*Korean J. Ecol.* 17(3) : 299~310, 1994

# Ecological Evolution by Competitive Exclusion: An Experimental Approach with Cellular Slime Mold, *Polysphondylium pallidum*

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# 경쟁배타에 의한 생태적 진화: 세포성 점균 *Polysphondylium pallidum*에 대한 실험적 접근

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## ABSTRACT

Intraspecific clonal interactions have important influences on a population structure of the cellular slime mold (CSM). This study was to investigate whether or not evolutionary change in a population could be induced by clonal competition, and to elucidate how various clones in a population evolve in a homogeneous environment of laboratory culture. The characteristic clones of *Polysphondylium pallidum* which had different resource consumption rates (RCR) and mating types I and II were selected for study. Investigation was conducted for 4 experimental time interval ($T_0$-$T_4$); one experimental time interval took almost 10~14 days from inoculation to havest of fruiting bodies. Two sets of 50 clones were cultured from 50 clones at $T_0$, and RCR variations of the population were compared between $T_0$ and $T_4$ for each set of clones.

Each clone of the CSM had a diverse resource consumption rate, or growth rate, in a homogeneous and limited Cerophyl agar plate despite the passage of 48~56 generations from the beginning of the experiment. Diverse clones with different growth rate could coexist in one site of the homogeneous agar plate as well as heterogeneous soil microenvironment.

When there was high clonal diversity of RCR, a clone in a population had high chances to encounter other clones with resultant increased clonal competition. In one set, 26 of 37 clones of mating type I were changed to mating type II for the 4 experimental time intervals, which indicated that the rate of competitive exclusion among clones during total experiment from $T_0$ to $T_4$ was 0.703. In another set, 31 of 37 clones of mating type I were changed to mating type II, having the rate of competitive exclusion 0.838.

The frequency of each of mating types changed by 0.93~1.29% in each successive generation. The competitive exclusion among clones occurred by 1.26~1.75% when approximately $2.6 \times 10^8$ bacterial cells were provided as food and thereafter one generation of myxamoebae of CSM elapsed at room temperature. This finding implicated that in the vegetative state of *P. pallidum* there was 1.26~1.75% probability of evolutionary change per generation changing from one clone to another clone.

**Key words**: Clonal diversity, Competitive exclusion, Evolutionary change, Resource consumption rate

## INTRODUCTION

The cellular slime molds (CSMs), amoebal predators of forest soil bacteria, show both animal-like (protozoan) and plant-like (fungal) characteristics. They represent a normal component of the microflora of most soils. The numbers and types of CSMs present in any particular soil are strongly influenced by a variety of environmental and nutritional factors (Singh 1946, Cavender 1973, Raper 1984).

Primitive phase consists of independent amoeboid cells termed myxamoebae, that feed upon bacteria and reproduce by binary fission until the available food supply is exhausted. When starved, the individual cells stream into central collecting points to form multicellular masses which become migrating pseudoplasmodia. These pseudoplasmodia later differentiate into fruiting structures consisting of both stalk and spore cells. Stalk cells die while spore cells can activate to yield single amoebae (Raper 1984).

In protozoan life cycles, as in the cycles of many other organisms, reproduction takes place without the intervention of meiosis and sexual fusion (Bonner 1947, Samuel 1961, Cotter and Raper 1968). However, there is a sexual cycle (Eisenberg and Francis 1977, Clark *et al.* 1973). Some CSMs show an alternative, sexual mode reproduction - the macrocyst. A zygote forms during development of macrocyst, thick-walled structure, and the meiosis occurs before germination of the macrocyst (Raper 1984).

Asexual reproduction, and local clonal growth in particular, exploits the fact that a successful genotype can be successful repeatedly in both time and space. Clones may persist for prolonged periods and they may disperse into various microhabitats. To the extent that different genotypes co-occur, the dynamics of a population, as coventionally assayed at the species level, is an aggregate of the dynamics of clonal populations and their interactions (Ketcham 1988).

Soil is very heterogeneous at the microhabitat scale in which CSMs function and bacterial prey are not uniformly distributed in the soil. Therefore each of CSMs has to adapt to diverse environments and evolve for their existence. Actually, natural populations of CSMs do contain a diversity of genotypes (Jacobson and Band 1987, Ketcham and Eisenberg 1989, Eisenberg *et al.* 1989).

The CSMs are a group of species that co-occur and utilize the same food resource; Se-

veral CSM species in soil are able to coexist because they have partitioned the total bacterial resources of the soil; interspecific competition shaped the genetic traits of species over evolutionary time with the ecological result that each species is predisposed to using some bacterial forms relatively well and others relatively poorly, i. e., there is resource specialization at the interspecific level (Horn 1971).

Diverse clones coexist as a result of interspecific clonal competition may recombinate during sporulation stage (Bonner 1958). Interspecific interactions have important influences on total community structure. There are several studies about the interspecific clonal interactions (Bonner and Adams 1958, Eisenberg *et al.* 1989, Horn 1971, Jacobson and Band 1987, Ketcham *et al.* 1988).

In laboratory cultures the CSMs are capable of growth on the same species of bacteria. This suggests that competition is an important factor in the CSM ecology. Also interspecific interactions have the evolutionary importance. Not only interspecific diversity but also clonal diversity within a species can be detected in one sample site. Therefore the role of intraspecific variation in the use of resource is an important factor for adaptation and evolution in a given environment and thus it must be carefully evaluated. Genetic diversity in natural population of CSMs can be assessed using a resource consumption rate (RCR) assay under common garden conditions (Ketcham 1988).

A relatively large number of very similar species occurs in the same habitat. The microdistribution of cellular slime mold species could be related to microhabitat differences, either in the physico-chemical environment of the soil or perhaps in the biological environment created by the growth of soil bacteria or the cellular slime molds themselves (Eisenberg 1976). Therefore very heterogeneous soil at the microhabitat scale makes diverse species and clones within a species coexist.

The purposes of this study were two folds: firstly, to investigate whether or not evolutionary change in a population could be induced by intraspecific clonal competition in a soil microbe, the cellular slime mold *Polysphondylium pallidum* which has worldwide distribution, and secondly, to elucidate how various clones in a population evolve in a homogeneous environment of laboratory culture by competition for one kind of the food.

## MATERIALS AND METHODS

### Selection of clones

Ten characteristic clones with different resource consumption rates (Horn 1971, Ketcham 1988) and mating types I and II (Eisenberg and Francis 1977, Francis 1975, 1980) of *P. pallidum* were selected from isolated clones which had been grown at a local woodlot in Delaware, USA. Soil for the isolation of *P. pallidum* clones was obtained by pushing a coring device (1.1 cm internal diameter) vertically into the soil to a depth of approximately 1.0 cm. Each soil core was transported to the laboratory in the test tube (Kuserk *et al.* 1977, Ketcham 1988).

**Bacterial resources**

The Bacteria (*Pseudomonas fluorescens*) were grown on lawns of NTGY (nutrient broth-tryptose-glucose-yeast extract; Raper 1984) plates for 24~36 hours. And then harvested bacteria were grown in NTGY broth cultures for several hours. Lawns were suspended as a slurry using a bent, sterile, Pasteur pipet and 14 ml of sterile phosphate buffer (0.0167 mol/L, pH 6.0); the harvest was collected into a single large test tube, 0.2 ml of *P. fluorescens* suspension in 9.8 ml of buffer, which were quantified by optical density (OD 660) of spectrophotometer and provided the same density (about $2.6 \times 10^8$ cells) of bacterial resources for all of the determinations made within a single experiment.

**Resource consumption rates**

A feeding front of CSM amoebae progressed at a linear rate through a uniform layer of bacteria on an agar surface; virtually all bacterial cells were consumed by the amoebae at the feeding front. Resource consumption rate of each clone could be represented by progressed feeding front. Resource consumption rate of each clone was inherently different which could be explained as genetic difference among clones (Ketcham 1988).

The bacteria were streaked in a cross fashion at the bare area of the center of the Cerophyl plate and then a single sorus of *P. pallidum* isolate was inoculated at the intersection of the bacterial prints. Inoculated plates were incubated at the room temperature (22 + 3°C).

The distance of feeding front movements along each arm of bacterial print were measured. After about 48 hours first marks were made underneath the Petri dish, using a metal scribe. A second set of lines was marked after an additional 48 to 72 hours. The distance between the two marks was measured. The rate of travel of the feeding front was calculated as the distance between the two marks divided by the elapsed time.

**Mating Type Characterization**

Spores of the strain under test were mixed with spores of a reference strain of mating type I or mating type II. Plates were incubated in the dark for 8~10 days at room temperature in a streak of freshly grown *E. coli* B/r on LP agar (Ketcham 1988) before they were inspected for the presence of macrocysts. Also each clone was cultured by itself under the same conditions to test for "selfers", since clones capable of macrocyst formation without a sexual partner are known in *P. pallidum* (Eisenberg and Francis 1977). Clones which do not form macrocysts with either reference strain after two trials is classified as nonmaters (Eisenberg and Francis 1977). There was no selfers nor nonmaters although mating type I and II existed in selected clones.

**Experimental design and analysis**

**1) Clonal mixes**

Selected original 10 clones were allowed to grow through layers of bacteria on the 40 Cerophyl plates - 4 replicates were made for each original clone. Average RCR was

estimated for all 10 clones respectively and plates were incubated undisturbed at room temperature until aggregation and fruiting activity were over. They then were harvested by washing their contents into graduated centrifuge tubes using 1% Triton X-100 in phosphate buffer and then mixed with a vortex mixer for 30 seconds. These spores in suspensions were counted using a hemocytometer. About 100 spores per clone were extracted.

1000 spores of original 10 clonal mixes per ml were diluted to 20 clones in 0.5 ml phosphate buffer which were spread with 0.5 ml bacterial slurry using a sterile, bent Pasteur pipet. All clones appeared were numbered, among which 50 clones were randomly chosen and transfered to 50 Cerophyl plates. Fifty clones of original mixes were saved into glycerol in freezer for final experiment.

For To experiment 1000 spores of 10 kinds of original clones in 0.5 ml phosphate buffer with 0.5 ml of the bacterial slurry were spread evenly on the surface each of 6 Cerephyl (Jones and Francis 1972) plate and then incubated until fructification.

### 2) Design for evolutionary change

Investigation for clonal change in a population with time was conducted for 4 experimental time interval ($T_0$-$T_4$); one experimental time interval took almost 10~14 days from inoculation to harvest of fruiting body.

At the beginning of $T_0$ mixed original clones of 6 plates were harvested and counted. Twenty spores in 0.5 ml phosphate buffer with 0.5 ml of bacterial slurry were spread on the 20 Cerophyl plates, respectively. After germination of spores appeared clones were marked with circles and numbered, 50 clones of which were randomly chosen and inoculated into the 50 Cerophyl plates and then incubated at the room temperature until all bacteria were consumed and the fruiting of the CSM was complete. Fifty clones of $T_0$ were saved into glycerol in freezer for final experiment.

Two experimental sets of 50 clones at $T_1$, $T_2$, $T_3$, $T_4$ were cultured from 50 clones at $T_0$ for the comparison of two populations. 0.5 ml phosphate buffer containing 1,000 spores with 0.5 ml bacterial slurry were spread on each of 6 Cerophyl plates for each set for investigation of intraspecific competition during one time interval. After one time interval two sets of 12 plates were harvested and counted. 20 spores in 0.5 ml phosphate buffer with 0.5 ml of bacterial slurry were spread on the 20 cerophyl plates; total 400 spores in each of two sets were inoculated. All clones appeared were marked. After each experimental time interval; $T_1$, $T_2$, $T_3$, $T_4$, 50 clones of each of two set were chosen randomly, which were allowed to grow on 100 plates and saved into glycerol in freezer as the clones of $T_0$.

### 3) Final comparison

Clonal diversities for RCR were evaluated repeatedly for 50 clones of original mixes, 50 clones of $T_0$, and two populations of 50 clones at $T_4$. RCR tests for all 200 clones were

conducted simultaneously after 4 experimental time interval. Also 200 clones were tested to determine mating type against standard strains with different mating types in a streak of freshly grown *E. coli* on LP agar.

## RESULTS

Selected ten clones of *P. pallidum* used for investigation of evolutionary change showed different resource consumption rates (RCRs) and two types of mating type Ⅰ and type Ⅱ (Fig. 1.).

The RCR could be expressed as growth rate of each clone on bacterial slurry, X (moving distance) cm/day, which was calculated based on measurements of moving distance in millimeter during a lapsed time. The number on a circle was the randomly numbered clone of isolate; numbers were served to distinguish clones. Clone 26 had the fastest growth rate showing mating type I, while clone 24 was the slowest one having mating type I. Four of ten originally selected clones were mating type Ⅱ. By mating type test each clone could be distingushed from others.

In order to investigate whether or not clonal change with time could take place and to observe how clonal change took place if selected original 10 clones were grown in a simple habitat, 10 clones were mixed in one agar plate. Actually three characteristic clones of *P. pallidum* with different RCRs in 1.1 cm diameter and 1 cm depth of one soil sample core were distinguished (Ketcham 1988). So there were much more possibilities that 10 different clones of CSM presented synchronously in heterogenous soil within such a size about 10 cm diameter as Petri dish.

Fig. 2 represented the change of RCR distributions for the clones after 4 time intervals. It was calculated that about 48-56 generations were elapsed, 12-14 generations of each time interval, from the beginning. In $T_4$ two populations, mating types Ⅰ and Ⅱ, were compared with the clones of $T_0$ since two lines of experiment was excuted for comparison of the difference between populations after $T_0$. The RCRs of 50 clones in original mixes were a little higher than those of original 10 clones, while the tendency of growth of the clones was similiar in that the proportion of mating type Ⅰ was 60%, and that mating type Ⅰ had either the fastest or the slowest growth.

At the beginning of the experiment, $T_0$, randomly chosen 50 clones were manifested by the expression of mixed clonal interaction, clones of mating type Ⅰ were a little more

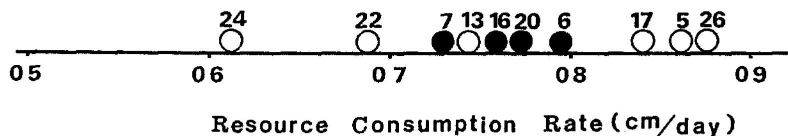


**Fig. 1.** RCR distirbution for the selected 10 clones. Each circle represents a *P. pallidum* isolate; open circle indicates mating type. Ⅰ, closed circle indicates mating type Ⅱ.

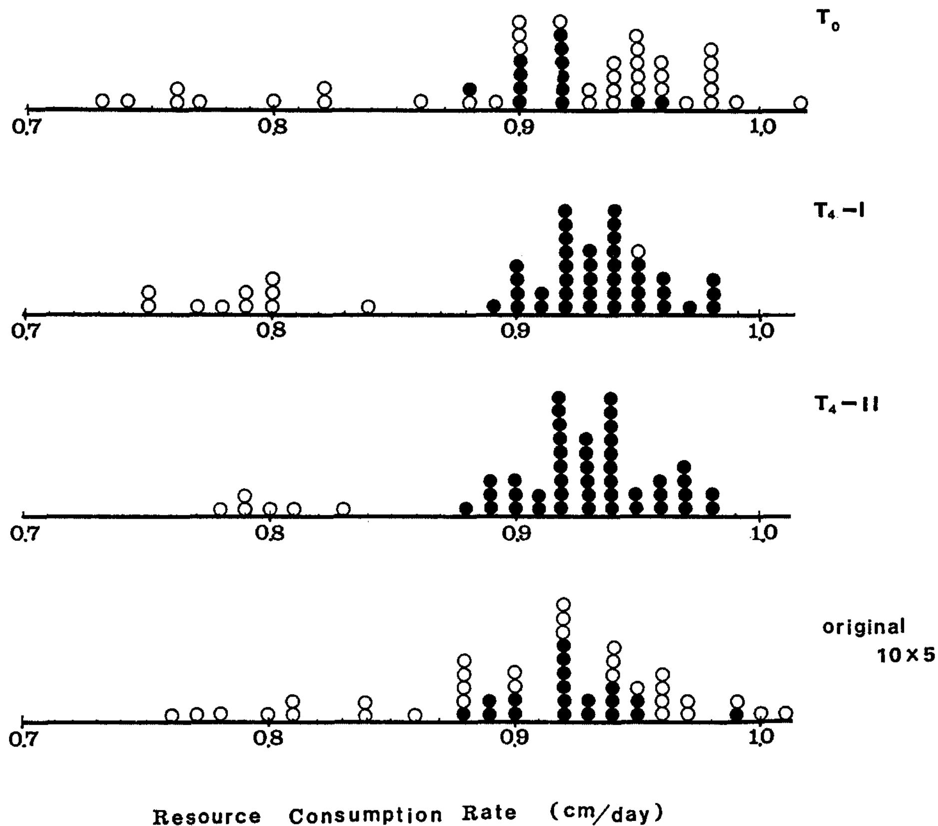


**Fig. 2.** Comparison of randomly selected 50 clones of original 10 clonal mixes, $T_0$, and $T_4$ for each set of clones ($T_4$-Ⅰ, $T_4$-Ⅱ). Each circle represents a selected clone of *P. pallidum;* open circle indicates mating type Ⅰ, closed circle indicates mating type Ⅱ. Each clone shows different resource consumption rates(RCR) and mating types Ⅰ and Ⅱ.

selected than original mixes although the tendency of RCRs distribution was similiar.

Statistical analysis by the ANOVA test revealed that clonal distribution of mating type Ⅰ at $T_0$ was significantly different ($P<0.001$) from that at $T_4$-A and B. Likewise, clonal distribution of mating type Ⅱ at $T_0$ was significantly different ($P<0.05$) from that at $T_4$-A and B (Table 1). These finding indicated that there were some clonal changes with time in mating types. As shown in Fig. 2, clonal hetrogeneities in $T_4$-A and B were different from $T_0$. In Table 1 clonal distribution for RCR in $T_0$ population was not significantly different from that at $T_4$-A ($p = 0.7185$) and at $T_4$-B($p=0.5946$).

Clonal RCR diversity was analyzed regardless of mating types at $T_0$ and $T_4$ using Simpson's Index(Simpson 1949). The results of the analysis were shown in Table 2.

According to Table 2 the population at $T_0$ had marginally higher clonal diversity for RCR than those at $T_4$-A and $T_4$-B, indication that the concentration of dominance was relatively low. Although the population at $T_0$ had higher evenness and lower dominance

**Table 1.** Results of ANOVA test for RCR distribution in the population of $T_0$, $T_4$-A and $T_4$-B. The numeral in parenthesis indicates levels of significance. N.S.: Not significant, *** P<0.001, *<0.05

| | Total | Mating Type I | Mating Type II |
|---|---|---|---|
| $T_0 \times T_4$-A | N.S. (0.7185) | ****(0.001) | *(0.0177) |
| $T_0 \times T_4$-B | N.S.(0.5946) | ***(0.001) | *(0.0342) |

**Table 2.** Simpson's diversity index computed for clonal distribution within the species *P. pallidum*

| | $T_0$ | $T_4$-A | $T_4$-B |
|---|---|---|---|
| Simpson's diversity | 0.93 | 0.92 | 0.91 |
| Inverse of Simpson's dominace | 14.76 | 13.03 | 11.56 |

than those at $T_4$-A and $T_4$-B, the differences were not signifcant by the ANOVA test.

The populations of $T_0$, $T_4$-A, and $T_4$-B had high diversity values over. 0.90. This figure suggested that the probability of ineterclonal encounter in a population (Hurlburt 1971) after 3 experimental time interval was still high. In other words the clonal competition could occur frequently with greater probability of competitive exclusion among clones having short clonal turnover time. Clonal interactions involving energy transfer, competition, and niche apportionment would still be complex in a population of $T_4$. As shown in Fig. 2 many clones of mating type I had changed to mating type II. This finding suggested that some competitive exclusion had happened among clones.

Table 3 represented the frequency of each of the mation type I and type II at $T_0$, $T_4$-A, and $T_4$-B. The net change of frequency per generation(dq/dt) could be expressed as follows(Futuyma 1979):

$$dq/dt = u(1 - q) - vq$$

The frequency of mation type I and type II in a population was represented as p $(=1-q)$ and q. The rate at which mating type I exchanged to mating type II was represented as u, namely, u is the probability of competitive exclusion in each generation. The reverse rate from mating type II to type I was represented as v. There was almost zero and negligible. The absolute increment in q was $u(1-q)$, since the frequency of

**Table 3.** The results of the frequency of each of the mating type I and type II (p and q), the change in frequency in each generation (dq/dt), the rate of competitive exclusion during experiment from $T_0$ to $T_4(\sum u)$, and the probability of competitive exclustion per generation (u) calculated based on Fig. 4

| | Mating type I | Mating type II | dq/dt | $\sum u$ | u |
|---|---|---|---|---|---|
| $T_0$ | p=37/50 | q=13/50 | | | |
| $T_4$-A | p=11/50 | q=39/50 | 0.009-0.0108 | 0.703 | 0.0126-0.0146 |
| $T_4$-B | p= 6/50 | q=44/50 | 0.0111-0.129 | 0.838 | 0.0150-0.0175 |

mating type I was $1-q$, and probability of competitive exclusion to mating type II was u.

At $T_0$, out of 50 random sampled clones, there were 37 clones of mating type I and 13 clones of mating type II. At $T_4$-A, out of 50 random sampled clones, there were 11 clones of mating type I and 39 clones of mating d types II, whereas at $T_4$-B, there were 6 mating type I and 44 mating type II. In one set($T_4$-A), 26 of 37 clones of mating type I were changed to mating type II, the rate of competitive exclusion among clones during total experiment from $T_0$ to $T_4$ was 0.703. In another set ($T_4$-B), 31 of 37 clones of mating type I were changed to mating type II, the rate of competitive exclusion among clones during total experiment were 0.838.

When changed value of q during experiment was divided by the number of generations (48~56) generations were elapsed during total experiment in this study), the frequency of each of mating type changed by 0.0093-0108 in the population of $T_4$-A and 0.0111-0.0129 in the population of $T_4$-B in each successive generation.

The probability of competitive exclusion among clones per generation was 0.0126-0.0146 in $T_4$-A and 0.0150-0.0175 in $T_4$-B. The competitive exclusion among clones occurred by 1.26~1.75% whenver one generation of myxamoebae of CSM elapsed at room temperature. Therefore, there was 1.26~1.75% probability of evolutionary change per generation changing from one clone to another by direct interclonal competition in resource limited system.

## DISCUSSION

The results of this experimnet for clonal evolution in a population was a little different from original expectation that clonal RCR diversity within a species growing in heterogeneous soil would be reduced to clonal RCR uniformity in homogeneous agar plate. Each clone had diverse RCRs in a simple and environment despite the passage of 48~56 generations from the begining of experiment. Diverse clones with different growth rate coexisted whether clonal habitat was heterogeneous or not.

A successful genotype can be successfully repeated in both time and space during local clonal growth. Clones may persist for prolonged periods and they may disperse into various microhabitats. Soil is very hetergeneous at the microhabitat scale and natural populations of CSMs do contain a diversity of genotypes. Therefore the role of intraspecific variation in the use of resource is an important factor for adaptation and evolution in a given environment.

When there was high clonal diversity of RCR and a clone in a population had high chances to encounter other clones, clonal competition in a population was actively happened. The intensity of clonal competition didn't change with time in homogeneous environment as heterogenous soil. Clonal diversity was hardly changed with time. Diverse clones seemed to coexist by partition of the bacterial resources with different RCRs, or

growth rates.

If two species are competing for a common resource which is in short supply, both species would benefit by evolving differences which reduce competition. The benefit involved is a higher average population size for each species and presumably a reduced possibility of extinction. But in many cases it may be impossible to evolve differences. If species A evolves to use smaller food items than species B, it may run into a third species, C, which also feeds on small food sizes. Thus species may be hemmed in by a web of other possible competitors, so that the option of evolving-to-avoid-competition is not feasible. There is only one option available to organisms caught in a competitive net-stay and fight. To fight in the broad sense means to evolve competitive ability (Krebs 1978). Organisms evolve competitive ability by becoming more efficient resource users and by developing interference mechanisms which keep competing species from using scarce resources in natural communites.

Direct interclonal competition of *P. pallidum* made more efficient resource users distributed broadly. Interclonal competition in resource limited environment raised the chance of competitive exclusion among clones and that induced evolutionary change of clonal distribution with time. Intraspecific clonal interactions might have important influences on a population structure.

26 of 37 clones of mating type I were changed to mating type II, the rate of competitive exclusion among clones during total experiment from $T_0$ to $T_4$ were 0.703 in one set, while 31 of 37 clones of mating type I were changed to mating type II in another set, the rate of competitive exclusion among clones during total experiment were 0.838. Therefore clones of mating type II selected at the beginning in this experiment seemed to have more efficient growth types than those of mating type I.

Evolutionary changes occurred rapidly in this CSM experiment implicating that the evolutionary time-scale approached the ecological time-scale. The frequency of each of mation types changed by 0.93~1.29% in each successive generation.

For investigation of ecological evolution the measurement for the change in frequency of a specific character during several generations is meaningful. Such change is rapid and can be easily detected(Langley and Fitch 1974). Intraspecific variation in the use of resource is an important factor for adaptation and evolution in population ecology (Ketcham 1988). So the RCR used in this experimnet is ecologically meaningful measurement by itself since the rate at which bacteria are ingested when an myxamoeba attacks a bacterial colony, and the test of mating type is helpful to distinguish the clones.

The clonal competition in a population actively occured which induced clonal turnover. The competitive exclusion among clones occurred by 1.26~1.75% when about $2.6\times 10^8$ bacterial cells were provided as food, and one generation of myxamoebae of CSM elapsed at room temperature. This finding suggested that in the vegetative state of *P. pallidum* there was 1.26~1.75% probability of evolutionary change per generation changing from one clone to another clone.

This study suggested that ecological evolution by competitive exclusion in a population occurred to the direction that increased competitive ability of a species against other species. In other words, clones in a population seemed to evolve to the direction that increased the competitive ability, because the clone which used the resource inefficiently was exchanged by the other clones which used the resource efficiently.


## 적 요

종 내의 클론 상호작용은 개체군 구조에 중요한 영향을 미친다. 본 연구는 클론간의 경쟁에 의해 개체군 내에서 진화적 변화가 일어날 수 있는 가를 조사하고, 균일한 실험실 배양 조건에서 어떻게 다양한 클론이 진화하는지를 밝히고자 하였다. 자원소비율과 교배형이 다른 *Polysphondylium pallidum*의 클론들이 선택되었다. 실험은 $T_0$에서 $T_4$까지 4번의 시간 간격을 두고 시행되었다. 접종 후 자실체를 수확할 때까지의 한 번의 실험 기간은 10~14일 정도 소요되었다. $T_0$의 50개의 클론으로 부터 일련의 실험기간 $T_1$, $T_2$, $T_3$, $T_4$에서 50 클론이 두 세트씩 만들어졌다. 개체군들의 자원소비율 변동은 $T_0$와 두 세트의 $T_4$ 클론들간에 비교되었다.

각 클론은 실험 시작 후 48~56 세대가 지난 후에도 균일하고 제한된 Cerophyl 배지 환경에서 다양한 자원 소비율 즉, 다양한 생장율을 보였다. 생장율이 다른 다양한 클론은 서식처가 이질적인 토양 미세 환경 뿐만 아니라 한 장소의 균질한 배양 접시 속에서도 공존할 수 있었다.

높은 클론 다양성은 한 개체군내의 클론에게 다른 클론을 만날 기회를 제공해 주었고 개체군 내의 클론 경쟁이 활발히 일어났다. $T_0$에서 $T_4$까지의 총 실험 기간동안 교배형 I 이었던 37개의 클론중에서 한 개체군은 26개가 교배형 II로 바뀌어 클론간에 일어난 경쟁배타율은 0.703였고, 다른 세트의 개체군은 31개가 교배형 II로 바뀌어 클론간에 일어난 경쟁배타율은 0.838이었다.

각 교배형의 출현 빈도는 연속적인 다음 세대마다 0.93~1.29% 만큼 바뀌었다. 2.6 × $10^8$ 밀도의 박테리아를 먹이로 제공해 준 실험실 환경에서 한 세대가 지날 때 마다 클론간 경쟁배타는 1.26~1.75% 만큼 일어났다. 이러한 발견은 *P. pallidum*의 생장기동안 매 세대마다 한 클론에서 다른 클론으로 변화하는 진화적 변화확률이 1.26~1.75%라는 것을 의미한다.


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(*Received* 9 *May*, 1994)