duced by two factors—the concentration of veliger larvae and the simultaneous presence of all three antibiotics in a culture dish. Although some pairing was evident if antibiotics were added individually, and a few pairs were observed in very high concentrations of larvae with no antibiotics, by far the highest number of pairs was observed in dishes with high concentrations of larvae to which the triple antibiotic mix had been added (Fig. 2). In this latter experimental situation a group of three adhering larvae, all swimming in unison, was sometimes seen (Fig. 1C).

Shelled animals of the planktonic stage were observed in pairs beginning with the first smooth hyaline valves of the straight-hinge veliger larvae until the beginning of the umbo stage at about 7 to 10 days. Unfortunately, it was not possible to maintain cultures beyond this time because of the germination of mold spores in the dishes. (Antifungal agents, as nystatin, candicidin, $HgCl_2$, griseofulvin, amphotericin, and Merthiolate, in concentrations nontoxic to larvae, were not effective as control agents.)

The exact site or mechanism of adhesion is not clear from microscopic observations alone (Fig. 1D). The specificity of the adhesion site suggests that mechanism and site are interrelated. Since pairing is observed in the earliest stages of shell formation and not in the trochophore stage, adhesion must be associated with changes that occur after the transitional period between trochophore and veliger larvae. In summary, these changes are as follows: the prototroch develops into a velum, the larva develops a rudimentary foot which proliferates a shell gland and a shell, the shell differentiates into right and left valves, digestive diverticula appear, the intestine acquires loops, the stomach develops a crystalline style sac, three sets of retractor muscles develop, and the anterior adductor muscle which functions in closing the shell appears anteriorly (*4*). The observation that some adhesion of larvae occurs in very high larval density even without antibiotics suggests that random contact between swimming animals allows an adhesive site to make contact. Obviously, this is apt to occur with greater frequency in high-density cultures. One possibility is that the antibiotic mix potentiates this effect by acting on the adhesive site. An alternative possibility is that antibiotics create a malfunction in the adductor muscle which causes greater numbers of animals to have open valves than in untreated larvae, thereby increasing the possibility of natural adhesive sites making contact.

Adhesion occurs anteriorly at the widest place in the valve. Paired larvae appear to be attached in a position in which the vela are facing each other (Fig. 1B). Since dorsal and ventral alignments are always the same in members of a pair or trio, the lateral views of paired larvae are of right and left valves. In trios the third member of the group seems to have the velum facing in the same direction as the second member (Fig. 1C). This arrangement becomes especially evident in older veliger larvae in which the left valve is larger and more convex than the right (Fig. 1E). The adductor muscles suggest themselves as a site of adhesion since the anterior adductor muscles of paired animals are facing each other and in trios the anterior adductor muscle of the third member is adjacent to the posterior adductor of the middle member. As the anterior adductor muscle develops prior to the posterior muscle, it could explain the specificity and high frequency of the anterior site of adhesion. Contrary evidence, however, is provided by paired fixed animals in which the visceral mass has contracted away from the edge of the shell and reveals no shell-to-muscle or muscle-to-muscle contact.

Veliger larvae normally produce a "sticky" secretion from the byssus gland in the foot at the time of setting. As most of the foot is made up of gland cells packed with secretions (*5*), it is possible that some precursor of this material may also have "sticky" properties that could provide attachment in high-density culture even at this early stage. Alignment of the pairs, however, would argue against the area of the rudimentary foot being the site of larval attachment.

The most promising hypothesis is that the newly calcifying shells are adhering to each other; more specifically, the valve margin of one larva adheres to the mantle epithelium of the other, or both mantle epithelia to each other. In this regard it is of interest to note that Nelson (*6*) ascribed a role to the mantle in the process of attachment of *C. virginica* to the substrate. Calcification consists of deposition of calcium carbonate (aragonite) (*7*) in an organic matrix. The organic matrix, from which protein fractions and an acid mucopolysaccharide have been isolated, is secreted by the mantle into the extrapallial space [in *C. virginica* the extrapallial space has access to the external media (*8*)]. Differences in specificity of regions of the mantle epithelium in the synthesis of particular proteins, as well as morphological differences in cells of the mantle [see review by Wilbur (*8*)], suggest that an adhesive material, possibly a mucopolysaccharide, is being produced by specific regions in the periphery of the mantle and that this adhesive is acted upon synergistically by the triple antibiotic mix.

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Control Factor of Nuclear Cycles in Ciliate Conjugation: Cell-to-Cell Transfer in Multicellular Complexes

Abstract. *Multicellular complexes of* Blepharisma intermedium *are regularly produced by treating doublet cells with the gamone of complementary mating type. Cells remain united without undergoing nuclear cycles of conjugation. However, if a cell of complementary mating type unites, nuclear cycles begin at the site of this union and propagate all through the multicellular complex.*

In the conjugation of ciliates, two cells temporarily unite and undergo a series of nuclear cycles including meiosis, fertilization, repeated mitosis, and development of new micro- and macronuclei. The whole process is a chain reaction initiated by the interaction between complementary mating types (*1–3*). If two such mating types of *Blepharisma intermedium*, 1 and 2, are mixed under appropriate conditions, cells unite in pairs after 2 hours of interaction (*4, 5*). Type 1 cells excrete gamone 1—blepharmone (*6*), which is a glycoprotein with a molecular weight of 20,000 and transforms type 2 cells so that they can unite. Type 2 cells excrete gamone 2—blepharismone (*7, 8*), which is calcium 3-(2′-formylamino-5′-hydroxybenzoyl) lactate and similarly transforms type 1 cells.

Transformed cells can unite in all three

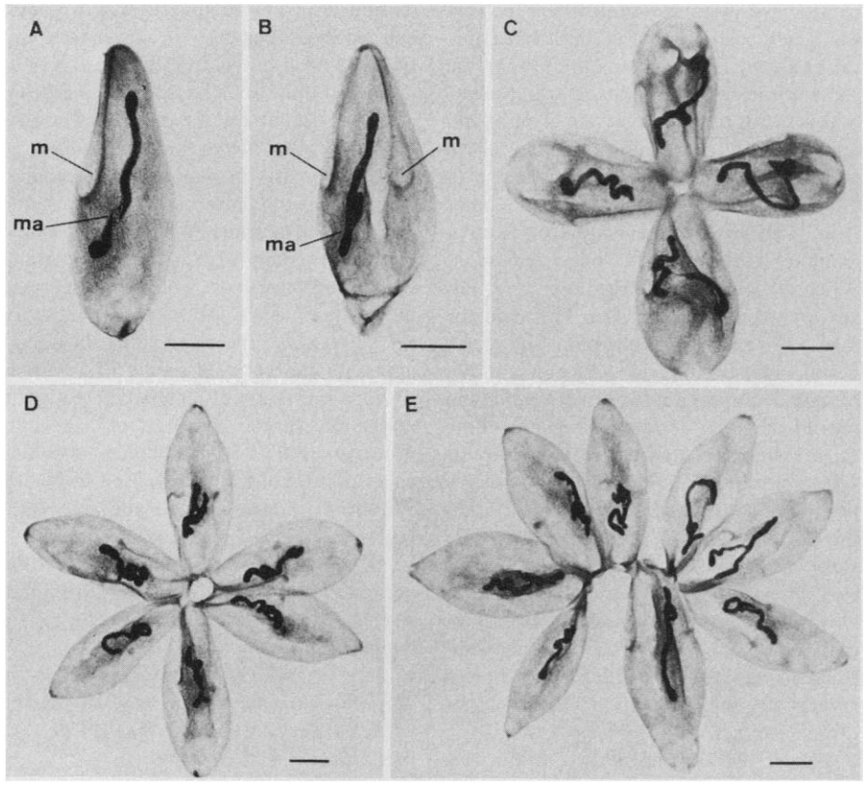

Fig. 1. Singlet, doublet, and multicellular complexes of *B. intermedium*. (A) Singlet cell; (B) doublet cell; (C) homotypic four-cell complex of doublet; (D) homotypic six-cell complex of doublet; (E) homotypic eight-cell complex consisting of seven doublets and a singlet (lower left) (*18*). Abbreviations: *m*, mouth; *ma*, macronucleus (scale, 50 μm).

possible combinations of mating types, but only heterotypic pairs (1-2) complete conjugation (*4, 5*). Homotypic pairs (1-1, 2-2) may persist for days if enough gamone is present, but they do not undergo nuclear cycles of conjugation. In both types of pairs, cells unite at the anterior part of the mouth. Since there is only one mouth per cell (Fig. 1A), each cell usually takes one partner. However, a morphological mutant, a doublet, has two mouths (Fig. 1B) and therefore two points of attachment. When doublets are treated by the gamone of complementary mating type, they unite side by side forming homotypic multicellular complexes (Fig. 1, C to E). Like homotypic pairs, they do not undergo nuclear cycles. I have added single cells of complementary mating type to the one end of these multicellular complexes and observed how nuclear cycles occur and propagate.

A red wild-type strain and a white albino strain of *B. intermedium* Bhandary (*9, 10*) were grown (*5*), concentrated, washed with and suspended in SMB (1.5 m*M* NaCl, 0.05 m*M* KCl, 0.4 m*M* $CaCl_2$, 0.1 m*M* $MgSO_4$, 2×10^{-3} m*M* EDTA, 2 m*M* sodium phosphate buffer, *p*H 6.8), and left for 1 day before use.

A doublet strain was obtained by fusing two cells. Homotypic pairs were induced in

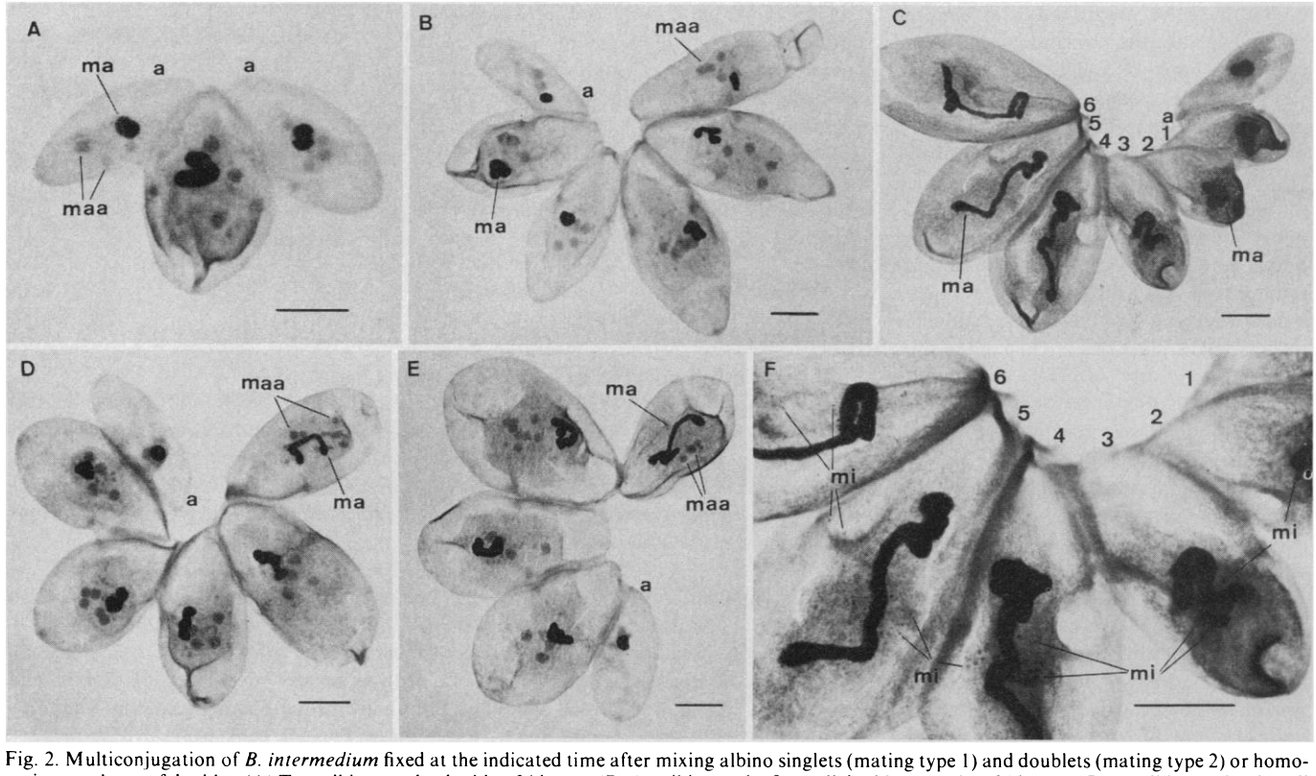

Fig. 2. Multiconjugation of *B. intermedium* fixed at the indicated time after mixing albino singlets (mating type 1) and doublets (mating type 2) or homotypic complexes of doublet. (A) Two albinos and a doublet, 36 hours. (B) An albino and a five-cell doublet complex, 36 hours. (C) An albino and a six-cell doublet complex, 14 hours. Macronuclear condensation is nearly complete in 1, hardly detectable in 6. (D) An albino and a five-cell doublet complex, 36 hours. (E) An albino and a four-cell doublet complex, 36 hours. (F) Detail of (C); micronuclear swelling in the first meiotic division is extensive in 2 and 3, less so in 4, not detectable in 6. Abbreviations: *a*, albino; *ma*, macronucleus; *maa*, macronuclear anlage; *mi*, micronucleus (scale, 50 μm).

red wild-type cells of one mating type by exposure to gamone of the other mating type in the presence of actinomycin S_3 (50 μg/ml) (Calbiochem), which does not inhibit the union of cells but prevents the separation of united cells. After 2 days, cells were fused in many pairs. When washed and cultured, some of them multiplied, keeping the fused condition. Three doublet clones of mating type 2 were thus obtained.

Homotypic unions of these doublets were induced in a mixture (98 : 1 : 1) of cell suspension, gamone 1 preparation (*11*), and 1 percent solution of bovine serum albumin (*12*). After 10 hours, when many homotypic complexes were formed, albinos of mating type 1 were added. Albinos started uniting to red complexes after 3 hours and remained united for about 2 days. Cells were fixed with Schaudinn's fluid 9, 14, 24, and 36 hours after the introduction of albinos, and were stained by the Feulgen reaction and light green.

Cells of *B. intermedium* have one long macronucleus and 10 to 30 micronuclei (about 1 μm in diameter) located near the macronucleus. In conjugation, micronuclei scatter in the cytoplasm, cells shrink, and nuclear cycles begin. The micronuclear cycles include three pregamic divisions: fertilization, repeated divisions of the fertilized nucleus, and differentiation of the division products to micronuclei and macronuclear anlagen. The macronuclear cycle includes the winding up, condensation, and degeneration of the old macronucleus and development of the new macronucleus from macronuclear anlagen (*13, 14*).

When a doublet unites with one or two albinos, all cells undergo macronuclear condensation and form macronuclear anlagen (Fig. 2A), indicating that nuclear cycles of conjugation regularly occur in doublet-albino mating.

In homotypic complexes to which no albinos unite, nuclei remain unchanged, except that micronuclei scatter in the cytoplasm and that the macronucleus winds up slightly in some cells (Fig. 1, C to E). However, if even a single albino cell unites to these homotypic complexes, most cells undergo macronuclear condensation and form macronuclear anlagen (Fig. 2B). When cells in a complex show different stages of the nuclear cycle, cells closer to the albino are in a more advanced stage (Fig. 2, C and F). It is therefore concluded that nuclear cycles begin in heterotypically united cells and propagate to other cells in the complex. This propagation is accompanied by a conspicuous shrinking of the cell (Fig. 2C), indicating that the cytoplasm is also undergoing a profound change.

The propagation of nuclear cycles is not mediated by the external medium, because the complexes without albinos never undergo nuclear cycles even if they are side by side with albino-united complexes for 36 hours. Thus an initiation factor of the nuclear cycle of conjugation which is produced by the heterotypically united cells and is transferred through cell unions may be postulated.

In some chain-type complexes in which an albino unites at one end, the macronuclear condensation is incomplete in cells at the other end, although new macronuclear anlagen are formed in the same cells (Fig. 2, D and E). This suggests that certain nuclear processes of conjugation might be controlled by different specific factors. In order to test this hypothesis, each factor should be isolated. A way to approach this problem would be to inject, by the microinjection method developed for *Paramecium aurelia* (*15*), fractionated samples from conjugating cells into a cell in the homotypic complex. The observed propagation of induced changes would be an assay of the activity of the factor. Such analysis would bring new insight into the regulation mechanism of the eukaryotic cell, especially the meiosis-initiating mechanism, because the first micronuclear cycle in ciliate conjugation is meiosis.

Doublets are known in other ciliates (*16*) including *P. aurelia* in which homotypic conjugation may occur (*2, 17*). However, in this species homotypically united cells also undergo all developmental changes of conjugation. The gamone-induced homotypic complex of *B. intermedium* is unique in that the process of conjugation stops at the stage of cell union, thus providing a test system for the isolation of factors that regulate meiosis and other nuclear and cytoplasmic events of conjugation and for analysis of their mode of transfer from cell to cell in multicellular complexes.

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Sleep Cycle Oscillation: Reciprocal Discharge by Two Brainstem Neuronal Groups

Abstract. *During the sleep cycle in cats, neurons localized to the posterolateral pole of the nucleus locus coeruleus and the nucleus subcoeruleus undergo discharge rate changes that are the opposite of those of the pontine reticular giant cells. The inverse rate ratios and activity curves of these two interconnected populations are compatible with reciprocal interaction as a physiological basis of sleep cycle oscillation.*

Neurons in the gigantocellular tegmental field (FTG) of the cat pontine brainstem show dramatic and state-specific discharge rate increases during desynchronized sleep (*1*). A tonic increase in discharge rate is detectable in the FTG population 5 minutes before the onset of desynchronized sleep (*2*), and phasic bursts of discharge by FTG neurons often lead the rapid eye movements of the fully developed desynchronized sleep episode by 250 msec (*3*). Neurons in most of the other tegmental nuclei share these properties of selectivity, tonic latency, and phasic latency with the FTG, although in diminishing degree as recording sites are distant from the FTG (*1–3*). These findings have led us to postulate that the FTG neurons may be a critical part of an executive system for desynchronized sleep and to consider pos-