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The Induction of Haploidy in *Tetrahymena pyriformis* Following X-irradiation.[1]

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SUMMARY. Vegetative clones of *Tetrahymena pyriformis*, variety 2, mating type II, were the recipients of various dosages of X-rays from 10,000 to 400,000r. One clone from the 400,000 level (UM7X), when mated with non-irradiated cells of various mating types in variety 2, failed to produce a migratory nucleus. A one-way transfer from the normal to irradiated mate occurred, resulting in the production of haploid exconjugants.

When haploids were crossed with diploids the former failed to produce a migratory nucleus. A one-way nuclear transfer from the diploid to the haploid occurred resulting again in the production of haploid exconjugants. When two haploids were crossed a clone resulted which showed 80 to 90 percent amicronucleate forms.

The mating type of the derived haploid is always that of the diploid parent. All derived haploids manifest physiological and morphological characteristics similar to those of the diploid except that the haploids possess a smaller micronucleus.

THE VARIETIES of *T. pyriformis* so far examined are normally diploid (2n = 10)(7,13). The task of determining the presence of recessive mutant genes requires several generations, whereas if autogamy, cytogamy, or haploidy could be induced, this information could be acquired in one generation.

During routine examination of thousands of clones of *T. pyriformis* under a wide variety of conditions nuclear reorganization in single cells has never been observed. Exhaustive efforts to induce autogamy in variety 1 have failed. Methods that have proven successful in *Paramecium*(2,12), have been fruitless with *Tetrahymena*. One is forced to the conclusion that if autogamy occurs naturally in *T. pyriformis* it is rare and that if the process can be induced some methods other than those which have been successful with *Paramecium* must be found.

Efforts were made to induce cytogamy by separating the two conjugants during the early stages of meiosis and before the interchange of gametic nuclei(8). The use of detergents, proteolytic enzymes, electric and heat shock, all failed to separate the animals. Vigorous mechanical agitation, however, did accomplish the separation but the "exconjugants", instead of continuing in meiosis as was hoped, either remained in the stage they had reached when separated or became amicronucleate. Approximately 40 percent of the cells lost their micronuclei and many of these mated with normal or other micronucleate animals. In any case, none of the separated cells survived. Apparently the process of separation inflicted irreparable damage to the cells.

The remaining alternative, the induction of haploidy, seemed to be the approach most likely to succeed in determining the genetic constitution of the organisms. Two possible advantages would result: (1) lethal and semilethal recessive genes could be eliminated, and (2) recessive mutations in the diploid could be revealed in crosses with the haploid, provided viable exconjugants could be obtained. This being possible, the genotype of parental stocks might be precisely determined and the analysis of the genetic system could be investigated more effectively than is possible at present. The purpose of this paper is to report the degree of success that has been obtained in producing haploid clones of *T. pyriformis* using X-radiation techniques and the subsequent fate of these haploids in crosses with other haploids and diploids.

MATERIALS AND METHODS

Variety 2, mating type II (strain UM7)[2] of *T. pyriformis* was used for the irradiation experiments. Animals taken in the log phase of growth were washed three times in doubly distilled water and loaded in 2 ml. nylon syringes. Each of the syringes to receive X-radiation contained approximately equal numbers of animals. The syringes were placed four at a time in the irradiation chamber, a modified form of the Wichterman chamber(16). Ice was packed around the syringes in the side chambers and changed at half-hour intervals. Temperatures were maintained by this method at 22-25°C.

X-radiation was obtained from G.E., X.P.T. high voltage tubes in alternate parallel, operating at 182 KV

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[2] Strain UM 7 was isolated by Dr. David Gruchy from a water sample taken from a small pond near Cape Codder Hotel, Woods Hole, Mass. in June of 1953.

and 25 MA and having an inherent filtration equivalent to 0.22mm. of copper. All deliveries were carried out at 6000r/min., measured in air. Samples of 1/10 ml. were withdrawn after X-radiation dosages of 10,000, 50,000, 100,000, 200,000, 300,000 and 400,000r and were transferred to tubes containing 5 ml. of peptone with antibiotic added (250 μg./ml. each of penicillin and streptomycin). All tubes were incubated at 25°C. after irradiation.

Single organisms, when transferred to drops of peptone in depression slides, failed in most cases to grow up into clones. Moreover, it is essential to be certain that nuclear transfer takes place between the pair members if the genetic constitution of the exconjugants is going to be known. In the past one member of the pair has been stained and the other member grown up into a clone. This method is laborious and fraught with technical difficulties, and moreover, one is never absolutely certain that the exconjugant clone produced resulted from a cell which went through meiosis as did the stained mate. To solve this problem a moist chamber technique was devised whereby one or several pairs were placed in a very small hanging drop preparation which could be observed under the phase-contrast microscope where the two-anlagen stage is clearly visible. A later improvement was to add culture fluid containing 100 to 200 cells in the bottom of the depression slide over which the drop containing the pairs was suspended for observation. The rapidly growing cells in the depression had a beneficial effect on the single cell isolates. Just what is responsible for the improvement in survival is not known.

For cytological studies, material fixed in Nissenbaum's fixative was stained according to Dippell and Chao's modification of the Delamater stain(14).

RESULTS

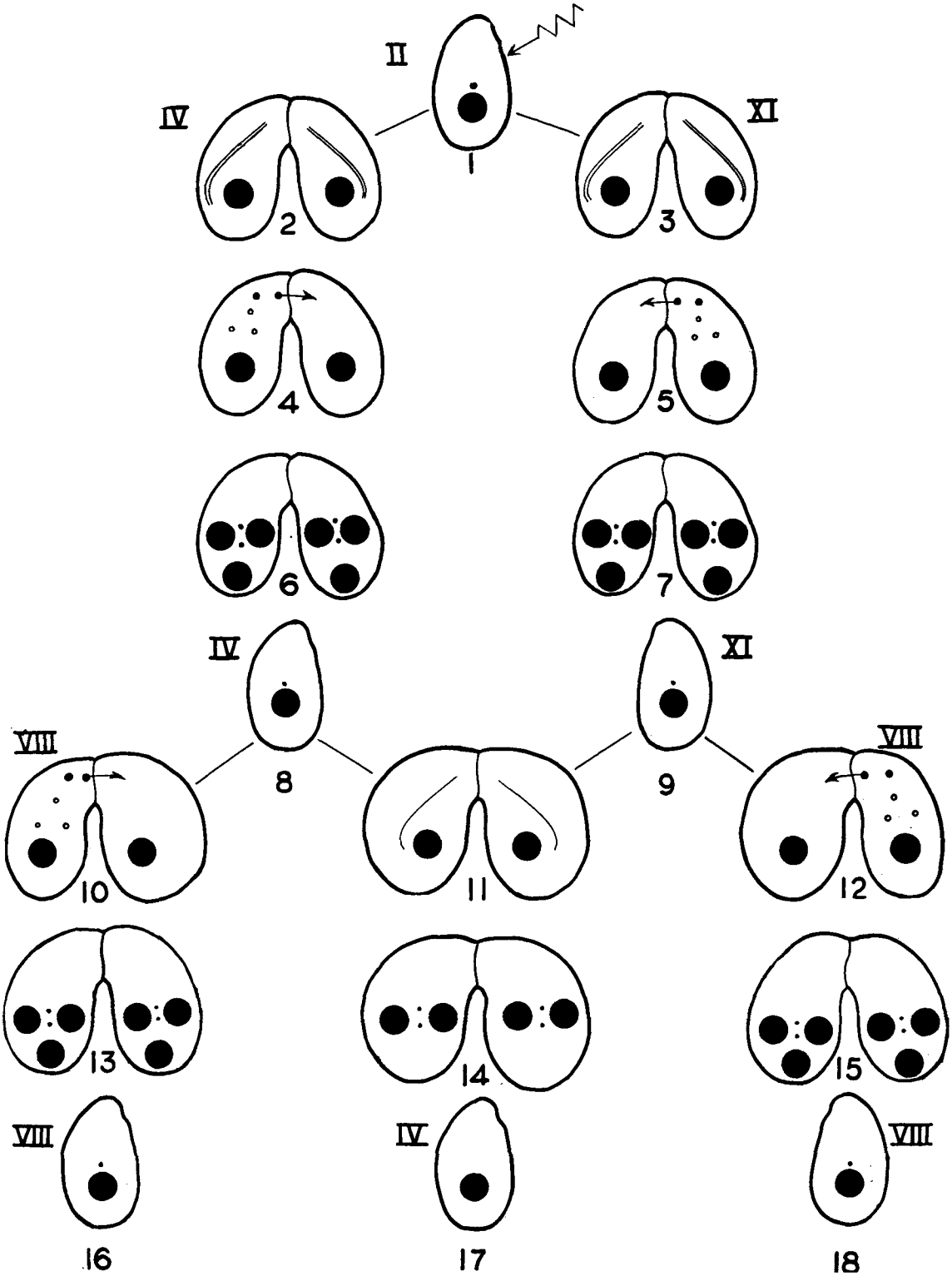
Matings of irradiated and non-irradiated animals

Three days following irradiation, 20 organisms from each radiation level (10,000 to 400,000r) were individually isolated into depressions containing peptone medium. The resulting clones were then transferred to tubes containing peptone, 48 hours after the original isolations were made. These clones were washed and mated with non-irradiated animals from variety 2, mating type III. A total of 120 clones were treated in this manner.

When each of the 20 clones isolated from each radiation level was mated with a non-irradiated clone, conjugation was greatly delayed. With increasing dosages of X-rays the refractory period was significantly increased. It varied from 6 hours with the clones which had been isolated from the 10,000r culture to as long as 24 hours for those isolated from the 400,000r level.

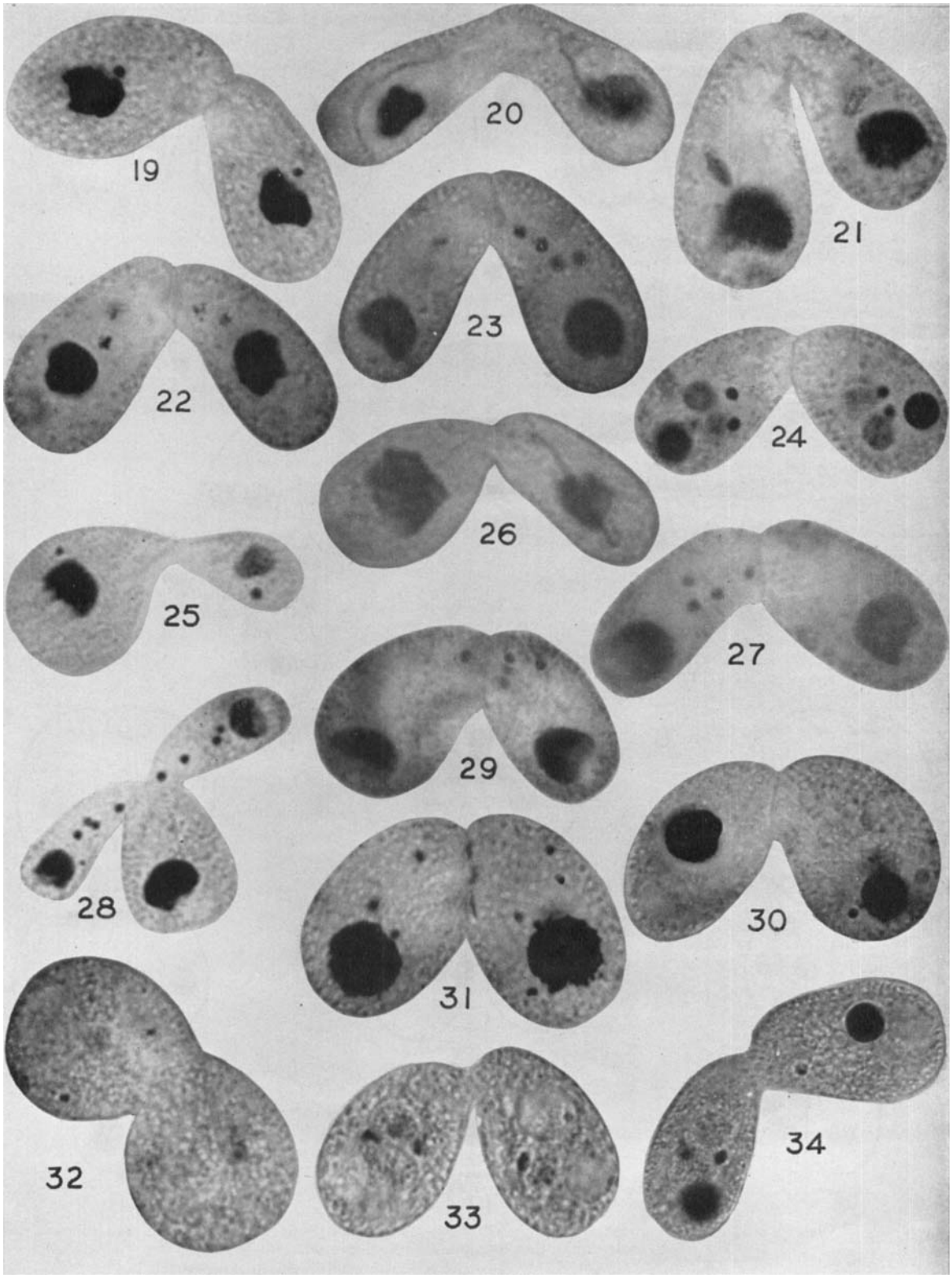
During conjugation, pairs were removed at ½-hour intervals, stained with aceto-orcein (1%) and examined under the microscope. Samples were studied up to and including the time when anlage stages were seen. Clones isolated at dosages from 10,000 to 300,000r, when mated with non-irradiated animals, failed to show significant abnormalities in nuclear behavior. However, one of the 20 clones isolated from the 400,000r group (designated UM7X), when mated with non-irradiated animals of mating type IV, showed striking nuclear abnormalities. The irradiated animal failed to produce a viable migratory nucleus (Figs. 1-5, 19-23). The non-irradiated mate, however, underwent meiosis producing stationary and migratory nuclei, the latter transferring to the irradiated animal. Since the UM7X mate formed no gametic nuclei no fertilization could take place; consequently the haploid nucleus in each animal developed parthenogenetically giving rise to macronuclei and micronuclei in each (Figs. 6, 7, 24). The nuclear components of both exconjugants were derived from the gametic nuclei of the normal mate and are genetically identical. Stained preparations demonstrate very clearly that a failure of the third prezygotic division in the irradiated mate had occurred and that the migratory nucleus of the normal mate passed to the irradiated exconjugant (Figs. 4, 5, 22, 23). The same events took place when clone UM7X was crossed to normal animals for all mating types in variety II except mating type II, since UM7X is of that mating type.

In normal conjugation the synkaryon divides twice following fertilization producing four similar nuclei, two located at each end of the cell. The macronuclei arise from the anterior nuclei and the micronuclei from the posterior nuclei. In the cross of irradiated with non-irradiated cells the hemikaryon also undergoes two successive mitoses giving rise to micronuclei and macronuclear anlagen. The macronuclear anlagen appear quite identical to those found in diploid exconjugants. Many abnormalities, however, are seen in the two-anlagen stages. Some animals possess one to three macronuclear anlagen and in others one animal may be completely devoid of chromatin. In a cross involving two diploids the macronuclear anlagen appear in both mates at about the same time, whereas in crosses of irradiated to non-irradiated the irradiated mate may show very early stages of meiosis when the normal mate has already reached the two-anlagen stage. By the time the conjugants separate both contain a full complement of nuclei although the micronuclei are somewhat smaller than is normally found in diploid cells, as one might expect since they presumably are haploid (Fig. 25). The macronuclei of the haploids are lighter staining than normals at separation but slightly later they take on the color inten-



Figs. 1-18. *Tetrahymena pyriformis*. Schematic drawing of the crosses which produced the haploids and some of the nuclear events that accompanied conjugation.

Fig. 1. UM7X, irradiated vegetative cell. Fig. 2. UM7X crossed with 2/IV. Fig. 3. UM7X crossed with 2/XI. Figs. 4 and 5. One-way nuclear transfer from diploid to X-irradiated cell. Figs. 6 and 7. Anlagen stages. Figs. 8 and 9. Vegetative haploid clones with mating types of diploid parent. Figs. 10 and 12. Haploids mated with diploid 2/VIII, with one-way nuclear transfer from the diploid to haploid shown. Figs. 13 and 15. Anlagen stages reached. Figs. 16 and 18. Vegetative haploid clones having mating type of the original diploid parent. Fig. 11. Two haploids crossed. Fig. 14. Anlagen stages lacking the old macronucleus. Fig. 17. Vegetative clone, mating type 2/IV showing 80-90% amicronucleate forms.



Figs. 19-34. *Tetrahymena pyriformis.* Photomicrographs of some of the nuclear events accompanying conjugation in the production of haploids.

Fig. 19. Irradiated (clone UM7X) and non-irradiated cell (2/IV) in conjugation. Fig. 20. Crescent form. Note the irregular pattern of the crescent in the right member, which is the irradiated conjugant. Fig. 21. First prezygotic division.

sity of normal diploids. This could mean that their ploidy is essentially the same as in diploids. This point is not clear at this time.

To test the percentage survival from crosses of irradiated and non-irradiated animals 50 to 200 pairs were isolated and allowed to produce clones from crosses between the irradiated clone UM7X (variety 2/II) and all other mating types in variety 2. No check was made for anlagen stages. The majority of pairs failed to produce clones and died while still paired. Only 1 to 2 percent of the pairs resulted in viable clones. Some pairs remained joined for as long as 72 hours, separated, underwent several fissions, and then died. In many cases pairs merely fused to form one single, large, abnormal animal which never underwent meiosis.

It soon became apparent as this investigation progressed that if clones of haploids were to be obtained, both members of the pair must be checked for anlagen to make certain that nuclear transfer had occurred. For this reason pairs were followed using phase-contrast microscopy and the hanging drop moist-chamber technique. In an effort to obtain viable haploid clones it was necessary to isolate many pairs, each of which was checked for anlagen stages to insure nuclear transfer. Twenty-four pairs, three to a drop, were followed for each cross between the UM7X and the remaining ten types in variety 2, making a total of 240 observations. Five haploid clones were obtained from the cross with mating type XI and one was obtained with IV. None of the other crosses yielded clones, although it is likely that with repeated matings haploids could be obtained from all of the mating types.

When the mating type of the haploid clones was determined it was always that of the original non-irradiated parent. The haploid derived from the cross of UM7X and mating type IV conjugated readily with all the mating types in variety 2 with the exception of IV, which indicated it was of that mating type. This haploid was designated 2/IVh#1. The five clones derived from the cross of UM7X with mating type XI conjugated well with mating types I, III, V, and VII initially and after repeated efforts finally conjugated with all mating types in variety 2 except XI, the mating type of the original non-irradiated parent. The five haploids are designated 2/XIh#(1)-(5).

All the derived haploids grew as well as the diploids on peptone and chemically defined media, and were somewhat larger than the diploids. The micronuclei were smaller than those of the diploid and, whereas the macronuclei stained less intensely than in the diploids when first formed, after several vegetative divisions these differences were no longer apparent. The haploids were more difficult to handle than their diploid parents. In routine centrifugation they swelled considerably in distilled water and a great many cytolyzed. This response necessitates lower speeds and more careful handling during centrifugation.

Matings of haploid and diploid animals

The next step was to study crosses between the derived haploids and normal diploids. In order to determine viability of exconjugants, haploids 2/XIh(1) and 2/IVh(1) were mated with the diploid 2/VIII. Mating type VIII was selected because preliminary observations indicated that a higher percentage of pairing with both haploids occurred with this mating type. One hundred pairs from each cross of the haploids with 2/VIII were isolated into single drops of peptone and permitted to grow into clones. Of the total number (200) only four produced clones. Many of the same abnormalities were observed here in these crosses as occurred in crosses of irradiated diploids with normal diploids. Many pairs died in the paired condition and some separated, underwent several fissions, and then died.

In order to determine the mating type and nuclear condition of the exconjugants sixty pairs from each of both haploid crosses were isolated and the nuclear condition checked microscopically. Three pairs were placed in each drop, rather than one, because survival is better where more organisms are present. Only drops in which all three pairs had reached the anlagen stage were retained for study. Examination under the phase-contrast microscope revealed normal anlagen stages in both mates as a general rule. Variants from the normal were seen. Some had one, three and even four macronuclear anlagen while others showed no nuclear structures whatever.

During the initial stages of conjugation a striking size difference between the conjugants was quite apparent, the haploid being larger in all cases (Fig. 25, 28). This size difference diminished as conjugation

Fig. 22. Second prezygotic division. Fig. 23. Second division completed by normal diploid, the irradiated animal at the left shows nuclear disintegration. Fig. 24. Anlagen stages reached. Fig. 25. Larger haploid with small micronucleus conjugating with diploid. Fig. 26. Crescent stage. Note the fine thread in the larger haploid (left animal). The thicker thread in the diploid is conspicuous. Fig. 27. Third prezygotic division beginning; haploid to the right shows chromatin fragments in the region of the attachment membrane. Fig. 28. Triples, two diploids at end of second prezygotic division paired with a haploid. Note fine thread-like crescent in haploid. Fig. 29. Nuclear transfer from the diploid (right) to the haploid (left). Fig. 30. Two haploids in conjugation, the one at the left lacks a micronucleus. Fig. 31. Conjugation between two haploids with accompanying nuclear fragmentation. Fig. 32. Amacronucleate haploids conjugating. Fig. 33. Faintly staining two-anlagen stages in haploids, note absence of old macronucleus. Fig. 34. Haploids in conjugation. The cell at the left shows normal anlagen stages whereas the cell on the right contains one micronucleus, one macronuclear anlage (posterior) and the old macronucleus.

progressed until by the time the anlagen stages were visible the cells were the same size. Stained preparations substantiated the haploid nature of the micronucleus which never develops beyond a two-thread stage. The crescent forms a thin single thread in the leptotene stage and its double nature is evident in the later stages. The four-thread stage (pachytene) is quite readily seen in the diploid mate in comparable stages (Fig. 26).

Abortive first pre-zygotic divisions were seen in the haploid which resulted in unequally distributed chromosomes on the spindle, the commonest being three at one end and two at the other. In the diploid a normal cytological sequence follows the crescent stage resulting in the formation of male and female gametes. The male nucleus migrates across into the haploid but no discernible nuclear transfer from the haploid to the diploid occurs (Figs. 8, 9, 10, 12, 25, 26, 27, 29). Fragments of chromatin in the haploid in many cases migrate to the area where normal nuclear transfer occurs. The hemikaryon in both exconjugants undergoes several divisions giving rise to two macronuclei and two small micronuclei (Figs. 13, 15).

Four clones were obtained from each cross and when checked for mating type all eight turned out to be mating type VIII, the mating type of the diploid parent in each case. The clones were designated 2/VIIIh(1)-(8). The growth characteristics and morphology of the cells were no different from the other derived haploids.

Mating of two haploids

The viability of exconjugants from crosses between the several haploids available was even lower than in the preceding crosses (less than 1%). From one series of experiments where haploids, 2/IVh(1) and 2/XIh(1), were crossed, 100 pairs were isolated of which only one survived. One member of this pair was devoid of chromatin, the other showed anlagen stages. A clone was established from this animal which proved to be mating type 2/IV and was given the designation 2/IVh#(2).

Repeated efforts were made to follow meiosis in the haploid pairs utilizing phase-contrast microscopy. Nuclear fragments could be seen moving about, but the sequence of events was so abnormal that it is not known whether or not meiosis was completed in any of the cases observed or that nuclear transfer occurred. It is true that most of the pairs showed anlagen stages, though very faintly, after 24 hours. The source of the nuclear material that gave rise to these stages is not known. The old macronuclei are not present in the conjugants by the time the macronuclear anlagen are visible.

A study of stained preparations revealed the formation of very faint crescents in both haploids. There were indications in some that an attempt was being made to undergo the first prezygotic division. Subsequent steps in meiosis were no more obvious in these preparations than in the living material. The nuclear complement usually consisted of two very faintly staining macronuclear anlagen and two small micronuclei (Figs. 11, 14, 33, 34).

The clone, 2/IVh#(2), resulting from the haploid cross, showed 80 to 90 percent amicronucleate forms as well as the occasional amacronucleate and enucleate organism. The haploid grows well on peptone and chemically defined media, conjugates readily with all mating types in variety 2 (except IV which is its own mating type), and manifests morphological characteristics very similar to the other haploids.

Conjugation between haploids of opposite mating type was generally good (50-60%). The first indication of conjugation usually occurred within two or three hours, which is similar to results obtained when diploids are mated. Moreover, mating took place between micronucleate haploids, amicronucleate haploids, amicronucleate and micronucleate haploids, and in a few cases between haploids in which no discernible chromatin could be seen in either member of the pair (Figs. 30, 31, 32).

DISCUSSION

For genetic studies with diploid organisms, homozygous stocks are highly desirable. Therefore, it is usually necessary to undertake a series of breeding experiments to insure the true genetic constitution of diploid stocks. The regular occurrence of autogamy in some ciliated protozoa has simplified enormously the problem of obtaining homozygous stocks in these organisms. The genotype of a clone is revealed simply by inducing autogamy, during which heterozygous genes segregate into the dominant and recessive homozygous classes in a ratio of 1:1. This is the closest form of inbreeding and is extremely useful in bringing to light recessive genes.

Fermor-Adrianowa(9) first described autogamy in the ciliate *Stylonychia pustulata*. It has been observed also in *Paramecium aurelia* by Hertwig(10) and Diller (5) and in *P. calkinsi* by Diller(6). Corliss(4) reports autogamy in one species of *Tetrahymena, T. rostrata*. This may be the first report of autogamy in a hymenostome ciliate and the first clear cut case among ciliates in general where the process occurs in the cyst stage. Exhaustive efforts to induce nuclear reorganization within single animals in *T. pyriformis* have been fruitless. The induction of haploidy, although a less satisfactory method than autogamy, does

warrant study because it is possible to determine the genotype of a heterozygous parent in one generation by rendering it hemizygous. This process has essentially the same potentialities as autogamy except that it is more laborious, in that it requires the mating of diverse mating types and the subsequent isolation of conjugating pairs. The resulting exconjugants are genotypically identical and, being haploid, respond immediately to their environment like homozygotes, because they possess only one genome in the micronucleus rather than the two characteristic of diploids. Haploids have been reported for *Paramecium aurelia* by Sonneborn(15) and Kimball and Gaither(11). The latter authors have induced haploids with X-rays. In the present investigation haploids in *T. pyriformis* have been induced employing X-radiation dosages of 400,000r. This dosage effectively disrupts the normal nuclear process, which proceeds no farther than the third prezygotic division in any case and in most the nuclear products become dissociated fragments before this division is reached. Vegetative mitosis is unaffected in these cells since growth is normal in all respects. It is only when the cell undergoes meiosis that difficulties arise. The cytoplasm of the cells descended from irradiated clones seems to be unimpaired because it receives the male gamete from the normal non-irradiated mate and permits the formation of the full nuclear complement. It is true that viability of such matings is very low, but it would seem that this must be accounted for by some explanation other than the deleterious effect of the radiations on the cytoplasm. It would seem more likely that the segregation of lethal genes or unbalanced genomes would account for the high mortality observed.

Haploids were obtained from matings of the irradiated cell with types IV and XI. Morphologically both haploids were identical to that of the diploid, except for a size difference, the haploid being the larger. The micronuclei were small in both and the macronuclear anlagen resembled the diploid in all respects. Just how the haploid nucleus can give rise parthenogenetically to a macronucleus which seems to contain DNA in equal amounts to the diploid is conjectural. It is true that whereas the macronuclei show no indication of haploidy the small micronuclei present real evidence that the DNA content is certainly less than that of the normal diploid. This is further substantiated by cytological evidence. During the first meiotic division chromosomes can be seen distributed unevenly along the spindle and only five unpaired chromosomes can be counted. There seems to be little doubt that the micronucleus is haploid although nothing can be said about the genetic constitution of the macronucleus.

When the haploids derived from the cross of irradiated with non-irradiated cells are mated with diploids, haploids result in a manner similar to the original cross. The steps in the formation of these haploids are similar to those reported by Chen(3) for *Paramecium bursaria* and by Sonneborn(15) for *P. aurelia*. The three prezygotic divisions in the diploid are followed by nuclear transfer of the male gamete to the haploid where it develops parthenogenetically to form the nuclear complement. In *P. aurelia* Sonneborn(15) reports that the normal number of micro- and macronuclei can develop from the hemikaryon but that many abnormalities occur. In some all the nuclei develop into supernumerary micronuclei, in others all develop into macronuclei, and in still others unequal numbers of the two kinds arise. In *T. pyriformis* there seems to be a higher percentage of haploids produced with normal macro- and micronuclear components. There seems to be some evidence also that the new nuclear complement may utilize the products formed from the degenerating macronucleus. This evidence is based on the fact that the degenerating macronucleus disappears much sooner in crosses of haploids with diploids than it does in normal diploid matings. Moreover, in crosses between haploids the degenerating macronucleus is rarely seen by the time the two macronuclear anlagen are visible. It would seem that the DNA which is being released from the degenerating macronucleus into the cytoplasm might well be used in the formation of the new macronuclear anlagen.

The haploids, both the ones derived by irradiation and those resulting from crosses with diploids, always possess the mating type of the diploid parent. This is as expected since the nuclear complements in both exconjugants are derived from the micronucleus of the diploid. There seems to be no doubt but that the micronucleus determines the mating type in these crosses. Sonneborn(1) reported that in crosses between amicronucleate and micronucleate *P. aurelia* the resulting haploids have the mating type of the micronucleate parent. However, the opinion was also expressed that in addition to the micronucleus, the macronucleus and cytoplasm might be important in determining mating type.

There seem to be no reports in the literature of exconjugants surviving from crosses between haploids. In the present study only one clone has been established from hundreds of isolated pairs. The lethality of such crosses, therefore, is extremely high as one might expect. Even though the nuclear events during meiosis were attempted by both members of the pair, the process was never observed in its entirety, although nuclear fragments were observed to accumulate at the membrane where nuclear exchange normally occurs (Fig. 31). The appearance of faintly staining

anlagen in many pairs is difficult to interpret. Obviously some sort of nuclear reorganization occurred resulting in a nuclear apparatus sufficiently adequate to support vegetative reproduction, because in cultures this haploid grows well although not as vigorously as the diploids from which it was derived. It is impossible to be certain if nuclear transfer took place. Only one member of the original pair survived and it turned out to be mating type IV, the mating type of one of the haploid parents. Further studies of these nuclear events with more refined techniques may clear up some of these points.

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Structure et Origine du Pédoncule chez *Chilodochona.*

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SUMMARY. The peduncle of the chonotrichous ciliate *Chilodochona* is composed of a bundle of protein fibers secreted by intracytoplasmic glandular ampullae. This process, very different from that involved in production of the peduncle in the peritrichous ciliates, also is found, although with a simpler result, among the Dysteriidae, a highly evolved family of gymnostomes closely related to the chonotrichs.

LES CHONOTRICHES sont des Ciliés gemmipares épibiontes qui se fixent sur les pièces buccales ou sur les lames branchiales de divers Crustacés au moyen d'une sécrétion élaborée en un point défini de la région antapicale du corps. Cette sécrétion est concrétée, chez *Spirochona* et *Heliochona* en un petit coussinet basal, tandis que chez *Stylochona*, *Chilodochona* et *Trichochona*, elle constitue un pédoncule allongé grêle et rigide.

Wallengren(12) a montré que le pédoncule de *Chilodochona* prend naissance dans le cytoplasme de l'Infusoire par un ensemble de fibrilles convergentes, et Guilcher(9) constate que celles-ci sont en rapport avec une série de vacuoles à paroi basophile. Ce fait permet de supposer que dans ce cas, comme dans celui de quelques Dysteriens(4) la matière constituant le pédoncule est sécrétée par un organite intracellulaire fonctionnant comme une glandule. Ce mode de formation du pédoncule fixateur est différent de celui que l'on connaît chez les Ciliés péritriches(1-3,5,7,11); de nouvelles observations ont pu être faites, à cet égard, sur *Chilodochona*.

MATÉRIEL ET TECHNIQUE

Chilodochona quennerstedti Wallengren se rencontre assez fréquemment à Concarneau et à Roscoff, fixé sur les endites des deux maxilles et les deux paires de maxillipèdes chez *Carcinus moenas* et *Cancer pagurus*. Il est aisé de séparer les poils portant de nombreux individus afin d'examiner les Ciliés à l'état vivant, ou de