opponent R/G model is more parsimonious, and naturally links both threshold and suprathreshold observations.

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10. When the blue peak rises high it obscures the green peak.
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14. This finding agrees with the results of P. E. King-Smith and K. Kranda [*ibid.* **21**, 565 (1981)].
15. See similar models by M. Ikeda [in (*7*)], S. L. Guth and H. R. Lodge [*ibid.* **63**, 450 (1973)], and C. R. Ingling and B. H. Tsou [*Vision Res.* **17**, 1075 (1977)].
16. Such a sliding would be expected from the model suggested by C. F. Stromeyer and C. E. Sternheim [*Vision Res.* **21**, 397 (1981)].
17. Fitting the model amounted to vertically translating the log sensitivities of the three mechanisms individually to minimize the squared deviation (summed over wavelength) between predicted and observed log sensitivity. For the R/G mechanism the crossover point was varied and then the red and green peaks were independently shifted vertically. Because of the isolation afforded by the low-frequency test, parameter estimates of the R/G mechanism were very robust. In particular, they were independent of assumptions about probability summation between the R/G and L mechanisms at the bottom of the sensitivity trough. We modeled probability summation by a power sum with an exponent of 4.0 [R. F. Quick, *Kybernetik* **16**, 65 (1974); (*6*)]. Below 500 nm, nonvisual pigments in the eye absorb strongly and alter the spectral sensitivity considerably from person to person; again the R/G parameter estimates were unaffected.
18. Supported by NSF grant BNS 79-24163 and NEI grant EY 01583.

9 July 1982

Synchronized Moulting Controlled by Communication in Group-Living Collembola

Abstract. *Group-living Collembola of the genus* Hypogastrura *coordinate their moulting by communication. Animals of different ages and moulting rhythms synchronized the moulting rhythms when combined in a single culture. This synchronization is apparently not dependent on external stimuli but is coordinated by chemical communication among these insects.*

In insects, moulting is commonly synchronized by external stimuli (*1*). Usually a stimulus applies only to one moult, such as when the diapause of a specific instar is terminated by a phenological cue. In group-living species of the collembolan genus *Hypogastrura*, I found that moults are synchronized within aggregates, each of which may contain hundreds of thousands of animals (*2*). Apparently moulting is coordinated by chemical communication and is independent of the animal's age.

Experiments were carried out with *Hypogastrura lapponica* and *H. socialis*, which were kept in chambers (3.5 cm in diameter and 3 cm high). A moist bottom layer of plaster of Paris and sand ensured favorable humidity in the cultures. Small pieces of bark covered with algae (*Pleurococcus*), which were replaced about once a week, served as food and shelter, and the animals usually moulted under them. The cultures were inspected every day, and if any animal had moulted since the previous inspection, the whole 24-hour period was included in the moulting interval of the culture.

The importance of communication between animals was tested in two ways: by splitting a synchronized mother culture and by combining animals from different mother cultures with different moulting rhythms. Each mother culture consisted of animals of the same age. In tests with combined cultures, the animals were chosen so that their size (age) differences were sufficient to allow them to be distinguished after mixing. Cultures to be compared were exposed to the same physical conditions in a climate room.

Figure 1 shows synchronized moulting of an undisturbed culture of *H. lapponica* at 10°C. Three stages of the moulting cycle—premoult, moult, and postmoult—were observed. During the premoult period, the animals are inactive and tightly aggregated, and the distal part of their legs and antennae become white as the old cuticle detaches from the new one. This stage lasts 2 to 3 days in each animal before moulting is completed. During the postmoult period (usually about a week) the animals feed or are active in other ways. Variations in temperature or light did not affect the synchrony of moulting.

A synchronized mother culture of *H. lapponica* was divided immediately after all members had completed their first moult and many, but not all, had started to feed on the bark algae. By further separating the feeding animals from those that had not yet started to feed, two cultures which, on the average, had completed the last moult at slightly different times, were obtained. If the feeding animals had not been separated from those not yet feeding, they would all presumably have continued to moult synchronously. After the separation, the two groups soon went out of phase (Fig. 1B).

New cultures were started by combining animals from several mother cultures. All individuals in the daughter cultures synchronized their moulting shortly after mixing. During the synchronization process, the postmoult period became prolonged in some daughter groups and shortened in others, but the duration of the premoult and moult periods was not altered (Fig. 2).

The moulting cycle appeared to be-

Table 1. Moult synchronization in *Hypogastrura lapponica* and *H. socialis* tested by combining groups that originally moulted at different times. Abbreviation: LD, light-dark cycle (hours).

Groups combined (*N*)	Animals per group (*N*)	Light regime	Temperature (°C)	Moult at synchronization
		Hypogastrura lapponica		
4	5	(LD, 17:7)	10	2
3	3	(LD, 17:7)	10	4–5*
3	7	(LD, 12:12)	10	3
2	7	Dark	15	2
		Hypogastrura socialis		
2	30 + 10	Light	15	2

*Before synchronization, about 50 animals hatched from eggs laid at the beginning of the experiment.

come synchronized by the second or third moult after the start of the daughter cultures, except in one case (Table 1). In that culture, the animals went through four or five moults before they first became synchronized (Fig. 2B). The fact that there were relatively few animals per group in this culture may account for the weaker interaction between the animals. One adult in this group laid about 50 eggs which hatched during the experiment, and all the animals in this group became synchronized as the new juveniles moulted for the first time (Fig. 2B).

Coordination of moulting was obtained even when cultures were kept in the dark, indicating that it was not dependent on visible stimuli. Moreover, the synchronization did not depend on whether the animals in a culture formed one group and gathered on one piece of bark or formed two groups and gathered on two pieces of bark at different sites in the culture chamber. This indicates that it is unlikely that the transfer of stimuli between animals requires direct body contact. The most likely possibility is that synchronization is controlled by chemical communication through pheromones, which have been shown to regulate aggregation and coordinated behavior in these as well as other *Hypogastrura* species (*3, 4*).

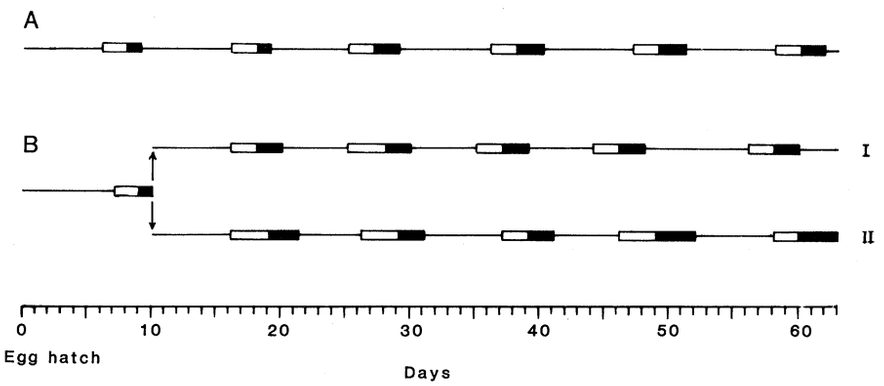

Fig. 1. The moulting cycles of the first six instars of *Hypogastrura lapponica*. Each culture was started from a single batch of eggs and kept at 10°C. (A) An undisturbed culture hatched from 36 eggs. (B) Two cultures formed by dividing a mother culture immediately after first moult. Group I had about 30 individuals that had started feeding before separation, and group II (18 individuals) had not yet started feeding. Open rectangles, premoult period; solid rectangles, period from first until last animal in a culture had completed moulting; horizontal lines, postmoult period.

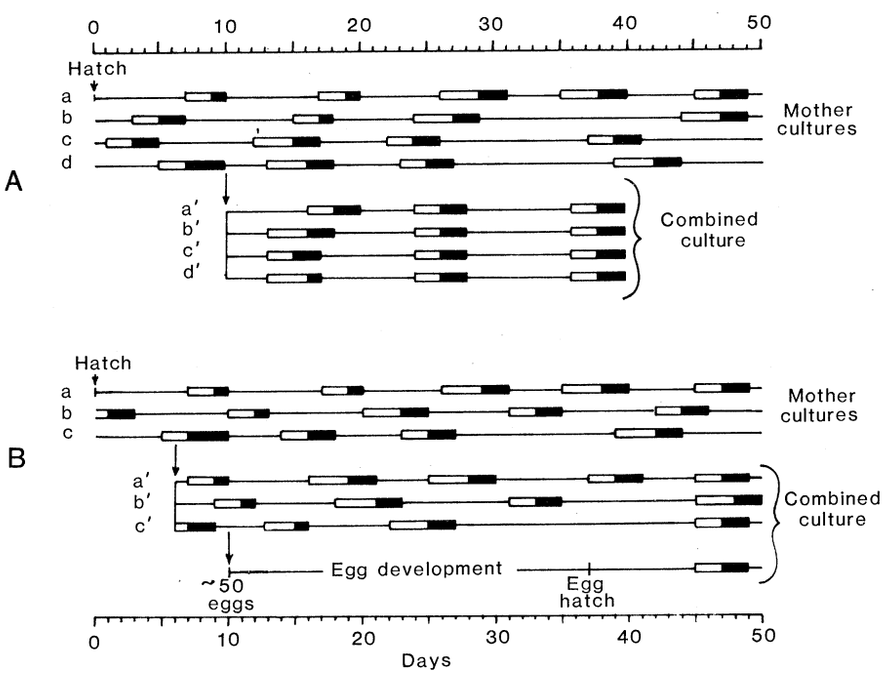

Fig. 2. Cultures formed by combining individuals from different mother cultures (the symbols are described in Fig. 1). (A) Each mother culture originally contained 20 to 50 animals of the same age. At the start of the experiment, the mother cultures were at various stages: *a*, egg hatch; *b*, 2nd instar; *c*, 3rd instar; and *d*, adults. One combined culture, with five animals from each of the mother cultures (denoted by *a'* through *d'*) was formed at the time indicated by an arrow. (B) A similar experiment but only three animals from each mother culture were combined. At the start of the experiment, *a*, egg hatch; *b*, 2nd instar; and *c*, adult. About 50 eggs were laid by one adult in the combined culture, and these hatched before the experiment was completed. The culture became synchronized as the new juveniles moulted for the first time and the original groups moulted for the fourth or fifth time. All cultures in (A) and (B) were kept at 10°C.

Only a few examples are known where chemical communication directly affects development in animals: in termites and Hymenoptera that have caste-forming colonies, pheromones are known to inhibit development into reproductive forms (*5*), and in some Orthoptera and Coleoptera reproductive maturity is synchronized by primer pheromones (*6*). Recently, a pheromone from adult *Dermestes maculatus* (Coleoptera), has been shown to inhibit larval development by delaying each moult (*7*). My findings provide evidence for chemical communication determining moult synchronization of all members of a conspecific insect group.

The adaptive significance of moult synchronization may be related to the group behavior of these animals. Many species of the genus are known to undertake coordinated migrations (*4, 8*), and since the animals are inactive for relatively long periods before moulting, coordinated migration may be possible only if the animals have a synchronized moulting cycle. This suggestion is supported by the winter behavior of the two species investigated. Independent of instar, they change into specific winter morphs with uncoordinated behavior that disperse on the snow surface (*2*). When specimens in the winter form are kept in culture, they do not synchronize their moulting. A long-time exposure to cold during winter is required for the change into the gregarious summer form, in which the animals return to synchronized moulting (*4*).

To some extent, the phenomenon described here may resemble the synchronization of development in parasitoids of different ages that are present in the same host (*9*). In that case, however, the animal development is controlled, at least in part, by hormones from the host. Together with the study of pheromones in *Dermestes* (*7*), my findings indicate that internal regulation of moulting may be an important mechanism in various kinds of group-living arthropods.

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30 June 1982

Myasthenic Globulin Enhances the Loss of Acetylcholine Receptor Clusters

Abstract. *Acetylcholine receptors are present in the sarcolemma of cultured skeletal muscle myotubes either as large clusters or in a diffuse distribution. Both the clustered and diffuse acetylcholine receptors are potentially removable from the membrane. Treatment of myotubes with globulin from patients with myasthenia gravis causes the loss of acetylcholine receptor clusters and the concomitant appearance of acetylcholine receptor microaggregates. The rate of acetylcholine receptor cluster loss is greater than the rate of acetylcholine receptor degradation, indicating that acetylcholine receptors are disrupted from clusters to form microaggregates before being removed from the plasma membrane.*

The distribution of acetylcholine receptors (AChR's) in cultured myotubes is not uniform. Areas of sarcolemma containing clusters of AChR's ("hot spots") and high sensitivity to iontophoretically applied acetylcholine (ACh) have been found both in vitro and in vivo (*1*). Studies of AChR turnover rate monitored with ^{125}I-labeled α-bungarotoxin (α-BTX), a neurotoxin that binds to the AChR essentially irreversibly, have demonstrated that the half-life of AChR's in myotube cultures is 18 to 24 hours (*2*).

To gain a better understanding of the process by which AChR's are removed, we have investigated AChR cluster morphology under conditions of enhanced AChR turnover. Antibodies to AChR from patients with myasthenia gravis (MG) were used to accelerate AChR degradation (*3*). We used a fluorescent conjugate of α-BTX to locate AChR clusters (*4*) in cultures of rat myotubes after incubating the cultures with antibodies from MG patients. Our studies demonstrate that the number of AChR clusters is greatly reduced in cultures incubated with immunoglobulin from MG patients (MG globulin). At the same time microaggregates (*4*) of AChR appear over a wide area of the sarcolemma. Both of these events occur faster than AChR degradation.

Experiments were carried out on cultured rat myotubes (*5*). Cells were plated in Falcon tissue culture dishes containing glass cover slips. Cultures were grown at 37°C under an atmosphere of 5 percent CO_2 and 95 percent air. The receptor distribution was examined by staining the cultures with a tetramethylrhodamine conjugate of α-BTX (α-BTX-TMR) (*6*). This technique is specific for detection of AChR's, allowing us to visualize AChR clusters. Cells first incubated with unlabeled α-BTX and then with α-BTX conjugate show a complete absence of label.

Cells were incubated for 1 hour at 37°C with $5 \times 10^{-8} M$ α-BTX-TMR, rinsed, and incubated with MG globulin or normal globulin for 0 to 6 hours. Other cultures were stained after the addition of MG globulin or normal globulin at 37° or at 4°C to determine (i) whether the distribution of microaggregates is similar to that seen when cells are stained before the addition of immunoglobulin and (ii) whether the redistribution of microaggregates can be seen when cells are stained at 4°C.

Cells were fixed in 2 percent buffered formaldehyde and examined in a Zeiss microscope equipped with incident-light fluorescence optics and with phase-contrast optics. Purified globulin from patients with MG that had high titers for antibody to AChR (demonstrated with radioimmunoassay) was used in all experiments (*7*). The number of myotubes containing intact AChR clusters (*4*) and the number of myotubes containing microaggregates (*4*) were counted in random microscopic fields. The number of intact clusters was defined as the ratio of the number of myotube segments containing intact clusters to the number of

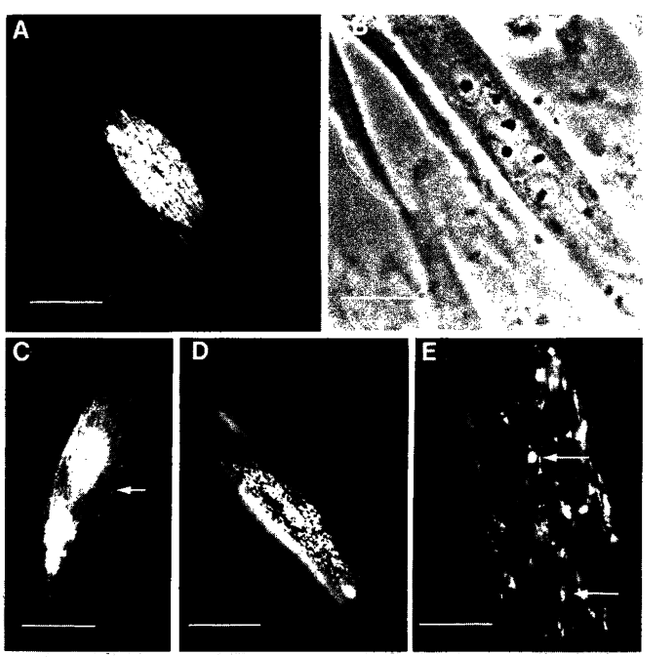

Fig. 1. Rat myotubes were grown on collagen-coated glass cover slips, washed, and stained with fluorescent α-BTX ($5 \times 10^{-8} M$) for 1 hour at 37°C. Cultures were incubated in normal globulin or MG globulin, fixed in 2 percent phosphate-buffered formaldehyde, and observed and photographed with a Zeiss fluorescent microscope. All micrographs were exposed, developed, and enlarged identically (×400). (A) Myotube incubated with normal globulin for 6 hours. An intensely fluorescent intact cluster is present. (B) Phase-contrast image of the myotube in (A). The fluorescent cluster seems to be localized over nuclei. (C) Myotube incubated with MG globulin for 5 minutes and fixed in formaldehyde. A few speckles (arrows) are close to the intact fluorescent cluster. (D) Myotubes exposed to MG globulin for 3 hours show an AChR cluster that is no longer densely packed. (E) Myotube incubated with MG globulin for 6 hours shows complete dissolution of the AChR cluster. Microaggregates 2 to 6 μm in diameter (arrows) are present. Scale bar, 20 μm.