

calcareous layer of the valve by a terminal, thin, flattened chitinous boss (3). The boss has a greater cross-sectional area than the muscle and is inset into the calcareous layer. Muscles are attached to the boss by interlacing of muscle myofibrils and boss tonofibrils. The bosses form the muscle scars on the valves, and coalescing of adjacent bosses form the so-called "sutured" muscle scars. In Fig. 1, the adductor muscle in the focal plane is the second from the dorsalmost. The muscle is terminally bifurcated, but the two bosses coalesce to form an apparently single boss at the point of attachment on the valve. Under high magnification ( $\times 600$  to  $\times 800$ ) the "suture" between the coalesced bosses is readily apparent.

In the two species studied, the basic difference between the trachyleberine "single" V-, J-, or U-shaped frontal scar and the hemicysterine frontal scars arranged in a more or less dorsal-ventral alignment of two or three elements is due to the nature of the transverse muscle termination and the position of the mandibular muscle. In the trachyleberine species *Actinocythereis vineyardensis*, each end of the transverse muscle bifurcates terminally into an anterior and a slightly larger posterior bundle (Fig. 1). Coalescing of the terminal attachment bosses of the bundles forms a V- or U-shaped frontal scar on the calcareous valves. The small mandibular muscle terminates in a contiguous dorsal position to the anterior bundle of the transverse muscle thus forming a small portion of the anterior side of the V- or U-shaped scar. Any minor variation in the attachment position of either the transverse or mandibular muscle accounts for the V-, J-, or U-shaped frontal scar found on the calcareous valves.

The hemicysterine species *Muellierina lienenklausii* bears three frontal scars with the lower two occasionally fused (4). In contrast to the anterior-posterior bifurcation of the transverse muscle in the trachyleberine species, the hemicysterine transverse muscle bifurcates into dorsal-ventral bundles approximately halfway between the body center and the body wall (Fig. 2). The mandibular muscle terminates adjacent and anterior to the ventral transverse muscle bundle (Fig. 2). Minor variation of the attachment of either the ventral transverse muscle bundle or mandibular muscle accounts for the fusion of the two lowermost scars or the distinct separation into three frontal scars.

Therefore, as similar muscles form the frontal scars of the trachyleberines and the hemicysterines, the nature of the bifurcation of the transverse muscle and the position of mandibular muscle attachment determines the frontal muscle scar patterns.

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## Sexuality in Chodatella

**Abstract.** *The unicellular green alga Chodatella longiseta Lemm. is described as reproducing solely by the production of autospores; however, it is also capable of oogamous sexual reproduction. Division of a single cell produces 8, 16, or 32 sperms which, upon release, attach to nonmotile cells. Fusion takes place, and a thick-walled resistant zygospore develops.*

Sexual reproduction has not been previously reported in *Chodatella*, a unicellular planktonic freshwater genus in the order Chlorococcales of the green algae. For this reason, as well as because of its unicellular condition, the mode of asexual reproduction (autospores), and the lack of any sort of motile cells, the genus has been considered a member of the Oocystaceae. Reconsideration of the classification of this organism is now imperative in view of the observation of sexual reproduction involving biflagellate gametes.

Unialgal cultures of *Chodatella longiseta* Lemm. were grown in soil-water medium (1) at room temperature under a bank of fluorescent lights. Such cultures produce biflagellate cells, each containing a chloroplast and lacking an obvious cell wall. The motile cells serve as sperm. Cells forming these sperm can usually be distinguished from those reproducing asexually. They first appear in a culture 4 or 5 days after inoculation and are generally smaller than cells forming autospores. As the chloroplast in such cells divides repeatedly, it usually becomes a very pale green and the pyrenoid becomes indistinct. After the sperms are complete, they are crowded toward the center of the cell, and the wall swells at the equatorial region. The sperms then break out of the cell wall and thin vesicle in which they have been enclosed during development. A single cell may form 8, 16, or 32 sperms.

The biflagellate swimmers attach to

cells indistinguishable from typical asexual *Chodatella* cells (Fig. 1), generally at the equatorial region, but sometimes at the poles. These same cultures also contain thick-walled cells, singly within spiny *Chodatella* cell walls (Fig. 2), which are later free in the culture medium. These thick-walled cells are assumed to be zygospores. Though not yet observed, actual fusion of the small biflagellate cells with the larger nonmotile cells can be concluded for two reasons. (i) In many instances the thick-walled cell has a small protuberance on one side (Fig. 2) in the usual position of attachment of the flagellated cells, as if the membrane surrounding the swarmer were incompletely incorporated into the nonmotile

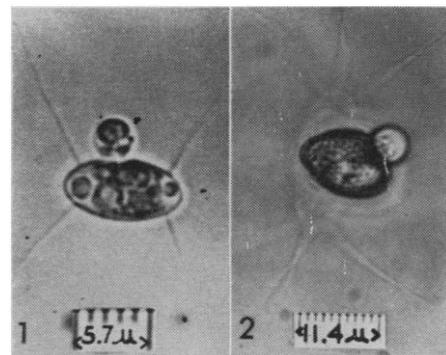


Fig. 1. Typical *Chodatella* cell with sperm attached at the equatorial region. Fig. 2. Single zygospore still within the spiny parental cell wall. The protruding portion of the zygospore wall indicates where the sperm was attached.

cell. The lumen within such a cell is continuous into the protrusion. (ii) Sometimes when the swarmers become attached the cytoplasmic contents of the nonmotile cell are released into the swarmer, thus indicating confluence of the two membranes. Such cells do not survive.

The thick-walled cells are resistant reproductive cells. A thick suspension of them was placed on watch glasses and allowed to dry thoroughly. The watch glasses were stored 4 months in the dark, culture medium was then added, and the preparations were placed in the light. Within 3 to 4 days *Chodatella* cells appeared, indicating, as did the original isolation of the organism from an old mud sample, the presence of a perennating structure. A thick-walled cell may represent a single resistant aplanospore produced by the protoplast of a vegetative cell, but the presence of flagellated cells which become attached to and apparently fused with nonmotile cells surely favors the interpretation that such a thick-walled cell is a zygospore. *Chodatella longiseta* not only reproduces asexually via auto-spores, but also is capable of oogamous sexual reproduction.

The classification in the Chlorococcales has long been problematical. Fritsch (2) questions the validity of maintaining the Coelastraceae separate from the Hydrodictyaceae for the mere lack of zoospore production by the former. Trainor and Burg's (3) recent discovery of flagellated isogametes in *Scenedesmus* emphasizes this recognized artificiality of classification in the Chlorococcales. The results of this investigation of a unicellular member of the Chlorococcales presents similar problems. *Chodatella* certainly cannot be retained in the Oocystaceae as defined by Smith (4), yet its relationship to the members of other families is not entirely clear. Hopefully, as more of the genera in the family are studied rigorously, the natural affinities within the group will emerge.

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## Neuronal Correlates of Behavior in Freely Moving Rats

**Abstract.** *Firing patterns of single neurons in the hypothalamus, preoptic area, midbrain reticular system, and hippocampus of awake, freely moving female rats were temporally correlated with exploratory sniffing and vibrissa twitching, feeding, lordosis, locomotion, and (or) arousal. These relationships were remarkably stable during continuous observations lasting many hours. During extended periods when certain of these movements were not performed, the correlated neurons showed no action potentials for minutes at a time. Electrical stimulation at certain recording sites elicited behavior patterns whose spontaneous occurrence was accompanied by neuronal activation. Self-stimulation was elicited from sites spontaneously activated during exploratory behavior.*

Evidence for the regional differentiation of functions in the limbic system-midbrain circuit is based mainly upon studies in which intracranial electrical stimulation, production of lesions, and hormone and drug implantation are used (1). Differential sensitivity of individual neurons in this region to neurohumors and to hormones and other normally blood-borne substances (2) implies that they function in homeostatic or behavioral mechanisms. We have analyzed the firing activity of limbic and midbrain neurons with respect to a wide variety of species-characteristic behavior patterns in an attempt to determine their functional differentiation (3). Other studies have identified neuronal correlates of components of limb movements, fighting, food-getting, and attention (4).

Electrodes, made of Diamel-insulated nichrome wire, 0.0025 inch (63.5  $\mu$ ) in tip diameter, were implanted into the lateral preoptic and hypothalamic areas, dorsal hippocampus, and midbrain reticular formation in each of 13 female rats. In six rats, activity was recorded in a total of 16 sites, as early as 1 day after operation (5).

Neuronal firing rate was greater in arousal than in slow-wave sleep in 50 to 100 percent of the units studied. The firing rate of some of these units (for example, Fig. 1, RF) was roughly proportional to the degree of arousal; it was lowest during slow-wave sleep (Fig. 1A), higher during quiescence (Fig. 1C), and highest upon startle (Fig. 1D). Activity tended to be high in paradoxical sleep (Fig. 1B), consistent with earlier studies (6). Electrical stimulation at this site (Fig. 3, 4160 RF) evoked moderate self-stimulation, but no feeding or escape behavior. Other neurons showed greater differentiation of function.

In one differentiated pattern, the neurons fired rapidly during any form of locomotion as well as when the rat

was startled, when it pulled away while the observer was holding the tail, when it groomed its face, and when its forepaws were held by the observer but not when the rat scratched with its hind legs. The unit fired very infrequently while the rat was standing very still but alert (Fig. 2, LH, F-I). Unit firing decreased dramatically when the rat stood still and started eating after approaching and sniffing food (Fig. 2F). This pattern occurred in 9 of 12 units during feeding and in 9 of 19 units during lordosis induced by manual stimulation of the flanks and perineum. Each of these neurons showed a comparable decline in activity when the rats stood still, but they became activated during locomotion. In contrast, the activity of the former "arousal" neurons was persistently high during all these wakeful states.

In a second, more highly differentiated firing pattern, two neurons were inactive except during vibrissa movement, which occurred during exploratory behavior or paradoxical sleep (for example, Fig. 1, POA). Eight additional neurons were activated during exploratory behavior (1 POA, 2 LH, 3 RF, and 2 HPC), but these were also active during any type of locomotion. A preoptic neuron whose activity was correlated with vibrissa movement was completely inactive during slow-wave sleep and quiet wakefulness and was not activated by startle (Fig. 1, A, C, D). It thus appeared to be independent of general degree of arousal. It did not fire when the rat lunged at and chewed on or withdrew from odorous substances presented on a cotton swab (Fig. 1, E-G). Chewing artifacts are seen at the end of Fig. 1, E and F; face-wiping movement artifacts are seen three times in Fig. 1G. Thus, the unit was not necessarily activated by odors to which the rat showed clear and opposite behavioral reactions, nor was it activated by nonspecific arousal. How-