hyperbranched variant is probably not an auxotrophic mutant.

Discussion and conclusions. The isolation of morphological mutants, easy to characterize by classical parameters, allows genetic and biochemical analysis of the branching processes. What has been possible in the case of hyperbranched mutants of *Neurospora* and *Podospora* is now possible for the protonema of *Ceratodon*. Even though it is too early to analyze these morphological processes at the level of regulation it is now possible to characterize them at the enzymatic level. This would elucidate the mechanisms controlling the deposition of lateral buds.

Cell walls have a primordial role during protonema morphogenesis and cell walls of Bryales possess glucane polymers. To ensure normal growth, cell wall component synthesis and deposition must be highly coordinated and the enzymes involved in cell wall polysaccharide synthesis must be regulated. Activities of glucane hydrolases and glucane synthetases should be studied to understand the regulation and the possible implication of their morphogenetic roles, in apical growth an protonema branching.

The mutant obtained allows analysis of the processes of caulogenesis. Is the blockage of this morphological stage in the mutant due to a need for certain growth substances, as in Bryophytes (β-indolylacetic acid, cytokinin, unknown growth factor) rather than to a deficient sequential enzymatic regulation as in certain mutants of *Penicillium baarnense*[12]? The preliminary examination does not give an explanation for the lack of budding in our mutant strain.

The only limitation to the genetic analysis of the morphogenesis of Bryales protonema lies in the difficulty of obtaining sexual reproduction in vitro. However the techniques used with protoplasts can overcome this obstacle. It is not clear, at this time, why the somatic hybrids have a different morphology from haploid strains[6-8].

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Synchronization of deposition of daily growth layers in the cuticle of the cockroach *Blaberus fuscus* by gating of moult

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Summary. In *Blaberus fuscus*, combined light/dark-warm/cold cycles induce a rhythm of moulting. Most of the moults take place in the middle of the dark cold phases. Because of a stable phase-relation between moulting time and endocuticle growth rhythm, the latter is synchronized with the environmental cycles, too; lamellated layers are deposited during the light warm phases, which correspond to the time of resting of the cockroaches. Therefore, the cuticle growth is internally synchronized with rhythmic locomotory activity and metabolic rhythms.

In many pterygote insects the endocuticle growth after moulting occurs in a daily pattern: on each day a lamellated and a non-lamellated layer are deposited[1]. In all probability this daily growth rhythm is controlled by an oscillator which is localized in the hypodermis[2-4]. In locusts[4,5] as well as in *Blaberus fuscus*[2,3], the cuticle growth rhythm satisfies 2 properties of circadian rhythms: free-running in constant conditions, and temperature compensation. In locusts, the deposition is light-sensitive[5,6]. In cockroaches, the growth rhythm cannot be influenced by constant light (*Periplaneta*[5], *Blaberus*[3]); it cannot be synchronized by LD cycles (*Blaberus*[3]) or shifted by light pulses of high intensity (*Leucphaea*[7]). Once started, the clock which controls cuticle growth cannot be influenced to change frequency and phase.

This point raises the question of the biological significance of the circadian rhythm of cuticle growth. Circadian rhythms allow organisms to entrain to environmental cycles and, more important, they allow an internal synchronization of rhythms of different physiological processes. Internal synchronization of these rhythms can be achieved by internal coupling meachnisms, or by external 'Zeitgebers'.

If the daily growth pattern cannot be synchronized by external 'Zeitgeber' or by internal synchronization, it must be assumed that the regular sequence of layers is of significance on its own, regardless of the time of day at which the layers are deposited (frequency control only).

We examined whether there is any evidence for a phase control of cuticle growth. There exists a stable phase

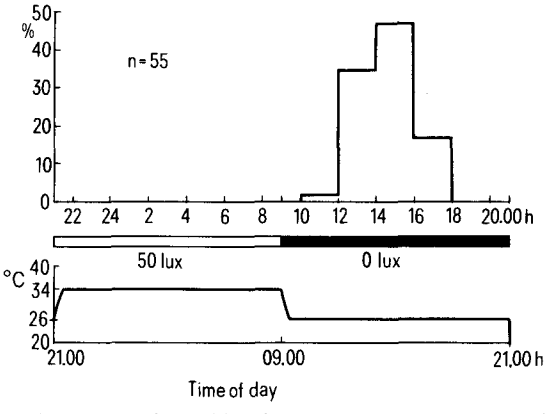

Figure 1. Pattern of moulting in a population synchronized by combined light/dark-warm/cold cycles. The animals prefer the middle of the dark/cold phase.

relationship between moulting time and the time of endocuticle deposition. If the moulting time of a population can be induced to occur at a distinct time of day, synchronization of cuticle growth would result automatically because of the fixed phase relationship.

Method. The animals were taken from a stock reared in the Department of Zoology, Münster (28 °C, LD 16/8 h). Parts of the tibiae of the middle and hind legs were fixed in buffered glutaraldehyde (3%, pH 6.2, 12 h), embedded in Epon (6:4) and cross-sectioned by an ultramicrotome (2–4 µm). The sections were analyzed with a polarizing light microscope. The time span between moulting and amputation was determined by observation of the moulting process or by the darkening patterns which allow the estimation of moulting time up to 4 h later[8]. During the dark phases the animals were observed in dim red light.

Results. In LD cycles (0/50 lux white light) and constant temperature moulting is aperiodic. If temperature cycles (26/34 °C) are applied in addition, the time span during which larvae of the last stage moult, is more and more shortened. After 11 days, moulting takes place preferentially during the middle of the dark cold phases (fig. 1). If a population is transferred into conditions of constant darkness and temperature, its moulting rhythm runs free, with a period somewhat longer than 24 h. It takes more than 20 days in constant conditions until the rhythm disappears, probably because of desynchronization within the population[9]. It can therefore be concluded that moulting is gated by an endogenous oscillator. It is unknown whether the oscillator is synchronized exclusively by the temperature cycles or by a combination with the LD cycles.

2 h after moulting, cuticle growth starts with deposition of

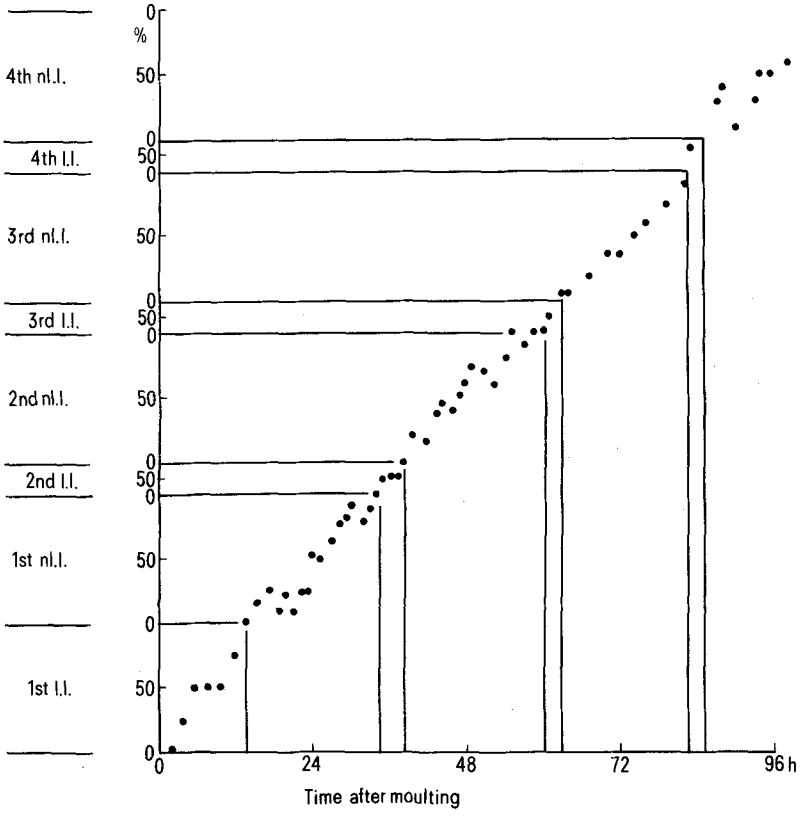

Figure 2. The course of cuticle growth for the 1st 4 days after moulting (in combined LD/temperature cycles: 0/50 lux, 26/34 °C). From the ordinate the number of the complete layers and the already deposited percentage of that layer, which is just growing, can be read off. Subdividing of the ordinate conforms to the relative thickness of the layers. l.l., lamellated layers; nl.l., non-lamellated layers.

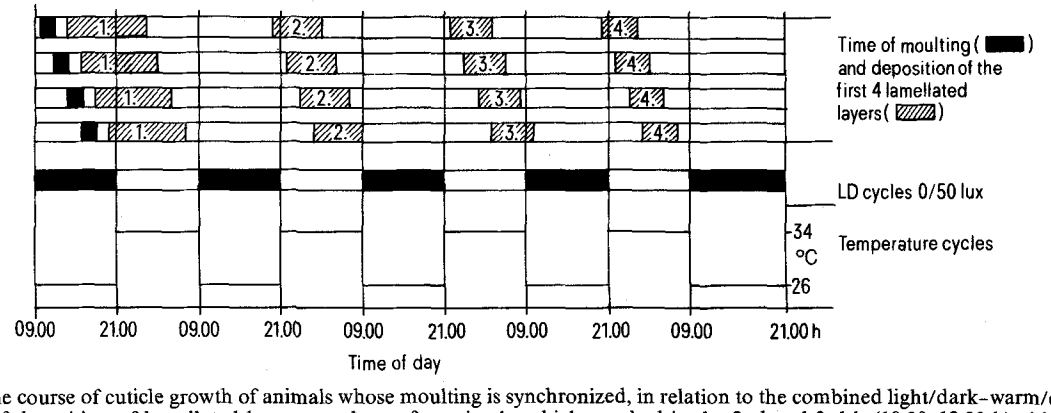

Figure 3. The course of cuticle growth of animals whose moulting is synchronized, in relation to the combined light/dark-warm/cold cycles. The times of deposition of lamellated layers are shown for animals, which moulted in the 2nd and 3rd h (10.00–12.00 h), 4th and 5th h (12.00–14.00 h), 6th and 7th h (14.00–16.00 h), 8th and 9th h (16.00–18.00 h) of the dark cold phases. In synchronized stocks moulting occurs during these times only (compare fig. 1).

the 1st lamellated layer, which is completed 11.5 h later. The growth of the non-lamellated layers takes about 22 h, that of the other lamellated layers almost 2 h. Neither arrests nor phases with enhanced growth could be observed (fig. 2).

Whereas in a synchronized stock moulting occurs during the dark cold phases, the 2nd and the following lamellated layers are deposited during the light warm phases. This fact is a consequence of the prolonged duration of the growth of the 1st lamellated layer (fig. 3).

Discussion. In *Blaberus fuscus,* synchronization of cuticle growth is achieved indirectly by the concentration of moults into a distinct time-span of the day. This gating of moult synchronizes the cuticle rhythm with the environment as well as with internal events and rhythms. Because the free-running period of the growth rhythms differs only very slightly from 24 h[3], a stable phase relationship of the cuticle growth rhythm to the environment is guaranteed, at least during the 1st days after gated moulting.

According to Neville[1] the deposition of lamellated chitin consumes much less energy than the deposition of non-lamellated chitin. In *Blaberus fuscus,* the lamellated layers are deposited during the light warm phases. Since this cockroach is a dark-active species, the less energy-consuming process takes place during the diurnal resting time. The same is true in the light-active locusts: *Schistocerca gregaria* deposits lamellated chitin during the dark phases[5]. Thus, phase controlled cuticle growth could be an internal adaptation to the circadian availability of metabolic and/or anabolic molecules in the hemolymph.

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Effect of trifluoperazine and calcium ions on gregarine gliding

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Summary. The drug trifluoperazine (TFP) inhibits the gliding motility of gregarine protozoans. This suggests that the Ca^{++} binding protein, calmodulin, plays a role in the motility process. However the presence of extracellular Ca^{++} was not required for gliding to occur.

The gliding movement of gregarine protozoans has fascinated biologists for many years, and various theories to account for their movement[2,3] have been proposed. We have recently suggested that the motility process involves mechanochemical force transduction occurring at or near the cell surface[4,5]. It was previously suggested[4] that the gliding process might not involve actin, however in more recent experiments we have clearly shown that the gliding motility is inhibited in a quantitative manner by the presence of cytochalasin B[6]. The importance of Ca^{++} ions in the regulation of many motility events, especially muscular contraction, has been clear for some time[7]. In striated vertebrate muscle troponin C plays the major role in Ca^{++} activation of the actin-myosin system. However, in many calcium activated intracellular systems another calcium binding protein-calmodulin-plays a pivotal role[8,9]. In the context of cell motility particular interest has focussed on the activation of myosin kinase by Ca^{++}-calmodulin.

Although external (extracellular) Ca^{++} is required for some motile processes e.g. heart muscle contraction, locomotion of *Amoeba proteus,* in other cases external Ca^{++} does not appear to be required e.g. locomotion of *Naegleria gruberi* amoebae[10], contraction of vertebrate striated muscle[11]. The intracellular level of Ca^{++} within cells would be expected to lie between 10^{-6} and 10^{-8} M[7], and alteration in this level has been shown to have profound effects, e.g. cilia reversal in ciliates[7]. The anti-psychotic drug trifluoperazine (stelazine) complexes selectively with Ca^{++}-calmodulin, thereby inhibiting Ca^{++}-calmodulin dependent enzyme reaction. It was decided to see if TFP would act as an inhibitor of gregarine locomotion and also study the effect of extracellular Ca^{++} on gregarine gliding.

The gregarines were obtained from the gut of meal worm larvae *(Tenebrio molitor)* and washed with Hedon-Fleig medium. (7.0 g NaCl, 0.3 g KCl, 0.1 g $CaCl_2$, 0.5 g $NaHCO_3$, 0.3 g $MgSO_4$ per l)[4]. Calcium free Hedon-Fleig solution was prepared by ommitting the $CaCl_2$ from the basic recipe given above. Measurements on gregarine motility were then carried out as descibed previously[4]. It was found that no significant difference inlocomotion rates was observed when Ca^{++} free or complete media were used. The speed of movement of gregarines in Ca^{++} free medium was found to lie in the range $2.2 \rightarrow 5.3$ µm/sec (7 moving individuals studied) i.e. about the same value as that found using the complete medium. To preclude the possibility that the water used contained trace amounts of Ca^{++} ion (e.g. at µM levels), experiments were also carried out using Ca^{++} free medium in the presence of the Ca^{++} chelating compound ethylene glycolbis (β-amino ethyl ether) N, N′-tetra-acetate (EGTA) at a final concentration of 10 mM. No alteration in motility was noted. Thus it

Inhibition of gregarine motility with TFP

TFP solution (final concentration)	Motility index* 0 min	20 min	40 min	60 min	80 min
0	100	107	105	100	107
10 µM	100	104	100	68	77
50 µM	100	73	41	23	9
100 µM	100	27	11	11	0

* Motility index $= \frac{\text{No. of motile gregarines at time t}}{\text{No. of motile gregarines at time 0}} \times 100.$