episodic mechanisms for the periodic cicada *Magicicada cassini*.

Synchrony in the snowy tree cricket is understandable in terms of the function of the song. Sexually responsive females come to the song, and the chirp rhythm is essential to their response (*15*). When males sing in proximity, synchrony allows the rhythm to remain evident to females outside the chorus. The chirp rhythm is so rapid that only proepisodic mechanisms can effectively preserve it. Individuals in choruses may have an advantage over solitary singers as to safety from acoustically orienting predators (*16*), and it is possible that females respond sooner or more readily to the louder more regular chirp of a chorus than to the song of a solitary male.

Except for two other tree crickets (*Oecanthus allardi* and *O. rileyi*), other insects known to have proepisodic mechanisms of synthrony have evolved them independently of the snowy tree cricket (*O. fultoni*). The mechanisms I have studied in other species are similar though not the same as for *O. fultoni* (*5*).

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References and Notes

1. Prior to 1960 [T. Walker and A. Gurney, *Fla. Entomol.* **43**, 10 (1960)] the snowy tree cricket was known as *Oecanthus niveus*.
2. See, for example, A. E. Dolbear, *Amer. Natur.* **31**, 970 (1897); J. McNeill, *Entomol. Amer.* **5**, 101 (1889); H. A. Allard, *Amer. Natur.* **51**, 438 (1917).
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5. Fireflies of the genera *Pteroptyx* (*3*) and *Photuris* [J. E. Lloyd, *Fla. Entomol.* **52**, 33 (1969)] and sound-producing insects of at least seven genera in the families Cicadidae (Homoptera), Gryllidae (Orthoptera), and Tettigoniidae (Orthoptera) [T. Walker, in preparation] synchronize rapid rhythmic signals with such perfection that they must use cues from one or more previous signals rather than from the contemporary one. Among other animals only human beings are known to have this ability (*3*).
6. The pulses within a chirp are delivered at two rates: The interval after the second pulse and every third pulse thereafter is slightly longer than the other intervals. The pulses are therefore grouped 2–3–3. The cricket produces long or short chirps by adding or dropping groups of three pulses.
7. In an individual from Franklin Co., Ohio, at 24.5°C, the average ($n = 43$) five-pulse chirp lasted 102 ± 3 msec ($\bar{x} \pm$ S.D.) with a following interval of 279 ± 14 msec for a period of 381 ± 14 msec, while the average ($n = 53$) eight-pulse chirp lasted 167 ± 4 msec with a following interval of 298 ± 15 msec for a period of 465 ± 14 msec.
8. First noted by M. W. Brooks [*Pop. Sci. Mon.* **20**, 268 (1882)] and recently discussed by B. C. Block [*Ann. Entomol. Soc. Amer.* **59**, 56 (1966)]. In eastern United States, the relation between chirp rate and temperature is chirps per minute $= 8.21T - 38.6$, where $T =$ temperature in degrees Celsius. Westward, the relation varies. The average pulses per second during a chirp approximates $2.24T - 4.16$; T. Walker, *Ann. Entomol. Soc. Amer.* **55**, 315 (1962).
9. Audio frequency doubler made by Alton Electronics Co., Gainesville, Fla., used between the tape recorder and microphone for one individual.
10. Female of *Montezumia modesta* in reply to male lisp at 25°C; J. D. Spooner, *Anim. Behav.* **16**, 200 (1968). The shortest delay that the snowy tree cricket demonstrated in the present experiments was approximately 90 msec.
11. Four chirps were spliced with blank tape to form a 6.7 second loop having chirps at 0, 2.2, 2.9, and 4.1 seconds. The loop ran continuously during a test.
12. The equipment used to produce artificial cricket songs is described in T. Walker, *Ann. Entomol. Soc. Amer.* **50**, 629 (1957).
13. When the period of the first chirp that the last broadcast chirp might not have influenced (by S or L responses) was compared with the mean period of the last 2 to 5 chirps prior to the beginning of the broadcast signal, ten of the first chirps were within 3.2 percent [$< \simeq 1$ S.D. (*7*)], five were within 6.4 percent ($< \simeq 2$ S.D.), and one was a two-pulse chirp evidently caused by an S response to the voiced announcement that the signal was off. Analyses of second and third chirps gave similar results.
14. R. D. Alexander and T. E. Moore, *Ohio J. Sci.* **58**, 107 (1958).
15. T. J. Walker, *Ann. Entomol. Soc. Amer.* **50**, 634 (1957).
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17. In the tests illustrated, 21 broadcast chirps fell near presumed natural five-pulse chirps. When the test chirp fell −50° to +83° out of phase with such a chirp ($n = 19$), the chirp remained five-pulse but the interval (normally 279 msec) usualy equalled or slightly exceeded the interval normal for eight-pulse chirps (298 msec) (*7*). Since the broadcast chirp was eight-pulse, the response was appropriate for achieving synchrony. In two cases the test chirp followed a five-pulse chirp by nearly 180°, and the response of the test cricket was to lengthen the period by nearly 180°.
18. Supported by NSF grant GB4949 and the Graduate School, Ohio State University. I thank D. J. Borror, J. E. Lloyd, and J. Buck for help and advice. Florida Agricultural Experiment Stations Journal Series No. 3329.

19 May 1969; revised 28 August 1969

Cellulosic Wall Component Produced by the Golgi Apparatus of Pleurochrysis scherffelii

Abstract. *The Golgi apparatus of a marine chrysophycean alga* Pleurochrysis scherffelii *Pringsheim produces wall fragments (circular-to-ellipsoidal "scales") which are released to the periphery by an exocytotic process involving the fusion of cisternae and the plasma membrane. The cellulosic component of the scales is a complex network of fibrils (10 to 25 angstroms in diameter) that resist treatment with strong alkali. Untreated washed scales yield galactose, ribose, arabinose, and traces of glucose; alkali-purified scales yield much more glucose. The fibrillar scale constituent shows a positive iodine dichroism of the intact wall, a positive zinc chloride–iodine reaction, breakage sites characteristic of highly crystalline cellulose, and solubility in Schweizer's reagent.*

Pectic cell-wall polysaccharides are formed within the Golgi apparatus and extruded in typical vesicles by an exocytotic membrane-flow mechanism (*1–4*). The same process is very likely for formation of hemicellulosic components (*5, 6*); however, it is generally agreed that cellulose is not made within the Golgi cisternae. Instead, the cellulose fibrils are thought to be formed outside of the plasma membrane from intracellular precursors (*1, 4, 6, 7*).

The difficulty of assigning to the Golgi apparatus the role of cell-wall formation comes principally from the lack of morphological evidence for equivalent structures within the Golgi cisternae in comparison with constituents of the cell wall *in situ*. Such morphological proof for the role of the Golgi apparatus in the production of the cell wall of a marine chrysophycean alga *Pleurochrysis scherffelii* (*8*) was recently demonstrated by Brown (*9*), who suggested that the cell wall was composed of cellulosic substances as indicated by a positive iodine dichroism and a negative spherite birefringence of the intact wall. Pectic substances were also demonstrated in the wall by a positive reaction with Ruthenium Red and uptake of Alcian Blue at *p*H 0.5. This report provides biochemical and additional morphological evidence that at least one component of the cell wall produced by the Golgi apparatus is composed of a cellulosic glucan.

Axenic cultures of *P. scherffelii* were grown in agar slants of von Stosch's medium (*10*) modified to include 4 grams of vitamin-free Casamino acids (Difco) in each liter of medium. Pure fractions of cell-wall fragments or "scales" were obtained from dissolution of mother-cell walls during zoospore production (*11*). The scale fraction was separated from the zoospores by centrifugation (1600*g*, 20 minutes), and the supernatant containing the scales was again centrifuged at 70,000*g* for 20 minutes. The resulting pellet consisted of circular-to-ellipsoidal scales (Fig. 1A). Whereas some of the scales produced an image of a distinct network of concentric microfibrils with an underlying layer of radial

ones, others revealed, in addition, some amorphous substances covering the scales. The fibrillar network resisted 10 minutes of boiling in water, but the amorphous material did not. Furthermore, the fibrillar network resisted 10 minutes of boiling in 5 percent KOH, although a progressive loss of order of arrangement occurred during this alkaline treatment as a result of the differential removal of the radial fibrils (Fig. 1B). When the fibrils of the scale fractions were subjected to 24 percent KOH for 12 hours or more, they lost all of the characteristic tertiary order of arrangement and consisted only of short rods about 1000 Å long.

For chemical analysis, a scale fraction, purified as described above, was treated with 0.5*N* H_2SO_4 at 100°C for 1 hour and then centrifuged at 70,000*g* for 20 minutes. The pellet was washed with distilled water until the washings were neutral. This partially hydrolyzed scale fraction was subjected to 24 percent KOH for 10 hours at 20°C, and then 2 hours at 100°C. Native cotton served as a control.

The resulting flocculent was thoroughly washed with distilled water and concentrated by centrifugation. Both this fraction and the washed untreated scales were hydrolyzed in 5*N* H_2SO_4 in sealed ampoules at 60°C for 15 hours, then neutralized with $BaCO_3$. The hydrolyzate was subjected to thin-layer chromatography (*12*), and the chromatogram was sprayed with anisidine phthalate (*13*) for detection of sugars. The alkali-purified scale hydrolyzate yielded glucose, but in a few cases traces of galactose were also detected. In contrast, the hydrolyzates of nontreated washed scales contained galactose, ribose, arabinose, and traces of glucose.

Our data indicate that the fibrillar network of the scales consists of cellulose or at least of cellulose-like glucans because of: (i) a positive iodine dichroism of the intact cell wall and a corresponding positive reaction of the alkali-purified material with zinc chloride-iodine; (ii) the appearance of breakage loci characteristic for highly crystalline cellulose known from other preparations (*14–17*); (iii) a resistance of the fibrillar component to boiling 24 percent KOH; (iv) solubility of alkali-purified fibrillar component in Schweizer's reagent (Cuoxam); and (v) thin-layer chromatography of the alkali-purified hydrolyzate showing that glucose is the predominant sugar.

Equivalent structure corresponding to the isolated negatively stained scales is shown in Fig. 2. The very same structures are observed within the cisternae of the Golgi apparatus (Fig. 3) thus demonstrating that the 10- to 25-Å concentric and radial fibrils are synthesized within the Golgi apparatus in the same arrangement that they show in the cell wall. This confirms directly, for the first time, the role of the Golgi apparatus in the synthesis of a cellulosic substance.

Scale production by the Golgi apparatus is known in other organisms, namely the algae of the classes Haptophyceae (*18*), the Prasinophyceae (*19*), and the Coccolithophoridae (*20*); however, a cellulosic component never has been reported from these members even though the morphology of certain of these scales is similar to those of *Pleurochrysis*.

Green and Jennings (*21*) reported

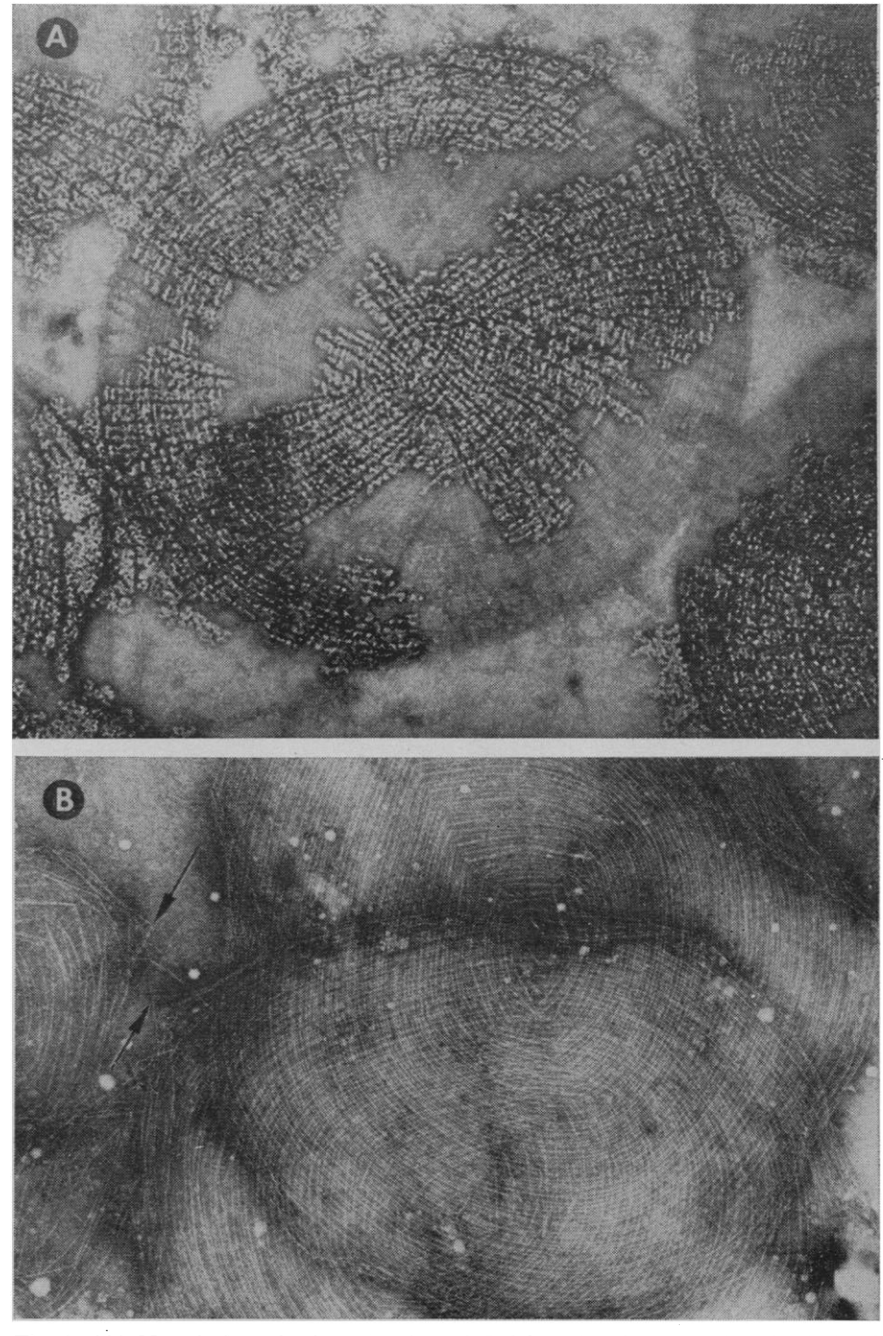

Fig. 1. (A) Negatively stained preparation of Golgi-produced untreated scales isolated from the cell wall of the marine alga *Pleurochrysis scherffelii*. The fibrillar network is cellulosic, while the more globular structures are probably pectin or other polysaccharide substances (× 72,500). (B) Scale fraction treated for 10 minutes with 5 percent KOH. Amorphous substance is lost and cellulosic fibrils become progressively deranged. Breakage loci, characteristic for highly crystalline cellulose, are shown at arrows (× 80,965).

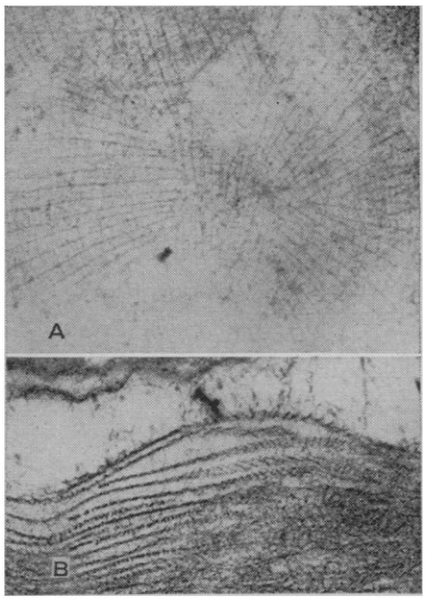

Fig. 2. (A) Grazing section of intact cell wall of material fixed in glutaraldehyde-osmium. The concentric and radial fibrils are identical to those observed with negative staining (× 83,075). (B) Cross section through an intact cell wall of fixed material. Some of the scales are sectioned obliquely and reveal the radial fibrils (× 69,230).

galactose and ribose as the major sugars of Golgi-derived scales of *Chrysochromulina chiton*, probably a close relative of *Pleurochrysis*. Because these authors did not use alkali-purified material, we believe that, in addition to cellulose, other dominant polysaccharides, probably of pectic nature, accounted for their sugar pattern. These same deductions also apply to *Pleurochrysis*. The noncellulosic components of the scales of *Pleurochrysis* are under study (*22*).

The fibrils of the scale cellulose are thinner than the 35-Å "elementary fibril" previously reported for cellulose (*1, 23*), thus augmenting the suggestion that the 35-Å limit does not hold true in general (*17*). As similar scales are produced in the algal classes mentioned above and perhaps in the Dinophyceae (*24*), at least one component of these may be cellulosic. It is not inconceivable that the "coccolith" structure is intimately associated with a cellulosic matrix because it has been shown recently that coccoliths derived from the Golgi apparatus in *Cricosphaera* are made on organic templates morphologically identical to the cellulosic moiety in *Pleurochrysis* (*20*). The morphology of the cellulosic component of the scale and its intimate association with the cisternal membranes suggest that its synthesis must be controlled by a structural template within the cisternal membranes. Because a cellulosic structure is polymerized and deposited within the Golgi cisternae, it may be that uridine diphosphoglucose (UDPG) and guanidine diphosphoglucose (GDPG), or both, as well as chain-synthesizing and chain-length-determining structures (*25*) occur on the internal surface of the cisternal membranes.

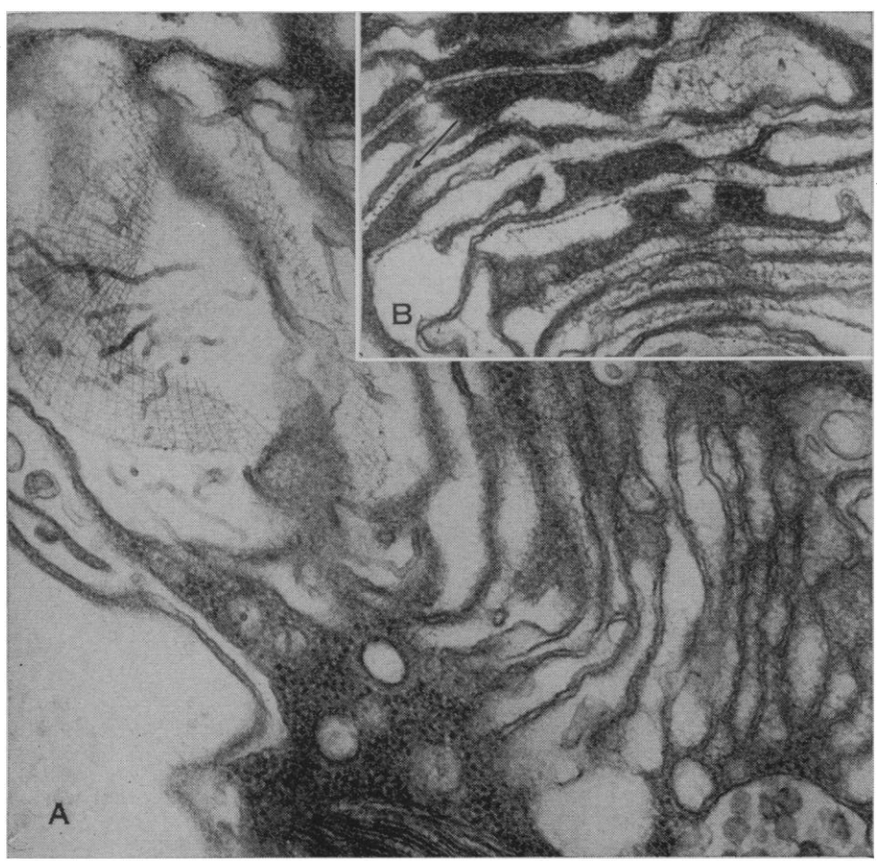

Fig. 3. (A) Section through fixed material showing the surface view of a scale within a distal Golgi cisterna (× 65,000). (B) Fixed material showing cross section of scales within the Golgi cisternae. The arrow denotes radial fibrils (× 65,000).

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26. One of us (R.M.B., Jr.) was supported by NSF postdoctoral fellowship, September 1968–69. Work supported in part by the Deutsche Forschungsgemeinschaft.

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10 June 1969