out into the cytoplasm), splitting of telophase nuclei; hemiploid nuclei (hemikaryons) are common. Repetition of such anomalies in both diploid and haploid nuclei must lead to hereditary diseases and death of exconjugants or their progeny, but this phenomenon is lacking in *P. putrinum* at normal feeding conditions in spite of a wide spectrum of aberrations. Genomic (subnuclear) replication within the Mi compensates these anomalies just like endomitosis in Ma makes possible chromatine extrusion by dividing nuclei, a normal process occurring in number of ciliates.

Nomenclature problem needs some remarks. At least for paramecia with giant Mi, the notions "haploid pronucleus", "diploid Mi" and "diploid synkaryon", commonly used for description of conjugation, are undesirable. There seems to be no sense in conventional recognizing each separate line or race as a "diploid" one. The terms must be used that imply a decrease or increase in chromosome number at meiosis or caryogamy without relation to the nuclear ploidy. The notions

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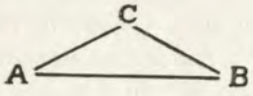
"euploid" and "hemiploid" or "hemizygotic" nuclei will be used below. In apomictic races, nuclear "isoploidy" or "euploidy" and not "diploidy" is constantly maintained.

IV. The system of mating types and syngens

Introductory remarks

Sonneborn 1938 isolated three stable lines of *P. putrinum* with complementary mt, conjugating following the scheme:



This scheme indicated the possibility of the existence of a multiple mt system in the species. These data were repeated, without further details, in several subsequent papers (partly Sonneborn 1957, 1971). Wichterman 1949 observed the mating reaction in *P. putrinum*. Diller 1948 isolated 15 selfing lines, two of which showed mating reaction after their mixture. My data (Jankowski 1962 b) are reported below. Bick 1966 showed the dependence of mating in *P. putrinum* (in populations or selfers?) from the density of cultures: mating begins in a log-phase and reaches its peak in a stationary one when 5–6% of cells are joined. Ammermann 1966 isolated in Donau stable and selfing lines of four mt of *P. putrinum*, and found fifth mt among caryonides; mt of 280 caryonides (from 35 pairs) was tested, but nuclear reorganization pattern was not determined by Ammermann. Exconjugant gives rise commonly to lines of a single mt (51% of caryonides), less commonly to different mt's, including non-parental ones (22%) and rarely to selfers (17%). Subclones isolated from selfing-lines are both of a stable and selfing kind. Ammermann concludes that mt system is similar in *P. putrinum*, *Tetrahymena* and *Stylonychia;* mt is determined in a non-genic way like in *P. woodruffi*.

Mating reaction

Selfing test preceeded an analysis of the mt system in *P. putrinum*. Large number of cells from fresh log-phase test-tube culture was removed into depression slides or Petri dish with or without feeding; stable lines die out from hunger without mating. The number of pairs in different selfing lines varies greatly from solitary pairs to mass mating; irrespective of their number, such lines are designated as selfers. They were tested further for Sg and MT only.

For determination of mt of stable lines, cells from fresh cultures devoid of food 1–2 days before crossing or those from stationary phase cultures were used. Starving cells do not mate, overfed ones may exhibit a weak mating reaction (sticking of two or more cells) with their following disjoining and mass asynchronous conjugation after 1–2 days.

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Sg 5 lines show weak agglutination or its complete lack in mixtures; mass conjugation starts actively after 1–3 days. This delay reminds the "waiting period" found in *Tetrahymena* (Elliott) and *Euplotes* (Katashima). Several lines of Sg 3, isolated still, do not mate with all other lines of the species and are regarded tentatively as belonging to a single mt of a separate syngen. A typical, rapid and active agglutination is peculiar for Sg 1, 2 and 4; large clumps of sticked cells are formed immediately after mixture of complementary lines. Ciliary movement becomes discoordinated; ciliary sticking, especially on the ventral body surface, is seen. Within 1.5–2 h the clumps break up into numerous firmly attached pairs and many still joining ones. Mating intensity reaches 70–80% and not 100% since the lines are mixed in different quantities, and some feebly united cells disjoin after their temporary agglutination. Serial preparations made at equal time intervals always show the belated formation of pairs during at least a day after the mating reaction, irrespective of its intensity, as well as some free cells not participating in mating. *P. putrinum* exhibits no circadian rhythm of mating found in *Euplotes* and green paramecium *P. bursaria* (Jennings, Wichterman, Cohen, Heckmann, Siegel et al.); conjugating ability of active lines seems to be coinciding in every hour of day and night.

Cells with a different mt differ in their dimensions also; this is evident at both inter- and intraclonal mating of *P. putrinum*. Doflein 1907 found 50% of "anisocopulae" (unequal sized partners) in a race of *P. putrinum;* Wichterman 1937 indicates that "preconjugants" of the species are smaller than vegetative cells. Similar differentiation was found by Vivier and Oger (see Vivier 1960) in *P. caudatum*, where the size difference is statistically significant. Selfing lines of *P. caudatum* exhibit two peaks in cell size explainable by the origin of two cell populations with different mt. Such anisogamy is evident in my races of *P. putrinum* also. Almost all pairs of amphi-, auto- and apomicts (M 6×M 12, LJ 2×LJ 17, CRn) shown in Fig. 4–38, are composed of mates with different length and/or width. Non-selective measurements of 72 amphimictic pairs reveals the typical length relation of partners 80–85 : 70–75 μ, and separate pairs exhibit much more significant differences (93 : 74, 93 : 77, 95 : 78).

Like in *Chilodonella uncinata*, the lines of *P. putrinum* may be successfully mated again after a week since the preceeding conjugation. The "natural epidemics" of mass conjugation in selfers, e.g. in CRn line, maintained in Petri dishes, take place regularly each two weeks after re-culturing of lines, when rapidly multiplying ciliates begin to suffer from the failure of the food. Immaturity period in *P. putrinum* is surprisingly short (7–14 days); this means that new mass conjugation may begin immediately after the end of a nuclear reconstruction phase in exconjugants that demands 8 cell generations. Joukowsky 1898 indicates a 6–7 days period for *P. putrinum*. Separate cells in my races reconjugate before the completion of reconstruction phase in all its stages, and sometimes even immediately after separation

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of mates. For the first time reconjugation in ciliates was traced on *P. putrinum* in all its stages including ex-reconjugants (Jankowski 1966 b).

Three groups of ciliates may be distinguished in relation to the duration of the immaturity period: A — species with a long period (2–8 months in *Tetrahymena pyriformis*, 3–14 in *P. bursaria*); B — those with a middle one (1–3 months, *P. caudatum*); C — those with a very short one or practically without it (1–2 weeks — *Chilodonella uncinata*, *P. putrinum*). Sonneborn found variation of this period in *P. aurelia* (group B) where it may be significantly abbreviated, sometimes permitting the reconjugation. Repeated mating, really, is common in B and C groups where it occurs at further shortening of the immaturity period to 1–6 days or its complete lacking; such extreme shortening is impossible in group A species.

The system of mt and syngens

The resulting scheme of mt analysis is presented in Table 4; it is based on a "standard mt series" selected at mating of hundreds of lines at different combinations. 114 lines of 309 isolated ones are selfers, other ones stable in the period of analysis, usually not exceeding one month since their isolation from nature. 3 distinct and 2 conventional syngens were recognized; Sg 1–4 include 8, 6, 1 and 7 mt respectively. Selfing is common in Sg 1, 2 and 4; a number of lines of CR, VK, QS, SB and other races do not mate with representative lines of Sg 1–4, and exhibit a feeble agglutination and delayed mass conjugation at intercrosses. None stable line of this group was found — I am sure, by occasion only. The group was designated conditionally as Sg 5 not differentiated at present into stable mt's, although selfers have a dominant mt and the complete set of mt's of this group may be detected at special research, which was not planned. Four lines of SZ race (17, 32, 41 and 45) are sexually isolated from other syngens and do not intermate; they are supposed to represent their own syngen (Sg 3) rare in research area.

From 309 studied lines of *P. putrinum* (100%), Sg 1 includes 113 lines (36.45%), Sg 2 — 59 (19.32%), Sg 3–4 71 (22.94%) and Sg 5 — 62 (20.00%). Sg 1 dominates in research area, and Sg 2, 4 and 5 occur with a nearly coinciding frequency.

Diller 1948, 1949, 1959 and Wichterman 1953 noted the common occurrence of selfing in *P. putrinum*, but Wichterman maintained a 12-years old culture of a stable line. Selfing is really highly peculiar for *P. putrinum:* each third line isolated from nature is selfing one (114 lines from 309 — 36.5%). This is, naturally, a mid-value for the species. Selfing is rare in Sg 1, more frequent in Sg 4, common in Sg 2, all lines of Sg 5 being selfers. It must be underlined that selfing in Sg 1, 2 and 4 is not an obstacle for a normal mating reaction with stable or selfing complementary line when selfers do not undergo an active intraclonal mating. The presence or absence of selfing in *P. putrinum* is independent from their nuclear reorganization patterns: selfers occur in all mixotypes (amphi-, auto- and apomictic races). Selfing seems to be independent from the culture age also: young fresh lines were analysed in limits of a month following their isolation. The lines, initially stable, almost

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Table 4

The standard stock series of mating types and syngens of *Paramecium putrinum*

Syngen		1								2						3	4							5	
Stock		OM 11	OM 17	OM 19	OM 23	M 5	ZN 72	ZN 13	M 41	YR 28	YR 47	YR 50	KS 1	LJ 27	LH 12	SZ 45	M 6	M 12	M 23	LR 6	LR 4	WH 17	WH 18	SB 9	SB 7
I	OM 11	—	×	×	×	×	×	×	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
II	OM 17	×	—	×	×	×	×	×	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
III	OM 19	×	×	—	×	×	×	×	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
IV	OM 23	×	×	×	—	×	×	×	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
V	M 5	×	×	×	×	—	×	×	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
VI	ZN 72	×	×	×	×	×	—	×	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
VII	ZN 13	×	×	×	×	×	×	—	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
VIII	M 41	×	×	×	×	×	×	×	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
I	YR 28	—	—	—	—	—	—	—	—	—	×	×	×	×	×	—	—	—	—	—	—	—	—	—	—
II	YR 47	—	—	—	—	—	—	—	—	×	—	×	×	×	×	—	—	—	—	—	—	—	—	—	—
III	YR 50	—	—	—	—	—	—	—	—	×	×	—	×	×	×	—	—	—	—	—	—	—	—	—	—
IV	KS 1	—	—	—	—	—	—	—	—	×	×	×	—	×	×	—	—	—	—	—	—	—	—	—	—
V	LJ 27	—	—	—	—	—	—	—	—	×	×	×	×	—	×	—	—	—	—	—	—	—	—	—	—
VI	LH 12	—	—	—	—	—	—	—	—	×	×	×	×	×	—	—	—	—	—	—	—	—	—	—	—
I	SZ 45	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
I	M 6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	×	×	×	×	×	—	—
II	M 12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	—	×	×	×	×	×	—	—
III	M 23	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	×	—	×	×	×	×	—	—
IV	LR 6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	×	×	—	×	×	×	—	—
V	LR 4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	×	×	×	—	×	×	—	—
VI	WH 17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	×	×	×	×	—	×	—	—
VII	WH 18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	×	×	×	×	×	—	—	—
—	SB 9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	×
—	SB 7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	×	×

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always remained as such during subsequent several years of cultivation. Selfing in initially stable lines is rare in *P. putrinum* (caryonides were not analyzed, however; vegetative clones are meant); there are only few such records in Sg 1 and 2. There was no need for replacement of a "standard stock series" of *P. putrinum* (Table 4) that remained stable during five years of its maintenance. Numerous sublines of CRn line, isolated during a 6-year period, were invariably selfers; the line itself, apomicts with an active and regular conjugation, is surely a hereditary selfer. Similar lines with hereditary instability of mt are known in *P. aurelia* (Bleyman).

Selfing tests and syngen determination in representative lines of 22 water bodies of Leningrad district and Kirishi region yielded some significant data on the structure of natural populations of *P. putrinum*. Race ZN includes 14 stable and 3 selfing lines (Sg 1), 1+0 (Sg 2). Race SB: 0+11 (5). Race H: 10+1 (1), 0+4 (5). Race CR: 0+9 (5). Race LR: 4+0 (1), 2+0 (4). Race T: 8+0 (1), 4+6 (2), 0+2 (5). Race NT: 10+0 (2), 0+10 (5). Race KS: 1+2 (1), 19+11 (2). Race LC: 5+0 (1). Race YR: 6+1 (1), 3+0 (2), 0+1 (5). Race OM: 20+4 (1). Race MR: 10+0 (4). Race A: 3+2 (1). Race M: 6+0 (1), 11+2 (4). Race LJ: 2+0 (1), 6+2 (2). Race LH: 1+0 (1), 3+0 (2), 10+2 (4). Race PR: 1+5 (4). Race HR: 2+0 (1). Race QS: 0+13 (5). Race SZ: 13+4 (1), 1+0 (2), 4+0 (3), 0+1 (5). Race WH: 1+0 (1), 4+1 (2), 2+6 (4). Race VK: 0+12 (5).

Animals of different syngens live commonly together in single water bodies (pond, lake, ditch etc.). Mt's within syngens are unevenly distributed throughout the region studied: Sg 1 includes mt I–VIII with 11, 25, 8, 33, 6, 5, 8 and 1 lines; Sg 2 includes mt I–VI with 8, 9, 8, 4, 6 and 2 lines; Sg 4 includes mt I–VII with 9, 14, 9, 12, 4, 7 and 3 lines respectively. The lines with mt VII and VIII in Sg 2 and mt VIII in Sg 4 were not still isolated and must be rare.

Stable and selfing lines are distributed throughout syngens as follows. Sg 1 with 113 lines (100%): 96 lines stable (84.96%), 17 selfers (15.04%). Sg 2 with 59 lines: 39 lines stable (66.10%), 20 selfers (33.90%). Sg 3: 4 stable lines were isolated only. Sg 4 with 71 lines: 56 stable (78.87%), 15 selfers (21.13%). Sg 5: all 62 lines are selfers.

Concluding remarks

P. putrinum is the second (besides *P. bursaria*) species of the genus with multiple mt system in various syngens; all other paramecia possess double system. Multiple system was indicated at first in *P. multimicronucleatum* by Giese and Arkoosh but Sonneborn and Barnett described a unique binary system with regular replacement of one mt by another during a day; the line exhibits different mt in dark and light.

Generally, mt system is well correlated with taxonomic subgenera of *Paramecium:* both species of *Helianter* have a multiple system, while *Cypreostoma* and *Paramecium* s. str. possess double one. Multiple mt system may be foreseen thus in (1) a new giant bi-micronucleate zoochlorella-bearing paramecium of *P. bursaria* group

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discovered by J. Dragesco in Cameroun, (2) in a giant peniculine *Neobursaridium gigas* derived from *P. bursaria* and (3) in lower peniculines, including species of *Frontonia*, *Disematostoma*, *Lembadion*, *Urocentrum*, etc. Double mt system may be foreseen (1) in all other new and rare paramecia, including *P. wichtermani*, *P. africanum*, *P. arcticum*, *P. pseudotrichium*, and (2) in *Faurella thermalis*, possible descendant of higher paramecia of "*aurelia*" type.

The multiple mt system is peculiar for ciliates generally — e.g., for *Tetrahymena*, *Colpidium*, *Glaucoma*, *Stylonychia*, *Oxytricha*, for many species of *Euplotes* besides *E. eurystomus* (it has, however, a system of "selfing suppression" types and not true multiple mt system). There are reasons thus to state that the lower range paramecia (*Helianter*) simply inherited their multiple system from other ciliates and that it was secondarily modified in the intermediate subgenus *Cypreostoma* and conserved without modifications by the higher range paramecia of "*aurelia*" group.

Six syngens of *P. bursaria* isolated still by Jennings, Opitz, and Chen in USA, England, China and Czechoslovakia include in sum 23 mt's (4, 8, 4, 2, 1 and 4). My material on *P. putrinum* coming from an extensively examined, but by territorium size restricted area (Leningrad District) yielded 3 good syngens with 8, 6 and 7 mt's and 2 conditional ones. Unlike in *Tetrahymena*, 8 seems to be a maximal number of mt's per syngen. Syngen set in a limited territory and within a local water body seems to be much more heterogenous in *P. putrinum* as compared with *P. bursaria*.

The abundance of selfing lines is a distinctive feature of *P. putrinum* in comparison with "rare selfers" — *P. bursaria*, *P. woodruffi*, *P. calkinsi* etc. Both the presence of selfing and the syngen structure in *Paramecium* show no relation to the occurrence of autogamy, unlike the view hold by Siegel 1956. Siegel ascribed autogamy to double-mt species and denied it in multiple-mt ones; but it is evident now that most of the higher range paramecia of a double-mt group are lacking autogamy (*P. caudatum*, *P. woodruffi*, *P. calkinsi*, *P. multimicronucleatum* etc.). Autogamy is not correlated with subgenera of *Paramecium* and possibly arised accidentally and independently in two unrelated groups, *P. polycaryum* and *P. aurelia*—→*P. jenningsi* complex. *P. aurelia* seems to be a descendant of non-autogamous *P. woodruffi* and not of an aberrant *P. polycaryum*.

V. Amphimictic conjugation

Introductory remarks

163 lines of *P. putrinum* from 309 (54%) ones reorganize their nuclei in amphimictic way; two kinds, obligate and facultative amphimixis are recognized. The facultative lines show no amphimixis, when mated with aberrant ones (automicts). All other isolated lines belong to auto- and apomictic groups; they hereditarily

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lost an amphimictic capacity. The nuclear cycle coincides entirely in both obligate and facultative amphimicts at inter- and intraclonal mating in both groups.

The time schedule of the nuclear events in crosses M 6×M 12 (MT A) was determined on preparations made during an agglutination phase and each 2 h further up to disjoining of most pairs. The duration of cell contact is dependent on the temperature: 26 h at 18–20°C, 2 days at 14°, near a week at 10°. There are, moreover, significant individual variations: cells disjoin in the interphase, prophase or anaphase Sk III or after III Sk fission. Clonal variation also exists; for example, two apomictic selfing lines with identical nuclear cycles, CRn and QS 3, remain joined near a day or 1.5 days respectively. According to the slides, most pairs of M race have the following approximate time schedule. 0 hours — joining of mates; 16^{30}, 17^{15}, 20^{20} — I, II and III Mi fissions; 21^{15} — pronuclear exchange and caryogamy; 22^{00}, 23^{30} — I and II Sk fissions; 25^{00} — cell separation; 26^{00} — III Sk fission; 39^{00} — nuclear differentiation (resorption of three nuclei in exconjugant, differentiation of anlagen and Mi). Exconjugant starts to divide within two days after conjugation.

Most pairs of all mixotypes of the species exhibit a slight degree of asynchrony in the nuclear behaviour between the partners; time difference in fission phases equals commonly to 3–10 min. Such asynchrony is too common and insignificant to be regarded as abnormality. In extreme cases, however, the difference reaches 10–20 min or even more.

The general scheme of amphimixis coincides well in my races and those of Diller 1934 and Wichterman 1937: the cycle includes 3 fissions of Mi, pronuclear formation, exchange and fusion of pronuclei and 3 Sk fissions (Fig. 4–20, Plates III–VII).

Prophase and I meiotic fission

After cell union, Mi moves away from the Ma and undergoes a slow swelling and structural differentiation (polarization). Chromatine bodies, gathered in longitudinal rows, are concentrated near one of the nuclear poles; the peculiar "light-green cup" with several chromatine grains shows a sharp swelling and enlargement on the other pole (Fig. 4 A–E). From the early beginning of its growth, Mi is surrounded by a light green zone; this structure with a distinct contour is revealed by light green, bromphenol blue, contains no DNA or RNA and may be identified on hematoxylin and unstained preparations due to its high refraction. The zone is shown only in some figures. It surrounds the Ma and its derivates on all stages of conjugation, including the division spindles. The nuclear membrane is invariably conserved in conjugants; I do not agree with Perez-Silva 1965: 217 who writes that in *Frontonia acuminata* "at the late prophase (Mi I) the nuclear membrane disappears, and a number of chromosomes are clearly observed". Since the spindles lie within a zone, some its part must be used for achromatic spindle formation. Large dense green polar cups are visible on both spindle poles (Fig. 5 W).

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Light green zone is not an artifact arising at fixation due to cytoplasmic shrinkage. It seems probable that in mated cells the nuclear envelope and matrix undergo the swelling, and all nuclear division proceed within an enlarged matrix. Centriolar cups are noted constantly on the ends of anaphase and telophase nuclei and also at one end of an interphase ones. At every stage of conjugation, the nuclei contain four main components: matrix, swollen envelope, chromosomes and dense polar cup on wide end of a chromosomal basket. Light green zone makes the matrix of anlagen in exconjugants, continuously surrounding all chromatine structures, and becomes finally the matrix of a new Ma. The continuity of the matrices of Ma and Mi may be considered as established feature in *P. putrinum*.

The growing Mi enters the parachute or bouquet stage in the early prophase Mi I. Like *Frontonia*, *P. putrinum* has no crescent stage. Chromatine granules are arranged in distinct longitudinal rows, occupying all the space of a growing Mi (Fig. 4 C–N). The nuclear dimensions reach 5.5–6×4 μ in the swelling stage, 11–12×6 μ in parachute stage, 14×7 μ in granular stage, and 23–25×4.5–5.5 μ in chromosomal stage; light green zone attains the size near 27–33×12–12.5 μ. The greatly elongated spindleform nucleus with longitudinal rows of chromatine bodies corresponds to a crescent stage of other ciliates. The poles of a light green zone with several chromatine granules are fixed on the pellicle, sometimes under the opposite body sides, and the spindleform Mi is hanging across the cell. The maximal elongation and adhesion of a zone is followed by condensation of a chromatine nuclear part without simultaneous condensation of nuclear matrix; very thin tangled chromosomes, still arranged in longitudinal rows, are seen now instead of chromatine rods and granules. The longitudinal arrangement is gradually lost, a tangle of thin chromosomes is seen; they start to enlarge and assume a more or less parallel disposition along the nuclear axis. The stage described is surely a spireme–zygoneme (Fig. 4 O–W; 5 A, B). Discrete chromosomes are not compressed in a ball, occupying a significant part of a light green zone.

The next stage is a typical pachyneme (Fig. 5 C, D, J, K); very thick and long discrete chromosomes of a more or less coinciding size in a set are seen instead of thin numerous bended ones of a preceeding stage. The number of thick chromosomes (47, 48 on Fig. 5 J, K) is equal to their number in hemiploid pronuclei of these lines (Fig. 12 A, B). No doubt, we see the bivalents — conjugating homologous chromosomes without a distinct slit among them. Pachyneme stage is a prolonged one, common on mass preparations. Thick bivalents are well seen at small magnification, while oil immersion must be used for thin filiform separate chromosomes of postgamic Mi fissions. Unlike the typical meiotic bivalents, those of *P. putrinum* are not shortened. Chen counted chromosome number of *P. bursaria* on this stage obtaining thus hemiploid and not diploid values. Egelhaaf regards similar stage in *P. bursaria* as pachytene also.

P. putrinum shows no distinct metaphase and classical tetrad plate; the transition from the late prophase to an early anaphase Mi I and formation of anaphase groups

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Fig. 4. Amphimictic conjugation, M 6 × M 12, Sg 4, MT A, Feulgen. Early and middle prophase Mi I

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Fig. 5. Amphimixis. Late prophase and anaphase Mi I

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is gradual and asynchronous (Fig. 5 E–W). There is no precision in chromosomal disposition within the groups. Bivalents disjoin gradually into thin paired (coupled) chromosomes scattered throughout the nucleus. Tetrads are recognizable, though atypical; they are formed asynchronously, and both thick bivalents and thin paired chromosomes may be observed in early prophase Mi I. Tetrad stage is rapid and badly expressed; Fig. 5 L shows surely a dyad formation. Unlike the tetrads, dyads are well seen; they differ in configuration within the nuclei. Their chromosomes are not shortened. Two anaphase groups of dyads are gradually formed; the buccal complex is resorbed at this stage and formation of the paroral cone (PC) begins. Paired filiform chromosomes are well seen on both poles of a spindle, and some belated dyads remain commonly in its equatorial part (Fig. 5 L–W). Dyads are still discernible in the late anaphase; telophase chromosomes are closely shifted one to another.

The axis of a I Mi division spindle has no definite direction at first; it is directed along the body in the late anaphase. Telophase daughter nuclei move to both cell ends, and are displaced then into an oral zone and deeply into the cytoplasm respectively. Anaphase chromosome groups are basket like, and telophase ones — rhomboid; chromosomes are longitudinally oriented and fill the entire nucleus (Fig. 6 A–K).

Prophase and II meiotic fission

There is no distinct interphase: the nuclei are not condensed into compact darkly staining oval bodies like those formed at all other Mi fissions. Telophase nuclei enter gradually into prophase Mi II, rising in size from 19–20×6–7 μ to nearly 35×4 μ. The nuclei become spindleform and polarized. Like in prophase Mi I, the elongated nucleus adheres by its poles to the pellicle; this process is followed by rapid compression and shortening of chromosome nuclear set without corresponding compression of a light green zone (matrix and envelope; Fig. 6 L–R).

Chromosomal tangle assumes a basket-like shape typical for prophase nuclei. Chromosomes lack the longitudinal orientation and look now like thin tangled filaments; their interlacing results in appearance of large amorphous chromatine clusters (Fig. 6 S–V). Elongation of both chromatine and light green zone may be considered as an early prophase Mi II, their condensation as mid-prophase. Late prophase is marked by a new elongation and widering of the nucleus and more discrete location of chromosomes.

Anaphase Mi II begins asynchronously without a distinct metaphase. A part of chromosomes moves away from the anaphase basket and is displaced to the opposite nuclear pole, where more and more chromosomes are gradually gathered (Fig. 6 W–Z, 7 A–C). All chromosomes are thin and typical, and show no paired disposition. Anaphase groups are indistinct or entirely not expressed, and chromosomes are distributed in disorder throughout the spindle; telophase nuclei produced by such spindles may bear a long chromosomal tail (Fig. 7 D–N). It seems quite

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Fig. 6. Amphimixis. Anaphase Mi I to anaphase Mi II

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probable that such uncoordinated distribution of chromosomes thoughout the spindle leads to uneven distribution of chromosomes between the daughter nuclei — to aneuploidy, if such concept is available to polyploid Mi derivates. Such abnormal spindles and all transitions to normal ones are seen commonly at all conjugation phases in M race of *P. putrinum*. The number of chromosomes in anaphase nuclei (near 50) much exceeds that of the filaments of achromatic spindles (5–8).

Both spindles of the Mi fission differ in their direction — across the body and at the angle to its axis. Telophase nuclei become rapidly condensed and intensively stained, entering a long interphase. Three daughter nuclei lying outside the cone degenerate in the mid-interphase assuming shape of small (3.5–4 μ) intensively staining bodies — pycnocaryons (Fig. 7 O–P). Pycnocaryons produced by extremely long telophase nuclei may have a "supplementary body" made by a condensed chromosomal tail. Light green zone is condensed and does not surround more the entire nucleus; it looks like a small dense sphere located in a pit made by curved nucleus. Pycnocaryons are resorbed during the final Sk fissions before cell separation; the nuclei become clear, vacuolized, weakly stainable; they undergo an instant rupture (due to vacuolization?) and are pulverized in the cytoplasm, sometimes after an extreme preliminary swelling (Fig. 7 Q). In some cases, that are only exception in *P. putrinum* (and rule for *P. caudatum*), preliminary resorption of pycnocaryons may be observed before pronuclear formation (Fig. 8 D). In typical cases, all three nuclei persist to the end of conjugation.

In some exceptional (pathological) cases, Mi degenerates in the late prophase Mi I or both its products in the interphase; such pycnocaryons attain the size near 10×9 and 6×5 μ respectively. Normal pycnocarya produced after II Mi fission reach 3.5–4 μ in diameter. Theoretically, these three kinds of pycnocarya must have 4 n, 2 n and n; their significant difference in size indicates the gradual decrease in DNA content and may be an unusual indirect evidence of a two-step meiosis in *Paramecium*.

From many reasons, pycnocarya are suitable for photometric studies: they are compact, of a simple and regular shape, have distinct contours; their relative ploidy (hemiploidy) is well known; unlike Mi, they do not synthesize DNA, much limiting thus the scattering of data in comparison with photometry of Mi on different phases of DNA synthesis. In other words, the study of pycnocarya should yield stability of results.

Hemiploid nuclei produced at the II Mi fission have near 50 chromosomes (41 and 47 on Fig. 12 A, B; a part of these is not seen in transversal optical section).

It is well known now that meiosis in its classical, or "metazoan" form was found in some ciliates only — in *Tracheloraphis*, *Nassula*, *Tetrahymena*, *Colpidium* etc. (Geitler, Devide, Ray, Raikov, Kovaleva etc.). Some separate stages of meiosis were noted in *Paramecium* by Calkins, Cull, Egelhaaf, Dippel, Nakata, Kościuszko et al., but the general course of meiosis differs greatly from

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the "metazoan" scheme. In *P. putrinum*, pachytene chromosomes are not condensed; classical tetrads are absent; anaphase groups of chromosomes are made asynchronously without distinct equatorial plate metaphase; dyads are not discernible in the interkinesis and so on. These deviations may be explained by the presence of very large chromosomes and by micronuclear polyploidy in *P. putrinum* which is surely a species character and not clonal (occasional) aberration in this species.

Prophase and III post-meiotic fission

Derivates of the II Mi fission enter the first typical (prolonged) interphase. They diminish in size to 8–11×4–4.5 μ; chromosomes become closely shifted together being still strictly parallel one to another (Fig. 8 G, H). Single nucleus within PC that survived the degeneration enters prophase Mi III, assuming a basket-like shape; it has relatively thick chromosomes. Both daughter chromosome groups are again made asynchronously without a distinct metaphase plate (Fig. 8, 9 A–K). A gradually increasing part of chromosomes leaves the basket and is aggregated near the opposite end of the nucleus. This process, which does not reminds us the classical mechanism of a mitotic spindle formation (distinct metaphase and synchrony in formation of daughter sets) is expressed especially well at all Sk fissions.

The III Mi spindle is oriented diagonally or almost transversally. One of the two daughter chromosome groups, that moves away into the cell interior, is much more compressed at the beginning of anaphase in comparison with that remaining within the cone (Fig. 9 E, F). Pronuclear differentiation is started thus in early anaphase Mi III: the nucleus outside the PC more rapidly enters the interphase. After the fission, two pronuclei occupy their classical sites in the cell, within a cone and deeply in the cytoplasm. Interphase (condensed) pronuclei are not morphologically discernible. The stationary nucleus lies transversally, while the migrating one — diagonally.

Cytoplasmic differentiation

The cytoplasm of the oral area in conjugating paramecia determines the nuclear fate: all the nuclei located outside the cone degenerate after II Mi fission; pronuclei made at III Mi fission are differentiated according to their sites in the cell. Sonneborn supposed an influence of the cone membrane or cytoplasm on the "surviving" nucleus. Such homogenous cytoplasm ("parorale Plasma"), according to Moldenhauer, was not known still in paramecium, but its description or figures may be found yet in Plate's and Hamburger's papers.

The cytoplasm of conjugating *P. putrinum* is heterogenous; haematoxylin, pyronine, bromphenol blue and light green reversal a "homogenous" paroral and vacuolized extraoral zones. The first one is composed of an immense number of smallest closely adjoining pyroninophile bodies and seems to represent dense accumulation of ribosomes. "Homogenous" zone occupies both the cone and all ventral contact area (Fig. 9 A, B). Cytoplasmic stratification starts in the late pro-

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Fig. 7. Amphimixis. Late anaphase Mi II, telophase (A–N, R); nuclear degeneration (O, P) and resorption (Q)

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Fig. 8. Amphimixis. Interphase to telophase Mi III

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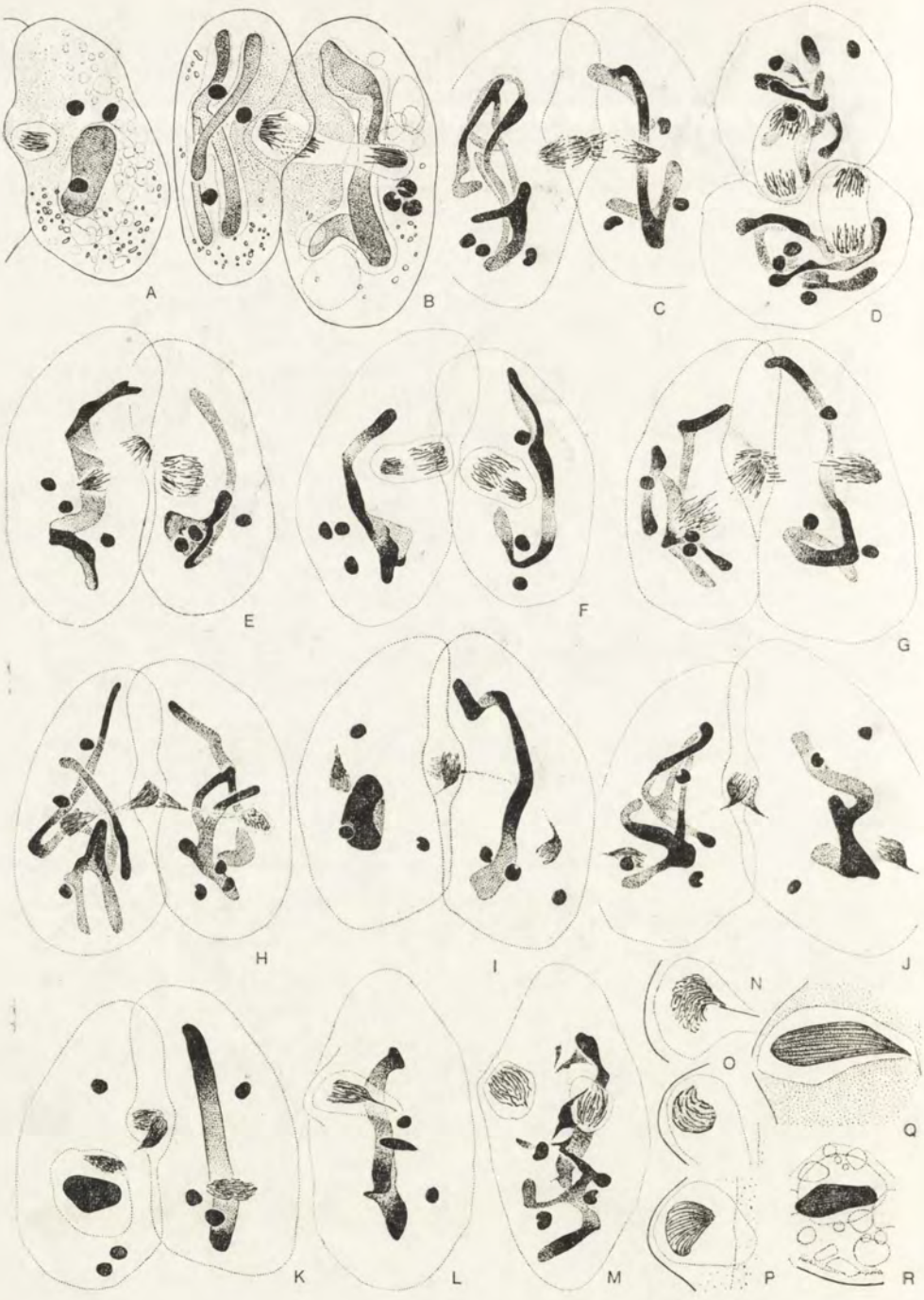

Fig. 9. Amphimixis. A–K, Q, R — conjugants; L–P — induced autogamonts. Cytoplasmic stratification and pronucleogenesis

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phase Mi I and disappears after nuclear exchange, when an active cyclosis is restored. The nuclei, Ma fragments and crystals are displaced now freely within the cell, and both layers are intermixed.

There are some reports of RNA accumulation in the cytoplasm of conjugating paramecia (Schubnikova, Gromova), explainable sometimes by the breakdown and resorption of an old Ma. Seshachar, e.g., reported an intensive RNA disposition within a specialized cytoplasmic zone in *Spirostomum* where pronuclei are formed. It seems that these authors dealt with simple cytoplasmic stratification and not with RNA synthesis and accumulation. At least in paramecia there is no source of RNA renewal in mates since (1) both RNA synthesis and extrusion are stopped until cell separation, and (2) Ma fragments are not resorbed in mates, "waiting" a completion of the nuclear reconstruction period in exconjugants, that demands several cell generations.

Somatic and buccal reorganization at mating

To reveal the mechanism of pronuclear interchange, the dynamics of the entire cell cortex was studied in impregnated conjugants, exconjugants and autogamonts (Jankowski 1972 c, d, e) and compared with that in vegetative dividers. Highly contradictory data were accumulated still in both areas in the literature. It is significant for us to know precisely: Whether old buccal complex disappears before or after the interchange? In what stage stomatogenesis begins? What is the structure and function of the paroral cone? etc. Very short summary of my data is presented below.

Buccal complex becomes rapidly resorbed (leaving a small remnant) in the late prophase or earliest anaphase Mi I, after a long lag period; the remnant disappears quickly after pronuclear exchange. Autogamonts, with their free ventral surface, reveal stages of resorption of the buccal complex and its overgrowing with clear kinetosome-free pellicle, a site of the paroral cone formation after I Mi fission. Pronuclei are exchanged actively in the second half of the interphase, pressing on the top of the cone and rupturing it. The narrow hole in the cone is a cause of the initial deformation of pronuclei and their spiraling movement. Conjugants, still firmly attached by their extremities, loose firm contact and disjoin in mid-ventral area, where wide slit becomes evident. Stomatogenesis begins on free surfaces immediately after exchange and cone dedifferentiation. Within several hours (anaphase Sk I — prophase Sk III) 3 strikingly large kinetosomal stripes (future UM basis, quadrulus and joined peniculi) appear on the pellicle, and giant triangular argentophile zone lies in the cone area. Further changes include the shortening and deepening of this complex, reconstruction of the entire somatic infraciliature, appearance of thick kinetodesms, formation of vestibular ciliature and, lastly, of a prevestibular zone. Striking resemblance between exconjugants and late vegetative dividers, with the entire somatic cell cortex reorganization in both, indicates

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a deep affinity of division and conjugation processes in paramecia. Conjugation is reorganization of both nuclei and cell cortex; this may be rule for all ciliates.

Pronuclear interchange

Once again, we enter a field of highly contradictory data; only some of these, related mostly to paramecia, may be mentioned briefly. According to Calkins and Cull, the pellicle in a contact area fuse and disappears after II Mi fission, and pronuclei move in the common cytoplasm. Wichterman, Schwartz, Seshachar, MacDougall, Dain et al. describe the passive exchange in anaphase or telophase Mi III when the elongated spindle presses against partner's pellicle. Moreover, Wichterman writes on "migrating spindle poles" instead of pronuclei. Grell 1967 reviewed the "active migration" theory, a view developed partly by Diller on *P. putrinum* and *P. caudatum*. According to Diller, pronucleus presses against the cone apex, with its subsequent breakdown, reversible stretching or dissolving, and enter partner's cell in the old pharyngeal zone. The nuclei are somewhat deformed and sometimes twisted during migration. Cytoplasmic currents and "intranuclear pression" (?) are significant for migration. Other authors hold that pellicular breakdown results in formation of a "cytoplasmic bridge" between two mates.

According to Inaba, pronuclei of *P. multimicronucleatum*, 8 μ large, are squeezed actively through one of the pellicular pores 1 μ wide: cytoplasmic currents are presumed to play some role in such process, resembling an amoeboid movement. In *P. caudatum* pronucleus breaks the cell membrane with its acute "perforatorium" and enters the oral opening of another partner (Moldenhauer). Vivier and Andre show a basket-like or amoeboid pre-migratory pronucleus with its widest edge facing the pellicle; one of its numerous chromatine "pseudopodia" is presumed to squeeze through a pore 2 μ wide, and the entire nucleus follows it. "Cytopharynx" is active still and bears normal ciliature. All this is an evident error: prophase or telophase chromosomal basket made after I or II Mi fissions (most probably after I one) was observed. Compact ovoid interphase nuclei migrate long after buccal resorption and cone formation in paramecia.

Pronuclear exchange is the commonest stage on mass preparations of amphimictic lines of *P. putrinum*. Three interconnected processes begin in the second half of prophase Mi I: (1) resorption of the oral (bucco-pharyngeal and vestibular) complex, (2) formation of a paroral cone, and (3) cytoplasmic stratification under the entire contact area. All these processes are terminated at anaphase Mi I; small amorphous buccal remnant persists temporarily, disappearing after caryogamy. Prolonged interphase begins after III Mi fission. Pronuclei are exchanged synchronously in the second half of the interphase, 30 min or less before Sk fission, rarely in the earliest prophase Mi IV (=Sk I), in any case after doubling of their DNA content — when a new nuclear division is soon to begin (Fig. 10, 11). Pronuclei migrate in a zone lacking both oral and preoral openings; local pellicular rupture is the only possible way of exchange. The nucleus must pass through two closely

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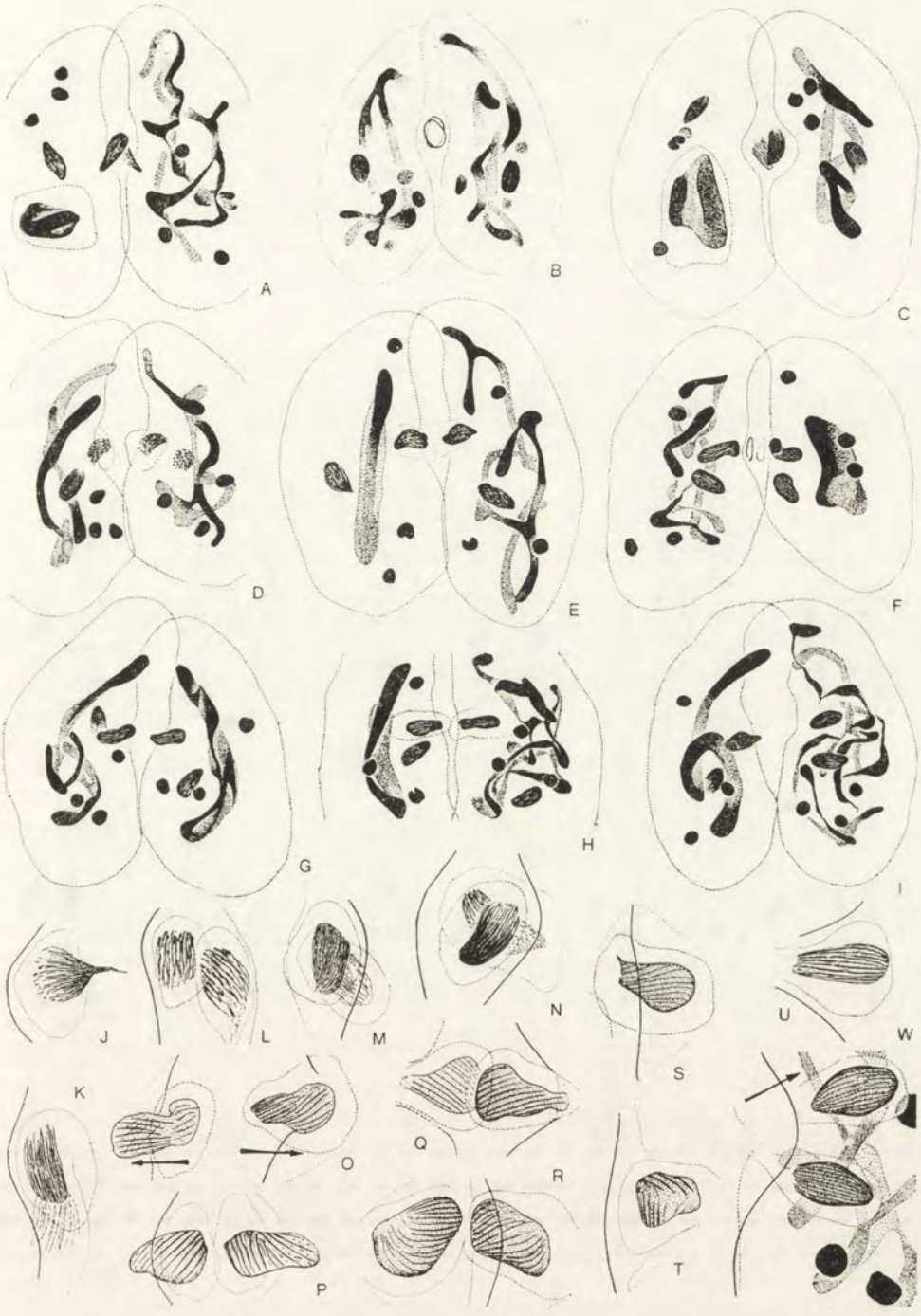

Fig. 10. Amphimixis. Pronuclear exchange

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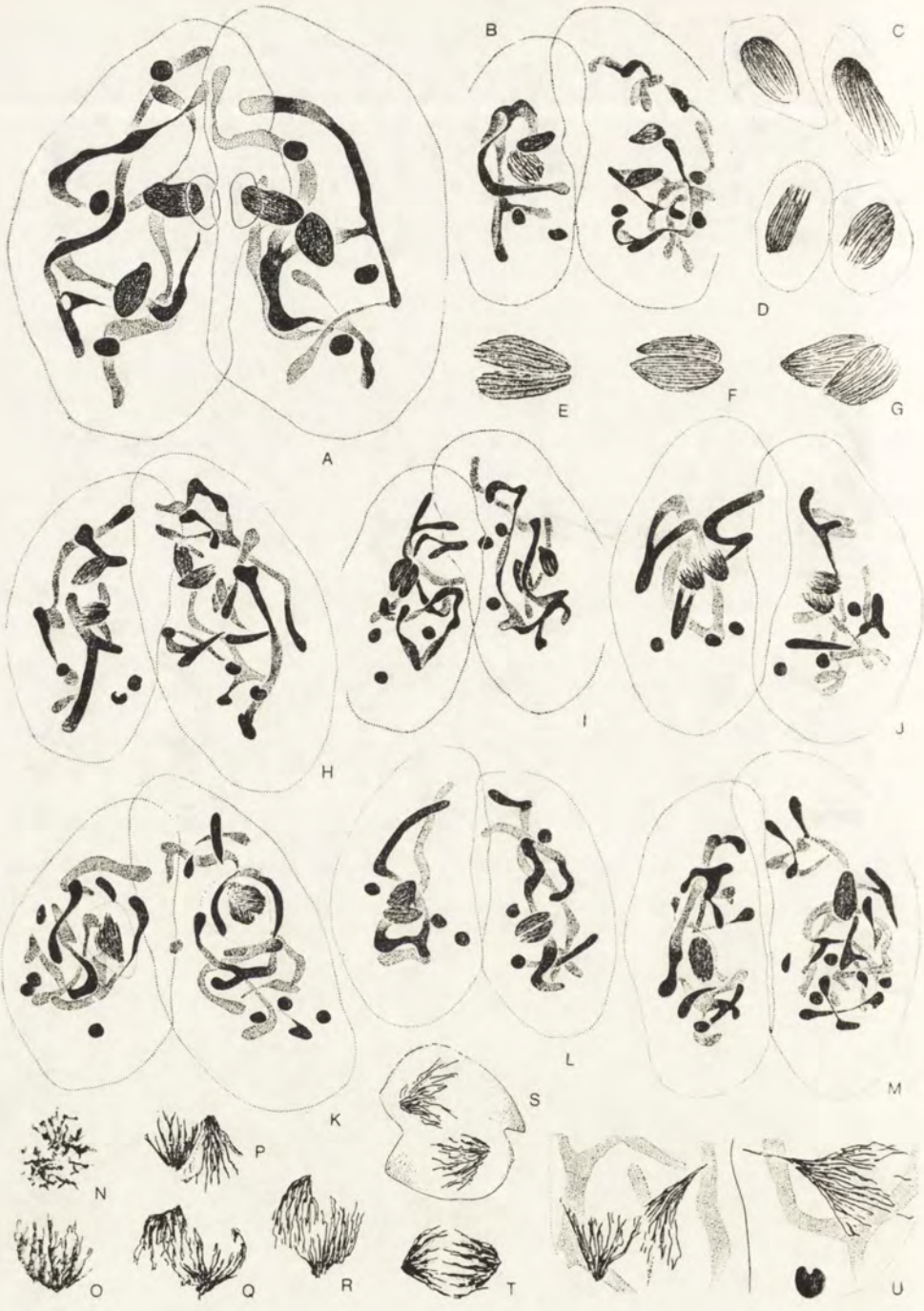

Fig. 11. Amphimixis. Caryogamy

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adjoining pellicles, its own and partner's one, since both cells have lost their buccal organelles and healed buccal openings. Both pellicles are smooth, free of organelles that can make the migrations difficult; trichocysts, cilia, infrakineties, argyrome, ciliary anlagen stripes and possibly rootlet ciliary fibers are resorbed.

After a long immobility period, migrating pronucleus bends slightly and begins to press against the left cilia-free cone wall adjoining to the partner (and not against the cone apex). The following stage must be studied with an elmiscope; impregnated slides show cone rupture, but it may begin from a widering of a pellicular pore. The nucleus seems to be too voluminous to enter a small invisible pore, however. Migrating nuclei undergo a series of peculiar deformations — become pyriform in shape, squeezing anteriorly and then medially; all this leaves impression that they pass through a moderately wide hole in cone wall. The nucleus screws itself in this hole and simultaneously "flows" into the partner's cell; chromosomes are parallel in pre-migrating nuclei and twisted in migrating ones. This partial spiralization helps the migration. The narrow apex of pronucleus enters the hole; it becomes wide, and the rest of pronucleus moves freely without deformation (Fig. 10, 11). Pellicular rupture after pronuclear migration may be seen in stained slides. Nuclear deformation is only temporary and is not descernible soon after exchange and caryogamy. Cytoplasmic currents were not found to play any role, so more that they are not seen in dense pyroninophile layer of the cytoplasm beneath contact area of living mates.

Caryogamy and synkarion fissions

After their exchange, migrating pronuclei restore rapidly their normal shape and approach the stationary ones, possibly due to a kind of chemotaxis, an active one-sided attraction. Caryogamy is initiated by the close contact and sticking of the light green zones, with their subsequent fusion (Fig. 11); both pronuclei, still independent in respect to chromosomes, are surrounded now by the common envelope and are lying in a joined matrix. As a rule, nuclei in identical growth stages (interphase or early prophase Sk I), and rarely interphase+prophase ones are fused (Fig. 11, 12). In some partners Sk formation is belated, beginning after a long delay in comparison with that in normal cells; both pronuclei separately enter a new prophase, still in joint matrix, and are fused ultimately before the formation of anaphase spindles. An extreme case, occasional non-fusion of pronuclei and their light green zones, belongs to individual aberrations in nuclear behaviour that are not analyzed in the present paper.

Two pronuclei fuse in various positions — parallel one to another or at acute or even right angles; they may contact by their wide poles only, producing both homopolar and heteropolar synkarya. Such variability is explainable by the fact that light green zones of approaching pronuclei come into contact in different position and immediately stick one to another without any further correlation of their position. Chromosomal groups within a joint matrix may maintain their

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Fig. 12. Amphimixis. Synkarya in optical sections, for chromosome counts (A–D); interphase, prophase, and early anaphase Sk I (E–U)

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independence during a long time, but the nuclear individuality is gradually lost before the early anaphase Sk I, usually in the late prophase or somewhat later, when the spindle formation begins. Prophase Sk I starts soon following pronuclear migration and fusion. Those chromosome groups that are not joined still under the common envelope may produce their own prophase baskets, usually asymmetrical. Such baskets are located sometimes one against another leaving erroneous impression of anaphase spindle (Fig. 12 J, K).

Chromosomes of prophase synkarya are commonly parallel one to another, and their total set may be easily counted on optical sections of the nuclei. Synkarya shown on Fig. 12 A–D possess 48+44, 44+41, 95 and 92 chromosomes respectively.

Equatorial metaphase plate is absent; a part of chromosomes is displaced from the basket to an opposite nuclear pole, and the entire nucleus is finally composed from parallel chromosomes (Fig. 12 L–U). Chromosomal baskets reach 7–8× ×9–10 μ in the early prophase Sk I, and near 11×10 μ in the late prophase. The distal ends of anaphase groups become condensed, and their medial parts are expanded (Fig. 13 E–N). Some belated chromosomes are scattered throughout the spindle (Fig. 13 N–Q). The spindles are longitudinally oriented, the daughter nuclei do not enter more into an oral zone (Fig. 13, 14). Anaphase groups become more and more compact at elongation of the spindles, and telophase ones look rhomboid. Sometimes they possess a long chromosomal tail. Telophase nuclei reach 11–14× ×4.5–6.5 μ in size.

Two daughter nuclei enter a new long interphase, undergoing diminution up to 9–10×4.5–5 μ; their chromosomes are strictly parallel one to another (Fig. 14 B–G). The light green zone is only slightly compressed in interphase nuclei. Each nucleus is distinctly polarized: one of its ends is acute and long while another one wide and blunt; it bears a light green cup participating in spindle formation.

Two nuclei in prophase Sk II stage assume again the shape of baskets measuring 5–6.5×6–7.5 μ. Anaphase groups of chromosomes are produced in a typical way: half of chromosomes retains its place within a basket, while others move away to an opposite (wide) nuclear pole making their own basket measuring 7.5–8×3–4 μ (Fig. 14 I–L). The spindles of II Sk division are elongated parallel to the body axis (Fig. 14 M–R; 15 A–M). Small (near 8×4 μ) telophase nuclei possess a typical shape and structure. After a long interphase (Fig. 15 N–Q) they enter prophase Sk III; chromosomal baskets reach 5.5–6 μ in length. Anaphase spindles are produced in a typical way (Fig. 15 R). Prophase and anaphase groups of II Sk fission are highly compact, only 6×3.5 μ large in a late anaphase; their light green zone becomes condensed (Fig. 16 M–T).

Distinct indications of an approaching cell separation are noted in the interphase following II Sk fission. Large slit separates both partners in their ventral area, and mates are joined with their anterior and posterior body parts, then only in their upper parts. As a rule, cells disjoin in the interphase after II Sk fission or in the

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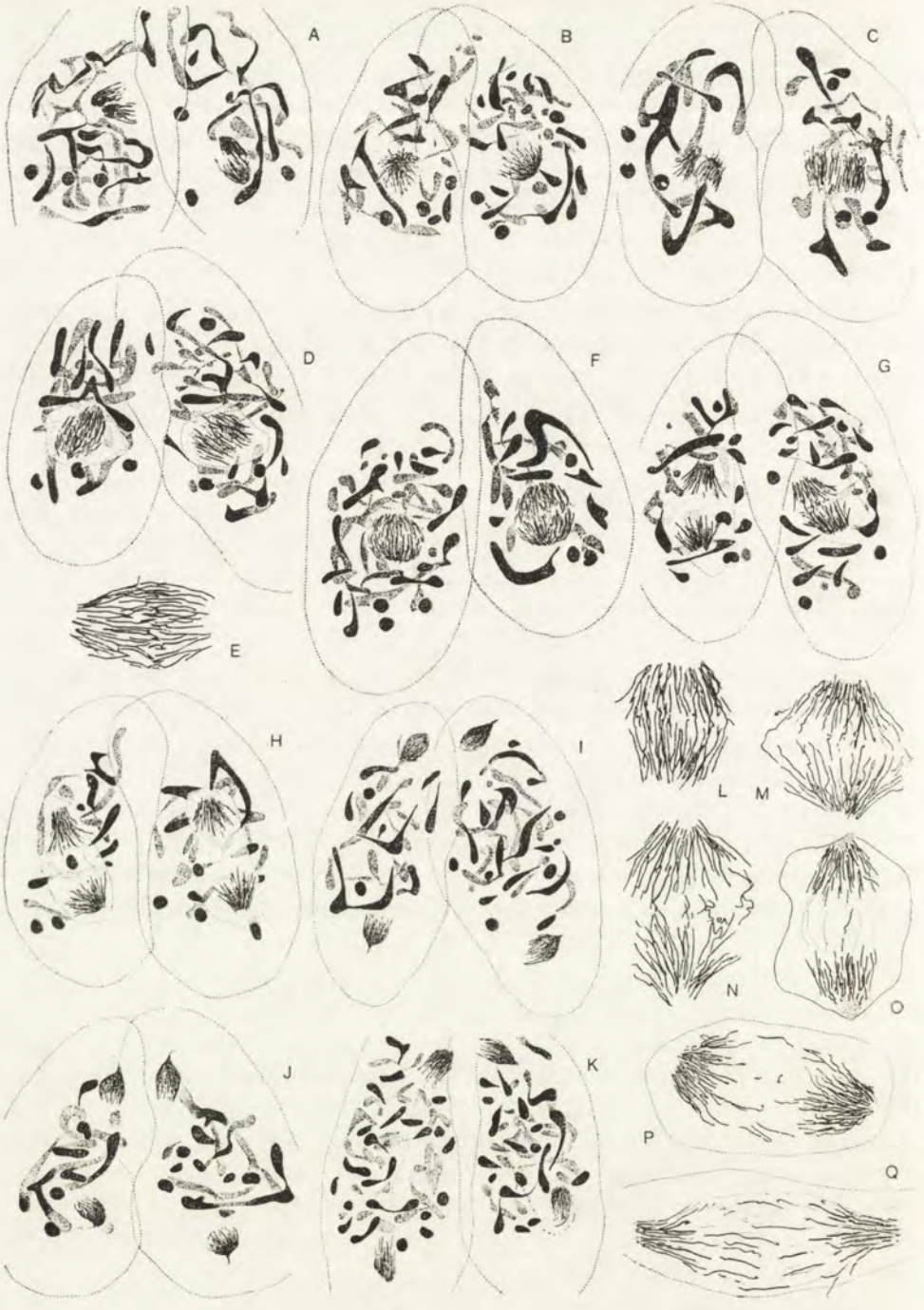

Fig. 13. Amphimixis. Prophase to anaphase Sk I

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early prophase Sk III when macronuclear fragmentation was accomplished. In some exceptional cases, the partners of M race disjoin in anaphase or even telophase Sk III (Fig. 16 D–L).

Exconjugants

The III Sk fission in M race starts usually in exconjugants (Fig. 16 C–L). The spindles have initially no definite orientation; they become longitudinally oriented after their extreme elongation. Four daughter nuclei move to the anterior and posterior body ends respectively. In most cases, Ma fragmentation was finished just prior to cell separation. Abnormal spindles composed of unevenly distributed chromosomes, filling the entire spindle, are common during fissions of synkaryon and its products.

Three nuclei from eight ones, received by exconjugant, undergo degeneration long before the I division of mate; other ones are differentiated into 1 Mi and 4 Ma anlagen. Early stages of their growth are shown in Fig 16 S–Z. Four analgen are distributed among the daughter cells at second division of an exconjugant, that gives rise to four caryonides. The fragments of the old Ma are also distributed between the daughter cells at 6–8-th divisions of exconjugant; they are resorbed only when the cell receives 1–3 fragments from the last division. Exconjugants are identical now with the vegetative cells.

Macronuclear fragmentation

Three general types of macronuclear behaviour may be distinguished in ciliates, and all these are represented within the genus *Paramecium*. Group I: Ma is dissolved in exconjugants without a preceeding fragmentation (*P. bursaria*). Group II: Ma becomes fragmented in conjugants, with subsequent resorption of fragments in late exconjugant generations (*P. putrinum*, *P. aurelia*, *P. woodruffi* etc.). Group III: Ma is fragmented and resorbed in exconjugants (*P. caudatum*, *P. polycaryum* etc.). Macronuclear breakdown in many other ciliates is not preceded by its branching. It must be noted that in different species of *Paramecium* mates are separating at different stages of the nuclear reorganization.

The main stages of macronuclear breakdown in M race of *P. putrinum*, typical for the species, are shown in Fig. 18 A–O. The first signs of branching are noted after 14 h following cell union, in the late prophase Mi I. Further processes occur in a rapidly increasing speed, and numerous small fragments are seen at the end of conjugation. Unlike many ciliates with a "hemictic" Ma breakdown into several blocks of different size (e.g. in hypotrichs, suctoria, also in a predecessor of paramecia — *Frontonia*), macronuclear branching and fragmentation in *P. putrinum* follows a definite pattern and is not a "chaotic" disintegration.

Macronucleus undergoes an extreme stretching, assumes a C-like shape and looks finally like a net before its fragmentation. Macronuclear width along the nucleus is different at all stages of its elongation and branching; thick zones serve

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Fig. 14. Amphimixis. Anaphase Sk I to anaphase Sk II

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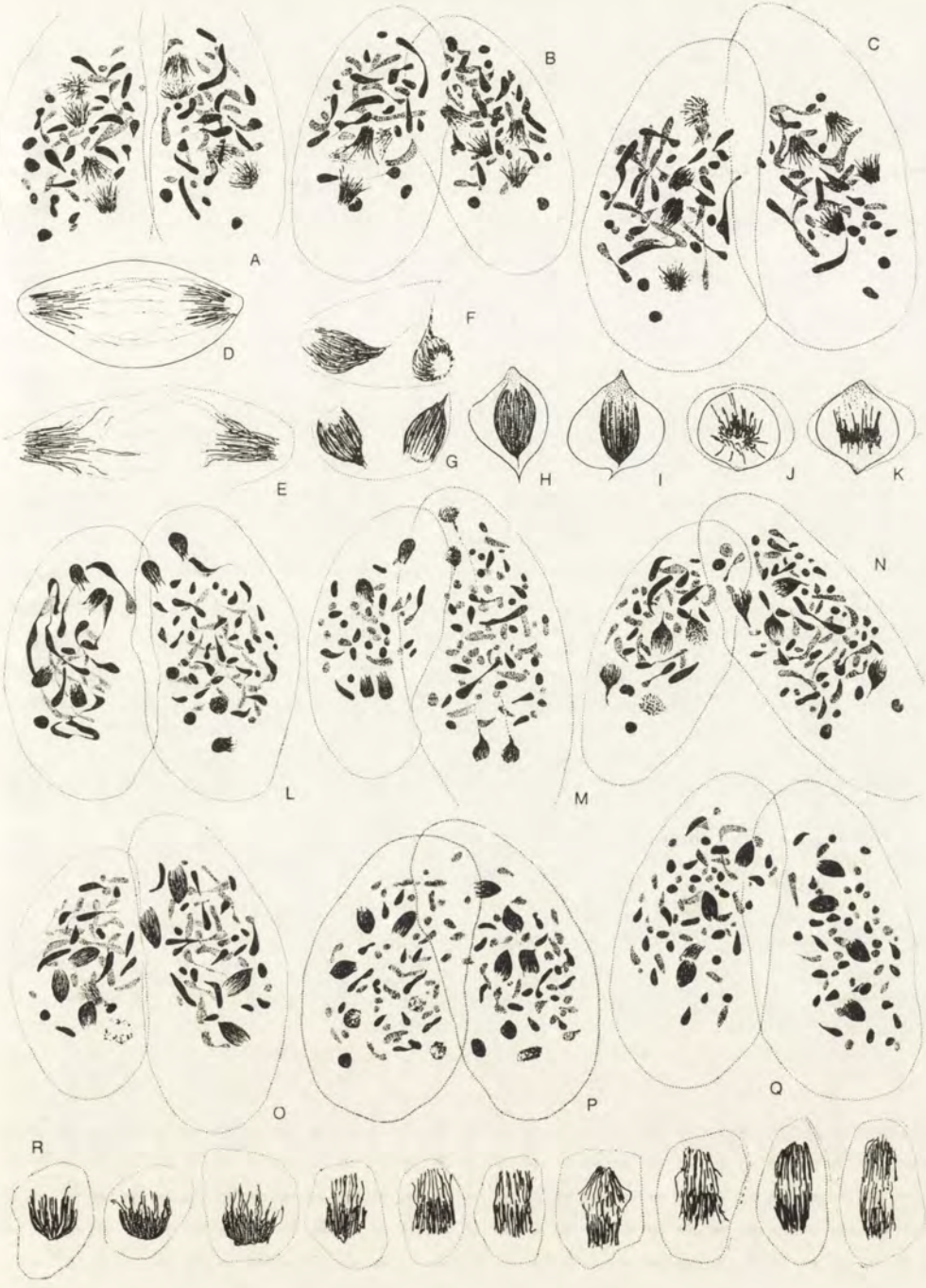

Fig. 15. Amphimixis. Anaphase Sk II to anaphase Sk III

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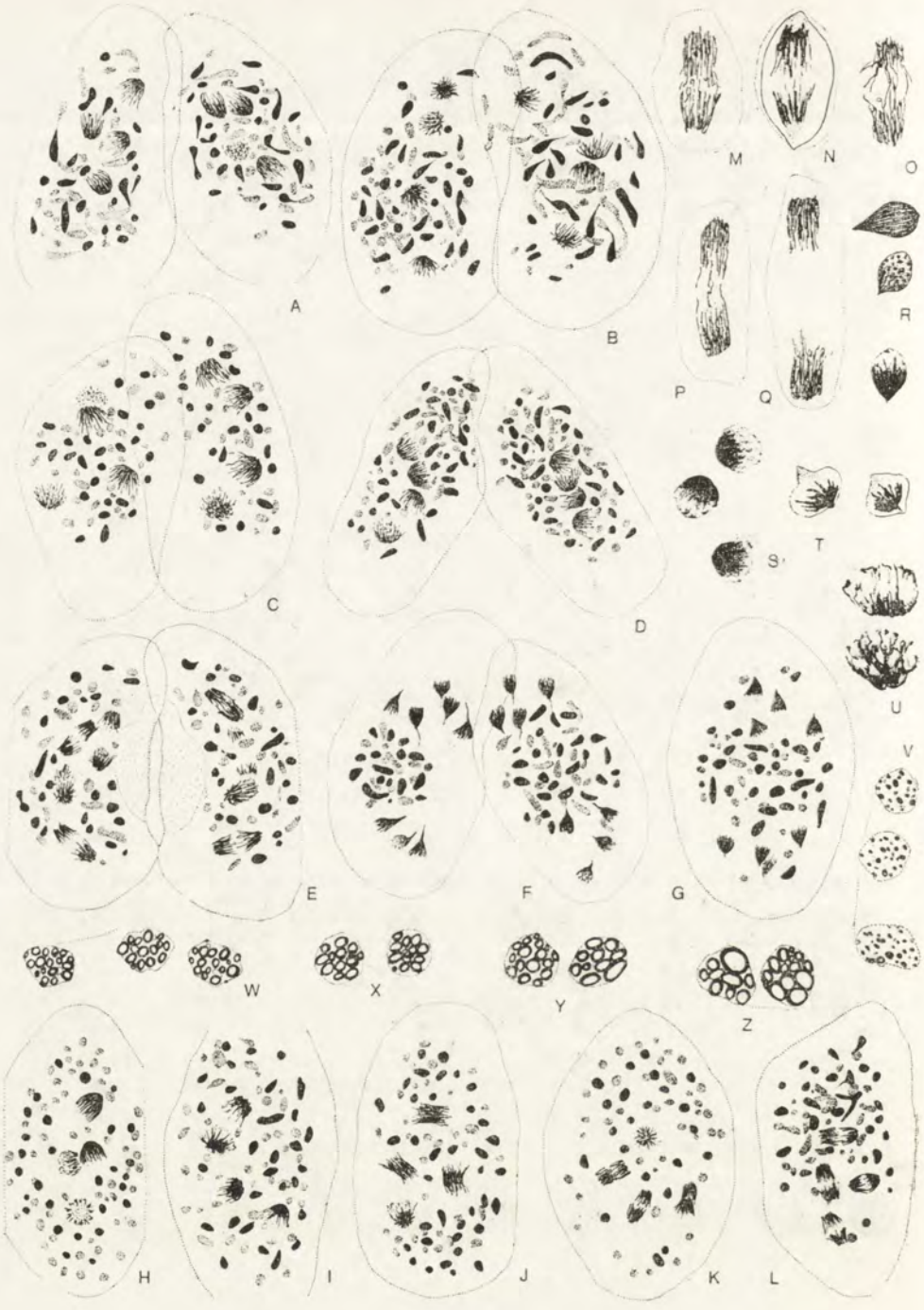

Fig. 16. Amphimixis. Anaphase to telophase Sk III, separation of mates (A–F); early exconjugants (G–L); last Sk fission and early anlagen (M–Z)

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as morphogenetic centers, producing new supplementary branches. Two such centers are reported at first; they gradually increase in number up to several dozens of centers encountered on final stages of conjugation. The narrowing and stretching of branches proceeds more rapidly outside these centers; after their extremal elongation (with width near 3 μ) they break away from the thickening zones. Their rupture takes place in the interphase following II Sk fission or in prophase Sk III; it proceeds more or less synchronously in the entire nuclear net. After the breaking of the net, its branches lose their acute shape and assume rod-like or ovoid outlines. The rods differ in both their length and width due to imperfect asynchrony of the rupture in different areas within the net. In sum, both small spherical bodies that accomplished their fragmentation and large branches continuing it are seen in mates prior to their separation. All fragmentation processes are stopped ultimately in exconjugants when RNA synthesis and nucleolar growth are initiated.

Estimation of the number of fragments in some races of *P. putrinum*, before I fission of an exconjugant, yields the following values: 52–81 (KS 20×YR 24); 59–104 (selfing line CRn); 70–112 (selfing line LH 24); 42–117 (OM 23×M 5); 61–117, commonly 64–96 (M 6×M 12); 63–122 (KS 12×SZ 7); 41–156 (LJ 1× ×LJ 4); and 65–182 (KS 1×KS 22). This indicates a significant individual and clonal variability in the number of fragments; a typical set includes near 60–120 ones. Such variation occurring in a single stage, may be explained by difference in micronuclear ploidy and in individual rates of caryorrhexy. Variation in the number of fragments occurs in other paramecia also, but there are some definite limits of variability for each species (Table 2); for example, the lower limit of that number in *P. putrinum* much exceeds that of *P. aurelia*, while the upper one exceeds that of all other species of the genus. There is, further, a definite correlation in the number and size of the fragments; they are large and scarce in *P. caudatum*, *P. calkinsi*, *P. aurelia* etc. and very small and abundant in *P. putrinum*. The number of fragments depends from the specific variation in caryorrhectic pattern: for example, in *P. calkinsi* macro-nuclear breakdown resembles hemixis and fragments are scarce; regular macronuclear branching and net formation preceeds its breakdown in *P. putrinum* and *P. woodruffi*, where fragments are more or less equal in size. The occurrence of the racial (clonal) variation in caryorrhectic pattern is noted below. The size of the fragments differs within the cell; this variation is lowest in *P. putrinum* (1–3 μ) and is more evident in other paramecia: 2–5 μ in *P. woodruffi*, 2–7 μ in *P. aurelia*, 3–9 μ in *P. multimicronucleatum*, 2–11 μ in *P. caudatum* and 3–16 μ in *P. calkinsi*.

Diller 1940, 1949 reported several cases of the interchange of macronuclear branches and fragments in conjugating *P. putrinum*, or their unilateral passing through the "cytoplasmic bridge". Such exchange was noted previously in *Collinia* (Collin, Schneider) and *Chilodonella* (MacDougall); its possibility in *Paramecium* was suggested by Sonneborn and Dippel. During the entire period of studies on *P. putrinum*, none case of such exchange was reported in my material. This process

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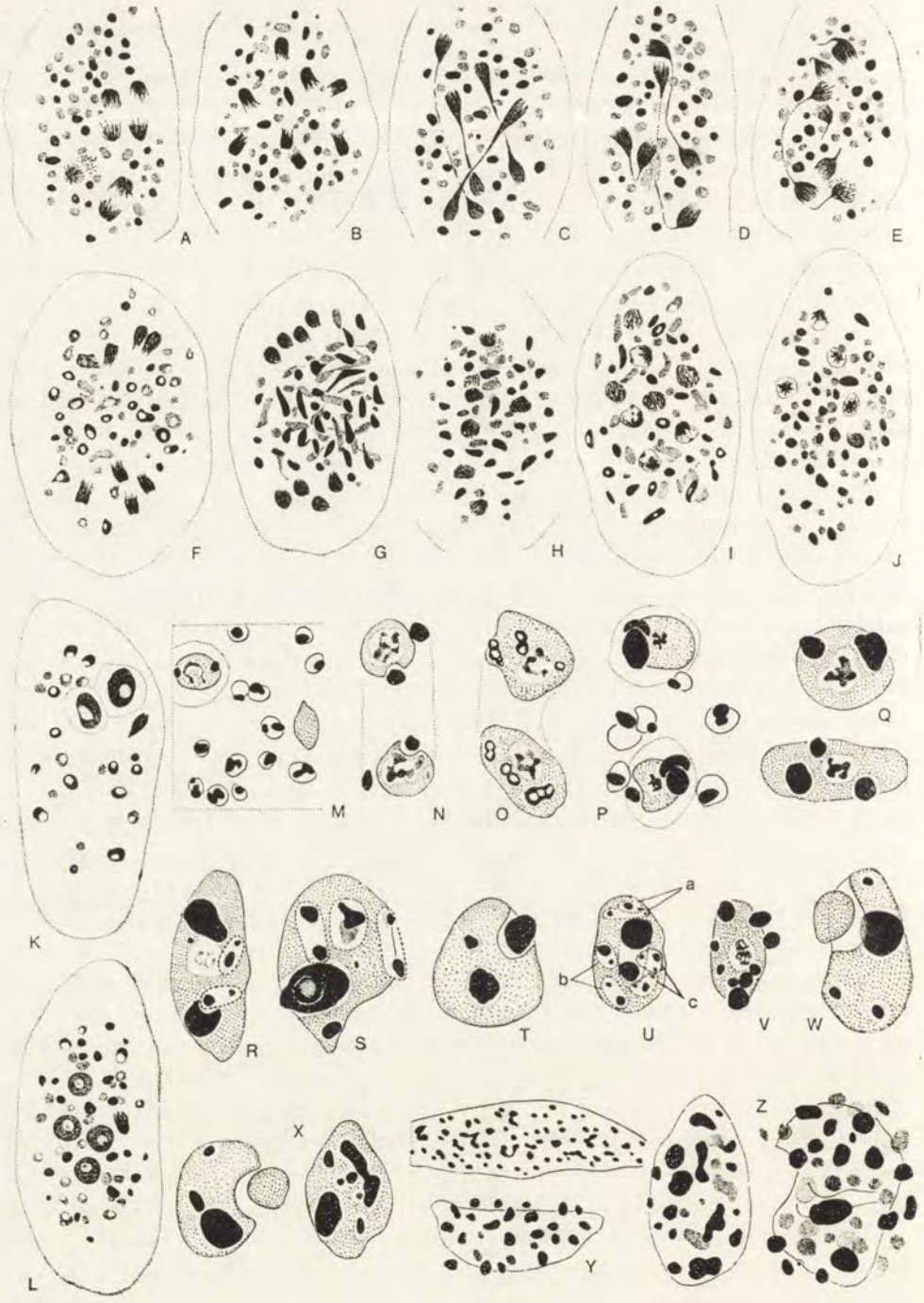

Fig. 17. Amphimixis. Last Sk fission and nuclear differentiation (A–L, Feulgen); nucleolar changes during nuclear reconstruction (M–Z, Unna). Note twin nucleoli in Fig. M–V

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Fig. 18. Amphimixis. Normal Ma breakdown (A–O); growth of Ma fragments at scarce feeding (P—R); delayed caryorrhexy (S–V)

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is impossible in *P. putrinum* since its Ma does not undergo the fragmentation in the moment of pronuclear exchange and cone rupture (Fig. 10, 11), and the hole that remains after the exchange is rapidly healed. The rise in temperature may lead to mass fusion of partners and formation of double H-shaped monsters in *P. putrinum*, usually with intermixing of their content through wide free contact area (Jankowski 1965 a), but this is simply a pathological phenomenon.

Various lines of *P. putrinum* reveal difference in correlation of Ma breakdown stages and nuclear divisions. For example, Ma becomes stretched in anaphase Mi I in a selfing line CRn and undergoes branching in a similar stage of VK lines. This indicates the existence of the racial difference in the rate of caryorrhexy. A significant number of lines of amphi- and automicts, but not apomicts, is characterized by a prolonged delay in Ma breakdown; Ma fails to undergo branching in some partners of these lines. This is abnormal instability of caryorrhectic pattern. A striking intraclonal variability of Ma breakdown is peculiar for many races of MT A and B; in some cells the breakdown proceeds with high or low speed as compared with the normal ones. This variation may be explained partly by discoordination of Ma breakdown and Mi fissions, and the difference in the rate of Mi divisions also. Figures 7–18 show many cases of asynchronous Ma breakdown in M 6×M 12 crosses; both mates within a pair differ in the rate of breakdown. In pairs with coinciding macronuclear stages, Mi may be seen in various stages of reorganization chain. For example, in the phase of pronuclear interchange, Ma may be rounded, elongated, branching and sometimes even fragmenting; in the other case, rounded Ma may be seen in pairs from the very beginning to nearly the end of conjugation. Race M, otherwise normal, differs in this respect from most other races of the species, and the mirror-like Ma breakdown is generally peculiar for *P. putrinum* as well as a definite correlation of Ma and Mi cycles. Intermixotype crosses seem to induce a great delay in Ma breakdown of the normal partner, although an automictic Mi cycle proceeds normally in both partners and conjugation is not pathological.

Nucleoli in dividing, fragmenting and regenerating Ma

A continuous extrusion of numerous large (4–6 μ) Nc is peculiar for interkinetic Ma of *P. putrinum*. This process is stopped in the really prophase of vegetative mitosis; the number of Nc become raised in elongating Ma parallel to decrease in their size up to 1.5–2 μ (Fig. 19 H–K). This indicates the beginning fragmentation of large Nc. Many Nc show a distinct bilobed or dumb-bell shape indicating their division. After the maximal stretching of the Ma, its Nc become scattered throughout the nucleus and stop fragmentation. Their rapid growth and extrusion into the cytoplasm is restored immediately after completion of cell division.

A similar dynamics of Nc was found in the branching Ma of conjugating cells, with a single difference: fragmentation proceeds until Nc will reach their minimal dimensions (near 1 μ) in the stage of I Sk fission. They reveal no further changes until cell separation (Fig. 19 L–Q). Nucleolar growth and formation of new Nc

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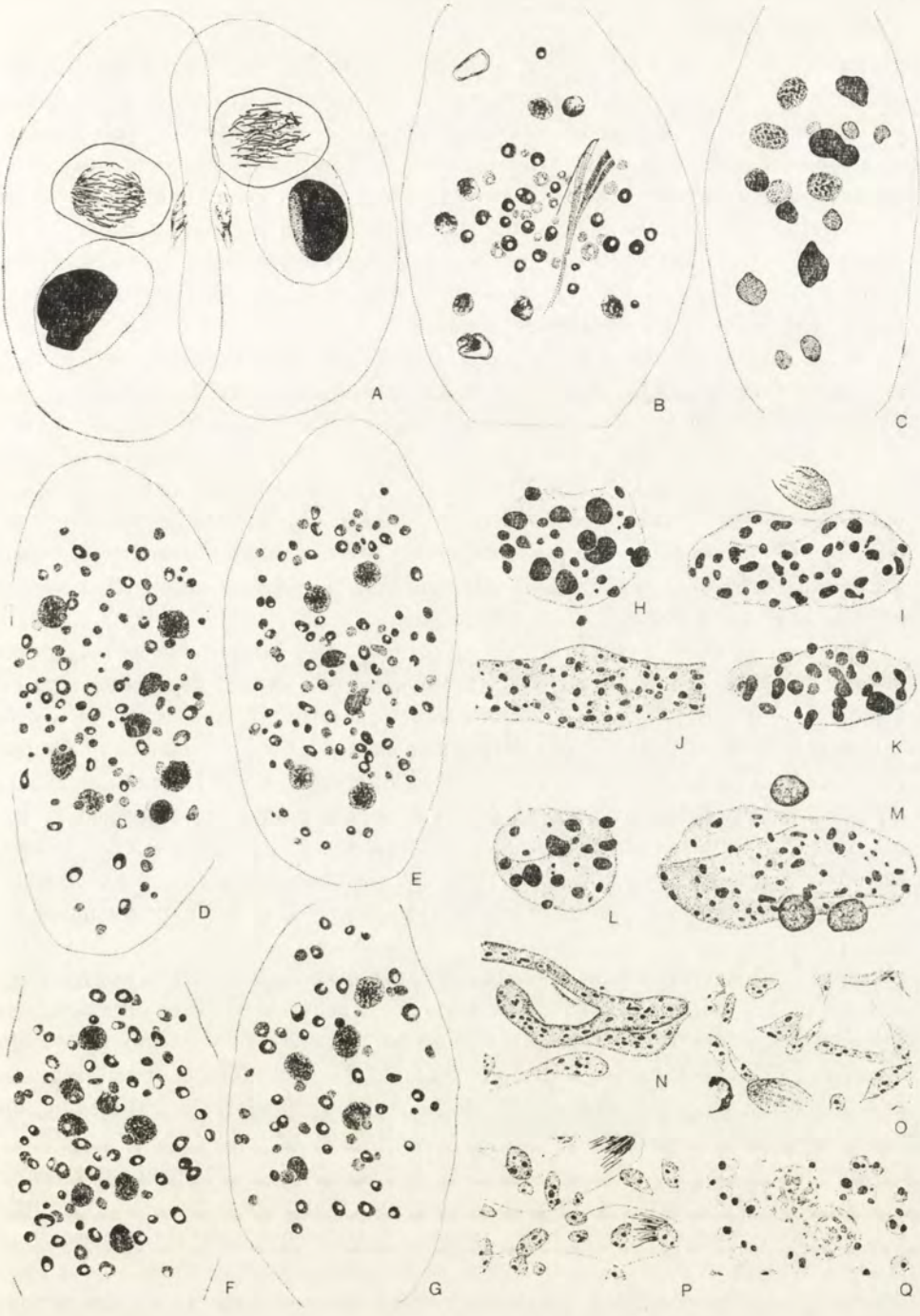

Fig. 19. Amphimixis. Buccal dedifferentiation and reappearance (A, B); Ma fragments after delayed breakdown (C); variation in the number of fragments (D–G); nucleoli in vegetative dividers and conjugants (H–Q, Unna)

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are restored with an extreme intensity after a complete Ma breakdown and disjoining of mates; like in dividers, initiation of RNA synthesis in mates is determined by cell separation. In pathological cases (formation of monsters by vegetative dividers or delay in separation of mates, with their possible subsequent fusion) Nc formation is invariably restored again, indicating removal of a blocking factor. Nearly all Ma fragments in exconjugants after their disjoining contain one, rarely two large Nc; their extrusion becomes common. After a great delay, due to its breakdown and blockage of RNA synthesis in conjugants, Ma restores its vegetative function in spite of its fragmented condition.

Nucleolar dimensions in exconjugant remain more or less constant only at their abundant food supply; when food is scarce, fragments are enlarged from 2–4 to 8–9 μ (Fig. 18 P–R). Since both chromatine zone and Nc are enlarged, there is a reason to suppose the existence of endomitosis in Ma fragments under these unfavourable conditions. Possibly the block of DNA synthesis in the old Ma is weakened in cells that receive scarce food. This "incomplete Ma regeneration" resembles the normal Ma regeneration occurring in cells that lost or inactivated their Mi derivates. In spite of their temporary growth, Nc become ultimately resorbed after several cell fissions.

Macronuclear regeneration is rare in normal culture conditions (it may occur in amicronucleate exconjugants or in those with pathological degeneration of all Mi products); this process may be induced experimentaly by destruction of Sk derivates by heating (Sonneborn) or, in our material, by X-irradiation in the late prophase Mi I (Kovaleva and Jankowski 1965, 1966 a, 1966 b). Radiation evokes the disturbance of Mi divisions without any visible changes in macronuclear behaviour; Ma fragments undergo a rapid increase in size and an active RNA synthesis, extruding a number of large Nc. The fragments become greatly enlarged even when the cell receives one or more abnormal Mi products — chromatine condensate of various size and shape, incapable for division and development and blocking cell division. Such cell die out in the culture in spite of the great activity of Ma fragments and endomitoses within these. Only those cells, that receive no deleterious Mi products, can survive after rare and occasional division of exconjugants; Ma fragments in these cells are distributed parallel to their subsequent cell divisions and undergo a great increase in size. Regenerating cells show normal feeding, division and conjugation.

As indicated above, Ma fragments are not resorbed in conjugants and exconjugants after cell separation; they are segregated among the progeny until a single fragment will remain in a cell; its resorption is initiated now. The entire segregation process requires at least 4–5 cell fissions. Contrary to the widespread, though not universally accepted opinion, fragments remain functional during all this period; RNA synthesis must occur with a high rate, judging from the common occurrence of the extrusion of large Nc. The fragment of Ma may be called a "mini-macronucleus" or "macronucleus in miniature" since Ma continues its RNA and partial

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DNA syntheses in its fragmented state. The tiny and numerous fragments of Ma in *P. putrinum*, its "mini-macronuclei", approach most closely Sonneborn's notion of "subnuclei" unlike giant scarce fragments in *P. aurelia* or *P. calkinsi*, but it is not clear now how these subnuclei maintain their integrity within the vegetative Ma.

Development of macronuclear anlagen

According to Raikov 1967, Ma development in ciliates includes four main stages: I — reappearance of distinct chromosomes; II — endomitosis, with possibility of polytenization; III — despiralization of chromosomes, the origin of a caryosome (caryosphere); an anlage becomes vesicular and looks achromatine; IV — Feulgen-positive stage, restoration of endomitosis and appearance of nucleoli; anlagen structure coincides now with that of the Ma. Paramecia lack II stage; caryosome appear at the earliest stages of the process from heterochromatine parts of chromosomes (Klitzke, Gromova, Egelhaaf, Saito et al.). Unlike many other ciliates, e.g., *Bursaria* or *Ephelota*, no structural evidence of endomitotic polyploidization was found in the anlagen of *Paramecium*.

Saito and Sato 1961 subdivided the process of Ma development in *P. caudatum* into five stages: I — formation of heterochromatine aggregates; II — their disintegration; III — "nucleolar" (caryosomal) stage; IV — "achromatine" stage and swelling of anlagen, and V — growth stage. Paired filiform chromosomes were detected by these authors at all stages of the growth including "structureless achromatine" one. After completion of a postgamic phase, the ends of chromosomes undergo swelling within the anlagen, giving rise to near ten rings made by fusion of heterochromatine parts of chromosomes. Chromatine filaments connected with such rings were figured by Klitzke also. An intranuclear caryosome appears after the gradual disappearance of the rings; an extreme swelling of an anlage leads to its seeming "dechromatization" and is followed by nucleolar formation, endomitosis and nuclear growth.

Contrary to these data, chromosomes were observed only in the earliest anlagen of *P. caudatum* (Puytorac et Blanc) and were not reported at all in *P. aurelia* (Jurand et al.). DNA synthesis was not linked by these authors with any structures of anlagen.

Ma development in *P. putrinum* proceeds in identical way in all studied races irrespective of their nuclear reorganization pattern (Fig. 16 R–Z, 17 M–Z, 20 A–U); the process differs greatly from that described in all other paramecia, and seems to include some stages not appearing in other ciliates. Eight nuclei, approaching body ends in telophase Sk III, are not morphologically distinguishable; they are scattered rapidly throughout the cell due to cytoplasmic currents. Interphase nuclei, undergoing diminution in size from 7–7.5 × 3–3.5 μ to 5.5 × 3.5 μ, look like clear spherules with a number of tangled chromosomes. They differ structurally from the interphase nuclei of conjugants — ovoid bodies composed of long parallel closely shifted chromosomes and wide dentate anterior pole bearing a light green cup. At their differ-

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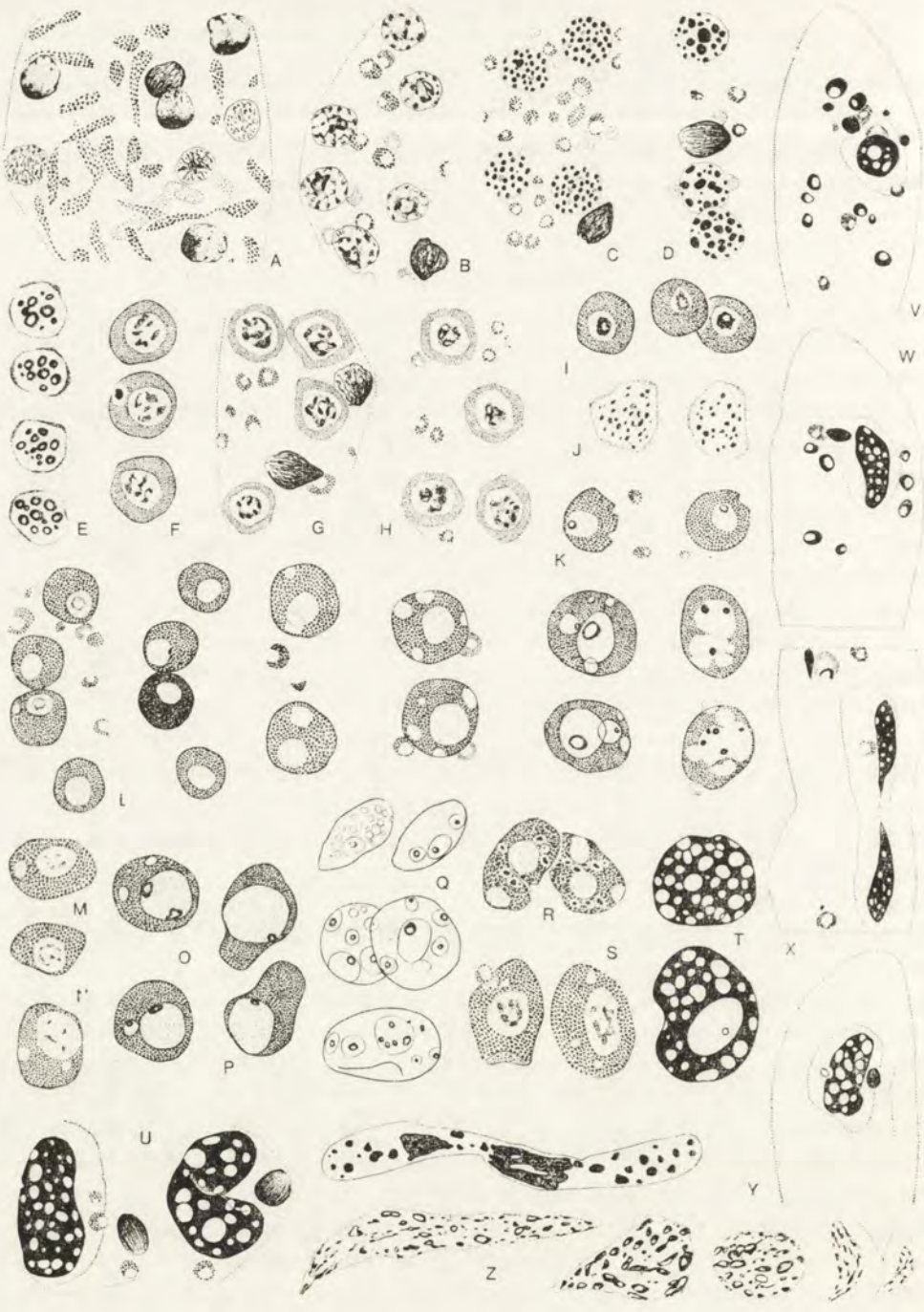

Fig. 20. Amphimixis. Growth of anlagen (A–U); III fission of exconjugant (V–X); vegetative cell (Y); fragmenting anlagen of reconjugants (Z)

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rentiation, four nuclei retain their peculiar spherical shape, and four other ones resemble Mi in the early I meioticp rophase: chromatine is concentrated at one of the nuclear poles, and the other one bears several chromatine clumps (Fig. 20 A). Three clear nuclei (from four) are stained more and more weakly and ultimately disappear; the fourth one becomes condensed and looks like a typical vegetative Mi, with its parallel chromosomes. It was not established, whether the anterior or posterior nuclei give rise to Ma anlagen in *P. putrinum;* the data of the previous authors indicate both possibilities — anterior nuclei in *Dogielella* (=*Cepedella*?) and *Tetrahymena* (Poljansky, Ray) and posterior ones in *P. aurelia* and *P. calkinsi* (Sonneborn, Nakata).

Dense regular chromatine clumps of somewhat different size are evenly distributed throughout the small early anlage measuring 5–6 μ only (Fig. 20 C). The hemiploid set of LJ 2×LJ 12 lines, used here for description of Ma development, includes near 70 chromosomes (62, 67) and euploid one near 130–140 chromosomes (116, 129, 134); unexpectedly, the anlage contains 20–30 clumps only. Their ring-like structure is sometimes evident. No filiform chromosomes were detected at all stages of Ma development. Chromatine clumps or rings start to increase in size — possibly by their fusion, since their number is decreased (Fig. 20 B–D). Their ring-like structure and size difference within a single anlage become gradually more evident. The rings seem to represent a transversal optical section through ellipsoid or spherical DNA-rich structures. At the early stage of development, during the origin and growth of these rings, anlage increases slightly from 5–6 to 6–7 μ only.

The next stage of Ma development appears suddenly: thin layer of clear cortical chromatine composed of very small granules becomes visible at the periphery of anlage; the rings, that were not interconnected previously, are replaced now by thick clear chromatine ribbons (Fig. 20 E–G). The anlage increases in size to 8–9.5 μ. This is a very rapid, transient and relatively rare stage. Chromosomal ribbons seem to be linked into a single net of unequal width. Some likeness of this net and polytenic net in spirotrichs must not be denied.

The peripheral layer with its "chromatine dust" becomes more and more thick, growing inside the nucleus that shows no distinct increase in size in this period; there is a simultaneous growth from all the surface of anlage. Small central condensate persists after the completion of this stage. Preparations leave an impression, that peripheral stripe is thickened due to some enigmatic process of a structural convertion of the chromatine of internal ribbons. Once again, some resemblance of this stage with the process of a sudden replacement of giant polytenic chromosomes by smallest chromatine grains, typical for definitive Ma in hypotrichs and heterotrichs, must be noted. Temporary polytenization of anlagen is not excluded in *P. putrinum.*

Very small "dusty" chromatine grains are seen at first in a new peripheral chromatine layer in the earliest stage of the reconstruction period; the inward extension of this layer is paralleled by the gradual increase of the granules. They are distinct

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and easily recognizable now at small magnifications (Fig. 20 I–K). After completion of the reconstruction phase, chromatine of the anlage does not differ more from that of a definite Ma and Ma fragments. First Nc appear in the beginning of a mentioned phase. The anlage, still spherical (6–6.5 μ) starts the slow increase in size and stains more and more intensively. A new granular chromatine does not fill all the anlage; small central hole with a remnant of heterochromatine ribbon remains within the nucleus; it gives negative RNA test and weakly positive Feulgen staining. The end of the reconstruction phase signifies the beginning of a period of an intensive DNA synthesis and gradual growth of anlage. Unequal intensity of DNA staining of different anlagen within the cell, rare case in ciliates, occurs in some exceptional exconjugants of *P. putrinum*; as a rule, all anlagen coincide in their staining intensity. The staining of a compact growing anlage of *P. putrinum* is so intensive, that separate chromatine granules are revealed with an increasing difficulty (in a schematized Fig. 20 this increase is not reflected for demonstration of anlagen structure).

All four anlagen are still spherical prior to I division of an exconjugant, and start their gradual elongation after that fission. After II cell fission, when single anlage remains within the cell, it differs from the definitive Ma by its more rounded shape and less voluminous size (Fig. 20 T). It has a normal structure and outlines during all future cell fissions, when segregation of Ma fragments is continued. The rudiment of a ribbon net — clear hole with chromatine ring — remains distinct up to II division of exconjugants, but is not more discernible among numerous nucleoli of an intensively staining Ma. A series of supplementary small DNA-containing rings, possibly nucleolar organizers, may be seen in the growing anlage at the late stages of its growth.

RNA synthesis in anlagen was studied in exconjugants of M race (M 6×M 12, Fig. 17 M–Z). Nc are absent during early stages of anlage development, when large heterochromatine clump in polarized nuclei or numerous clumps and rings are seen; moreover, Nc are absent in the moment of the origin of a "reconstructive" peripheral zone. Quite similarly, Nc are absent at polytenization stages of chilodonelles and spirotrichs. Nc appear during the period of the growth of this zone (a period of anlage reconstruction), when DNA synthesis begins judging from the increasing intensity of the nuclear staining. It seems probable that in anlagen, unlike Ma fragments, both RNA synthesis and DNA accumulation after reconstruction period are started simultaneously, while in fragments RNA synthesis preceeds that of DNA and usually takes place in the absence of the latter one. Reconstructed anlagen contain at least 2 Nc (Fig. 17 M, N); new Nc appear parallel to the growth of primary ones. Like in *P. bursaria* (Egelhaaf), twin Nc are common in *P. putrinum;* they are outlined on Fig. 17 S, U. Nucleolar extrusion is still rare. Nc appear within a new granular anlagen zone, possessing the structure of a normal vegetative Ma, without visible link with a central zone and its heterochromatine remnant. As a rule, Nc are not very numerous, but may reach a large size before the first

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fission of anlage (Fig. 17 Q–X). This fission proceeds just like in vegetative dividers, with nucleolar fragmentation and distribution throughout the entire Ma. Nc are not numerous and are initially very small in daughter nuclei (Fig. 17 Y, Z).

The structure of Ma anlagen was studied in reconjugants also (Fig. 20 Z), since they do not undergo degeneration and, moreover, are copying the behaviour of the normal Ma, stretching and breaking up into a number of small fragments. Many chromatine clumps and rings of different size and shape, and sometimes large chromatine bodies are seen within such anlagen. Thin filiform chromosomes of the usual type were not detected.

Whether there exists any connection between the resorption of fragments and growth of Ma anlagen? According to MacDonald, DNA from an old Ma of *Tetrahymena* may be incorporated into growing anlagen of two new Ma, when exconjugant receives scarce feeding. Seshachar, Dass, Bhandary et al. admit the possibility of such phenomenon at normal cultural conditions also. It must be recalled that in the species studied by these authors (*Blepharisma*, *Frontonia*, *Tetrahymena*) an old Ma is resorbed without fragmentation long before the moment of convertion of anlagen into a definite Ma (at cell fission). These two processes do not coincide in time in *P. putrinum*, where anlagen become definitive Ma prior to III cell fission, and fragments are resorbed after six or seven cell divisions. The possibility of incorporation of DNA from the resorbing fragments by growing anlagen is thus entirely excluded in *P. putrinum*. According to Raikov 1967, such incorporation is generally doubtful.

Ma fragments start to extrude large Nc after separation of partners. All cytoplasmic DNA must be obtained at first from the fragments of an old Ma, since Nc formation within four anlagen will begin before the I fission of exconjugant only. After this fission, anlagen contain large Nc, but they are rarely extruded into the cytoplasm; in this period Ma fragments remain the main source of cytoplasmic RNA, and smaller quantity of RNA is produced by growing anlagen. After II cell fission, when anlagen are structurally similar to the definitive Ma, both RNA sources (all Ma fragments and single large anlage) are active still and seem to extrude an equal quantity of RNA. Further fission result in decrease of the number of fragments per cell, with corresponding decrease of their synthetic significance. Thus old RNA is equaled by a new one and is ultimately replaced by new RNA in VI–VII and further cell generations of exconjugant. This duplicity of RNA may result in prevailing of the old phenotype traits in the early progeny of exconjugant.

In sum, several distinct stages of anlage development were recognized in *P. putrinum* (without an attempt of correlation of these stages with those of Raikov, Saito et al.): I — polarization of telophase nuclei; II — appearance and equal distribution of chromatine clumps; III — the growth of ring-like structures and descrease in their number; IV — the sudden origin of a "dusty" peripheral chromatine layer and replacement of rings by supposedly polytenic ribbons; V — the inward extension of peripheral zone and convertion of ribbons into chromatine

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granules of usual type; VI — the end of conversion, with conservation of a central remnant area; restoration of RNA synthesis; VII — gradual growth of anlage, endomitosis and an intensive RNA synthesis with nucleolar extrusion. This unusual process differs significantly from that in all other paramecia; for example, single chromatine aggregate and no rings are formed in *P. bursaria*, achromatine stage occurs in *P. caudatum* etc. Ma development in various paramecia may follow their specific patterns. "Reconstruction" phase found in *P. putrinum* (stages IV–VI) seems to be lacking in all other ciliates.

Following Saito and Sato, the rings in *P. putrinum* may be regarded to as heterochromatine clumps. Since they are abundant in *P. putrinum* (unlike the anlage of *P. caudatum*), its nuclear set must include many additional B-chromosomes composed by heterochromatine or there must be extensive zones of heterochromatine in euchromosomes.

The functional significance of macronuclear fragmentation

Several types of correlation of the nuclear behaviour and the number of Ma anlagen may be recognized in ciliates.

Pattern I: breakdown of Ma and numerous anlagen. This is a commonest pattern, e.g., in most peritrichs where seven anlagen are usually produced; old Ma breaks up after its regular branching (Rosenberg, Popoff, Brouardel, Müggе et al.). Examples from other orders are *Bursaria* (Poljansky), *Frontonia* (Bütschli, Vimala Devi), "*Parachaenia*" — *Ancistrocoma* (Kofoid and Bush). *Paramecium putrinum*, *P. woodruffi*, *P. caudatum*, *P. multimicronucleatum* and *P. africanum* belong to this group. At least four anlagen are produced in all these species; they undergo a slow increase in size demanding at least two segregating fissions. Ma fragments are distributed among the daughter cells and serve as RNA source until the end of the growth and segregation of anlagen.

Pattern II: breakdown of Ma and two anlagen. This combination is more rare; it was encountered in *P. aurelia*, *P. calkinsi*, *P. jenningsi* and some other ciliates.

Pattern III: no fragmentation of Ma and two anlagen. Common condition in *Spirostomum*, *Climacostomum*, *Colpidium*, *Dogielella*, *Tetrahymena*, *P. bursaria* etc. (Maupas, Bütschli, Devidè, Poljansky, Seshachar, Ray et al.). The anlagen become definitive Ma after I cell fission and thus must grow rapidly.

Pattern IV: no fragmentation and one anlage. Usual condition in many orders, e.g., in various hypostomes (*Chilodonella*), hymenostomes (*Loxocephalus* of *L. luridus* type), suctoria (MacDougall, Tuffrau, Grell et al.).

Pattern V: fragmentation of Ma and one anlage. Relatively usual pattern, peculiar mainly for spirotrichs (*Euplotes*, *Stylonychia*, *Metopus*, *Nyctotherus*, *Brachonella*), for various suctoria, some hypostomes, chonotrichs etc. (Grell, Kormos Collin, Noland et al.). Only *Nyctotherus* possesses a regular Ma branching before its fragmentation, like in *P. putrinum;* in other forms Ma breakdown resembles

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hemixis — simple disintegration of the nucleus into several amorphous blocks of varying size.

Pattern VI: no fragmentation and numerous anlagen. Atypical pattern occurring in some hymenostomes (*Dexiotricha*, *Cyclidium*) and astomes, where seven anlagen are usually produced.

The review presented above reveals no strict correlation between Ma behaviour and anlagen number, but one can see a distinct tendency to the occurrence of Ma breakdown in species with 2, 4 or more anlagen and its lack in those with 1–2 anlagen. Some atypical patterns (e.g., in group VI) occur in a limited number of phylogenetically related orders. Large number of anlagen is linked usually with complicated branching of Ma, while low number is accompanied with its "hemictic" breakdown.

With rare exceptions (Seshachar et al.), Ma fragmentation is regarded as an evidence of degeneration of an old Ma before the development of a new one (Mügge, Diller, Vimala Devi, Alonso, Pèrez-Silva, Guilcher et al.). Possibility of Ma regeneration from its fragments does not contradicts, in fact, the degeneration hypothesis since fragments may be regarded as potentially functional. In suctoria, according to Guilcher, these "unnecessary" fragments may be extruded through tentacles. Willis 1948 formulated the degeneration hypothesis in the most deciding way. According to Willis, Ma of *Legenophrys tattersalli* becomes a necrotic nucleus in the early stage of conjugation; this is evident from its breakdown. Achromatine cavities and vesicules, appearing in Ma fragments, indicate a pycnosis preceeding an ultimate disintegration of fragments. The chromatine of fragments was designated as "necrochromatine".

Sole significance of fragmentation is evident when some fragments are fused with a new anlage, rising its ploidy by such unusual way (Bütschli, Turner, Kidder, Rao); however, most recent authors deny the possibility of such fusion (Katashima, Wichterman, Diller, Siegel, Heckmann et al.).

It is quite evident, in the case of *P. putrinum* and other paramecia, that the fragments of an old Ma are actively functioning structures capable for the synthesis of both RNA and DNA. The "achromatine cavities" in "necrotic Ma", mentioned by Willis, are RNA-nucleoli. Since the growth and segregation of Ma anlagen is slow and requires a series of cell fissions, fragments remain the principal site of RNA synthesis and extrusion. I have suggested that fragmentation is needed for supply of daughter cells with RNA source at every division of an exconjugant. Willis had not studied RNA dynamics and proposed an erroneous "necrochromatine" explanation of structural changes in Ma.

Since fragmentation serves for supplying the nearest exconjugant progeny with RNA source, it occurs mainly in species with large number of anlagen (pattern I). Those with two anlagen make an intermediate group (patterns II and III). In both last cases, anlagen become definitive Ma after the I cell fission, and thus no RNA source (and correspondingly no fragmentation) is needed in exconjugants. The

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species with pattern II have derived from paramecia possessing four anlagen (*P. woodruffi*); it seems possible that they simply inherited an ancestral trait (fragmentation) without correlation with modified (reduced) number of anlagen.

Pattern IV is simply a replacement of an old Ma by new one before I fission of exconjugant. Ma remains functional in all cases, including the entire (fragmentation, hemictic breakdown and simple "surviving" without any visible changes (e.g., old Ma continues the nucleolar extrusion in exconjugants of *P. bursaria*, group III). In a definite moment, possibly at the beginning of stomatogenesis, in toto resorption of an old Ma is initiated.

Pattern V is a similar replacement of an old Ma with a new one, but with its preliminary breakdown before resorption. May be, this is the sole case when caryorrhexy indicates the nuclear destruction.

Pattern VI needs a special cytological study: peculiarities of RNA synthesis in Ma anlagen must be investigated in each species of the group. Irrespective of the large number of anlagen, Ma is resorbed in toto, without distribution of its parts between the daughters. There are some data (Russo, Dain, Lucas, Behrend et al.) on the intensive RNA synthesis in anlagen of these groups (e.g. in *Dexiotricha* and *Cryptochilum*) — a process that makes unnecessary Ma breakdown and segregation.

Concluding remarks

The scheme of the amphimictic nuclear reorganization in *P. putrinum* differs from those of other paramecia and, paradoxally, most significantly from the most related species, *P. bursaria*. Only *P. putrinum* among paramecia lacks the crescent stage in prophase Mi I and possesses a parachute stage; unlike *P. bursaria*, none daughter nucleus degenerates after I Mi fission; unlike *P. caudatum*, *P. woodruffi*, *P. jenningsi*, *P. calkinsi* and many races of *P. bursaria*, there is no degeneration of one daughter nucleus after I Sk fission. 4 anlagen are produced in *P. putrinum* instead of 2 in *P. aurelia*, *P. bursaria*, 4 of *P. caudatum* and *P. woodruffi*, and 8 of *P. africanum*. Ma undergoes branching and fragmentation from the beginning of conjugation, while it may undergo breakdown after cell separation (*P. polycaryum*) or remains intact in *P. bursaria*. Unlike *P. caudatum*, partners of *P. putrinum* disjoin only after completion of postgamic phase. There are many other differences. In its general scheme, amphimictic conjugation in *P. putrinum*, including both Ma and Mi behaviour and separation stage, coincides most closely with that of *P. woodruffi*, belonging to another subgenus (*Cypreostoma*) derived from *Helianter*.

The knowledge of the schemes and details of the nuclear reorganization processes is extremely significant at present for studies in *Paramecium* taxonomy. None of nine species studied still in respect to conjugation has coinciding dynamics of Ma and Mi, taken in their unity. The entire identity of amphimixis in "*P. trichium*" of Diller and *P. putrinum* of my material is thus a significant evidence of their synonymy.

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Highly endemic ciliate fauna was discovered recently in warm and tropical countries, including many new paramecia — *P. jenningsi*, *P. pseudotrichium*, *P. africanum*, *P. ugandae*, *P. bursaria*-like species with giant cell size and two small Mi and all these, together with Doroszewski's *P. arcticum*, must be subjected to detailed cytological analysis of conjugation. The main attention must be directed on the *P. arcticum* — *P. woodruffi*, *P. jenningsi* — *P. wichtermani* — *P. ugandae*, *P. multimicronucleatum* — *P. africanum* and *P. polycaryum* — *P. pseudotrichium* complexes, requiring more decisive separation.

VI. Automictic conjugation

Introductory remarks

P. putrinum lacks the natural autogamy although it was admitted by Diller 1957. However, the lines with unique automictic conjugation are common in nearly 1/3 of isolated races of the species. Nuclear dynamics coincides entirely in both stable and selfing partners; superficially, this is a typical conjugation with cell separation after near 23 h following their mixture. Nuclear reorganization is described and illustrated below on LJ 2×LJ 17 lines (Sg 2, MT C).

Only one kind of automixis was recognized still in the species; it was believed to be linked with apomixis I (Jankowski 1962 a). In many pairs of MT B and C, coming from both intercrosses and intraclonal mating, two mates within a pair showed a distinct difference in their reorganization processes; it was supposed that Mi divides at first in one partner, failing to divide in another one; the next nuclear fission proceeds synchronously, involving two hemiploid nuclei in automict and single large belated nucleus (Mi in the late prophase) of apomict. The following nuclear sequence in a presumed apomictic partner was considered to be identical with apomixis I, described below. A thorough cytological restudy of meiosis in all mixotypes, made after 1965, revealed the linkage of another processes — automixis I+automixis II and not automixis I+apomixis I. First nuclear division occurs synchronously in both mates, but its products are joined again in automixis II.

Automixis I includes 2 meiotic Mi fissions, caryogamy and 3 Sk fissions; automixis II includes the first Mi fission, caryogamy and 4 Sk fissions. Three types of pairs are invariably found on the slides: those with automixis I in both cells, with automixis I and II in each cell, and with automixis II in both cells. Two kinds of automixis are quite alike at most their stages, except for meiotic one, and due to their constant linkage they will be described together (Fig. 21–30; Pl. VIII–XI).

Nuclear events at automixis I and II

The I Mi fission in automicts, still not separable into two categories, proceeds just like in amphimictic partners of other races. After cell union, Mi is detached from Ma and becomes polarized, entering a prolonged prophase Mi I (Fig. 21).

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Fig. 21. Automictic conjugation, LJ 2×LJ 17, Sg 2, MT C, Böhmer and Feulgen. I meiotic fission

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Fig. 22. Automixis. Anaphase Mi I to prophase Mi II

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The nucleus is passing through the parachute stage, typical for the species, when the main part of chromatine clumps is aggregated near one of the nuclear poles Slow nuclear swelling begins; polarized Mi reaches 11×10 μ in size. Mi becomes flattened and somewhat condensed at its further differentiation, assuming peculiar leaf-like outlines. Polarization gradually disappears, and small weakly stainable chromatine clumps become evenly scattered throughout the nucleus. 1–2 vacuoles may be noted sometimes within the nucleus. The clumps seem to represent the optical sections of bended chromatine ribbons filling the entire nucleus.

The next stage of the growth may be called "chromosomal": we deal here with distinct chromosomes and not with clumps and ribbons of unclear nature. Thin tangled chromosomes are revealed; at further enlargement of the nucleus they look as points or commas and not like spherules on their optical sections. A leaf-like Mi attains the size near 10×5–6 μ, "chromosomal" one near 15×7 μ in the beginning of its growth and up to 18×10 μ in the late prophase Mi I. Chromosomes are located more freely in the growing Mi. They undergo a sudden thickening in the late prophase Mi I, fusing possibly into bivalents (Fig. 21 Q–S). Thick bivalents are much less numerous than thin chromosomes of preceeding stages; paired nature of bivalents is not evident at first. Metaphase stage is missed, and anaphase groups are formed asynchronously. The active formation of dyads, long paired chromosomes, take place in the early anaphase Mi I when the formation of two daughter groups is initiated (Fig. 21 T, U; 22 F–H). Thick chromosomes are recognizable still in mid-anaphase Mi I, but only thin ones, possibly paired, are seen within a stretching spindle (Fig. 22 J). The spindle is directed diagonally; one large daughter nucleus enters the cone, and other one remains deeply within the body (Fig. 21, 22).

Telophase nuclei are not condensed; like in typical meiosis, no true interphase follows, and both interkinetic nuclei enter soon a prophase Mi II stage (Fig. 23 A–C). Telophase chromosomes of I meiotic spindle are located parallel one to another and may be easily counted on optical sections since they are not too closely shifted together; Fig. 22 O–Q show 116, 129 and 134 chromosomes (some of these may be outside the optical section). Small nuclei produced after meiosis in automicts I contain 62, 66 and 67 chromosomes, surely a hemiploid set (Fig. 25 M, N). The chromosomes in telophase Mi I and interkinesis must be at least partly interconnected. Prophase nuclei resulting from I Mi fission are much larger than those of II and III Mi divisions.

Further course of the nuclear events depends entirely on the fate of the prophase nucleus located outside of the cone. Nuclear reorganization will follow automictic I pattern, if this nucleus will not change its position; it will follow an alternative way, if this nucleus will stick to another one located within a cone. Since interkinesis is abbreviated at this stage, "free" existence of prophase nuclei is short lasting and relatively rare on preparations. In this stage, fusion of a light green zones and joining of their chromosomal groups is observed approximately in 1/3 to 1/2 of partners, showing no detectable dependence from the reorganization pattern of another mate.

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The nucleus located outside the cone is attracted to a paroral (conal) one or comes into an accidental contact with it; their light green zones are fused in contact area, and two prophase baskets of chromosomes become surrounded ultimately by a common envelope (Fig. 23, 24).

Automixis II, an interrupted meiosis, occurs too commonly in mates to be simply an occasional phenomenon; approaching of the nuclei leaves an impression of a purposeful process that may be due to an unilateral attraction (by chemotaxis?) of the extraoral nucleus by paroral one. The last nucleus must lie much below the fusion area. Giant prophase nucleus, in all respect different from normal amphimictic synkarya, is composed by two large prophase euploid nuclei and must be tetraeuploid; synkaryon is too large to fit within a cone. It may be supposed that, since meiosis is continued, synkaryon is made by two sets of dyads separating at its I fission. In sum, preliminary caryogamy is possible after the I Mi fission in automicts, preventing formation of pronuclei and excluding completely a hemiploid nuclear phase.

Two nuclei of automict I, still typical for amphimicts, and single Sk of automict II enter synchronously an anaphase Mi II stage; the intercalary nuclear union does not leads to any delay in nuclear division. The spindles of both partners are located diagonally; half of the daughter nuclei (two small hemiploid nuclei in automict I and one euploid one in automict II, Fig. 24, 25) enter the oral area and remain deeply within the body respectively. The parallel location of spindles at II Mi division does not occurs in amphimicts, where single daughter nucleus enters a cone. The cone, accomodating usually single hemiploid nucleus only, includes a single paroral nucleus of automict I, another one being located parallel to it, in its close proximity. Chromosome groups reach $8\times12\,\mu$ in anaphase Mi I, near $8\times5\,\mu$ in anaphase Mi II and $6–6.5\times2.5–3\,\mu$ in telophase and interphase Mi II. All nuclei of partners produced after II Mi division enter a long "classical" interphase: they become darkly stained and condensed, their chromosomes are strictly parallel one to another. Only those nuclei that are located in the oral area, in and near the cone, "survive" the interphase, the others degenerate in a typical way during the second half of the interphase, producing two small ($4\,\mu$) pycnocarya in automicts I and single large ($7\,\mu$) one in automicts II (Fig. 25 N–T).

Shortly after completion of the nuclear degeneration, the large euploid nucleus of automict II enters slowly a new prophase; thus process is paralleled by a preliminary caryogamy in automict I. An abundant stained material reveals all stages of the gradual rapprochement and fusion of two hemiploid nuclei in the oral area of automict I, preceeded by a typical "contact reaction" — fusion of their light green zones along the contact area, with subsequent union of two chromosomal sets. In amphimictic races, pronuclear migration occurs in the late interphase, but subsequent fusion of pronuclei may be significantly delayed. A similar variation is peculiar for automicts I, where pronuclei fuse in the late interphase or in the early, rarely middle prophase Mi II (Fig. 25 C–T). Both pronuclei uniting into synkaryon

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Fig. 23. Automixis. Telophase Mi I and prophase Mi II. Union of 2 prophase nuclei during automixis II

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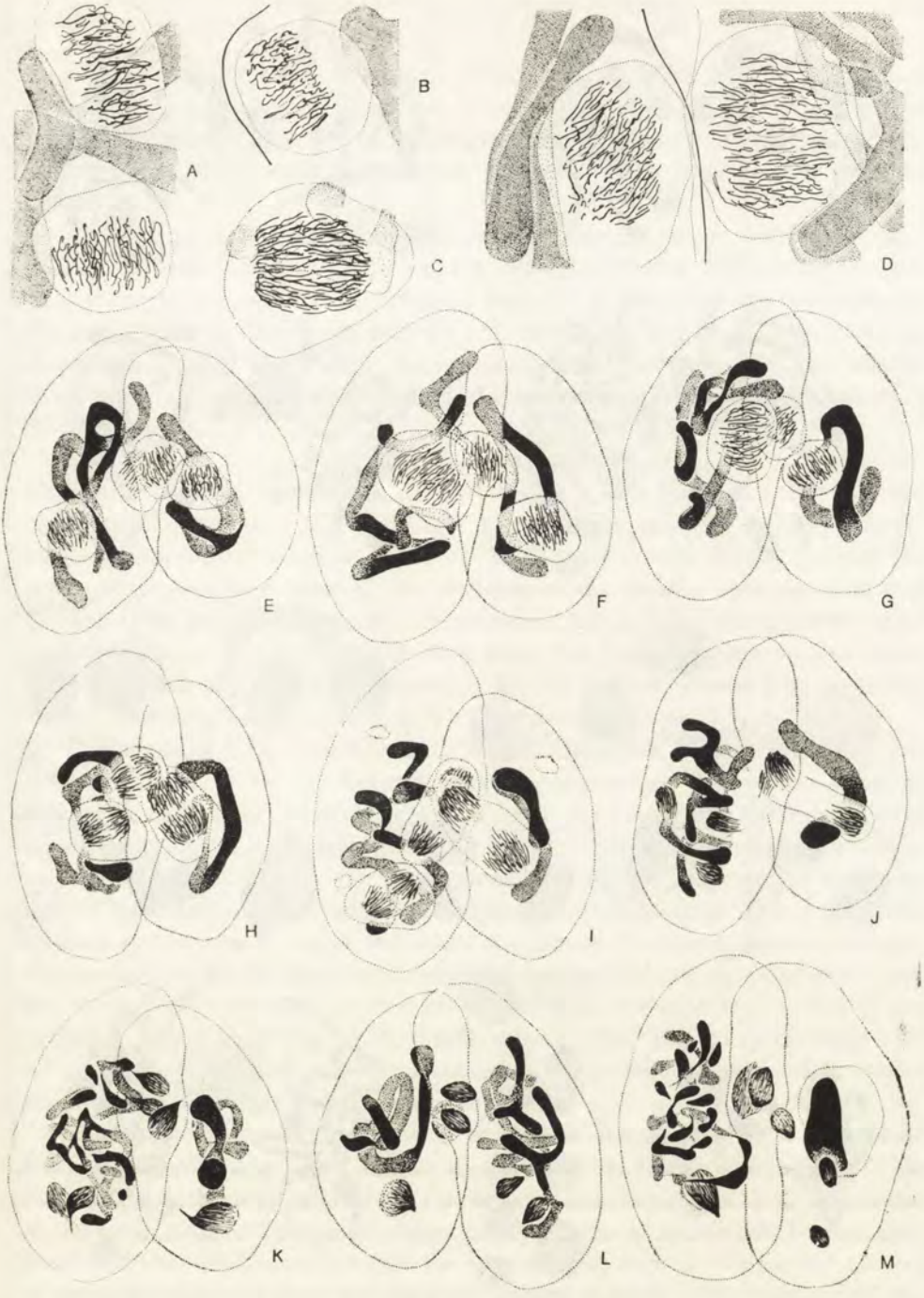

Fig. 24. Automixis. Early anaphase Mi II in automicts II (A–C) and automicts I (D); II Mi fission (E–M)

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Fig. 25. Automixis. Anaph ase Mi II to prophase Mi III (A–H, S, T); caryogamy in automicts I (I–L); nuclear deg eneration (M–P); pycnocarya of automicts II and I (Q, R)

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may be in different stages of growth (interphase+prophase, Fig. 27 A–L). Such asynchrony is common in *P. putrinum*. Two chromosomal baskets remain distinct within a synkaryon, but late prophase groups come closer together and are ultimately united into a single prophase set. The resulting Sk is too large to fit in the cone.

All subsequent processes follow an entirely identical way in both automicts I and II, but numeration of Sk divisions is naturally different. The number and size of pycnocarya are significant markers of the kind of nuclear events undergone by the cell, until their resorption prior to cell separation. Two small pycnocarya indicate automixis I, single large one — automixis II. Pycnocarya are very distinct in vivo, thus pairs with any needed kind of automixis may be selected at examination of living mates.

Mi undergoes five subsequent divisions at both types of automixis, and its homology may be easily established; III Mi fission is I Sk fission in automict I and II Sk fission in automict II (Fig. 26, 27). Metaphase stage is omitted, and chromosomes composing a prophase basket are distributed asynchronously between the poles of the anaphase spindle. The direction of the spindles coincides with that of I and II Mi fissions in both kinds of automicts, and also with that of III Mi fission in amphimicts — diagonally to the body axis. One large daughter nucleus enters the cone, while the other one remains in the cell interior. A new long interphase begins; anaphase nuclei reach near $9 \times 12\,\mu$, interphase ones 11×6–$7\,\mu$.

The nuclei of both automicts after III Mi fission are located identically, and the stage shown at Fig. 27 U (prolonged counterstanding of two large nuclei in partner's cones) is very peculiar for automictic process. Although the entire nuclear set of automicts in this stage strikingly resembles that of amphimicts in a similar stage (interphase following III "postmeiotic" Mi fission), no cases of migration and of secondary cross or selfing caryogamy were encountered during the whole research period even as simple individual aberrations. At least in the races studied, the products of III Mi fission, differentiated into paroral and extraoral nuclei just like hemiploid pronuclei of amphimicts proved to be not capable to exchange and syngamy. Euploidy of "pronuclei" differentiated after prelimiary caryogamy in all partners seems to be a factor responsible for prevention of repeated nuclear fusion, as well as their mutual interchange.

A long "counterstanding stage" is finished in the second half of interphase following III Mi fission. In a definite moment, not far from prophase Mi IV, the nuclei come back inside the body of the same partner (Fig. 27 V; 28 G–I). It is just in the same stage as hemiploid migratory pronuclei of amphimicts become activated and start their migration into the partner's cell. Paroral "migratory" nucleus of automicts does not show any signs of "contact reaction" with "stationary" one after its exist from the oral zone, although their occasional touching, never followed by sticking and fusion, was occasionaly observed. After a short delay, two nuclei in each cell enter prophase Mi IV. The IV Mi fission is in all aspects similar to II Sk

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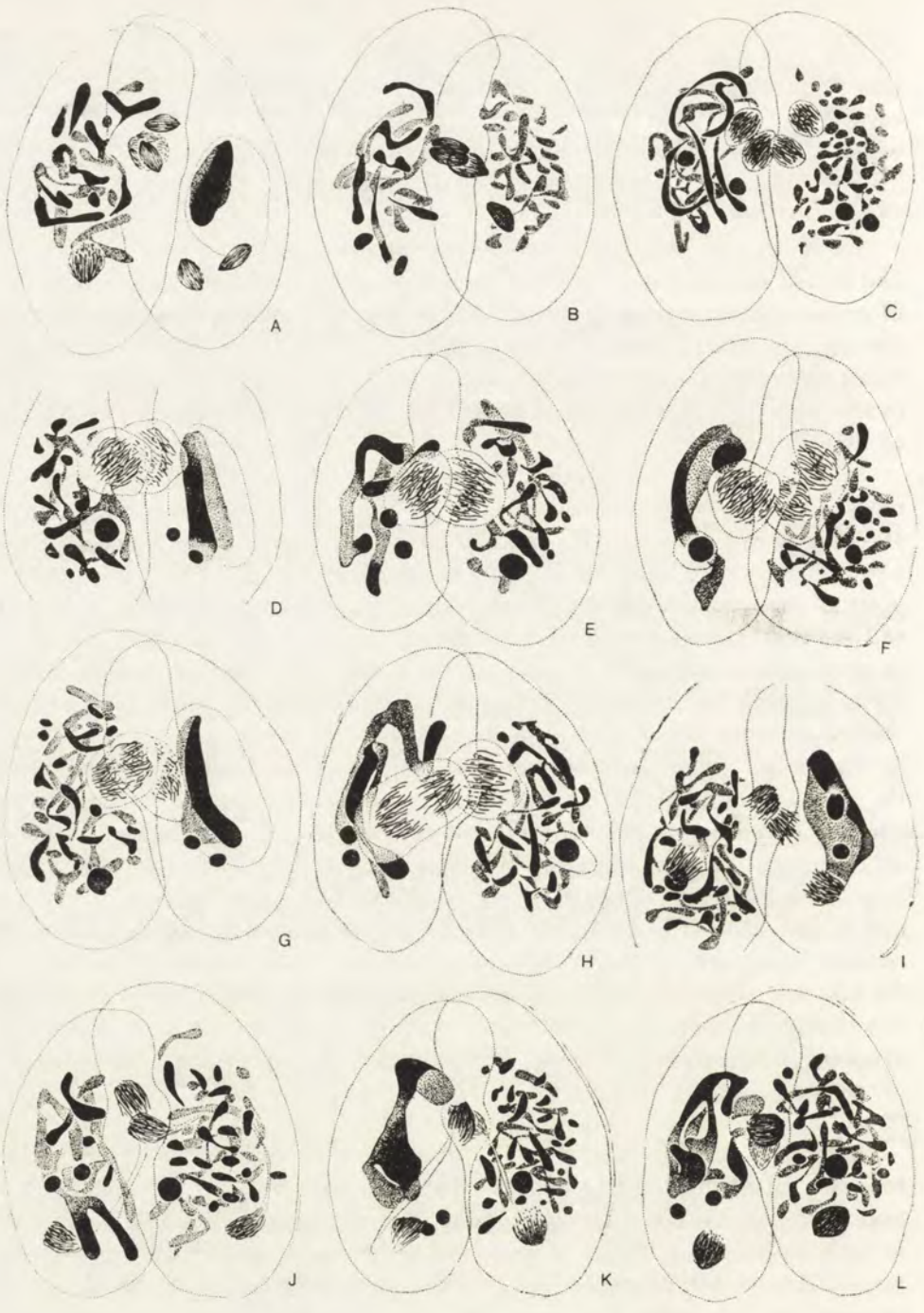

Fig. 26. Automixis. Telophase Mi II to telophase Mi III

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fission of amphimicts. Large prophase nuclei are lying usually one above another; anaphase groups are formed gradually, and their size (near 8–9 × 7 μ) is much smaller when compared with that of preceeding Mi fissions; chromosomes are more closely shifted one to another. Early anaphase spindles have no definite orientation at first, while the late ones are elongated parallel to the body axis. Two daughter nuclei move to anterior and two to posterior body ends; all four nuclei, near 8 × 5 μ large, are scattered then within the cell, being located frequently one above the other (Fig. 28, 29 A–L).

The new, last (V) Mi fission begins after a long interphase; this is III Sk fission in automicts I and IV one in automicts II. Four nuclei increase in size and assume the typical shape of prophase baskets; their spindles are directed along the body (Figs. 29 L–W, 30). Partners disjoin usually in the early prophase Mi V, and this division is accomplished in exconjugants. Polarization of chromatine occurs in four nuclei, presumed Ma anlagen; other nuclei are composed of numerous thin tangled chromosomes forming a ball. Some typical stages of the growth of anlagen are shown in Fig. 30 J–R; the entire process of Ma development in LJ race, typical for the species, was described in a preceeding chapter.

Concluding remarks

"Amphimictic" conjugation described in *P. putrinum* by Doflein 1916 may now be identified as one of the aberrant reorganization patterns, namely automixis I. In Doflein's race, two small pronuclei are located within a cone (atypical site for amphimicts) and are not differentiated; two pycnocarya prevail in his figures (3 pycnocarya are shown several times on Doflein's figures, but the third one seems to be a folding site of Ma branches or simply Ma fragment). It is not surprising that none typical stage of pronuclear migration and caryogamy was described by Doflein. Figure 294 D and E in Doflein–Reichenow's 1953 treatise illustrate Sk division stages and not III Mi fission differentiating pronuclei. Figure F shows the nuclear approaching in cone area but not their exchange. Figure G shows an automictic and not amphimictic Sk. Automictic races of *P. putrinum* are very common in our isolations, and it is quite probable that just such automictic race was occasionly isolated by Doflein.

The discovery of automixis in *P. putrinum* as racial (inherited) and not individual (occasional) process puts forward the problem of the existence of doubtful cytogamy in ciliates. Wichterman 1940 noted the absence of pronuclear interchange in living *P. caudatum* located in a microcompression chamber; the migratory pronucleus leaves its own partner's pellicle in the interphase following III Mi fission and moves back into the cytoplasm, with subsequent fusion to stationary pronucleus of the same cell. Such "double autogamy" or "cytogamy" was considered to be an intermediate process between the typical amphimictic conjugation and autogamy in singles. Unfortunately, the study of living material was overvalued and cytogamy was not proved on stained slides. Wichterman suggest-

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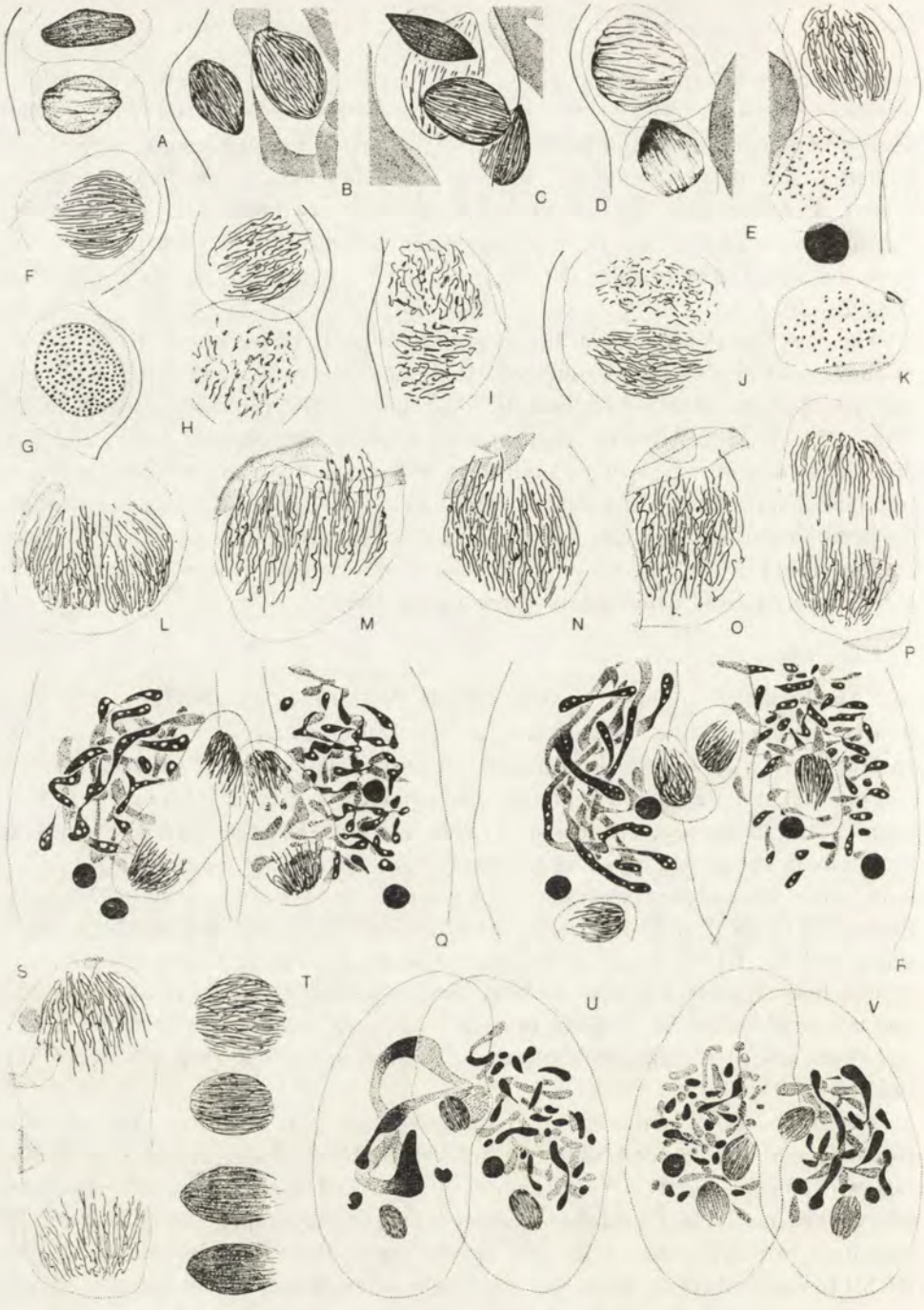

Fig. 27. Automixis. Caryogamy in automicts I, III Mi fission and nuclear departure back inside from the pellicle

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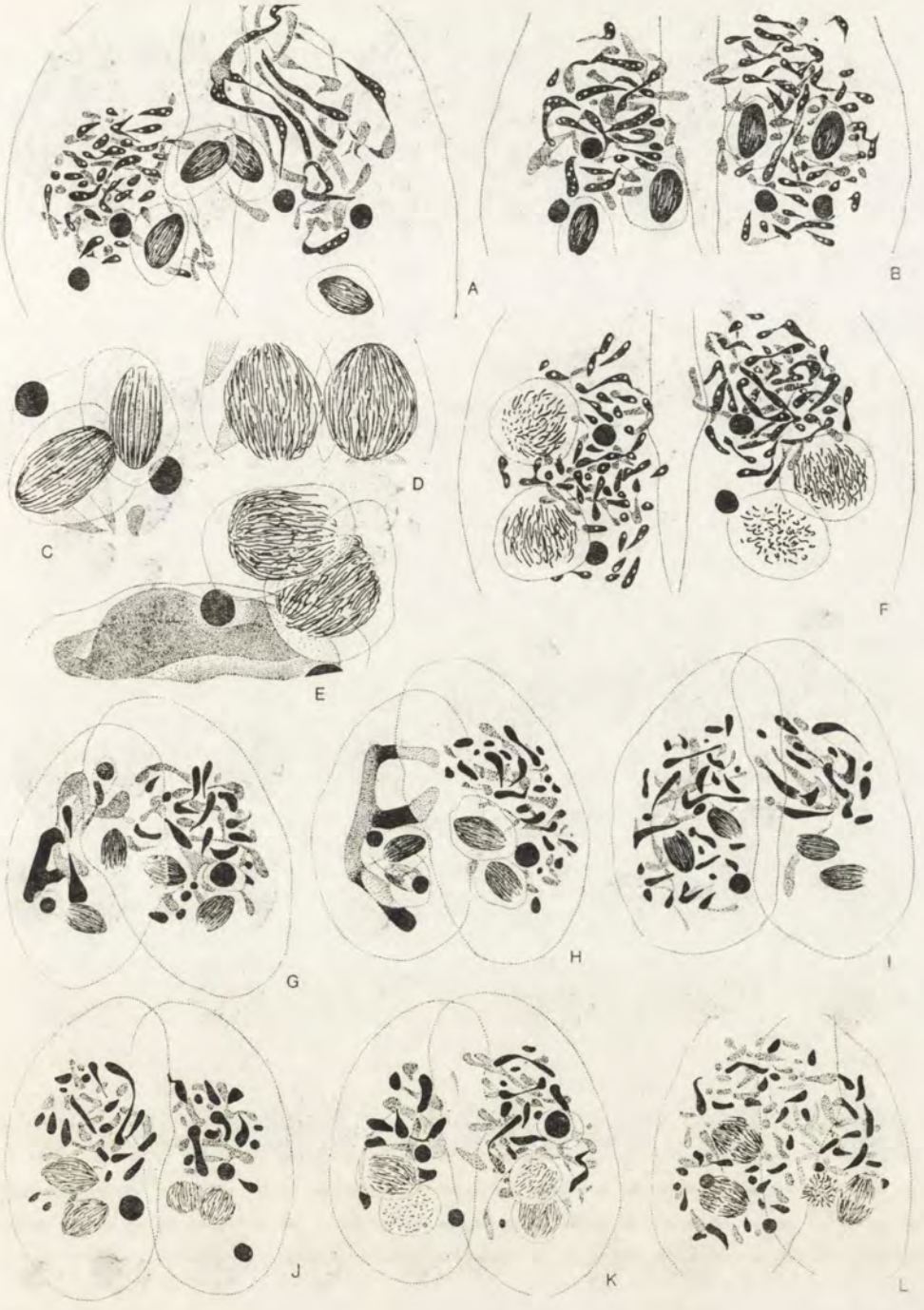

Fig. 28. Automixis. Interphase to early anaphase Mi IV

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Fig. 29. Automixis. Anaphase Mi IV to telophase Mi V

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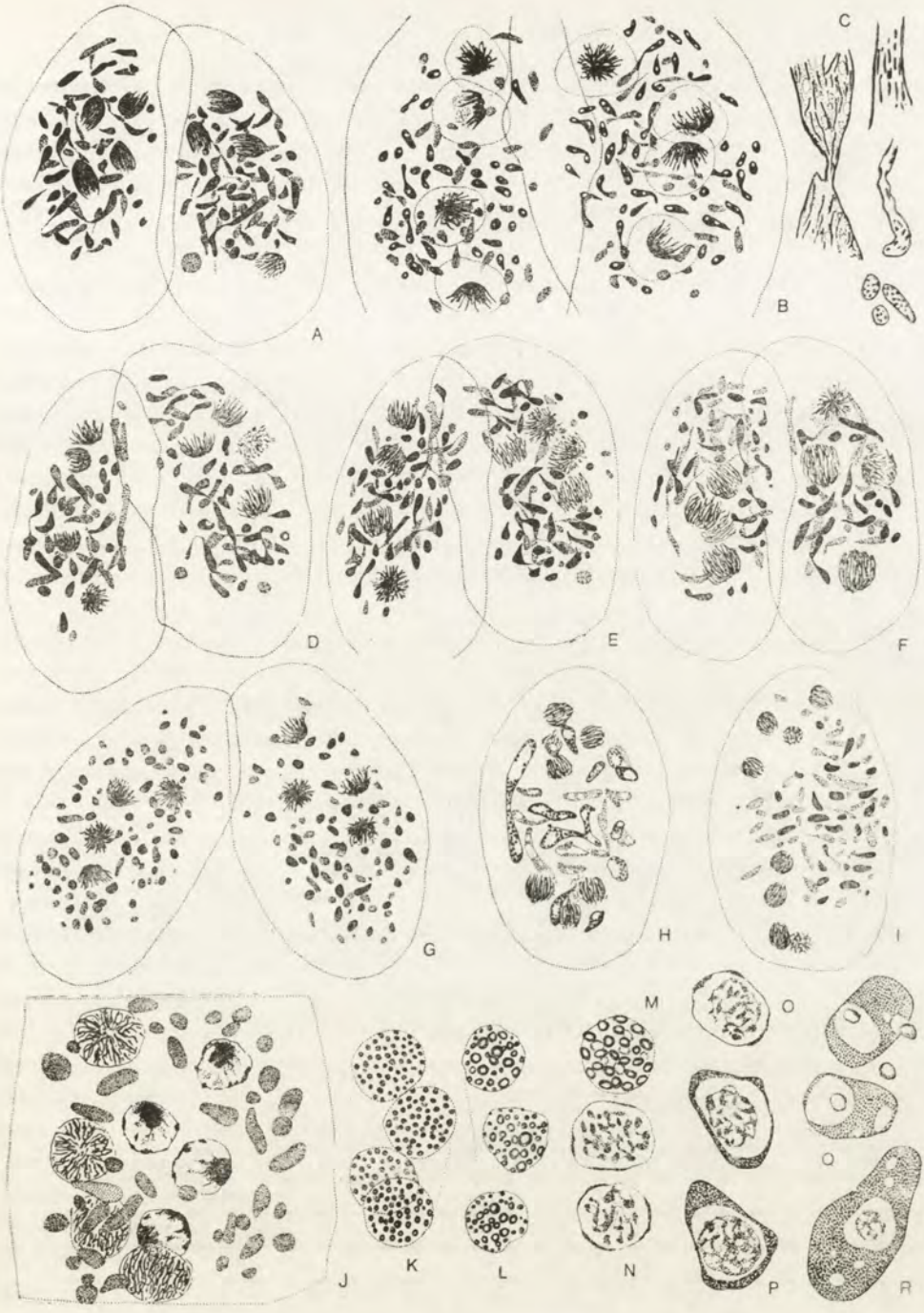

Fig. 30. Automixis. V Mi fission, cell disjoining, some peculiar stages of Ma development

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ed the occurrence of cytogamy in selfing lines and of amphimixis in intercrosses of stable ones; however, it is evident now that the mode of induction of the nuclear reorganization (Mi activation) has no influence on the type of reorganization processes. Even in *P. putrinum* with its set of reorganization patterns, cross and selfing conjugation are cytologically identical within limits of mixotype. During many years I tried to isolate cytogamic races of *P. caudatum*; a number of isolated races, including many selfing lines, showed invariably the typical pronuclear interchange, common on mass preparations. Cytogamy may be an individual aberration or result of compression (Porter showed the disturbance of physiological processes in compressed *P. aurelia*). I don't know report on the isolation of cytogamic lines in *P. caudatum* and I could not find hereditary basis for it, when all three pregamic fissions and the typical pronuclear differentiation occur. On the contrary, such basis exists in *P. putrinum* where the pregamic phase shows significant modifications preceeding the 3rd differentiating micronuclear fission: these modifications include atypical orientation of spindles, atypical set of pycnocarya and intercalary formation of synkarya by euploid and hemiploid nuclei after I and II meiotic fissions. Large euploid nuclei, resulting from III Mi fission, are no more capable for exchange or repeated caryogamy. Unlike amphimictic races of *P. putrinum*, *P. caudatum* etc., pronuclei appearing after II Mi fission in automictic races are not differentiated into migratory and stationary ones, while "pronuclei" made after I Mi fission represent large chromosomal balls instead of compact ovoid bodies, that naturally can not pass through the partner's pellicles. Migration in automictic races of *P. putrinum* is impossible even as individual aberration.

The notion "cytogamy" must not be applied to automictic processes in *P. putrinum* described here, because Wichterman's scheme of cytogamy has nothing common with automixis I and II involving hereditary modifications of meiosis itself, while only postmeiotic phase is modified in Wichterman's material. However, cytogamy in Wichterman's sense is quite common as an individual aberration in various ciliates, and is simply one of the large series of possible aberrations. There are both cytological (Diller, Chen, Hiwatashi, Kormos, Miyake et al.) and genetical evidences of an occasional cytogamy (Kimball, Powers, Sonneborn); its dependence on the external conditions, (e.g. temperature) was demonstrated. Autogamy in one partner may occur during crosses of normal and amicronucleate lines (Horvath), at some kind of a triple conjugation (Chen, Hiwatashi, Kormos, Miyake, Jankowski), and presumably during interspecific crosses of *Blepharisma* (Inaba). Diller and Mitchell suggested the possibility of cytogamy as a specific character in *P. polycaryum* and *P. jenningsi* respectively, although this was not still proved, especially in little studied *P. jenningsi*. The sole reliable description of mass (not individual) cytogamy was presented by Katashima 1959. All the lines of *Euplotes eurystomus*, isolated in Japan, were selfers; the cells were weakly joined and underwent automixis during selfing, but there was a firm contact and amphimixis during interclonal crosses. Selfing is

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presumed to play here the same role as autogamy in *P. aurelia*. Cytogamy in *Euplotes*, a facultative phenomenon, involves the normal pregamic phase and thus has nothing in common with automixis in *P. putrinum*.

VII. Apomictic conjugation

Introductory remarks

20% of isolated lines of *P. putrinum* inherit an aberrant reorganization pattern: 4 equational (isoploid) Mi fissions not interrupted by caryogamy and nuclear migration and followed by the typical nuclear reconstruction phase in exconjugants. Single large nucleus degenerates after I Mi fission, while the other one undergoes 3 isoploid divisions producing 8 nuclei (1 Mi, 3 resorbing nuclei and 4 Ma anlagen). This pattern, inherited invariably at vegetative divisions and after conjugation, is designated further as "apomixis I". Diller 1949 described a more simplified nuclear reorganization in a North American race: 3 fissions of Mi yield 8 nuclei of exconjugant. Such process, designated below as "apomixis II", was never encountered in my races in spite of extensive search in this direction.

Nuclear dynamics in vivo

In addition to hundreds of mass stained preparations, the process was traced also in ten living pairs of a selfing line CRn in Commandon–Fonbrune's vaseline oil chambers; a series of observations was made also on some separate phases of the nuclear cycle. As example, one record (February 28–29, 1959) is presented below.

21^{40} — Partners are firmly joined as a selfing pair. 9^{15} — A swollen clear Mi enters I fission lasting near 20 min. One large daughter nucleus enters the oral zone, other one remains deeply inside the body. The buccal apparatus has been nearly entirely resorbed in this phase, and mineral crystals are gathered in a peculiar group near the posterior body end. 11^{40} — Both nuclei are in a long interphase. The extraoral nucleus decreases in size, loses its longitudinal striation and looks homogeneous. Fluid vacuole surrounding the nucleus may indicate its dehydration. The nuclear contours become more and more distinct; entire nucleus assumes a very high refraction and is easily noticeable at lowest magnifications. 13^{45} — The paroral nucleus starts a new division lasting near 18 min. The axis of the spindle coincides with that of I Mi fission, and both daughter nuclei undergo a similar differentiation, being located inside the cone and deeply in the body. Paroral nucleus retains its place and does not migrate into another partner; its slow increase in volume is initiated. 14^{32} — Paroral nucleus starts to move into the cytoplasm of the same partner, undergoing no deformation. The nucleus is very large and distinct, and the absence of migration in CR race (unlike the aberrant QS race, where it may occur as occasional aberration) was proved by observations on numer-

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ous living and stained pairs. Two large functional nuclei and one pycnocaryon are seen now inside the cell. After several minutes (14^{38}) paroral nucleus of another partner moves back from the pellicle inside the cell, also remaining in its partner. Both functional nuclei within each partner do not approach each other and do not fuse with these (such aberrations, were recorded, however, on stained slides). 16^{21} — Both nuclei enter synchronously a new, III Mi fission, lasting near 21 min and yielding four clear longitudinally striated oval nuclei with acute edges. They have no definite place at first, and are slowly displaced by cytoplasmic currents within the cell. 16^{46} — The nuclei have occupied a more regular position, one above the other along the body axis. 18^{10}. First signs of the approaching (separation) of cells are well expressed. Large slit separates them in the oral zone, and mates remain sticked by their anterior and posterior body parts. Mineral crystals are scattered throughout the cell by cyclosis. Large pycnocaryon loses the clearness of its outlines, becomes clear and is quickly (during 8 min) resorbed within the cytoplasm. 20^{42} — The last (IV) Mi fission has been initiated. The slit separating both mates increases in size. 20^{47} — Partners are disjoined, their shape is modified: cells are small, with wide rounded ends and marked deformation in the contact area. Vestibular and prevestibular areas are not discernible. Somatic cilia in the contact area and buccal ones are still not restored; the cells are weakly mobile and do not feed. Morphogenesis proceeds rapidly, however; the oral ciliature sinks inside the body, vestibulum and prevestibulum soon reappear. Exconjugant assumes the normal shape and starts an active feeding after several hours following cell separation.

Time intervals between Mi divisions vary in different pairs at coinciding cultural conditions; slight asynchrony (several min) is the commonest feature in CR race, and a significant one (15–20 min) may represent an abnormality.

Cytological study of apomixis I

Nuclear processes coincide entirely in different apomictic races of *P. putrinum* (CR, SB, QS, VK etc.), and races differ mainly in their clonal "spectra" of aberrations in nuclear behaviour. Apomixis I is illustrated below on selfing line CRn (Sg 5, MT D) (Fig. 31–38; Pl. XII–XIV).

Prophase Mi I is extremely prolonged, just like in amphi- and automicts. Mi moves away from the Ma and begins its slow growth; chromatine polarization and separation of wide typical light green zone are initiated from the earliest stages of the nuclear growth (Fig. 31). Mi passes through the parachute stage and becomes spindleform and clear; chromatine ribbons are converted into numerous thin tangled chromosomes. Mi dimensions reach now 32–36 × 13–16 μ. Chromosomes are gathered together within a wide transversal (equatorial) stripe making its external layer; this is probably a pre-metaphase stage. During the straightening of bended chromosomes, the nucleus becomes wide and rounded, and chromosomes are distributed throughout it. Long filiform chromosomes are distributed asynchrono-

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Fig. 31. Apomictic conjugation, selfing-line CRn, Sg 5, MT D, Böhmer. Prophase Mi I

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usly among two daughter anaphase groups without a distinct metaphase stage. The classical signs of meiosis, recognizable in both amphi- and automicts, were not demonstrated in apomictic races. Apomicts lack the stage of wide bivalents corresponding in their number to haploid set of chromosomes; none stage may be regarded as tetrads, dyads etc. We deal surely with modified mitotic and not meiotic fission. I did not find this result to be ultimately proved, however, and I could not exclude categorically possibility of conjugation of chromosomes; I Mi fission in apomicts needs special repeated study.

The axis of I Mi spindle is directed diagonally; two daughter nuclei, located within PC and deeply in the body, enter a typical prolonged interphase instead of a transient interkinetic stage peculiar for all meiotic races of the species (Figs. 32, 33). Interphase chromosomes are located very closely together; darkly staining nuclei, reaching 15×8–$9\,\mu$ in size, assume a leaf-like shape. In the second half of the interphase large extraoral nucleus becomes degenerated; after its significant compression, the nucleus looks spherical or crescentic, and is stained now with extreme intensity. Pycnocaryons are equal to vegetative Mi in their size (near $7\,\mu$, Fig. 32 Y, Z), and this is perfect indirect evidence of the euploidy of degenerated nucleus. Pycnocaryons of apomicts much exceed small hemiploid pycnocarya of meiotic lines (amphi- and automicts I) and coincide with those of automicts II where their euploidy is also evident. In some aberrant cells of CR race, Mi undergoes degeneration in the second half of I prophase producing a giant chromatine clump containing much more DNA than that made after I Mi fission; such difference in size may be expected both in meiosis and mitosis, since after I Mi fission of amphi- and apomicts both daughter nuclei must be euploid, and prophase nucleus must contain the DNA quantity four times exceeding that of hemiploid nuclei.

The interphase between I and II Mi fissions is very long in apomicts and very short in meiotic mixotypes where no DNA synthesis occur; this indicates the mitotic nature of II Mi fission in apomictic lines. The paroral nucleus assumes a basket-like shape in prophase Mi II, measuring near $15\times 15\,\mu$. Polarized telophase nucleus enters the cone in a typical position (with its wide end facing the pellicle) or, less commonly, in inversed one; this occasional asymmetry in the nuclear position has no influence on the subsequent nuclear events.

Anaphase Mi II is not preceeded by a distinct metaphase; two anaphase groups are made asynchronously and contain numerous long thin chromosomes (Fig. 32; 33). One of the daughter nuclei enters the oral zone, another one remains deeply inside the body; both functional nuclei coincide entirely in their shape and sites within the cell after I and II Mi fissions. Interphase nuclei reach $15\times 18\,\mu$ after the I Mi fission and near 12×8.5–$9.5\,\mu$ after II fission.

Interphase paroral nuclei of both partners retain for a long time their previous position within the cell; long counterstanding stage of giant nuclei (Fig. 34 F–H) is very peculiar for apomicts as well as for automicts II. Although the nuclei are differentiated into paroral and extraoral ones and resemble pronuclei of amphi-

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Fig. 32. Apomixis. Anaphase Mi I to interphase (A–S); nuclear degeneration (T–X); pycnocaryon and vegetative Mi (Y, Z)

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Fig. 33. Apomixis. Telophase Mi I to anaphase Mi II

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Fig. 34. Apomixis. Anaphase Mi II to interphase (A–H); beginning of nuclear departure back inside the cell (I); II Mi fission (J–Q)

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micts made after III Mi fission, they are not generally capable to exchange and fusion. However, unlike automicts II, some exceptions of this kind were reported in apomictic races isolated in USA (Diller) and in USSR. It must be definitely underlined that this is only an expression of individual variability of the nuclear processes in some lines, atypical for most mates of these lines, for other apomictic lines and for apomictic mixotype generally.

Near half an hour before III Mi fission, the paroral nucleus leaves its site and moves slowly inside the body of the same partner undergoing no deformation. After its reversal, both functional nuclei are not in contact and their light green zones are not normally united (Fig. 34 I, 35 A–E). Once again, caryogamy and formation of giant synkarya may be noted in some aberrant mates of lew lines. In typical mates, both nuclei are lying separately and deeply inside the body, usually one above the other; interphase stage is continued some time and is followed by synchronous prophase of Mi III (Fig. 35 F–P).

The spindles of III Mi fission are stretched along the cell; telophase nuclei are scattered throughout the cell, never entering the cone (even as individual aberration) and producing no pycnocarya (although this is possible in some aberrant cells) (Fig. 36). Anaphase baskets of chromosomes are smaller (near $10 \times 9\ \mu$) than those of the previous Mi divisions, and their chromosomes make a more compact group; two telophase nuclei reach the anterior and posterior body ends respectively, but all nuclei are intermixed in the early interphase. Their axial location, one above the other, is common (Fig. 37 D). The nuclei, reaching $9.5–10 \times 5.5–6.5\ \mu$ in the early interphase, are diminished to $7.5–8 \times 5\ \mu$ at their maximal compression; they look ovoid, with dentate anterior end.

Prophase and anaphase of the last IV Mi fission are shown in Fig. 37 and 38. Anaphase baskets reach only $6–8 \times 6–6.5\ \mu$ in size; the spindles are stretched along the body. 4 relatively small nuclei move to each body end, and all 8 nuclei become scattered then throughout the cell. Pycnocaryon is resorbed during an interval between two last Mi fissions, parallel to the swelling of 4 prophase nuclei. As a rule, IV Mi fission is started and accomplished in conjugants, and 8 nuclei are seen in each one of disjoining partners; less commonly, cells may separate during IV Mi fission. Nuclear reconstruction in exconjugants follows a typical pattern (Fig. 38 H–M).

Chromosome counts made in anaphase of I, II and IV Mi fissions yield 118, 124 and 109 chromosomes; this proves the equational (isoploid) nature of Mi divisions in apomicts. The addition of Bouin's fluid in insignificant, non-lethal quantity to conjugating cultures results in chromosomal condensation and abnormal fragmentation of Ma (Fig. 38 U–Y). The counts yield 101 and 108 chromosomes, the values approaching those obtained at counting of normal chromosomes. It seems that the technique of artificial chromosomal condensation could be applied for chromosomal counting in paramecia.

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Figure 38 N–R present an abnormal pattern of Ma breakdown occurring in some CRn cells at normal conditions. Large clear cavities surrounded by peripheral chromatine layer may arise in early or, more commonly, in the late stages of Ma branching and fragmentation; atypical premature resorption of some fragments was also noted (Fig. 38 S, T). Ma anlagen pass through a typical chain of growth processes, including stages of chromatine condensation, heterochromatine clumps, reconstruction and endomitotic growth.

The problematic process: apomixis II

Diller's 1949 race resembles closely my apomictic races in the size of vegetative Mi and its products, and this facilitates the comparison of reorganization processes. According to Diller 1949, 1959, several american races of *P. putrinum* reveal an atypical "abbreviated" conjugation — apomixis with a more simple scheme than that described above. There are 3–4 Mi fissions; as a rule, they are not interrupted by degeneration, exchange and fusion of large nuclei. No attempt of separation of individual aberrations from the normal process was undertaken by Diller, and thus no definite scheme of the process was presented. Diller regards Mi of his lines as "diploid" nucleus; abbreviated conjugation was supposed to include meiosis and formation of "haploid" nuclei, with their possible subsequent division or splitting. Synkaryon formation is sometimes omitted, and this leads to a progressively increasing hypoploidy resulting in the amicronuclearity and death of the line. "One-step meiosis" (at single fission of the Mi) and "parthenogenetic division of haploid nuclei" must be compensated by some mechanism providing the "diploidy" of exconjugant nuclei. Several alternative hypotheses were proposed, including: (1) endomitosis, resulting in the nuclear diploidy; (2) alternation of abbreviated and normal conjugation; (3) alternation of abbreviated and "polyspermic" conjugation, rising again the nuclear ploidy: (4) possibility of omission of meiosis (Diller 1949). Diller 1959 found that new selfing lines of the species reorganized their nuclei at abbreviated conjugation and had ultimately died out.

Judging from the study of a related process in my races and from Diller's perfect figures, I am inclined to exclude the first three hypotheses of Diller. None alternation of aberrant and normal conjugation can not occur; endomitosis is impossible in pronuclei (hemikarya), while fusing into synkaryon seems to be improbable. I identify Diller's process as apomixis, a series of isoploid nuclear divisions with the entire omission of meiotic phase (chromosome reduction) and not as modified or pathological amphimixis. Migration (usually unilateral) and fusion of large nuclei are simple individual aberrations both in my and Diller's races. The occasional or "progressive hypoploidy" is entirely impossible in apomicts; moreover, there is a distinct tendency to hyper-polyploidization as a result of abnormal fusion of 2 euploid nuclei in some per cent of partners. With some hesitation, I designate Diller's cycle as "apomixis II": Diller indicates the occurrence of 3–4 Mi fissions and of rare formation of pycnocarya, and it might be, that he dealt

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Fig. 35. Apomixis. Nuclear departure without interchange; interphase to anaphase Mi III

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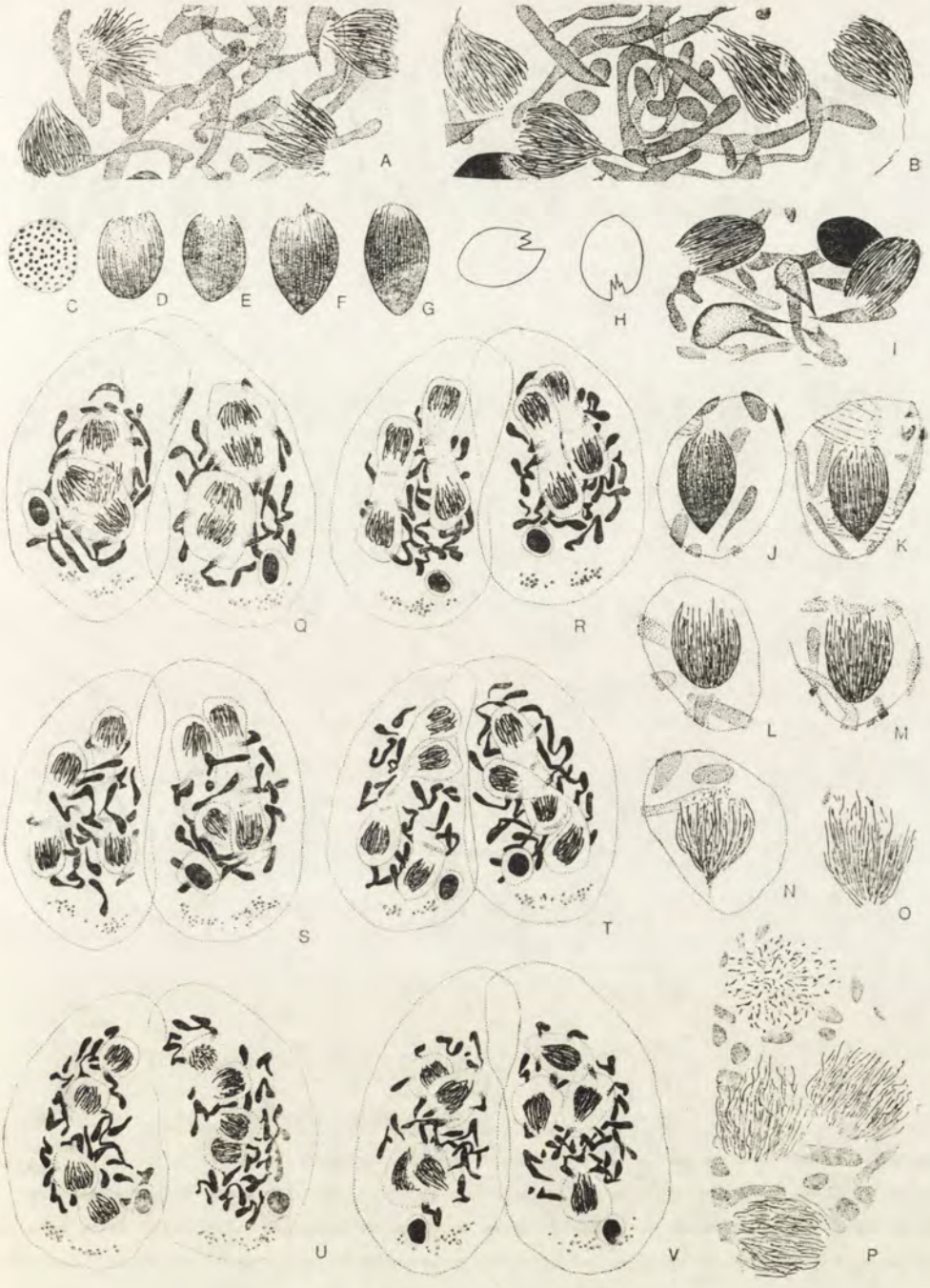

Fig. 36. Apomixis. III Mi fission to early prophase Mi IV

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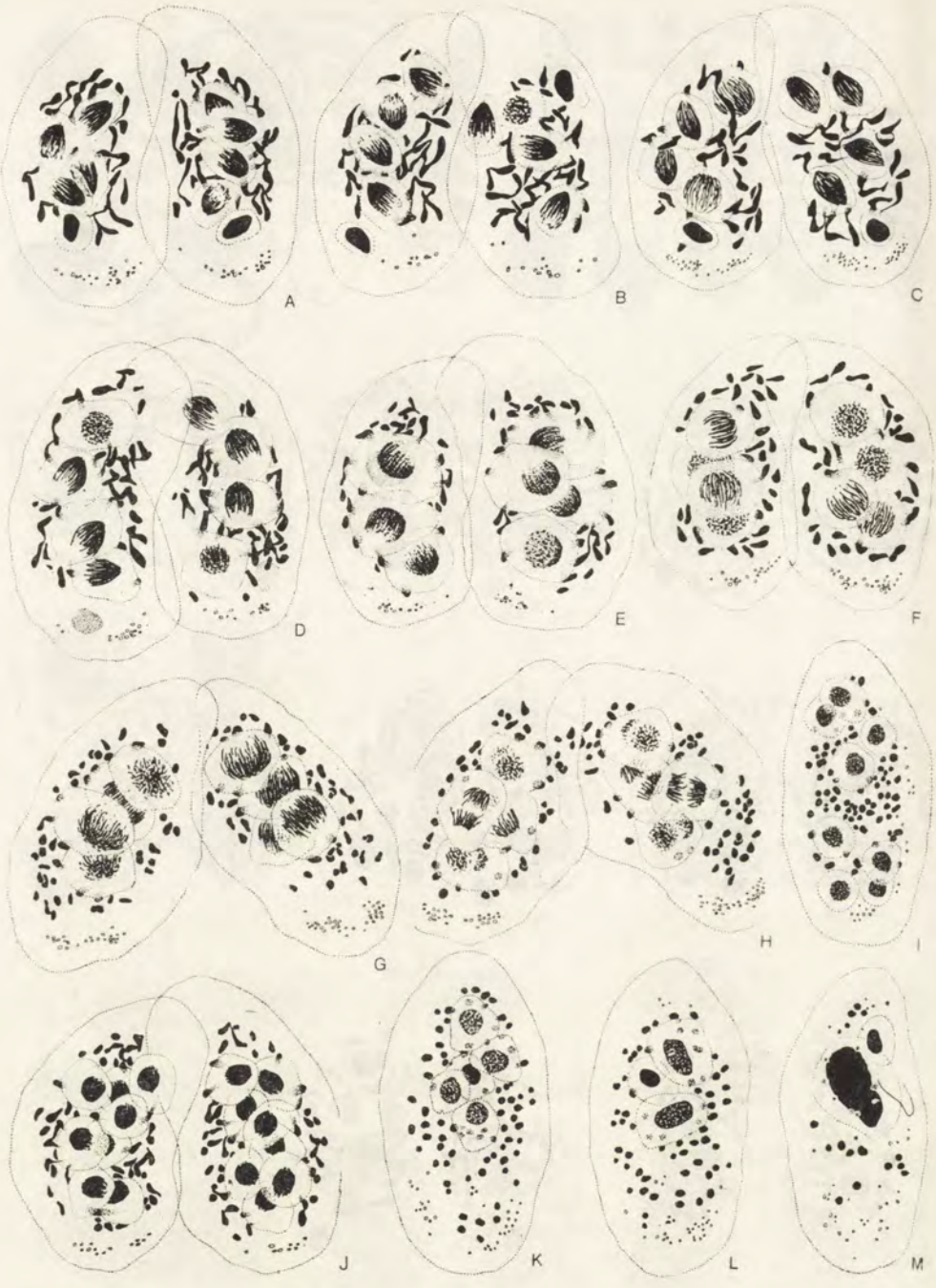

Fig. 37. Apomixis. Interphase, cell separation, IV Mi fission and exconjugants

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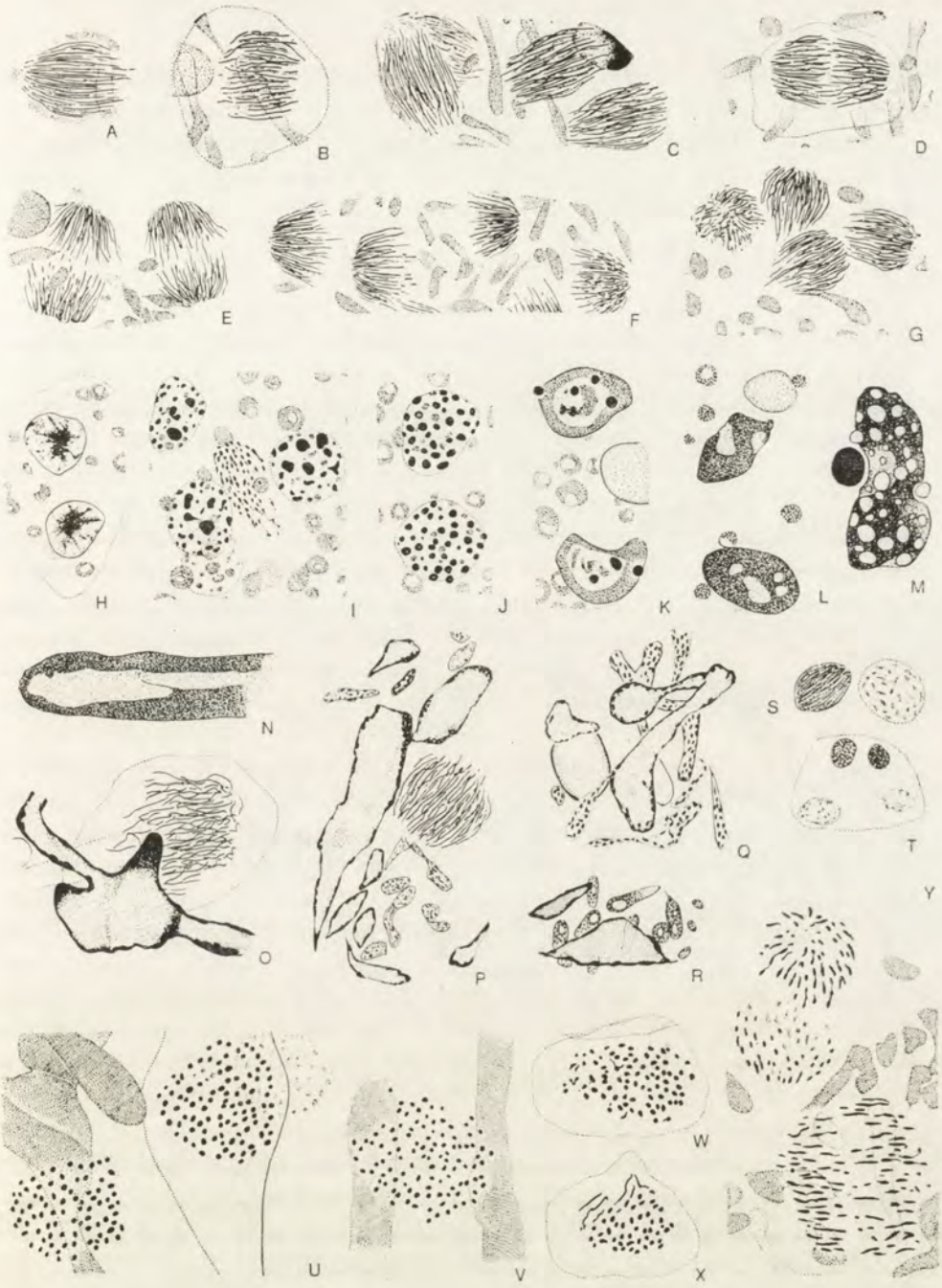

Fig. 38. Apomixis. IV Mi fission and peculiar stages of anlagen growth (A–M); abnormal Ma fragmentation (N–Q); resorption of pycnocarya and fragments (S, T); chromosomal shrinkage after prolonged action of diluted Bouin's fluid (U–Y)

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with what I must designate as "apomixis I". Omission of one Mi division is impossible in apomicts even as an individual, and so more as a mass aberration; thus 3 or 4, and not 3–4 Mi fissions must occur in Diller's material. Before reinvestigation of this, I tentatively admit that there are normally three divisions and no Mi degeneration after first fission occurs.

Concluding remarks

According to a generally accepted view, apomixis (or apocaryomixis) is a multiplication process omitting the caryogamic phase (nuclear fusion into Sk) or syngamy (gametic fusion into zygote; Rostand, White, Suomalainen et al.), that is a secondary omission of the sexual process. Apomixis is widespread among plants and animals and occurs in two forms — ameiotic (diploid) and meiotic (haploid) one. The term "apomixis", as a rule, is not used for ciliates, being replaced by the term "parthenogenesis" bearing in almost all cases no relation to the processes described (e.g. "parthenogenetic fissions of the Mi"). Parthenogenesis implies the development of the egg without its fertilization and may be applied for stationary hemikarya not fused with migratory ones developing androgenetically. Thus "apomictic" and not "parthenogenetic" conjugation is recognized in ameiotic isoploid races of *P. putrinum*. It may be noted also, that "conjugation" should mean simply the cell union for reorganization of their nuclei, irrespectively of the character of processes involved.

Near 60 indications of "endomixis" (apomixis) were published in the present century, including my own description of such process in *Cyclidium*. Woodruff and Erdmann, who proposed the term, designated in this way a series of equational Mi divisions accompanied with breakdown and replacement of an old Ma. The analysis of all published descriptions of "endomixis" (Jankowski 1965 d), omitted here, reveals a number of processes that have nothing in common with its original scheme, e.g. hemixis, autogamy in singles, exconjugant reconstruction cycles and even nuclear replacement in dividing *Loxodes*. In many cases, "endomixis" too strikingly resembles exconjugant nuclear changes, although their identity was not definitely proved; e.g. Davis described fusion of two cells in *Trichophrya* with subsequent development of a new Ma directly from the products of I Mi fission (a typical exconjugant phase in *Suctoria*). Although *P. putrinum* seems to be the first evident case of endomixis in its original sense, I avoid usage of this confusing term, synonymic with "diploid" (isoploid) apomixis.

"Haploid" (hemiploid) apomixis occurs commonly in ciliates as individual aberration accompanying the normal amphimictic conjugation. Hoyer and De Horne supposed the resorption of stationary pronuclei and "parthenogenetic" divisions of migratory ones in *P. caudatum* and *Colpidium*, but such processes are unknown in these ciliates. Suzuki 1957, in a careful study of *Blepharisma japonicum*, found that "parthenogenetic" conjugation determined by culture age and feeding conditions may occur in his race: meiosis is missed, all Mi fissions are

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isoploid, their number is abbreviated from 6 to 5; II Mi fission yields diploid nuclei undergoing three further divisions. Diploid pronuclei, produced during the process, are not capable to exchange and caryogamy. In spite of the general interest in conjugation of *Blepharisma* (Weisz, Bhandary, Seshachar, Inaba et al.; my slides), this process was not reported by other authors.

In sum, euploid apomixis was proved for *P. putrinum* only, where it is inherited by many lines of MT D and E, uncapable for any other of kind the nuclear reorganization. Unlike *Blepharisma*, this is an obligate and not facultative reorganization pattern.

VIII. The system of mixotypes

Mixotypes, a new intraspecific differentiation

Although the nuclear reorganization in *P. putrinum* may follow one of five patterns described above, the species can't be subdivided directly into five groups with corresponding reorganization processes. There are numerous cases when a single line may conjugate in a different manner, and this complicates the intraspecific differentiation. Bearing in mind this pluripotency, the species may be subdivided into five groups (an occasional coincidence) uniting lines with a definite reorganization pattern or with their definite linkage. These groups, having no analogy in other protozoa, were designated as "mixotypes" (Jankowski 1962 a). The groups with combination of several reconstruction modes were named at first as "synmixotypes", but this term was not further used.

Mixotype (MT) A includes lines with a single amphimictic reorganization pattern; they may be called "obligate amphimicts". Many lines of CR, QS, VK and other races are obligate apomicts (MT D) incapable to any other reorganization process except apomixis I. Judging from Diller's data, apomixis II is inherited in some american lines, and is not alternated with other processes; these lines constitute an arbitrary fifth mixotype (MT E).

Mt B and C are previous "synmixotypes" (note that literal designations of all mixotypes are changed in comparison with previous articles). MT C includes lines of automicts, incapable for amphi- or apomixis. A prolonged study indicates that automixis I and II do not exist separately, as "pure" reorganization patterns; at least in my races they are invariably linked together. Their linkage in Mt B at intermixotype conjugation is a significant supporting evidence of impossibility of their separate existence. Irrespectively of the nature of the activating factor (cross and selfing, intra- and intermixotype conjugation), the slides show invariably three types of pairs — those with automixis I in both partners, automixis I and II in each partner respectively, and with automixis II in both mates. Preparations of T 4 × T 7 lines, studied for non-selective distribution of these patterns, yielded the proportion 141:243:116, that is near 1:2:1. This may take place if in the moment of mixture of two lines, nearly half of the cells of each line is capable to automixis I, and other

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ones to automixis II. It seems possible, that at every cell fission one daughter cell shows the first tendency while the other cell — a second one, but it seems more probable that the kind of automixis is determined occasionally during conjugation. Automixis II is due to the union of two interkinetic nuclei after I Mi fission, and automixis I — after II Mi fission; automixis I will follow, if there will be no contact of the nuclei after preceeding division. Nuclear sticking and union after I Mi fission may take place at their occasional contact, since large prophase spheres are not compact ovoid interphase nuclei capable for movement. The alternative hypothesis consists in ascribing a limited chemotactic attraction of extraoral sphere by a paroral one. Irrespectively of the nature of a mechanism providing caryogamy in automicts II, it may be concluded that MT C races are "destined" for automixis I, and this process may be modified at occasional, non-obligate fusion of the products of I Mi fission.

Mt B includes lines with a most complicated combination of different reorganization processes. Three such patterns are possible. The lines belonging to MT B exhibit only typical amphimixis at their intercrosses within their mixotype. The same lines may be mated with those of MT C, obligate automicts; cytologically this conjugation is entirely identical with that within MT C, that is the same linkage of automixis I–II may be revealed. In other words, lines of MT B at definite conditions may restraint from amphimixis and reorganize their nuclei by automixis, like their partners. In sum, amphimictic lines of *P. putrinum* may be subdivided into two groups, obligate (MT A) and facultative ones (MT B), capable for changing of their reconstruction process from amphimixis to automixis. It is naturally admitted that realization of intermixotype crosses at all possible combinations will further complicate the system of mixotypes.

Beginning the study of *P. putrinum*, I expected that this species is represented in nature mainly by amphimictic lines, and that lines with aberrant nuclear reorganization are simply an exception. Incidentally the first line of the species (CRn) isolated in 1958 was apomictic selfer. It is evident now, after study of 22 races, that aberrant lines are as common as amphimictic ones. From 309 lines (including selfers), where mixotype was determined, MT A includes 47 lines (15%), MT B — 106 (34%), MT C — 73 (26%), and MT D — 62 (20%); MT E was proposed to include Diller's races. In addition, there are several lines (5%) with pathological conjugation, supposedly of MT B or C, and several non-conjugating lines of Sg 3. The relation of total amphimicts (MT A+B) to aberrant lines (MT C+D) is 49:46 (in per cents), near 1:1; the relation of obligate amphimicts to all other lines (MT A:MT B, C, D) is 15:80, near 1:5.5. These data lead to a striking conclusion that lines of *P. putrinum* capable to amphimixis only are very rare in nature. As a rule, samples from a single water source yield a number of aberrant lines.

Description of an aberrant conjugation by Doflein 1916 (his "amphimicts" are automicts I) and its repeated reports by Diller 1948, 1949, 1959 indicate that aberrant mixotypes occur widely outside the research area. Since MT E with apo-

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mixis II, established according to Diller's description, was not found in Leningrad District in spite of an extensive special search, it remains not clear whether all mixotypes are known now and whether similar studies in other remote places may yield more new mixotypes of the species.

Intermixotype mating

The system of mixotypes is correlated at least with some syngens: Sg 1 and 2 include both MT B and C each, Sg 4 includes MT A, and Sg 5 — MT D. One of these, Sg 4, seems to be "pure" for MT A, not participating in elaboration of a system of aberrant cycles, while all other Sgs may be expected to include the entire mixotype set. More extensive collections must be made to check this possibility.

Definite mt are not correlated with definite mixotypes within Sg 1 and 2; for example, the standard set of lines of mt I–VIII in Sg 1 may be doubled in 2 mixotypes: (1) MT B, lines OM 11, OM 27, OM 19, ZN 30, M 5, ZN 72, ZN 18 and M 41; (2) MT C, lines ZN 22, SZ 4, OM 6, OM 23, LH 4, SZ 20, ZN 13 and ZN 3. This parallelism indicates the possibility of intercrosses of complementary lines differing in their mixotypes. Since I expect to find all mixotypes in these syngens, they may be intercrossed, theoretically, in all combinations. However, only one combination (MT B×C, amphimicts ×automicts) was possible in the research period; since only automictic pairs are revealed in these crosses, an aberrant reorganization pattern becomes dominant, suppressing an amphimictic one, and this domination may give some indications on the possible way of the origin of mixotypes. In some way the partner of MT C induces restraint from amphimixis in MT B mate, and this influence must be rendered in the very beginning of conjugation since both I and II Mi fissions are cytologically different in auto- and amphimicts. The switching of reorganization process in MT B partner must occur in prophase Mi I, possibly soon after the union of mates.

Several such inductory mechanisms may be supposed — ciliary or pellicular interaction, or exchange of at least small quantities of the cytoplasm in prophase Mi I. It is well known that the pellicle of paramecia and other hymenostomes has a sieve appearance, being penetrated by a number of channels and pores; very small quantity of the cytoplasm is exchanged in the early stages of the union, and significant quantities may be exchanged after pronuclear migration (Chen, Sonneborn, Elliott, Vivier, Harrison, MacDonald et al.). Various cell constituents (Ma fragments, mitochondria, zoochlorelles, kappas, viruses suggested by Bomford) may participate in a passive exchange. Pathological intra- and intersyngenic crosses (e.g., old × young paramecia, or lines with incompatible cytoplasm) is an indirect evidence of the cytoplasmic exchange at the early prophase stages of meiosis (Chen, Ray, Nagel et al.). Our case with intermixotype crosses is principally similar to all crosses mentioned above, with sole difference that this is not a pathological phenomenon. The cytoplasm of an aberrant mate must exert some "directory" influence.

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Homology of micronuclear divisions and the origin of mixotypes

The pregamic phase of ciliate conjugation includes sometimes 2 or 4 Mi fissions instead of the typical number of three. *Euplotes*, for example, has 1 premeiotic, 2 meiotic and 1 postmeiotic fissions (Turner, Siegel, Heckmann, Wichterman et al.); 2 fissions were found in other ciliates by Finley, Grell, Moldenhauer et al. According to Suzuki 1957, "parthenogenetic" (isoploid) conjugation in *Blepharisma japonicum* involves reduction of the number of Mi fissions from 6 to 5; II fission does not results in chromosome reduction and yields 2 diploid nuclei, dividing three times. *P. putrinum*, with its striking instability of meiotic phase, belongs to a group (*Hymenostomatida*) where such atypical modifications of meiosis are unknown.

Let us compare the schemes of five reorganization patterns in *P. putrinum* (Table 5); one can see reduction of the number of fissions from 6 to 5, 4 and 3; one can't imagine more simplified reconstruction process than apomixis II, coinciding with a normal postgamic phase (that involves 3 fissions of Sk). Nevertheless, the origin of aberrant cycles is not explainable by simple deletion of Mi fissions; some definite pregamic fissions are preserved, but significantly modified, with corresponding shortening of all subsequent reorganization phase.

Each Mi division in amphimicts leads to a definite, stable pattern of disposition of the daughter nuclei within the cell, and their sites determine their fate. I and II Mi fissions are preserved in automicts I, producing hemiploid nuclei, but the spindles of II fission are diagonal, producing thus 2 paroral and 2 extraoral nuclei. 3 daughter nuclei degenerate after II Mi fission in amphimicts, and 2 in automicts; paroral nuclei fuse into Sk occupying atypical site within the cell. Its products are normally differentiated again into paroral and extraoral nucleus, and the first one starts its reversal movement in the same moment as hemiploid migratory pronucleus in amphimicts. This coincidence is not occasional; all 3 pregamic Mi fissions are conserved in automicts I, but the second and third one are modified and Sk formation is premature.

Similar comparisons indicate that all 3 pregamic fissions are preserved in automicts II also, but caryogamy occurs here after I Mi fission, and the following events are simply a copy of such nuclear position, that is peculiar for amphimicts. In both kinds of automixis III Mi fission is preserved, but it leads to differentiation of 2 giant euploid nuclei uncapable for exchange and fusion. There is a typical two-step meiosis in automicts I, but there may be different opinions in respect to automicts II. Since the cytology of I Mi fission coincides entirely with both kinds of automixis, it may be supposed that the union of 2 prophase nuclei does not hinder the completion of meiotic changes within a chromosome set (separation of individual chromosomes after crossing-over) and that we deal here with modified meiosis not leading to haploidy (or hemiploidy) of the daughter nuclei. Due to union of both products of I meiotic fission, 2 euploid nuclei instead of 4 hemiploid ones

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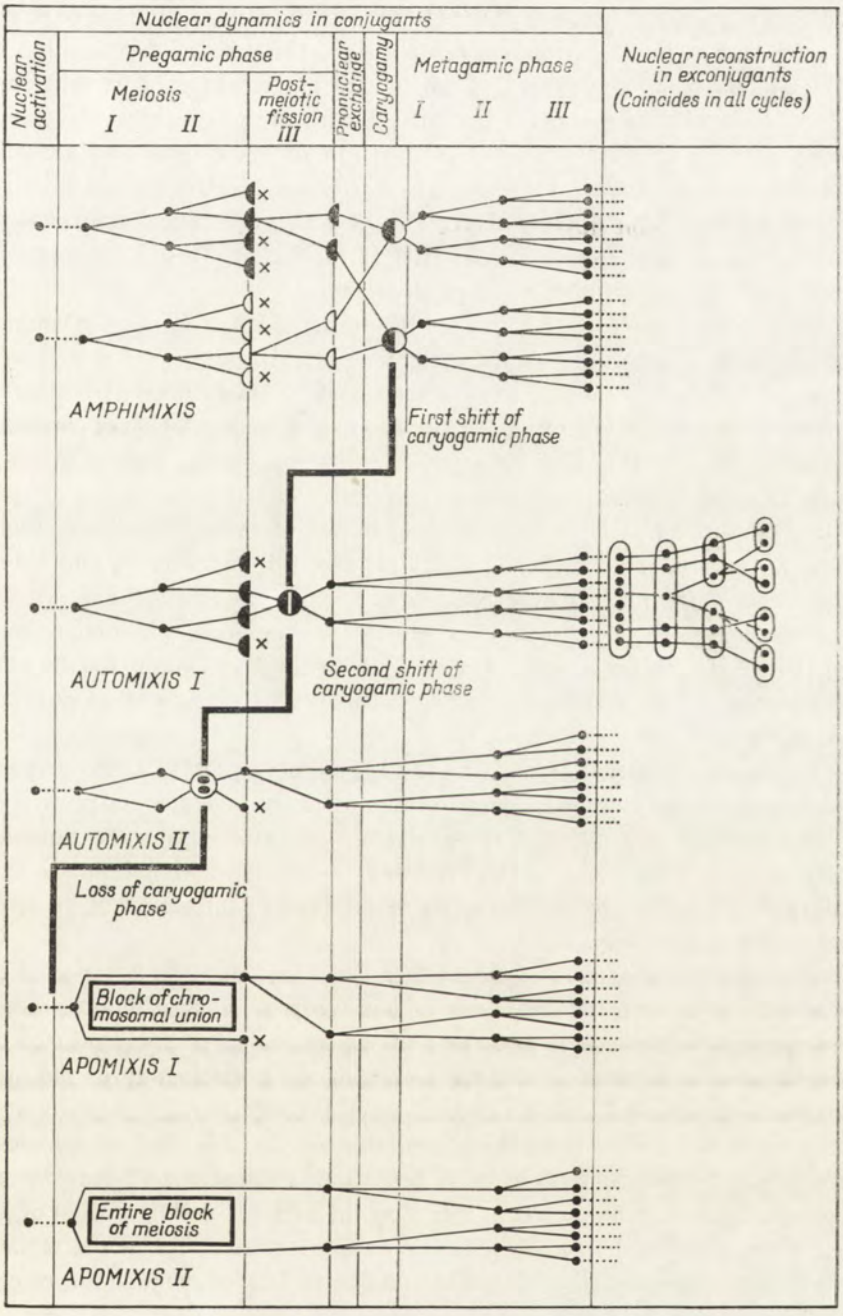
Table 5

Comparison of the schemes of amphi-, auto- and apomixis

Designations: ● – euploid nuclei; ◖◗ – hemiploid nuclei; ◐ – amphimictic synkaryon; ◑ – automictic synkaryon; ⊛ – union of 2 euploid nuclei during meiosis; ●×, ◖× – pycnocarya.

The linear arrangement of reorganization patterns does not represent their supposed phylogenetic sequence.



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are produced at the next Mi fission. In my opinion, there is no reason to deny the meiotic nature of these 2 fissions since "meiosis" means both crossing-over and hemiploidization, and the last process is unnecessary due to autogamic union of the nuclei.

Bearing in mind the direction of the spindles, nuclear sites within the cells, absence of a short-lasting interkinesis after I Mi fission, the time of degeneration of the daughter nucleus and the period of the inward movement of a paroral one, one can homologize II Mi fission of apomicts I with III postmeiotic Mi fission of amphimicts. This is evident also at comparison of apomixis I and automixis II where III "differentiating" Mi fission is also retained. Unlike automicts, some apomictic mates exhibit nuclear aberrations of a high theoretical interest — migration or fusion of large euploid nuclei after II Mi fission. This is a direct evidence of the homology of the above named divisions.

I Mi fission in apomicts I combines the traits of I and II ones of amphimicts (long prophase, degeneration of the daughter nucleus), but does not lead to hemiploidization of nuclei. I think that meiosis (2 first Mi fissions) is replaced here simply by an isoploid Mi fission, probably due to lack of copulation of chromosomes in prophase Mi I, with natural omission of a linked subsequent II Mi fission.

I Mi fission in apomicts II proceeds identically with the second one of apomicts I; it leads to differentiation of the daughter nuclei, sometimes capable to migration or fusion. This fission corresponds to III Mi fission in amphimicts and automicts. The absence of the nuclear degeneration in apomicts II is quite natural, since II Mi fission (leading to the nuclear degeneration in amphimicts, automicts and apomicts I) is lost in this mixotype. Apomixis II differs from apomixis I by omission of the equational Mi division, replacing 2 meiotic Mi fissions of amphi- and automicts.

It was noted above that automixis I and II are linked processes, and preparations show usually many pairs with these processes in both mates respectively. This parallel course of two different reorganization processes shows the homology of Mi fissions in a most deciding way. All three divisions of Mi take place synchronously and are homologous; they differ in the time of formation of Sk after I or II Mi fission respectively.

The results of comparison of the nuclear cycles lead us to an unexpected conclusion about the unifactorial difference of five types of reorganization processes in *P. putrinum*. Although this hypothesis must be somewhat complicated (see below), it constitutes a starting point for the search of the possible way of the origin of mixotypes. Automixis I may be derived now directly from amphimixis at modification of II Mi fission (hereditary alteration of the direction of spindles and, correspondingly, of nuclear sites and behaviour). Automixis II may be derived independently from amphimixis at modification of I Mi fission (fusion of its derivates) while apomixis I from amphimixis or from automixis I at the replacement of the first 2 fissions by single isoploid one due to lack of conjugation of chromo-

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somes. Apomixis II may be derived from apomixis I at further omission of the introductory equational Mi division. It is significant to note that III postmeiotic "differentiating" division is invariably preserved in all five types of reorganization processes, and meiosis only is subjected to hereditary alterations (Table 6).

Table 6

Homology of micronuclear fissions at 5 inherited nuclear reorganization patterns in *Paramecium putrinum*

 — amphimictic and automictic euploid and twice euploid synkaryon

Reorganization pattern	The number of micronuclear divisions	Micronuclear divisions				
		meiotic		postmeiotic	postgamic	
Amphimixis	6	I	II	III	IV	V VI
Automixis I	5	I	II	III	–	IV V
Automixis II	5	I	II	III	–	IV V
Apomixis I	4	I^1		II	–	III IV
Apomixis II	3	–		I	–	II III

[1] The I Mi fission is isoploid one being equivalent to both meiotic fissions of amphimicts and automicts

It is evident now that *P. putrinum* is characterized by an extreme instability of gene system controlling the normal course of meiosis; some inherited aberrations (mutations) in this system resulted in the origin of lines with two kinds of automixis and two more kinds of apomixis. "Unifactorial" hypothesis explains well the way of reorganization process from amphimixis to automixis I or II and vice versa: sole change in the pregamic phase is sufficient for such switching. This hypothesis explains also the origin of apomixis II from apomixis I, of apomixis I from amphimixis, as well as of automixis I and II independently from amphimixis. Nevertheless, it explains only one of many problems. It remains still unknown, how the replacement of reorganization process in MT B may be induced by contact with automictic cells; what is the role of the cytoplasm and the mechanism of gene-cytoplasm interchange; why automixis I and II do not exist in pure state, as clonal traits; why apomixis I and II are not linked, and so on. It remains completely unclear now, why aberrant mixotypes are so widely distributed in nature, being spread on extreme territories, possibly throughout the species range; moreover, they occur even more commonly than obligate amphimicts. If *P. putrinum* as a species arised

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at mutation in one cell and then became widespread, the hereditary instability of meiosis might appear on the earliest stage of development of the species, and territorial expansion of the species throughout the moderate climate zone could be paralleled by expansion of aberrant mixotypes. A world-wide distribution of auto- and apomicts may be explained also by existence of a similar mixotype system in the ancestral species of a small frontoniid, simply retained by *P. putrinum*. The third hypothesis is a genetic one: if aberrant cycles really dominate above the amphimictic one at intermixotype crosses, and are inherited in the progeny of such pairs, then the gradual spreading of mixotypes all over the world might be due to a slow convertion of amphimicts into auto- and apomicts, initiated by some mutant cells.

Intraspecific differentiation in *P. putrinum* and species problem

The species structure in *P. putrinum* is highly complicated and seems to be unique among ciliates. The system of mt, grouped into syngens, is linked with a system of morphotypes (subspecies) and mixotypes (groups of races with different reorganization patterns). All these subdivisions constitute a morphological entity, and the species, sharply limited from other paramecia, is easily determinable due to a number of peculiar traits.

Whether the "biological species" may be recognized in the species, that is predominantly non-amphimictic; whether *P. putrinum* is true taxonomic species? According to Sonneborn 1957, the taxonomic species of *Paramecium* and *Tetrahymena* represent a complex of biological species, named syngens, that are too similar morphologically to give them latin names (although such proposals were made by some authors). Although we understand usually the "syngen" as a group of complementary mt, Sonneborn introduced this term in much wider sense, as general notion corresponding to the previous "biological species" and equaly applicable to amphimicts, automicts, and agamonts. The syngen is determined according to the mating ability in amphimicts and according to other, specific traits in agamonts; no general criterium of syngen exists. Sonneborn's idea was directed against both denying the existence of species in *Protozoa* and equaling of each stock to the species. Since "syngen" is applied irrespectively of the reproduction modes, this notion is equally applicable to *P. putrinum*.

Syngen in its wide sense, like the taxonomic species, has undergone an irreversible way of evolutionary differentiation, but, unlike that species, it reveals still no detectable morphological differences or only some insignificant ones. When the young species obtain morphological characteristics also, syngen coincides with description of the taxonomic species and there is no need for further use of this notion. The species *Paramecium aurelia* and *Tetrahymena pyriformis* are now in the state of superspecies (coenspecies, or species complex), including 2–16 syngens, that are reproductively isolated and differ in their physiology (mainly in thermostability) and geographical distribution. Syngen is not a subspecies, variety or form, primarily morpho-taxonomic notions. Hairston, who did not accept

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Sonneborn's idea, proposed to consider the previous species of paramecia as separate genera, and their syngens as species receiving binary latin names; Elliott finds sufficient to refer these as "*T. pyriformis* I, II ...".

Following Sonneborn, both morphological differentiation and the occurrence of amphimixis must not be overvalued as main species criteria in protozoa. Although two steps in species evolution (agamic and amphimictic species) are generally recognized, three such steps may be distinguished in animals (Jankowski 1966 d): primary-agamic, facultative-amphimictic (agamo-amphimictic) and obligatory-amphimictic species, when amphimixis becomes the sole possible way of species existence. Ciliates fall into an intermediate group, since they are reproduced by fission or budding, and amphimixis (conjugation) occurs relatively rarely and serves for the nuclear and cortical reorganization only, but not for reproduction. Conjugation is unknown in many common, well studied ciliates (e.g., in most colpodas) and is very rare in others — *Spirostomum*, *Stentor*, *Woodruffia* etc.). Paramecia may be cultured during years without conjugation. The significance of amphimixis in intermediate group must not be overvalued and equaled to that in higher animals. Orthodox classification of species into agamic and amphimictic ones seems to be not correct. It is just this contrast that resulted in opposition of "tentative" (agamic) species to "real" (amphimictic) one; the gradual three-step evolution of the species indicates very eloquently the limited significance of amphimixis and the heterogenous and artificial nature of "amphimictic" or "real" species.

The discovery of secondary auto- and apomictic lines of *P. putrinum* indicates once more the relative significance of amphimixis as species criterion in ciliates. Irrespective of a kind of reorganization processes, syngens in this species represent true reproductively isolated biological species.

The significance of mixotypes for the species

Should mixotypes be regarded as adaptive differentiation? In higher animals, intraspecific differentiation bears generally adaptive character (Mayr, Zavadsky et al.); the more varieties and races exist, the more are possibilities for the flourishing of the species and its many-sided adaptation to the medium. There may be a seasonal, ecological, functional, nutritional and other kinds of divergence, and such variation makes impossible the elaboration of a universally applicable system of intraspecific categories. On the contrary, the intraspecific polymorphism in *Protozoa* in many cases is not explainable by definite ecological and geographical factors (Poljansky 1957). The origin of the groups of complementary mt in ciliates (syngens in the narrow sense) is not a result of adaptation of some races to the medium, or result of their isolation; it may be imagined as unrolling in the time and on extreme space of some complicated system of supersexual differences between the groups of hermaphrodite lines. Syngen system is not medium-induced differentiation pattern. It is enough to recall that syngens include a definite, multiple number of mt (2 to 16) and that mt system of one syngen is paralleled by that of another

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one, making possible the aberrant intersyngenic conjugation between complementary mt analogues.

In *P. putrinum*, both systems of syngens and mixotypes can't be regarded as adaptive differentiation: once again, mixotypes represent the blind unrolling in time and space of the system of the nuclear reorganization patterns, which origin was made possible by instability of genes controlling the normal course of meiosis in primarily amphimictic races. As result of this development, aberrant lines occur now not more rarely than amphimictic ones. In spite of their abundance, the species does not suffer from its restraint from amphimixis: it is flourishing in countries with suitable, moderate climate, and is abundant partly in our reaserch area. Obligate auto- and apomicts, for example selfing lines KS 14 (MT C) and CRn (MT D) are perfectly cultivated in laboratory, yielding mass cultures free of any signs of hereditary diseases in spite of mass non-amphimictic repeated conjugation occurring at each change of the medium.

Theoretically, in case of diploid animals, restraint from amphimixis must have negative consequences: the deep reconstruction of the nuclear apparatus becomes impossible; lethal mutations must be homozygotized, etc. Situation with ciliates is contradictory, however. Jones and Diller, cultivated apomictic races of *P. putrinum* in USA, noted their lowered viability and death respectively, but nothing similar was recorded in my races maintained in cultures during many years. It may be recalled that *P. putrinum* has polyploid and not diploid Mi, thus homozygotization of mutations in micronuclear genes is impossible in both automicts II and apomicts with their isoploid nuclear reorganization. It seems pertinent to recall also a number of works showing the possibility of negative influence of amphimictic conjugation on healthy lines of ciliates. The rise of viability is not a universal result of amphimixis; low reproduction rate, nuclear anomalies, aberrant cortical morphogenesis and other diseases of the progeny may lead to significant or total death of exconjugants after cell separation (Jennings, Sonneborn, Lynch, Corliss, Warren, Chen, Ray, Nagel, Nanney, Kościuszko, Suzuki et al.). Lethal conjugation is explained usually by incompatibility of the cytoplasm or genomes and occurs sometimes without any detectable anomalies in the nuclear behaviour (e.g. in *Tetrahymena chironomi*, as described by Corliss). In sum, the loss of amphimixis must not have only inevitable negative results for *P. putrinum*.

According to Faurè-Fremiet 1953, the main significance of reorganization processes in ciliates consists in the replacement of an old nucleus, that may accumulate unbalanced genomes and may become aneuploid at cell fissions; such replacement may be realized during conjugation, autogamy, "endomixis" of *Loxodes*-type, hemixis and Ma regeneration from its fragment (Faurè-Fremiet, Sonneborn, Corliss, Schneller, Nobili et al.). From this viewpoint, auto- and apomictic conjugation is still useful for the lines of *P. putrinum*, incapable of single-celled reorganization, since it leads to the nuclear and cortical reconstruction and by no means represents a pathological phenomenon.

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The new Ma in auto- and apomicts, and not only in amphimicts of *P. putrinum*, must not correspond entirely in its genome set to an old one. Too commonly we encounter the uneven distribution of chromosomes within anaphase spindles, loss of chromosomes by dividing nuclei; there are many dozens of anomalies in the nuclear behaviour, and some of these may be of especial genetic significance, e.g. unilateral migration of euploid nucleus into another partner.

In the review of the data on complexity of species structure in *Protozoa* Poljansky 1957 noted that "The most complicated state of intraspecific differentiation among protozoa occurs in ciliates. Unfortunately, the problem of species structure was not studied here in enough details. Nevertheless, the accumulated data indicate a diversity of intraspecific groups in ciliates. One of the possible causes of a complicated intraspecific polymorphism may be the occurrence of a variety of kinds of reproduction and of reorganization of the nuclear apparatus, constituting the source of variability". The results of the study of species structure in *P. putrinum* may be regarded as an additional evidence in favour of this statement. Both known (syngens and morphotypes, linked with syngens) and new kinds of intraspecific differentiation (mixotype system, linked with morphotypes and syngens) appeared here just on the basis of hereditary modifications of conjugation.

Paramecium putrinum — a proposed new object for laboratory research

The circle of main laboratory objects among ciliates is wide enough now (separate species of *Paramecium*, *Tetrahymena*, *Euplotes*, *Blepharisma*, *Stentor*, *Dileptus*, *Urostyla* and many others): 3 species of *Paramecium* from near 15 known ones are main objects of cytological and genetic research — *P. aurelia*, *P. bursaria* and *P. caudatum*. Many paramecia have some traits that make difficult the cytogenetic studies; for example, *P. aurelia* has very small chromosomes; autogamy in this species may change both pheno- and genotype without knowledge of investigator; *P. bursaria* has too long immaturity period; its Ma remains intact in mates and pycnocaryons are resorbed on various stages of conjugation, thus correct determination of reorganization stages is difficult. *Tetrahymena* lacks an active mating reaction after mixture of complementary lines.

According to my experience, *P. putrinum* shows a successful combination of useful traits that make easy the study of the species and indicate its value for research in ciliate morphology, morphogenesis, cytology and genetics. The species is widespread in nature and may be easily cultured in laboratory. Its kinetome topology is much simpler than that of all other paramecia, especially higher ones; the number of kineties is small, buccal and vestibular cavities are shallow, ciliary oral stripes are easily visible. This simplifies the study of the kinetome and buccal dynamics at both fission and conjugation. Ma contains very large nucleoli, and their behaviour may be traced easily in dividers and conjugants in Ma, its fragments and anlagen. Ma undergoes the regular branching and fragmentation during conjugation, and this feature helps the identification of reorganization phases. Small

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dimensions of the species are compensated by the presence of very large Mi, while most other paramecia exhibit an inverse relation. Meiotic stages may be traced in details in large chromosomes; the approximate number of chromosomes may be counted on optical sections of both euploid and hemiploid nuclei. Large size of the Mi derivates simplifies the study of the nuclear dynamics and detection of nuclear aberrations. Moreover, nuclear events may be traced in living pairs.

The species shows an active and immediate mating reaction; immaturity period is practically absent, and caryonides may be tested for mt already after a week following their isolation (this period is measured by months in *P. bursaria* and *Tetrahymena*). Selfing lines are common, constituting near 30% of isolations; thus it is easy matter to find a stable or selfing line within a single sample, according to the design of the work. Since autogamy is absent in *P. putrinum*, stable lines maintain by years their original phenotype and genotype. Autogamy may be induced at wish by one of existing techniques, e.g. by multi-agglutination (Jankowski 1965 c). In addition, reconjugation and multiconjugation may also be induced and studied in *P. putrinum*.

Apart from all these advantages, the existence of a system of nuclear reorganization patterns is the most significant trait of the species. Only *P. putrinum* among studied ciliates includes many lines with regularly inherited amphi-, auto- and apomixis. Intermixotype crosses (amphimicts × automicts, and possibly all other combinations) are possible within the syngens, with replacement of amphimictic process by an aberrant one. This means that one and the same line may be induced to conjugate in various ways, according to the wish of investigator! Selection of partners with definite reorganization processes may be performed in vivo in conjugating wild samples or laboratory cultures; it is enough to note the number and size of pycnocarya. One can also made a guaranteed selection of pairs with automixis I and II in three possible combinations.

Unlike pycnocarya of *P. caudatum* and *P. bursaria*, those of *P. putrinum* are remarkably stable until the end of conjugation and thus may be used for cytophotometric research: they do not synthesize DNA, and their relative ploidy in all mixotypes is known. Such studies may supplement those made on vegetative Mi. Both Mi and hemiploid pycnocarya may be compared cytophotometrically with Ma fragments, and I anticipate the decisive prove of micronuclear polyploidy in *P. putrinum* by such comparisons.

Both partners may be easily markered in vivo by chinese ink or vital stains, but there are also some natural markers; e.g., lines with normal and clear Ma occur in Sg 1 and 2; Ma breakdown is greatly delayed at intermixotype crosses in one partner, and this may be noted in living cells.

Some perspectives

It is difficult now to realize all the perspectives that may give to investigators the occurrence of amphi-, auto- and apomixis in a single ciliate species and the

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possibility of intercrossing of such lines. The research possibilities will become more wide when amicronucleate lines, too rare in nature, will be isolated or experimentally produced in each mixotype. For example, mating of amicronucleate auto- or apomicts with normal ones may be used for Ma regeneration; amicronucleate amphimicts may be crossed with normal automicts, with Ma regeneration in the first ones. It is very interesting to know whether amicronucleate automicts induce restraint from amphimixis in MT B cells, like normal ones. Many similar items await special research.

The number, composition and distribution of syngens and mixotypes must be analyzed further in areas covering the species range. It remains to isolate all mixotype set in each syngen and to perform intermixotype crosses at all possible combinations (B×D, D×C etc.) in addition to B×C crosses, realizable now. All intermixotype crosses must be repeated twice using amicronucleate partners in all possible combinations, to reveal the role of nuclei in redirection of reorganization processes. A special cytological study must be made anew on the first Mi fissions in apomicts I to exclude ultimately the possibility of conjugation of chromosomes. Apomixis II of Diller must be cytologically studied again: Diller noted the possibility of nuclear degeneration, and it remains to be solved whether this is simple aberration or too simplified description of apomixis I. The lines used by Diller seem to be cultivated now axenically by Dr Lilly's group in New York.

In addition to an extensive cytological problematics, *P. putrinum* allows the genetic studies of a kind unrealizable in other paramecia. For example, one may compare the inheritance of main traits (mt, selfing activity and nuclear reorganization pattern) at amphi- and automixis realized both at intercrosses and selfing. Mixotype inheritance after intermixotype crosses is a large new field of research.

Ammermann 1966 made first genetic studies on *P. putrinum* (mt inheritance in caryonides) without detection of the nuclear reorganization pattern in studied lines, at least with no mention to it. The rules of mt inheritance are surely different in amphi-, auto- and apomixis; in this respect, I must underline the leading idea of the present research: cytological (cytogenetical) studies on *P. putrinum*, at least determination of mixotype and prevailing anomalies in Mi behaviour, must precede genetic ones.

It remains to hope that *P. putrinum*, the most primitive and cytogenetically the most interesting species of the genus, will receive in future the same attention as *P. aurelia*, *P. caudatum* and other main objects of cytogenetic research among ciliates.

Acknowledgements

I express my gratitude to Professor G. I. Poljansky, for constant guidance at all stages of this research in the Leningrad State University (Chair of Invertebrates) and at post-graduate course in the Institute of Cytology Academy of Science USSR (Laboratory of Protozoan Cytology), 1959–1965.

I am grateful to the members of the Editorial Board of this Journal for acception of this oversized article in its full length.

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IX. Summary

Short morphological sketch of *Paramecium putrinum* is presented, its synonymics and phylogeny are discussed. The kinetome and nuclear apparatus of vegetative forms were studied; heteroploid series of Mi is described. Polyploidy of Mi is regarded as species character and not simple clonal aberration in 5 species of *Paramecium* with massive Mi.

3 distinct syngens with 8, 6 and 7 mating types and 2 provisory syngens were found in the research area. Selfing lines of the species are widely distributed in nature; at least in syngen 5 selfing appears to be an inherited character.

Nuclear reorganization in conjugants may follow one of 5 ways, depending on the mixotype of both partners. Amphimixis, automixis I and II and apomixis I and II are distinguished as hereditary, not occasional processes.

Amphimixis was studied in special details. It includes 6 Mi fissions (meiosis, pronuclear differentiation, their exchange and fusion, 3 metagamic divisions); 3 hemiploid pycnocaryons are formed after II Mi division. Nuclear changes were traced step-by-step, some typical stages of meiosis were detected, chromosome number was determined. Pronuclear migration was studied in details; unusual pattern of anlage development was found. The study of Ma fragmentation and RNA dynamics indicates that Ma fragmentation serves for providing the exconjugant progeny with RNA source.

Automixis I includes 5 Mi fissions — meiosis, fusion of 2 undifferentiated pronuclei in the oral cone and 3 fissions of a synkaryon. Meiosis proceeds normally, but directions of II Mi spindles are hereditary modified and only 2 pycnocaryons are formed.

Automixis II, with unique modification of meiosis, includes 5 Mi fissions; 2 euploid nuclei are united in II Mi prophase. One euploid daughter nucleus degenerates after I fission of such synkaryon, and the remaining one divides three times.

Apomixis I includes 4 isoploid Mi fissions with degeneration of one large euploid nucleus after I Mi fission. Apomixis II includes 3 isoploid fissions without nuclear degeneration; it is established here after Diller's 1949 description.

A new intraspecific differentiation — mixotype system — was discovered. Mixotype is a group with a definite nuclear reorganization pattern or with their definite combination. MT A includes obligate amphimicts, MT B — facultative ones, MT C — automicts with coupled automixis I+II, MT D — apomicts I, MT E — apomicts II. Intermixotype mating (MT B×C) is possible, with automixis in all partners. The origin and significance of mixotype system is discussed.

The successful combination of many useful traits and the existence of a mixotype system makes *P. putrinum* perfect new object for research in protozoan cytology and genetics.

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РЕЗЮМЕ

Приведен краткий очерк морфологии *Paramecium putrinum*, обсуждается синонимика и филогения вида. Изучен кинетом и ядерный аппарат вегетативных форм, выявлен ряд гетероплоидии Ми. Полиплиодия Ми у 5 видов парамеций с массивными Ми признается видовым признаком, а не клональной аберрацией.

В районе работ выделено 3 четких сингена с 8, 6 и 7 типами спаривания и 2 условных. В природе широко распространены селфинг-линии вида; по крайней мере в Сг 5 наследуется постоянная нестабильность типа спаривания.

Реорганизация ядер при конъюгации может пойти одним из 5 путей, в зависимости от миксотипа партнеров. Различаются 5 наследуемых (не случайных) реорганизационных процессов — амфимиксис, автомиксис I и II, апомиксис I и II.

Амфимиксис изучен особенно подробно. Имеются 6 делений Ми (мейоз, дифференцировка пронуклеусов, их обмен и слияние, 3 метагамных деления); дегенерируют 3 гемиплоидных пикнокариона после II деления Ми. Шаг за шагом прослежены изменения ядер, найден ряд типичных стадий мейоза, определено число хромосом. Детально изучен процесс миграции пронуклеуса, выявлен необычный способ развития зачатков Ма. Изучена фрагментация Ма и нуклеолярный цикл у конъюгантов и экскоңъюгантов. Фрагментация Ма служит для снабжения потомков экскоңъюганта источником РНК.

Автомиксис I включает 5 делений Ми — мейоз, преждевременное слияние 2 недифференцированных пронуклеусов в ротовой зоне и 3 деления синкариона. Мейоз протекает типичным образом, но наследственно изменены направления веретен II деления Ми, образуются лишь 2 пикнокариона.

Автомиксис II — уникальная модификация мейоза — включает 5 делений Ми; 2 ядра объединяются в профазе II мейотического деления; синкарион составлен эуплоидными ядрами. После I деления синкариона дегенерирует одно крупное эуплоидное дочернее ядро, оставшееся делится трижды.

Апомиксис I включает 4 изоплоидных (эквационных) деления Ми с дегенерацией одного ядра после I деления Ми. Апомиксис II с 3 эквационными делениями, без дегенерации ядер, устанавливается по описанию Диллера.

Установлена гомология делений Ми при всех 5 способах ядерной реорганизации. Выявлена новая форма внутривидовой дифференцировки у инфузорий — система миксотипов, групп с разным способом реорганизации ядер или с определенным их сочетанием: MT A (облигатные амфимикты), MT B (факультативные амфимикты, способные к автомиксису I и II), MT C (автомиксис I сцеплен с автомиксисом II), MT D (апомиксис I) и MT E (апомиксис II). Возможна меж-миксотипная конъюгация MT B × MT C, с отказом от амфимиксиса у партнера из MT B. Обсуждается происхождение системы миксотипов и значение ее для вида.

Благодаря удачному сочетанию ряда признаков и наличию системы миксотипов *P. putrinum* признается удобным новым объектом для исследований по цитологии и генетике простейших.

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X. REFERENCES

Ammermann D. 1966: Das Paarungssystem der Ciliaten *Paramecium woodruffi* und *P. trichium*. Arch. Protistenk., 109, 139–146.

Bick H. 1966: Populationsökologische Beobachtungen über das Auftreten sexueller Prozesse bei Süsswasserpolypen und Ciliaten. Zool. Anz., 176, 183–192.

Borchsenius O. N., Skoblo I. I. and Ossipov D. V. 1968: Polimorfizm mikronukleusov *Paramecium caudatum* Ehrb. I. Morfologičeskij tip interfaznych jader. Citologija, 10, 227–235.

Bütschli O. 1873: Einiges über Infusorien. Arch. mikrosk. Anat. 9, 657–678.

Bütschli O. 1876: Studien über die ersten Entwicklungsvorgänge der Eizelle, die Zelltheilung und die Conjugation der Infusorien. Abh. senckenb. naturforsch. Ges., 10, 1–250.

Bütschli O. 1889: *Protozoa*. III. Abtheilung: Infusoria und System der Radiolarien. In: H. G. Bronn's Klassen und Ordnungen des Thier-reichs 1, 1098–2035.

Cheissin E. M. and Ovtchinnikova L. P. 1964 a: A photometric study of DNA content in macronucleus and micronucleus of different species of *Paramecium*. Acta Protozool., 2, 225–236.

Cheissin E. M., Ovtchinnikova L. P. and Kudrjavtsev B. N. 1964 b: A photometric study of DNA content in macronucleus and micronucleus of different strains of *Paramecium caudatum*. Acta Protozool., 2, 237–245.

Claparede E. et Lachmann J. 1858–1861: Études sur les Infusoires et les Rhizopodes. Mèm. Inst. natn génev., 5, 1–260; 6, 261–482; 7, 1–291.

Diller W. F. 1934: The conjugation of *Paramecium trichium*. Anat. Rec., 60, suppl., 92.

Diller W. F. 1940: Nuclear behavior and reproduction in ciliated *Protozoa*. In: Contr. Zool. Labor. Univ. Pennsylvania, Philad., Univ. Calif. Bicent. Conf., 81–95.

Diller W. F. 1948: Nuclear behavior of *Paramecium trichium* during conjugation. J. Morph., 82, 1–31.

Diller W. F. 1949: An abbreviated conjugation process in *Paramecium trichium*. Biol. Bull., 97, 331–343.

Diller W. F. 1957: Nuclear behavior and reproduction in ciliated *Protozoa*. Bioscience, 28, 217–234.

Diller W. F. 1959: Possible origin of hypoploidy in *Paramecium trichium*. J. Protozool., 6, Suppl., 19.

Doflein F. 1907: Beobachtungen und Ideen über Konjugation der Infusorien. S. B. Ges. Morph. Physiol. Münch., 23, 1–8.

Doflein F. 1916: Lehrbuch der Protozoenkunde. 2. Aufl., Jena.

Doflein F. und Reichenow E. 1953: Lehrbuch der Protozoenkunde. 6. Aufl., Jena, 249–252.

Dogiel V. A., Poljansky G. I. and Cheissin E. M. 1962: Obščaja protozoologija, Izd. Nauka, Leningrad 1–592.

Ehret C. F. 1967: Parathene theory of the shapes of cells. J. theor. Biol., 15, 263–272.

Fauré-Fremiet E. 1953: L'hypothèse de la sénescence et les cycles de réorganization nucléaire chez les ciliés. Rev. suisse Zool., 60, 426–438.

Gillies C. G. and Hanson E. D. 1968: Morphogenesis of *Paramecium trichium*. Acta Protozool., 6, 13–31.

Golikova M. N. 1969: Polimorfizm mikronukleusa u nekotoryh svobodnoživuših infuzorij. Uspehi Protozoologii Tezisy dokladov III Mežd. Kongressa Protozoologov, Leningrad 1969, Izd. Nauka 24–25.

Grell K. G. 1967: Sexual reproduction in *Protozoa*. In: Research in Protozoology (ed. T. T. Chen), Oxford, 2, 148–213.

Jankowski A. W. 1960: Processy konjugacii *Paramecium trichium* Stokes. I. Amfimixis i avtogamija. Citologija, 2, 581–588.

Jankowski A. W. 1962 a: Processy konjugacii *Paramecium putrinum* Clap. et Lachm. II. Apomiktičeskije reorganizacjionnyje cykly i sistema miksotipov. Citologija, 4, 434–444.

Jankowski A. W. 1962 b: Processy ... III. Množestvennaja sistema tipov sparivanija u *Paramecium putrinum*. Zh. obšč. Biol. 23, 276–282.

Jankowski A. W. 1965 a: Processy ... IV. Individualnaja izmenčivost jadernych processov pri apomiktičeskoj konjugacii. Citologija, 7, 55–65.

Jankowski A. W. 1965 b: Processy ... V. Vozvrat k amfimiksisu v miksotipe B. Dokl. Akad. Nauk SSSR, 163, 523–525.

Jankowski A. W. 1965 c: Processy ... VII. Jadernyje processy pri vnekonjugacionnoj avtogamii, inducirovannoj novym metodom "množestvennogo sparivanija". Acta Protozool., 3, 239–262.

http://rcin.org.pl

Jankowski A. W. 1965 d: Sistema tipov sparivanija, singenov, miksotipov i sposoby reorganizacii jadernogo apparata u *Paramecium putrinum* C. et L. Cand. biol. sci. diss. Institute of Cytology Acad. Sci. USSR, Leningrad, 1, 1–400: 2, 1–196.
Jankowski A. W. 1965 e: Sistema tipov sparivanija, singenov, miksotipov i sposoby reorganizacii jadernego apparata u *Paramecium putrinum* C. et L. Avtoreferat cand. diss. Leningrad, 1–22.
Jankowski A. W. 1966 a: Processy ... VI. Indukcija i citologičeskoje izučenije trojnoj konjugacii. Citologija, 8, 70–79.
Jankowski A. W. 1966 b: Processy ... VIII. Jadernyje processy pri povtornom sparivanii — rekonjugacii. Zool. Zh., 45, 818–829.
Jankowski A. W. 1966 c: Processy ... IX. O "nekrochromatine" i funkcionalnom značenii fragmentacii makronukleusa. Citologija, 8, 725–735.
Jankowski A. W. 1966 d: Problema celostnosti biologičeskogo vida. In: Filos. Voprosy Sovrem. Biologii, Leningrad, 155–176.
Jankowski A. W. 1969 a: Predlagajemaja klassifikacija roda *Paramecium* Hill, 1752 (*Ciliophora*). Zool. Zh., 48, 30–40.
Jankowski A. W. 1969 b: Citologičeskoje izučenije amfimiktičeskoj konjugacii *Paramecium putrinum*. Uspehi Protozoologii Tezisy dokladov III Mežd. Kongressa Protozoologov, Leningrad 1969, Izd. Nauka, 44–45.
Jankowski A. W. 1972 a: Jadernyj apparat *Ciliophora*. I. Poliploidija mikronukleusa u infuzorij kak vidovoj priznak. Genetika, 8, 78–84.
Jankowski A. W. 1972 b: Povtorenije filogeneza v ontogeneze infuzorij. In: Voprosy Evoljucii, Novosibirsk, 2, 95–123.
Jankowski A. W. 1972 c: Stomatogenez pri delenii *Paramecium putrinum*. Doklady Akad. Nauk SSSR (in press).
Jankowski A. W. 1972 d: Processy ... X. Reorganizacija kinetoma pri konjugacii. Citologija (in press).
Jankowski A. W. 1972 e: Kinetome dynamics in *Paramecium putrinum* C. et L. (manuscript for Arch. Protistenk.).
Jankowski A. W. 1972 f: Abnormal nuclear behavior in *Paramecium putrinum* C. et L. (manuscript for Arch. Protistenk.).
Joukowsky D. 1898: Beitrag zur Frage nach den Bedingungen der Vermehrung und des Eintrittes der Konjugation bei den Ciliaten. Verh. Nat. Mediz. Ver. Heidelberg, 6, 17–42.
Katashima R. 1959: Mating types in *Eiplotes eurystomus*. J. Protozool., 6, 75–83.
Kovaleva N. E. and Jankowski A. W. 1965: Vlijanije ionizirujuščego izlučenija na processy jadernoj reorganizacii *Paramecium putrinum*. I. Zh. obšč. Biol. 26, 176–189.
Kovaleva N. E. and Jankowski A. W. 1966 a: Vlijanije ... II. Acta Protozool., 3, 239–262.
Kovaleva N. E. and Jankowski A. W. 1966 b: Jadernyje processy pri konjugacii paramecij, predvaritelno oblučennyh rentgenovskimi lučami. Citologija, 8, 80–89.
Perez-Silva J. 1965: Conjugation in *Frontonia acuminata* Ehrenberg. Progress in Protozoology, Abstr. Second int. Conf. Protozool. Excerpta med., London, 216–217.
Plate L. 1888: Protozoenstudien. VIII. Die Conjugation von *Paramecium putrinum* Cl. et L. Zool. Jahrb., Abt. Morph., 3, 135–200.
Poljansky G. I. 1957: O vnutrividovoj differencirovke i strukture vida u prostejših. Vestnik Leningrad Univ., Ser. Biol., No. 21, 45–64.
Poljansky G. I. and Raikov I. B. 1960: Rol poliploidii v evoljucii prostejših. Citologija, 2, 509–518.
Raabe Z. 1964: Zarys Protozoologii. Warszawa, PWN, 1–283 (See p. 167–168).
Raikov I. B. 1967: Kariologija prostejših. Nauka, Leningrad, 1–259.
Raikov I. B. 1969: The macronucleus of ciliates. In: Research in Protozoology, Pergamon Press, Oxford, 3, 1–128.
Raikov I. B., Cheissin E. M. and Buze E. G. 1963: DNA content of macronucleus and micronucleus in *Paramecium caudatum*, *Nassula ornata* and *Loxodes magnus*. Acta Protozool., 1, 285–300.
Roque M. 1961: Recherches sur les Infusoires ciliés: les Hyménostomes Péniculiens. Bull. biol. Fr. Belg., 95, 431–519.
Saito M. and Sato H. 1961: Morphological studies on the macronuclear structure of *Paramecium caudatum*. III. Zool. Mag. Tokyo, 70, 81–88.
Siegel R. W. 1956: Mating types in *Oxytricha* and significance of mating type system in ciliates. Biol. Bull., 110, 352–357.
Sonneborn T. M. 1938: Mating types in *Paramecium aurelia*. Proc. Am. phil. Soc., 79, 411–434.
Sonneborn T. M. 1949: Ciliated *Protozoa*: cytogenetics, genetics and evolution. A. Rev. Microbiol., 3, 55–80.

http://rcin.org.pl

Sonneborn T. M. 1957: Breeding systems, reproductive methods, and species problems in *Protozoa*. In: The species problem, AAAS symposium, ed. by E. Mayr, Washington, 155–324.
Sonneborn T. M. 1971: Methods in *Paramecium* research. In: Methods in Cell Physiology. Acad. Press, New York, 241–339.
Stokes A. C. 1885: Some new Infusoria. Am. Nat., 19, 433–443.
Stokes A. C. 1888: A preliminary contribution toward a history of the freshwater Infusoria of the United States. J. Trenton Nat. Hist. Soc., 1, 71–319.
Suzuki Sh. 1957: Parthenogenetic conjugation in *Blepharisma undulans japonicus* Suzuki. Bull. Yamagata Univ., Nat. Sci., 4, 69–84.
Vivier E. 1960: Contribution à l'étude de la conjugaison chez *Paramecium caudatum*. Ann. Sci. Nat., Zool., 12 sér., 2, 387–506.
Wenrich D. H. 1926: The structure and division of *Paramecium trichium* Stokes. J. Morph., 43, 81–103.
Wenrich D. H. 1928: Eight well-defined species of *Paramecium* (*Protozoa*, *Ciliata*). Biol. Bull.. 56, 390–401.
Wichterman R. 1937: Conjugation in *Paramecium trichium* Stokes (*Protozoa*, *Ciliata*) with especial reference to the nuclear phenomena. Biol. Bull., 73, 397–398.
Wichterman R. 1940: Cytogamy. A sexual process occurring in living joined pairs of *Paramecium caudatum*, and its relation to other sexual phenomena. J. Morph., 66, 423–451.
Wichterman R. 1949: The collection, cultivation and sterilization of *Paramecium*. Proc. Pa. Acad. Sci., 23, 151–180.
Wichterman R. 1953: The biology of *Paramecium*. New York.
Willis A. G. 1948: Studies on *Lagenophrys tattersalli* (*Ciliata*, *Peritricha*, *Vorticellina*). II. Quart, J. micr. Sci., 89, 385–400.
Woodard J., Gelber B. and Smith H. 1961: Nucleoprotein changes during the mitotic cycle in *Paramecium aurelia*. Expl. Cell Res., 23, 258–264.

EXPLANATION OF PLATES I–XIV

1–9: The kinetome of *Paramecium putrinum*, line M 6. Chatton–Lwoff and Klein

10–16: Nuclear apparatus, lines OM 23 (10), M 5 (11), CRt (12), CRn, monster and normal cell (13, 14), T 3 (15), HR 22 (16). Feulgen and Böhmer

17: Vegetative division, line CRn. Böhmer

18–63: Amphimictic conjugation, lines M6 × M 12, Sg 4, MT A. Feulgen and Böhmer

64–98: Automictic conjugation, lines NT 3 × NT 12, Sg 2, MT B. Böhmer

99–122: Apomictic conjugation, selfing-line CRn, Sg 5, MT D. Böhmer

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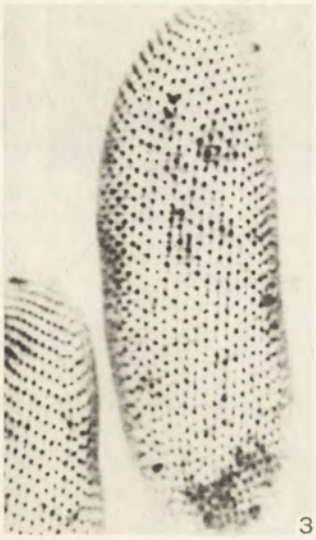
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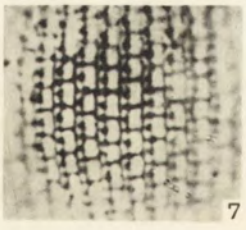
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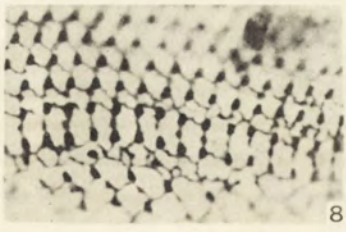
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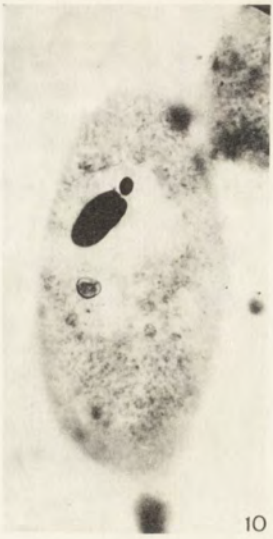

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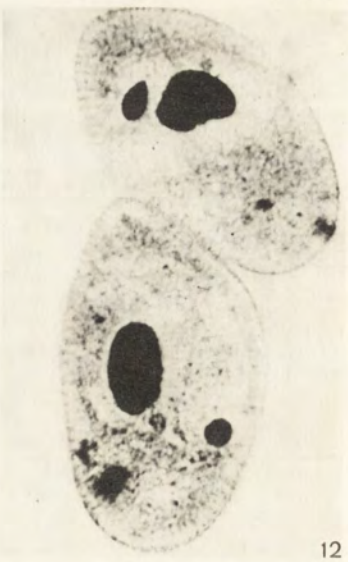

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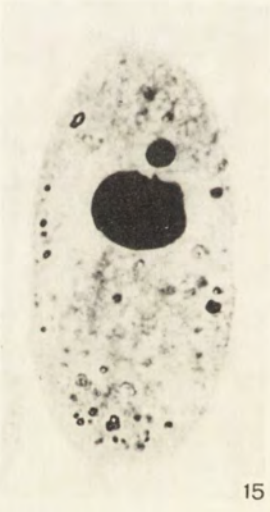

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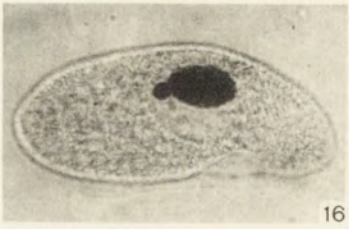

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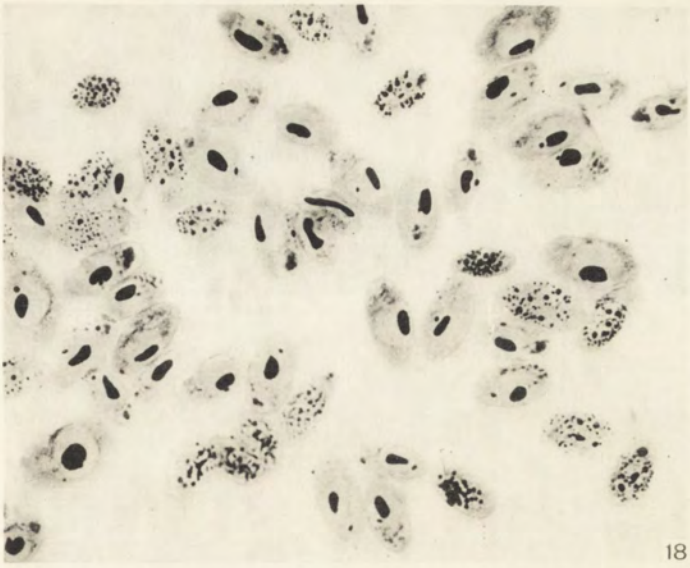
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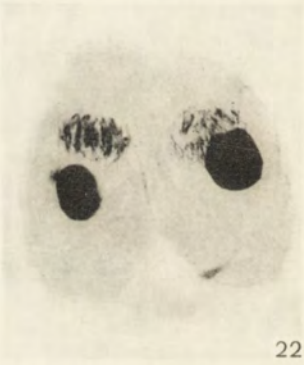
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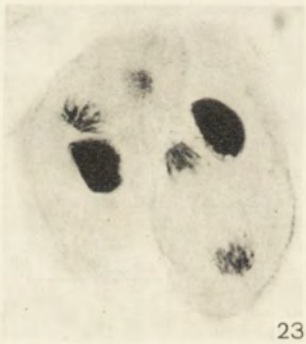
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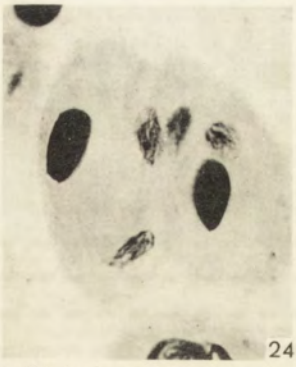
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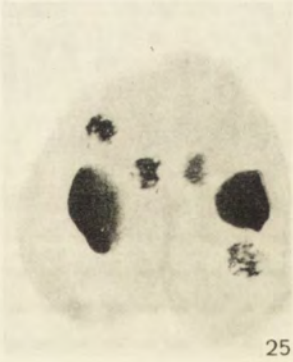
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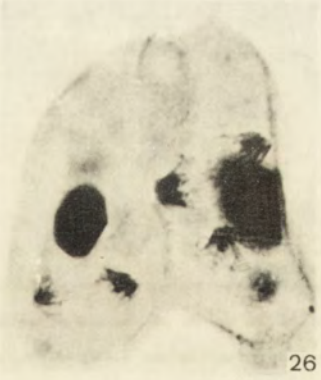
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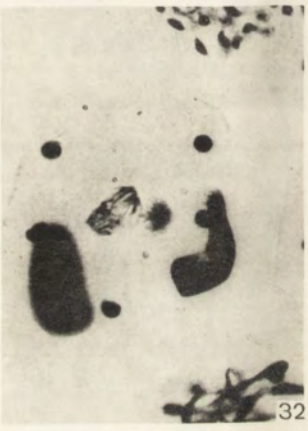
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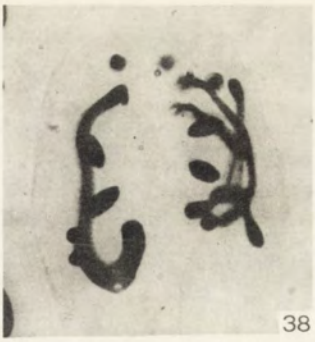

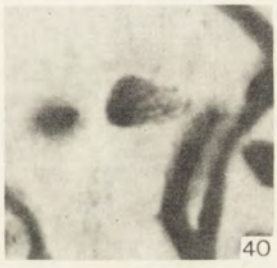

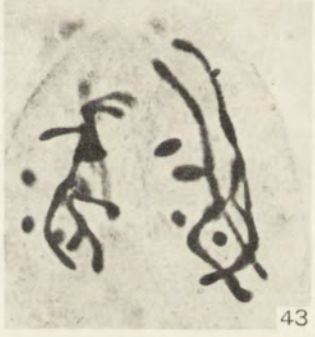

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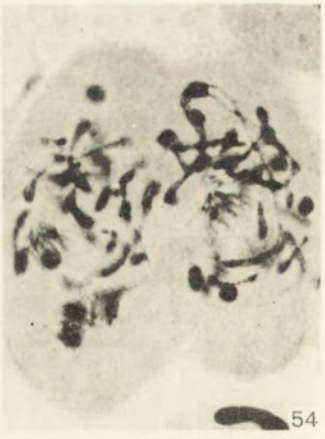
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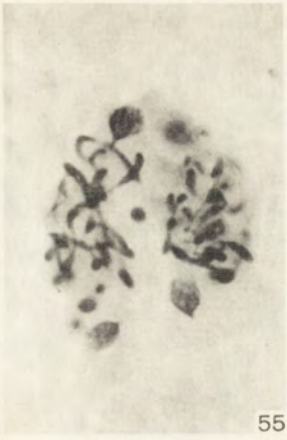
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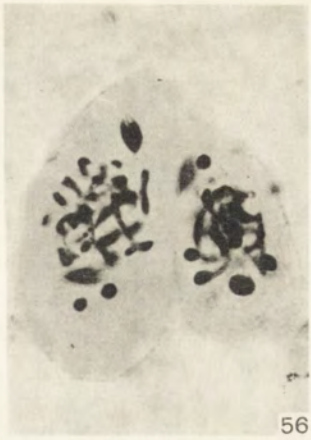
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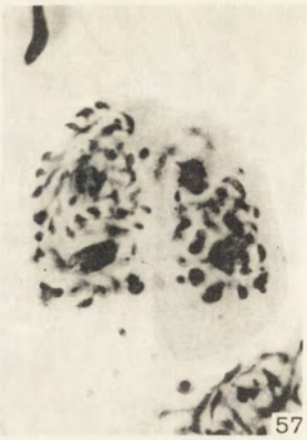
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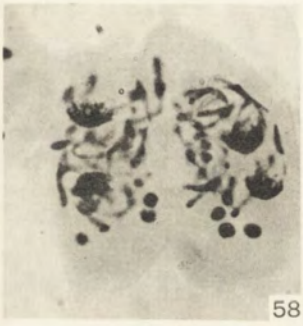
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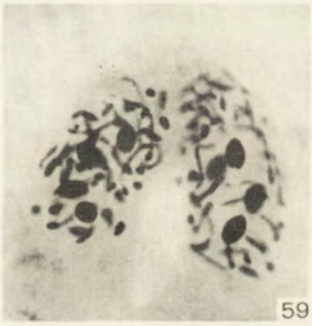
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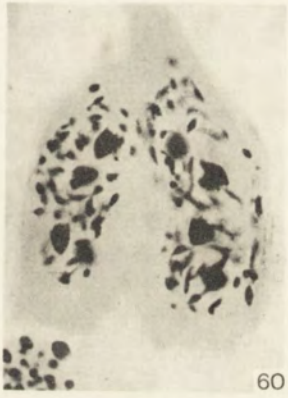
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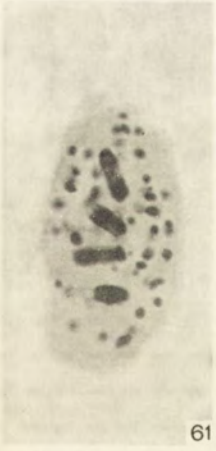
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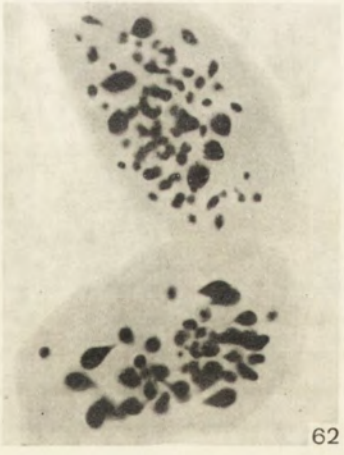
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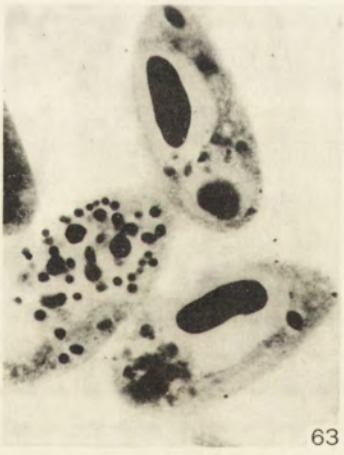
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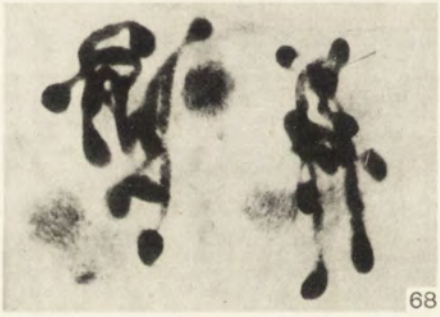
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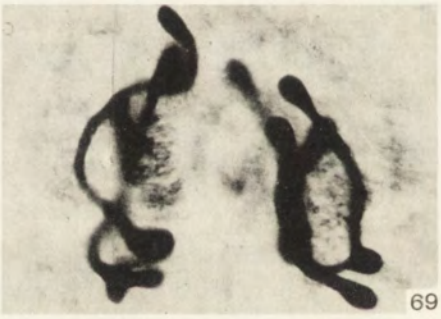
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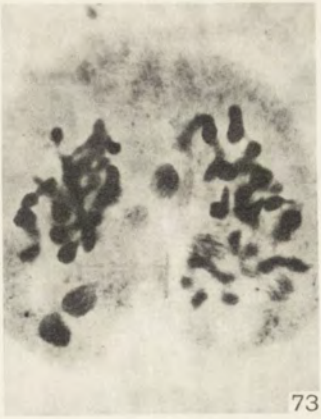
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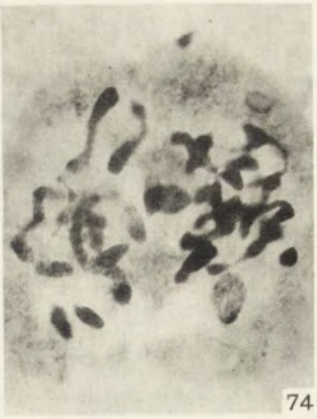
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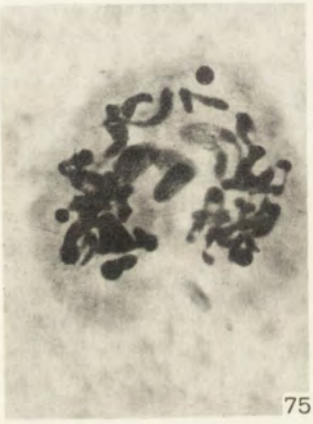
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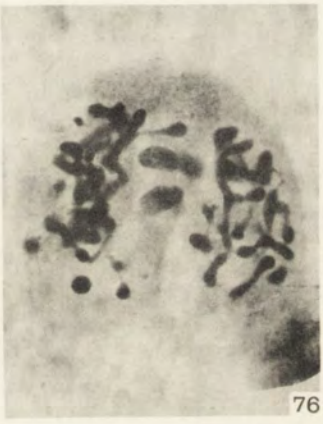
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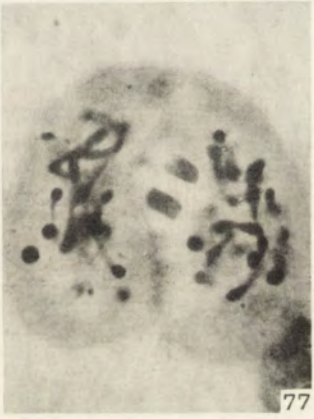
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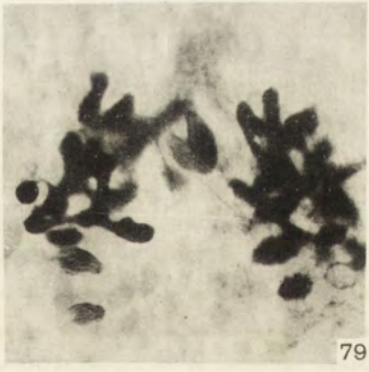
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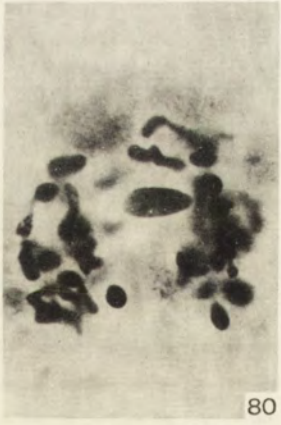
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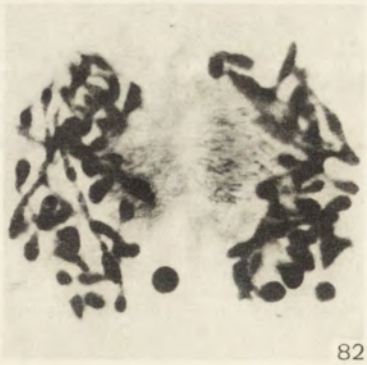
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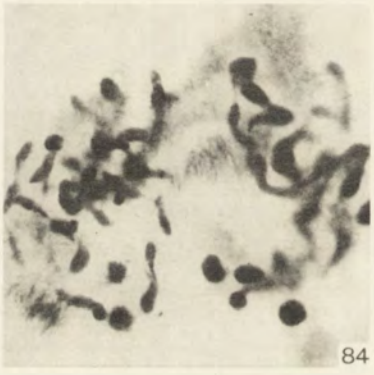
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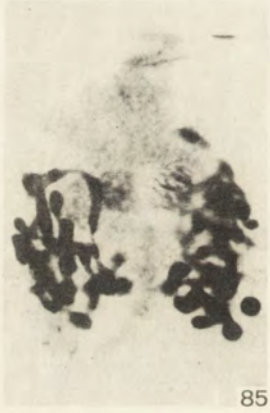
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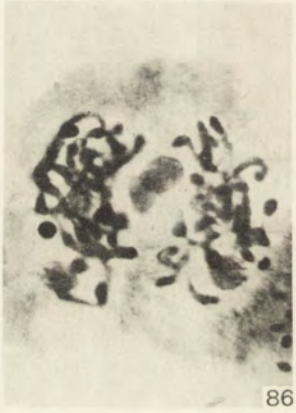
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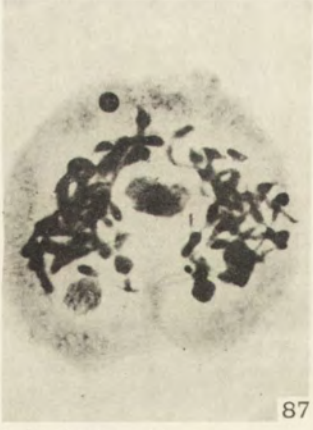
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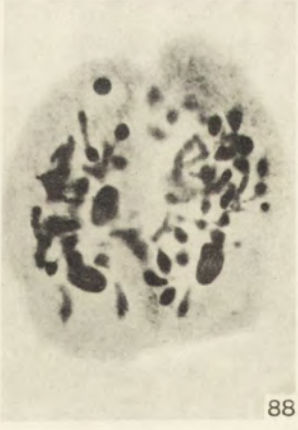
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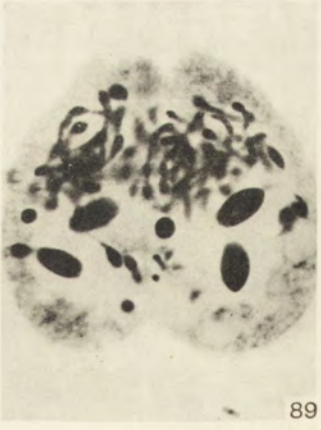
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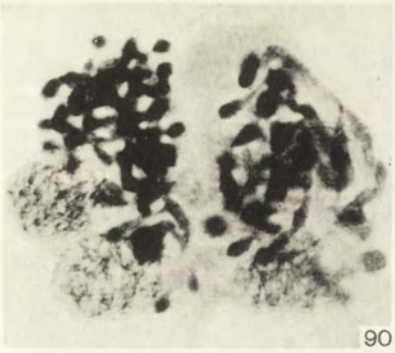
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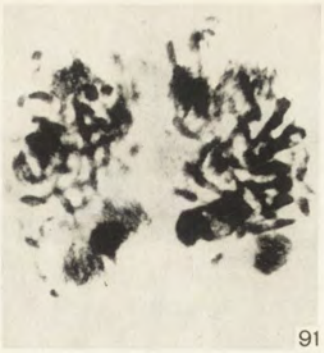
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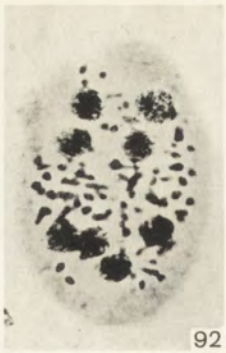
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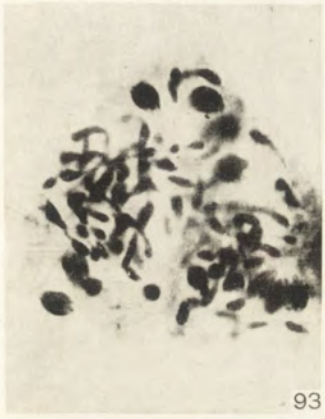
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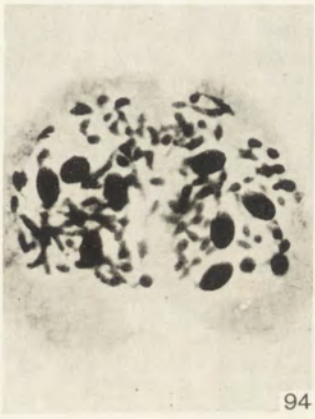
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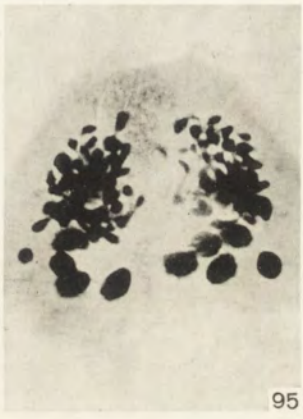
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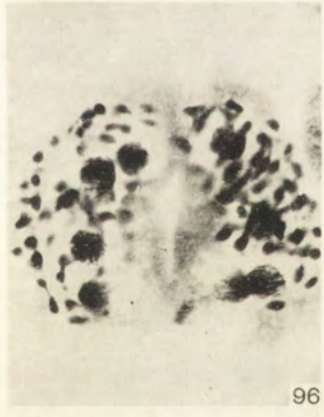
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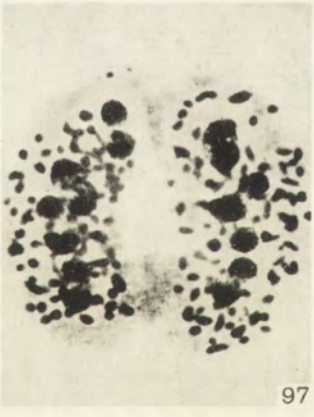
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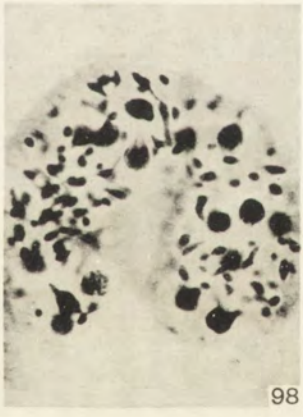
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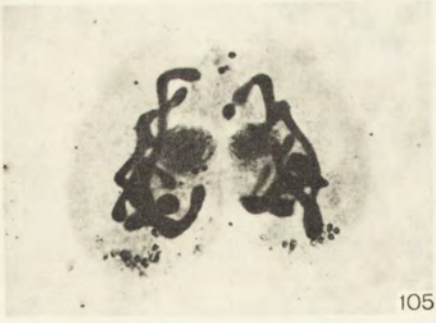
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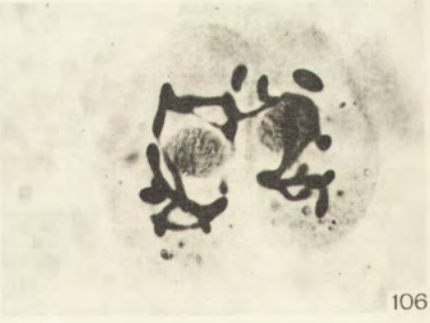
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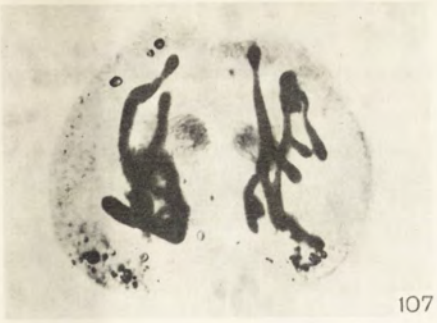
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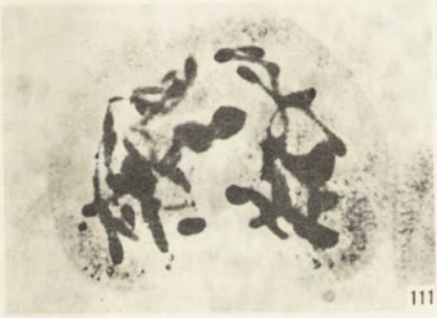
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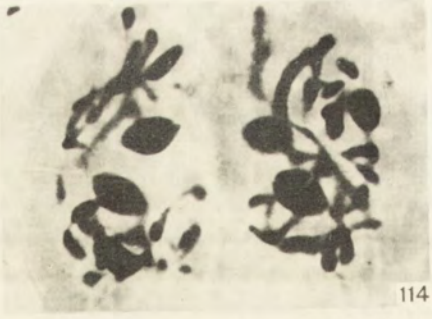
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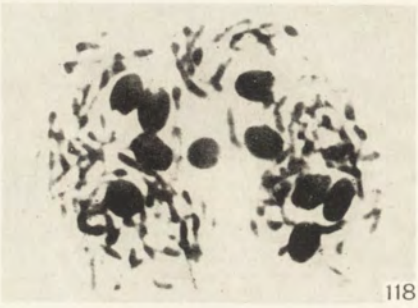
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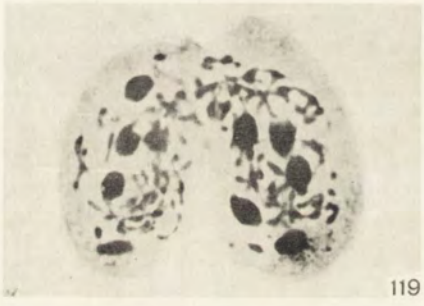
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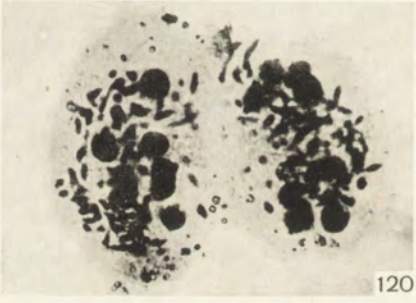
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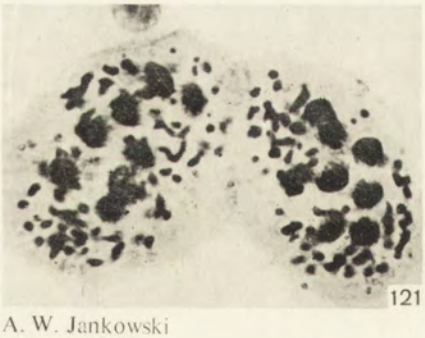
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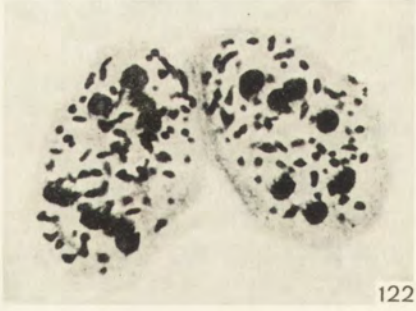
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PROTISTOLOGICA

Tome VIII

Fascicule 3

Fascicule 4

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ANNALES DE LA STATION BIOLOGIQUE DE BEESE-EN-CHANDESSE*

Numéro 1

Numéro 2

Numéro 3

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* Protozoological papers there are mentioned only. Faunistical papers where among others Protozoans are mentioned – are omitted.

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Numéro 5

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Fasciculi praeparati:

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