

months. During that time the bud remains enclosed in bud scales and develops short shoots and lateral primordia, but no internode elongation occurs on either the terminal or lateral buds until the following spring. In contrast, young trees produce lateral primordia and short shoots, which quickly begin to elongate internodes and complete their development.

Three-year-old loblolly pine may sequentially produce in one growing season as many as four temporary buds that begin to elongate almost immediately. The final quiescent bud that will overwinter is not formed until September (4). Therefore, lateral primordia which might be potentially reproductive may form too late in the growing season to allow the lengthy process of strobilus differentiation to be completed. The material that flowered in this study was induced to form a quiescent bud that persisted for longer than 2 months during favorable spring conditions. Material of similar age that flowered after topworking also set buds early in the growing season, just like the large adult material to which they were grafted (4).

The transition to the adult reproductive phase in loblolly pine thus appears to be at least in part a function of loss of ability to elongate several buds during the growing season. Reproductive competence in young material would therefore be masked by bud set too late to permit the differentiation of reproductive structures.

Flowering in woody plants is a long, drawn-out process easily reversible in its early stages; a decline in vegetative growth usually precedes it (1). I therefore propose that a critical event in the transition to the adult reproductive phase in loblolly pine and possibly other temperate-zone woody plants is the change in vegetative growth behavior that results in the formation of quiescent buds earlier in the growing season. This change then allows sufficient time for the slow process of initiation and differentiation of reproductive structures to occur during good growing conditions. Whatever causes phase change would therefore act directly on vegetative growth and would only indirectly affect reproductive development.

This hypothesis is further supported by the observation that stimulating vegetative growth by frequent watering and fertilizing resulted in no female flowering and greatly reduced male flowering on 2-year-old potted grafts of 50- to 60-year-old mature scion material. These grafts were vegetatively more vigorous than

similar grafts of the same clones which were simultaneously water-stressed or given photoperiod and temperature shock as previously described. The ramets receiving the last two treatments have formed a heavy crop of both male and female strobili.

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Light-Stimulated Morphogenesis in the Fruiting Myxobacterium *Stigmatella aurantiaca*

Abstract. When the fruiting myxobacterium *Stigmatella aurantiaca*, a gliding prokaryote, is starved on an agar surface, the cells form multicellular aggregates resulting from morphogenetic movements. In the presence of incandescent light, each aggregate develops into a structurally complex fruiting body, possessing a stalk and several sporangia. In contrast, this pattern of development is not seen when cultures are incubated in the dark. The cells form irregular interconnecting aggregates, which rarely develop into fruits. However, aggregates formed in the light will develop into fruits even if placed in the dark, suggesting that the light produced a relatively stable alteration in the phenotype of the cells.

The fruiting myxobacteria are typical of other Gram-negative bacteria in structure and physiology, but differ in that multicellular interactions are a prominent and important part of their life cycle (1). These organisms exist as swarms of individual cells embedded in a secreted extracellular matrix material commonly referred to as slime. When the cells are

starved on an agar surface, cell movements result in the construction of multicellular aggregates and fruiting bodies of genetically determined shape. Their life cycle makes the myxobacteria invaluable for the study of cell interactions and morphogenetic movements. We have isolated a dispersed-growing strain of *Stigmatella aurantiaca* that retains its fruiting ability even when repeatedly subcultured in liquid. The new strain, designated DW-4, was isolated as a natural variant from a nondispersed growing strain (CCf). We have devised conditions to produce rapid and synchronous fruiting of strain DW-4 (2). A typical fruit of *S. aurantiaca*, strain DW-4, is approximately 50 μm tall and possesses a stalk supporting several sporangia (Fig. 1).

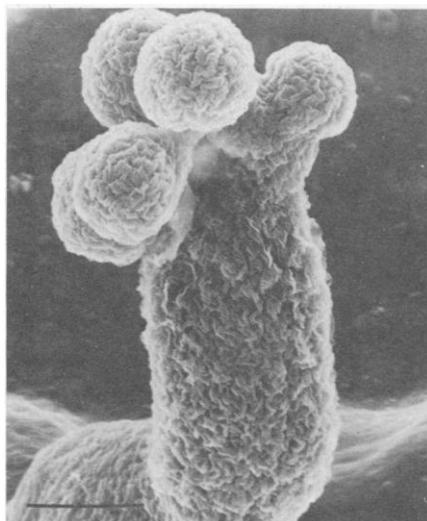


Fig. 1. Fruiting body of *S. aurantiaca* strain DW-4. Small blocks of agar containing fruiting swarms were mounted on specimen stubs, air-dried, and coated with metal in a vacuum evaporator. The first coat was carbon, followed by a mixture of gold and palladium (60:40). Specimens were examined with an Autoscan (ETEC Corp.) scanning electron microscope. The bar represents 20 μm .

We report here that, in the absence of light, the cells only form irregular aggregates and rarely develop fruiting bodies. However, if aggregates form in the light, fruits develop even if the cultures are placed in the dark.

The myxobacteria were grown vegetatively in shaking liquid cultures containing 1 percent Bactocastone (Difco) and 8 mM MgSO₄ at 30°C. Under these conditions the cells grew exponentially until the cell numbers reached 4 × 10⁸ per milliliter. Cultures harvested when the cell concentration exceeded 1 × 10⁸ per milliliter displayed the greatest dependency on light for development. When the cell number reached 1.2 × 10⁸ to 1.4 × 10⁸ per milliliter, the cells were harvested by centrifugation and washed once with Bonner's salts solution (3) containing 0.5 g of CaCl₂ · 2H₂O per liter. Washed cells (2 × 10⁸), suspended in 5 μl of Bonner's salts, were placed on Bonner's salts agar plates prepared 2 days before use, and incubated at 30°C at an intensity of 215 lux of incandescent light. The cells aggregated in 10 hours, and formed stalks in 12 to 14 hours and sporangia after 17 hours. Approximately 200 fruiting bodies developed from each 5-μl portion of resuspended cells. During the entire process, there was no detectable growth or increase in the number of cells, respectively (2). Consequently, we conclude that morphogenesis is due entirely to the movement of the cells. The morphology of developmental stages of the parent strain CCF has been described (4).

A different developmental pattern was observed when the cultures were incubated in the dark. The cells aggregated in 10 hours, forming elongated, interconnected ridges (Fig. 2). The number of sporangia that developed in the dark varied from 0 to 10 percent of control samples incubated in the light. However, the number of sporangia that developed in the dark was often 30 to 40 percent of the number developing in the light when the cells were harvested from cultures whose cell density was between 0.8 × 10⁸ and 1.1 × 10⁸ per milliliter. The few sporangia that did develop in the dark were not borne on stalks but rested directly on the ridges. Figure 3 is a photograph of aggregates that formed in the light and which developed into fruiting bodies. Cells fruit normally if allowed to aggregate in the light and are then transferred to the dark. This suggests that the light may have induced a relatively stable change in the phenotype of the cells.

Photoinducible morphogenesis has been reported in a number of eukaryotic

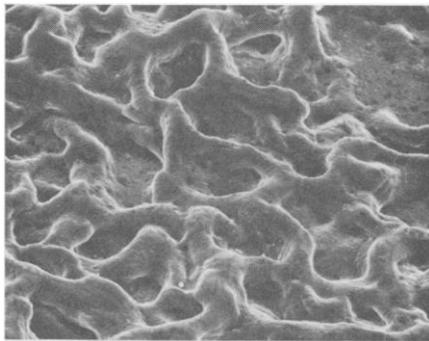


Fig. 2. Development in the dark. Scanning electron micrographs of ridges formed in the dark. Cells were incubated in the dark at 30°C for 24 hours. After 12 hours the ridges had formed. The bar represents 20 μm.

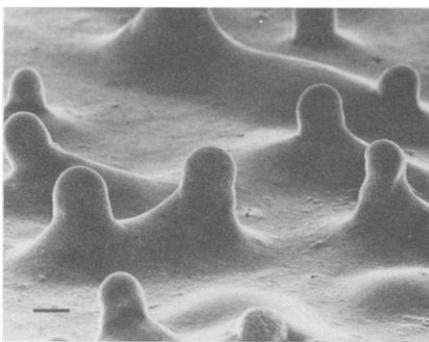


Fig. 3. Development in the light. Scanning electron micrographs of fruiting swarms that formed in the presence of light. Plates were incubated in 215 lux of incandescent light. Temperature was held constant at 30°C. The photograph represents samples 12 hours after the initiation of development. The bar represents 20 μm.

microbial systems, including both acellular and cellular slime molds, and fungi (5). Except for a brief statement by Coucke that light stimulated fruiting body formation in the myxobacterium *Sorangium compositum* (6), we were not

aware of any other publications of photomorphogenetic effects in nonphotosynthetic bacteria. However, it is known that myxobacteria do respond to light in other ways. Aschner and Chorin-Kirsh reported that certain species of myxobacteria are negatively phototactic (7), and Burchard and Dworkin reported light-induced lysis and carotenogenesis in the myxobacterium *Myxococcus xanthus* (8). The action spectrum for carotenogenesis in *M. xanthus* suggests that a heme compound may be the photoreceptor (9). We have found that red light effectively promotes fruiting body formation in *Stigmatella*. We anticipate that the use of light to experimentally alter the course of development will be a valuable aid in analyzing the molecular bases underlying morphogenetic movements and may also lead to the discovery of new pigments involved in morphogenesis in prokaryotes.

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Suppression of Chronic Allergic Encephalomyelitis: Relevance to Multiple Sclerosis

Abstract. *The expression of chronic relapsing experimental allergic encephalomyelitis in strain 13 guinea pigs was suppressed with a single series of injections of myelin basic protein in incomplete Freund's adjuvant. The suppression appeared permanent, and subsequent rechallenge with central nervous system antigen failed to elicit exacerbations.*

Chronic relapsing experimental allergic encephalomyelitis (EAE) is a model for demyelinating disease (1) that simulates in many ways the clinical and pathologic courses of the human demyelinating disease, multiple sclerosis (MS) (2). The application of acute EAE as a direct

model of MS is limited due to its short-term monophasic pattern, fatal outcome, and microscopic lesions. These are in contrast with the chronic relapsing course and grossly visible central nervous system (CNS) lesions of MS (2). Injections of myelin basic protein (MBP),