

Transfer of nuclei from a parasite to its host

(*Polysiphonia/Choreocolax*/microspectrofluorometry)

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ABSTRACT During the normal course of infection, nuclei are transferred via secondary pit connections from the parasitic marine red alga *Choreocolax* to its red algal host *Polysiphonia*. These "planetic" nuclei are transmitted by being cut off into specialized cells (conjuctor cells) that fuse with an adjacent host cell, thereby delivering parasite nuclei and other cytoplasmic organelles into host cell cytoplasm. Within the foreign cytoplasm, planetic nuclei survive for several weeks and may be active in directing the host cellular responses to infection, since these responses are seen only in host cells containing planetic nuclei. The transfer and long-term survival of a nucleus from one genus into the cytoplasm of another through mechanisms that have evolved in nature challenge our understanding of nuclear-cytoplasmic interactions and our concept of "individual."

Parasitic organisms have evolved many specialized mechanisms for invading their hosts. However, no example yet has been reported of the regular introduction of nuclei of a parasite into cytoplasm of living cells of its host, leading to modification of the metabolism of the host cell to the benefit of the parasite. Such an interaction would presumably require a most intimate coordination of host and parasite metabolism. Here we report evidence of such behavior in the parasitism of the common filamentous marine red alga *Polysiphonia* by its parasite *Choreocolax*,‡ a distantly related species of red algae.

The red algae (Rhodophyta) are widespread eukaryotic plants that occur as one of the three dominant divisions of algae found in subtidal and intertidal communities. Parasitic taxa constitute >15% of all known genera of the major class Florideophyceae (3). The parasitic red algae parasitize only other red algae. Whereas the host plant is photosynthetic, many parasites, such as *Choreocolax*, are colorless; plastids occur in the cell of this parasite, but these do not normally develop thylakoids or pigmentation (4). The parasite fixes little if any CO₂ (3) and relies upon its host for nutrients. Thus, *Choreocolax* is obligately dependent on its red algal host and can only be cultured in association with its host (3).

Like its host *Polysiphonia* and most other red algae, *Choreocolax* has a triphasic life history (1, 2, 5): (i) a haploid gametophyte generation, which produces gametes; (ii) a diploid carposporophyte generation, which arises from the zygote and proliferates *in situ* within the tissues of the gametophyte to produce diploid carpospores; and (iii) a diploid tetrasporophyte, which produces haploid tetraspores by meiosis.

Both *Polysiphonia* and *Choreocolax* grow by divisions of uninucleate apical cells. As in other florideophycean red algae, septum formation is incomplete at cell division, leaving a centrally located cytoplasmic connection between the two daughter cells. The contiguous cells remain connected by the

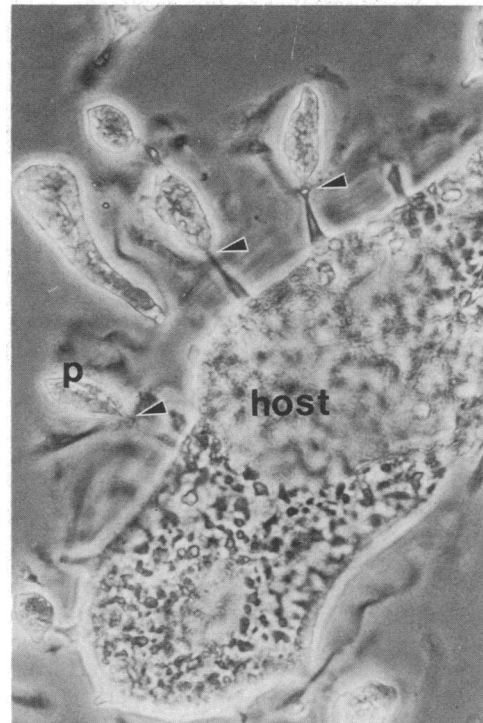


FIG. 1. Secondary pit connections (arrowheads) between a cell of the host *Polysiphonia confusa* and the parasite *Choreocolax* (p), fixed in 3:1 95% ethanol/glacial acetic acid. (Squashed; phase contrast, $\times 200$.)

infurrowed cell membrane, and the pore is secondarily plugged by a glycoprotein plug (6, 7). Traditionally, the junction has been called a primary pit connection.

In addition, cells of both *Polysiphonia* individuals and *Choreocolax* individuals are capable of forming "secondary" pit connections, which link adjacent cells of the same individual not derived from the same mitosis; a nucleus is transferred from one cell to another when a secondary pit connection forms (8, 9). Secondary pit connections are also conspicuous between the cells of *Polysiphonia* and its parasite *Choreocolax* (Fig. 1). These conditions are a feature common to all red algal parasite-host associations (8). Therefore, the question arises—are nuclei transferred between parasite and host just as they are between cells of an individ-

Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; P-nucleus, "planetic" nucleus.

‡P. Kugrens (1) recently separated the Pacific *Choreocolax polysiphoniae* Reinsch (the experimental material employed in the present study) from the Atlantic-type species. He named the Pacific form *Leachiella pacifica* Kugrens. We have not adopted this nomenclature change as we feel that a reexamination of the type material [which has not been examined since H. H. Sturch in 1926 (2)] must be made before this change is effected.

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ual, and if so, what happens to these nuclei and what are the consequences of this transfer of genetic information?

MATERIALS AND METHODS

Polysiphonia confusa Hollenberg was collected intertidally from Pebble Beach, CA (Stillwater Cove, Monterey Co.), and haploid and diploid plants were established in uni-algal cultures. The parasite on its host was collected from the same site and grown in bi-algal culture with its cultured host by methods previously described (10). All cultures were

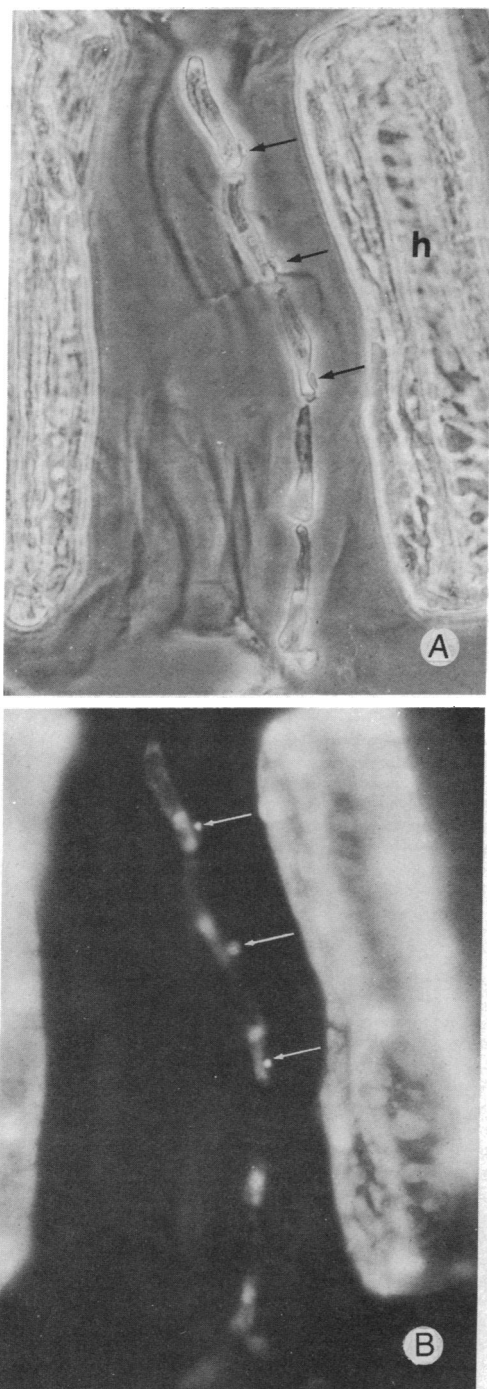


FIG. 2. (A) A filament of parasite cells growing between two host (h) cells. Several small conjunctor cells are indicated by arrows. (Squashed; phase contrast, $\times 160$.) (B) Same field as A, with arrows showing DAPI-stained planetic nuclei within the conjunctor cells. (Epifluorescence microscopy, $\times 160$.)

grown in dishes containing enriched seawater (PES) and incubated at 12°C in a 12:12 light/dark cycle. The incubators contained warm white fluorescent lamps providing ≈ 50 microeinsteins of irradiance per sec per cm^2 .

Slides were prepared for examination and microspectrophotometry by fixing excised samples 1 hr in 3:1 (vol/vol) 95% ethanol/glacial acetic acid and soaking the fixed samples 1 hr in 50% (wt/vol) chloral hydrate to soften the tissue. Squashes were made on gelatin-coated slides. The coverslips were removed after freezing in liquid N_2 , and the slides with the attached squashed and frozen cells were immediately immersed in 3:1 95% ethanol/glacial acetic acid, passed through an alcohol series to 100% ethanol, and air dried. Dried slides to be stained were first rehydrated in 200 mM KCl for 5 min and then stained with 4',6-diamidino-2-phenylindole (DAPI; $0.5 \mu\text{g}/\text{ml}$) in McIlvaine's pH 4.1 buffer (11). After 30 min, the coverslip was sealed with nail polish. Such slides, if kept in a humid atmosphere in the refrigerator, were stable for 2-3 days. Prior DNase treatment (11) removed the DAPI-staining material.

Microspectrophotometry was performed as described by Coleman *et al.* (11), but with the addition of a Zeiss 46 79 60 (KP 500 short pass-interference filter) to suppress autofluorescence from phycobilin pigments not removed by the fixation process. For each measurement, an adjacent nonnuclear area was also measured and subtracted as background. A fluorescent mineral standard was used to check instru-

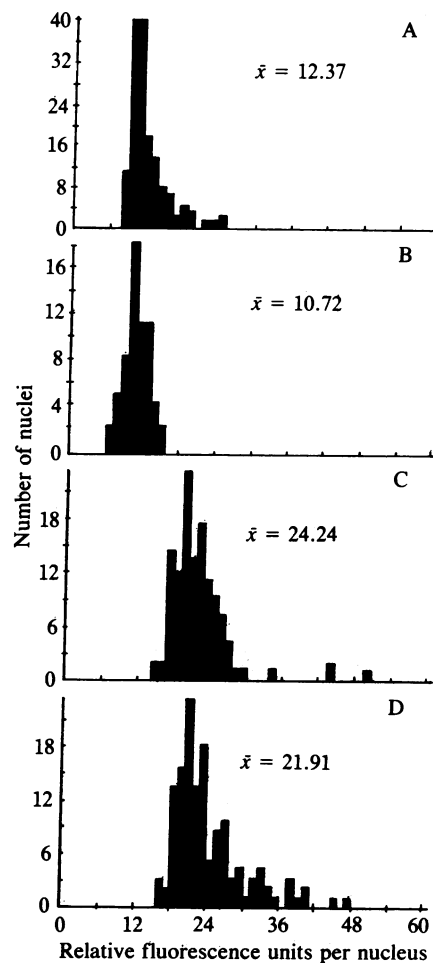


FIG. 3. Histograms of DAPI-stained nuclear fluorescence of *Choreocolax* planetic nuclei. (A and B) Haploid planetic nuclei within conjunctor (and vegetative) cells of *Choreocolax* (A) and after entering the host's cytoplasm (B). (C and D) Diploid planetic nuclei within the conjunctor (and vegetative) cells of *Choreocolax* (C) and after entering the host's cytoplasm (D). \bar{x} = mean.

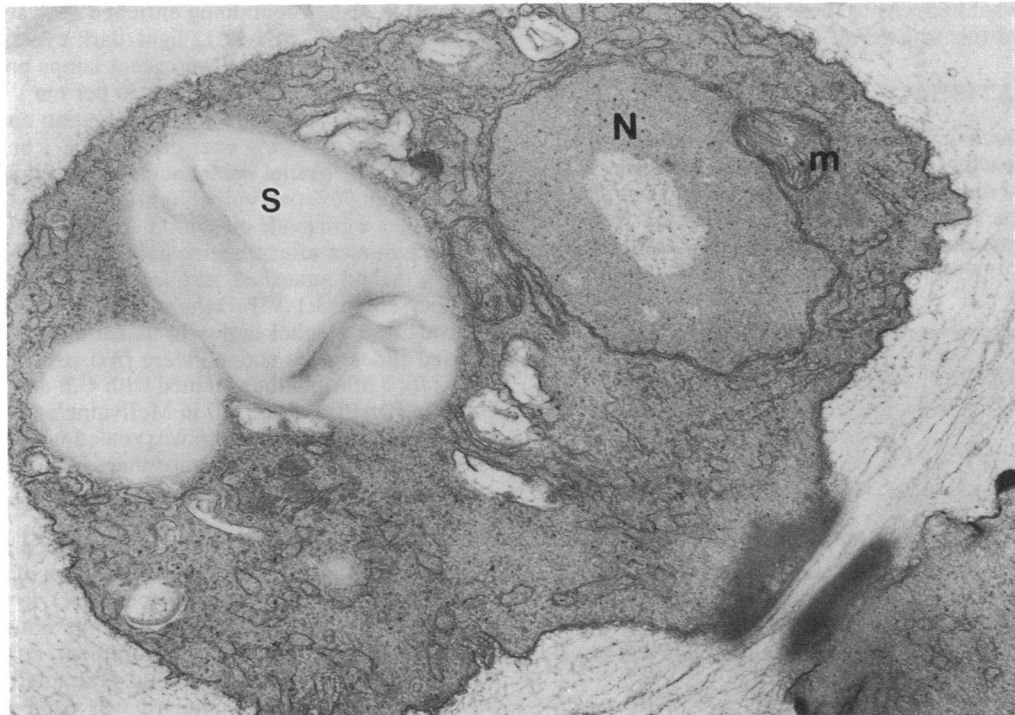


FIG. 4. Transmission electron micrograph of *Choreocolax* conjuctor cell prior to its fusion with an adjacent host cell. The condensed conjuctor cell nucleus (N) is evident, as are numerous mitochondria (m), endoplasmic reticulum, and starch (S). ($\times 36,000$.)

ment stability (11), and each slide was compared to a similarly fixed and stained slide of *Choreocolax* sperm cells (5) to standardize all readings.

Freshly collected field material as well as cultured plants were fixed for transmission electron microscopy in 4% glutaraldehyde buffered with 0.1 M cacodylate buffer (pH 6.6)

(1 hr at 3°C) and post-fixed in buffered 1% osmium tetroxide. The material was dehydrated and embedded in Spurr's medium, sectioned, stained with uranyl acetate and lead citrate, and examined with a JEOL 100 B and a Philips 201 transmission electron microscope operated at an accelerating voltage of 80 kV.

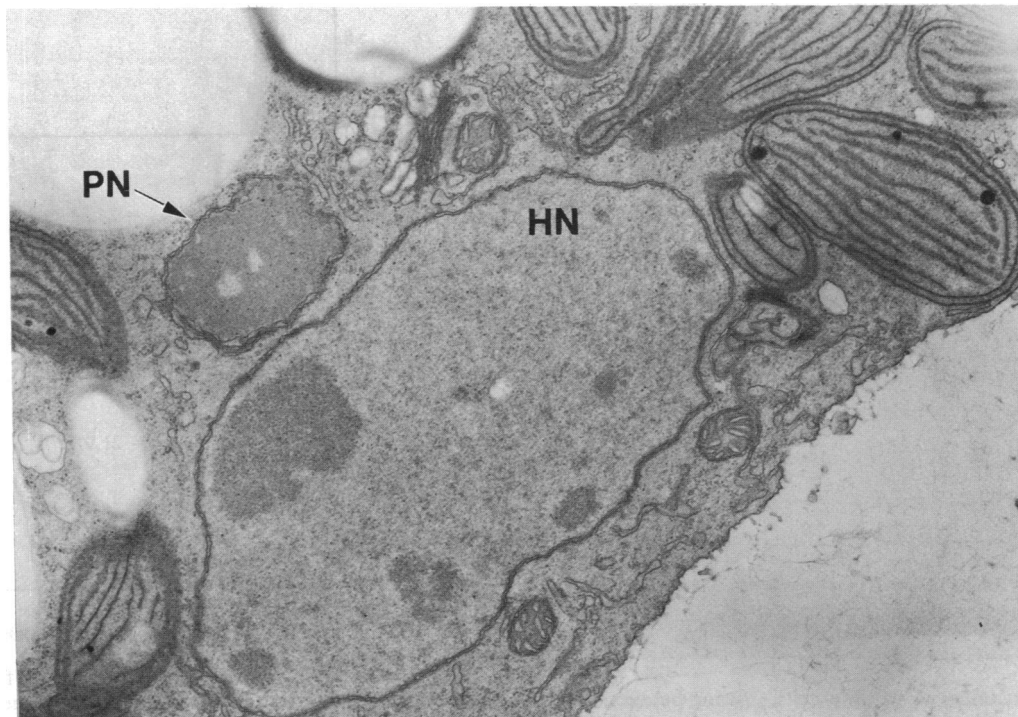


FIG. 5. Transmission electron micrograph of P-nucleus (PN) of *Choreocolax* adjacent to a host nucleus (HN), both within the cytoplasm of a host cell. ($\times 18,400$.)

RESULTS AND DISCUSSION

To examine whether nuclei are transferred between *Choreocolax* and its host *Polysiphonia*, cultured *Polysiphonia* plants (both haploid and diploid) were inoculated with parasite tetraspores (haploid) or carpospores (diploid), and the infection was allowed to progress until the tissue of the parasite erupted from the host's surface and produced reproductive structures. In nature and in culture, both tetraspores and carpospores of the parasite successfully infect haploid and diploid *Polysiphonia*. A tetraspore will produce a mature *Choreocolax* plant bearing gametes in 5–6 wk. Carpospores are formed ca. 2 wk after fertilization, and carpospore infection results in a tetrasporophyte bearing tetraspores within another 5–6 wk.

The parasite *Choreocolax* invades *Polysiphonia* by growing between host cells and forming many secondary pit connections with them. Apically dividing filaments of parasite cells, adjacent to host cells, produce conjuctor cells by asymmetric cell division from the side of the parasite cell, which is adjacent to the host cell (Fig. 2A). The conjuctor cell contains a highly condensed nucleus (Fig. 2B), which we have termed a "planetic" (Greek *planètēs* = wandering) nucleus (P-nucleus). Microspectrofluorometry of DAPI-stained cells reveals that each P-nucleus has a DNA content corresponding to the basic haploid or diploid level of the haploid or diploid cell from which it was derived (Fig. 3). The conjuctor cell (Fig. 4), which remains attached to the mother cell by a primary pit connection, fuses with an adjacent host cell, thereby transferring the P-nucleus as well as the other cell contents (ribosomes, mitochondria, proplastids, and endoplasmic reticulum) into the cytoplasm of the host (Fig. 5). Upon fusion and nuclear transfer, the host and parasite cells remain attached through the secondary pit connection (Fig. 1). These developmental processes are summarized in Fig. 6.

Numerous parasitic cells adjacent to a single host cell may form secondary pit connections with the host cell, resulting in a host cell containing hundreds of P-nuclei (Fig. 7). The identity of these P-nuclei is shown by their DNA content as

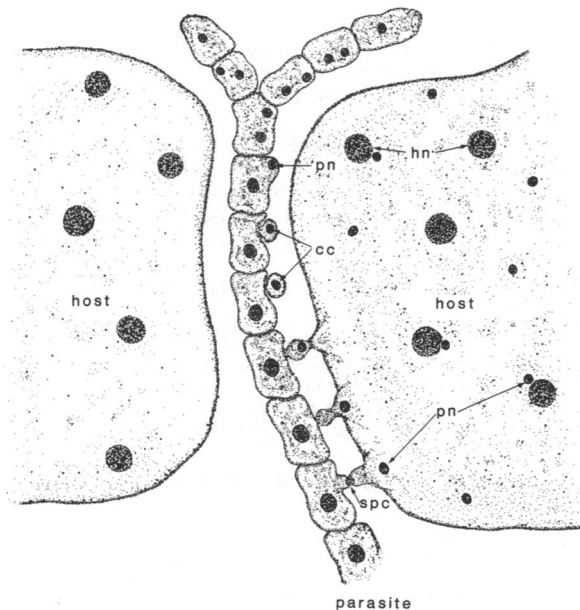


FIG. 6. Diagram of developmental events of conjuctor cell formation and planetic nuclear transfer. An apically dividing filament of parasite cells is growing between two host cells and is in the process of forming several secondary pit connections (spc). In one host cell, several P-nuclei (pn) have been "injected," and the host nuclei (hn) have increased in size and DNA content.

measured by microspectrofluorometry (Fig. 3) as well as their size and morphology. We have no evidence that the P-nuclei divide within the host's cytoplasm. Relative DNA values of P-nuclei do not double, and no P-nuclei in division were observed. Furthermore, the fact that there is an approximate 1:1 correlation between the number of P-nuclei and host-parasite secondary pit connections suggests that the P-nuclei are relatively stable and neither divide nor degenerate. Many of the nuclei reside within the cytoplasm of the host for nearly the entire duration of the infection cycle (i.e., spore infection to reproduction, ca. 5–8 wk), since the first cell cut off from the infecting carpospore or tetraspore rhizoid forms a conjuctor cell that immediately transfers a P-nucleus to a host cell (Fig. 8).

It is still uncertain whether the P-nuclei are active in directing cellular activities within the host. However, dramatic cytoplasmic changes are evident in both haploid and diploid host cells that receive P-nuclei. The infected cells (i) enlarge up to 20-fold, (ii) may divide, (iii) form a thickened cell wall, and (iv) undergo cytoplasmic alterations that result in a decrease in size of the large central vacuole and a concomitant increase in cytoplasmic components. Host nuclei increase in number and in DNA content, mitochondria and plastids multiply, and photosynthetic storage products accumulate. Host cells lacking the P-nuclei, even those adjacent to parasitic cells or to infected host cells, do not show any of these responses.

Nuclear transfer during secondary pit connection formation within an individual was first described nearly a century ago (12) in the red alga *Polysiphonia*. This observation, which has been continuously cited ever since, has not been examined critically since this original paper because traditional histological stains are generally unsatisfactory when applied to red algae (5). Furthermore, despite numerous ultrastructural studies of red algae, only two papers have included electron micrographs of nuclei in conjuctor cells. Both studies concerned parasite-host interactions, and neither study discovered the fate of the nuclei after transfer to the host cell. Peyrière (13) suggested that membrane-bound, electron-dense bodies seen in host cells were transferred nuclei; however, no evidence that the bodies contained DNA

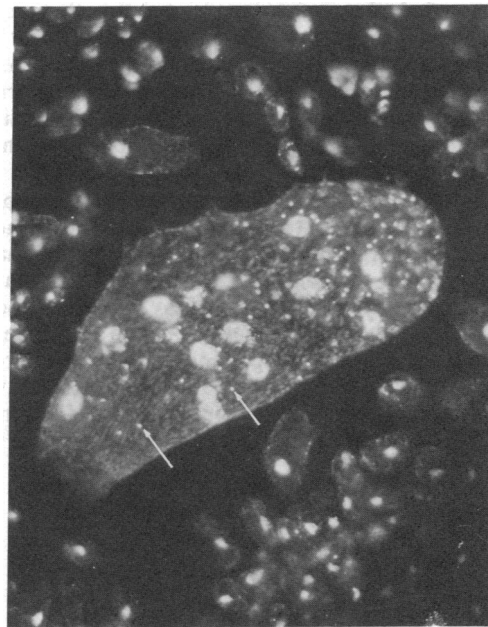


FIG. 7. Many small haploid planetic nuclei (arrows) of *Choreocolax* occur within the cytoplasm of a multinucleate host cell. This host cell was isolated within the tissues of the parasite reproductive tissue. (Squashed, DAPI stain; epifluorescence, $\times 64$.)

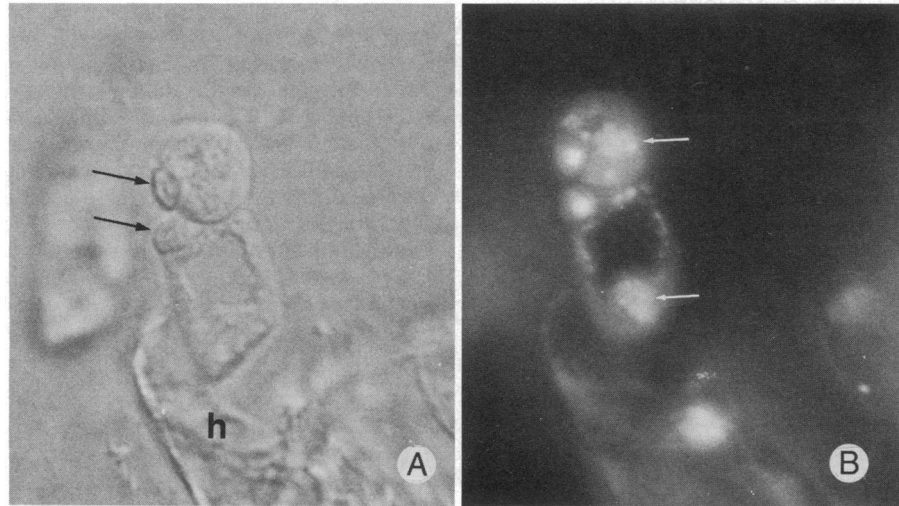


FIG. 8. Phase contrast (A) and epifluorescent (B) image of the apical cell cut off from the host-penetrating rhizoid of a germinating tetraspore. The conjuncture cells are indicated by arrows in A, and the bright haploid P-nuclei are evident in these cells in B. The arrows in B indicate the vegetative nuclei of the apical cell and the rhizoid. The surface portion of a host cell (h) is in the plane of focus. (Squashed, DAPI stain; oil immersion, $\times 560$.)

was given to support this assertion, nor were they surrounded by any distinguishable double membrane. She suggested that the parasite nucleus was subsequently eliminated from the host cell after transfer. Quirk and Wetherbee (14), failing to find parasite nuclei in the enlarged host cell by electron microscopy, suggested that they were degraded.

The limitations of electron microscopy in the studies of Peyrière and Quirk and Wetherbee have been overcome by using the recently developed DNA fluorochrome DAPI combined with epifluorescence microscopy (5, 8, 9). The dye has been used to show that secondary pit connections represent sites of nuclear transfer between cells. Furthermore, the DAPI-DNA fluorescence of nuclei reveals characteristic substructure and is proportional to the amount of DNA. Host and parasite nuclei can be readily distinguished by their size, appearance, and relative DAPI-DNA fluorescence. Using DAPI staining, we have observed the same nuclear transfer in every red algal parasite-host association that we have examined, including those that occur between closely related (i.e., same taxonomic family) parasites and hosts (*Asterocolax-Polyneura*, *Gardneriella-Agardhiella*, and *Plocamio-colax-Plocamium*) and those between hosts and parasites of different red algal orders (*Harveyella-Odonthalia* and the present study).

The phenomenon of secondary pit formation accompanied by nuclear transfer seems to be characteristic of the normal development of a large number of red algae and one that has just been extended to red algal parasite-host associations. This intriguing cellular mechanism may provide important clues as to the evolutionary affinities of the red algae. Perhaps the most similar known phenomena in nature are heterokaryosis found in higher fungi, and the cell fusion which occurs at fertilization in sexual reproduction of organisms. Comparisons among these processes should provide a fertile field for future research.

Even more interesting is the problem posed by the introduction and survival of a nucleus in foreign cytoplasm. If, as seems likely from these data, the parasite *Choreocolax* takes over host cells by injecting long-lived, functional nuclei, this represents a new and remarkable regulatory mechanism of parasitism and one that raises a great variety of questions concerning nuclear-nuclear and nuclear-cytoplasmic interactions and their control.

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