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The Balance of the Storage and Decay of DNA by Producers and Decomposers in the Ecosystem of a *Zoysia japonica* Grassland

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잔디초지 생태계에 있어서 생산자와 소비자에 의한 DNA의 축적과 분해의 평형

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ABSTRACT

An investigation was performed to reveal the relation between the storage and decomposition of the litter DNA of a *Zoysia japonica* grassland on Mt. Kwanak. The loss constant k of litters was 0.167. The times required for the decomposition of half, 95% and 99% of accumulated DNA on the grassland floor were 3.8, 16.6 and 27.6, respectively. The amount of DNA which is turned to living organism in the ecosystem is higher than that of crude protein. In the case of crude protein, the decay constant k was 0.181. The times needed for the decomposition of half, 95% and 99% of accumulated crude protein on the *Z. japonica* grassland floor were 3.8, 16.6 and 27.6 years, respectively.

Key words: *Zoysia japonica*, Mt. Kwanak, Litter DNA, Crude protein, Decomposition, Accumulation.

INTRODUCTION

The ratios of the production and decomposition of DNA in the litters on the grassland and forest floors index to evaluate the informational conditions of the ecosystems. The annual production of DNA in the litters is depended on an ecosystem of grasslands and forests. The genetic characteristics of its ecosystem are expressed by the genetic informations of its producer, consumers and decomposers, and the ecological selection of the environmental factors.

The authors calculated the decay parameter k of DNA and crude protein in the litters on a *Z. japonica* grassland ecosystem on the north-west side of Mt. Kwanak, and used this

k estimating the time stayed with the fragments of DNA and crude protein in the soil horizons of L, F, H and A_1. The direct object of the present study was to reveal the relation of the production and decomposition of the *Z. japonica* litters and to trace the amount of DNA and crude protein.

EXPERIMENTAL METHODS

1. Field method

Litters of *Zoysia japonica* were collected from 0.25 square meters of the ground surface in the *Z. japonica* grassland on Mt.Kwanak at the end of the growing season, from October to November, 1996. Annual production of the litter and the accumulation of humus of the L, F, H and A_1 layers were measured.

2. Laboratory method

1) Free DNA purification

Soil samples of 30g from L, F, H and A_1 layers were dried at 60℃ for 8hours, mixed with DNA extraction buffer [Tris 50mM, EDTA 40mM, pH 7.8, cellulase 0.5g, peptidase 2ml(1 unit)] to give a final volume of 1,000ml, and shaked at 120rpm for 10hours.

After the shaking treatment, glass wool filtration was performed. Then, 40ml of 3M NaOAc(pH 6.5), 20ml of 20% SDS were added to filtrated solution and mixed. After centrifuging at 6,000rpm for 15 minutes, equal volume of TE-saturated phenol (phenol : chloroform = 1 : 1) to sample was added. The mixture was then extracted twice by using TE-saturated phenol at 6,000rpm for 10 minutes. The supernatant were taken and equal volume of ethanol was added.

Mixtures, not vortex mix, were incubated for 30minutes on ice and precipitated with absolute ethanol at 6,000rpm for 15minutes. Each of the pellet of crude nucleic acid was dissolved in 5ml of TE-buffer and collected to 20ml of total volume. This was divided and transferred to four tubes. Each tube contained 5ml of sample. Then, 2ml of 20% PEG was added to sample, mixed, and put on ice for 10 minutes. After incubated, samples were centrifuged at 10kg for 10 minutes. Supernatant was taken and transferred to a new sterile tube.

5M NaCl of 1.5ml was added to each supernatant and incubated at 4℃(ice) more than 1hours. Centrifugation at 10kg for 10minutes precipitated DNA at the bottom of tube, and this pellet was resuspended in 1ml of TE buffer. If not dissolved well, this procedure was performed at 60℃.

2) Total DNA purification

Soil samples from B(leaves), L, F, H and A_1 layers of 1g were dried at 60℃ for 8 hours and mixed with 25ml DNA extraction buffer(Tris 50mM, EDTA 40mM pH 7.8, cellulase 1

unit, pectinase 1 unit) in tube by shaking at 120rpm for 8 hours at 25℃. After the shaking treatment, samples were grinded with liquid nitrogen 20min still all powder, added the skim milk powder solution 2ml(0.1g/25ml H_2O) and shaked vigorously. Centrifuge at 6000rpm for 15min. The supernatant was collected, add 2ml of SDS solution(0.3% SDS in 0.14M NaCl, 50mM NaOAc, pH 5.1) and shaked. After centrifuging at 6,000rpm for 15min, the supernatant mixed with equal volume of equilibrated phenol(pH 7.8).

The aqueous phase was recovered by centrifugation and precipitated with two volume of ethanol at −20℃ for 1 hour. The pellet of crude nucleic acid was obtained by centrifugation at 13,000 rpm for 15min at −4℃, added TE buffer 1ml. It was added 0.5ml of PEG(M.W 8,000), deposited in ice for 10min and centrifuged at 13,000rpm for 15 min. The supernatant was moved to new tube, added 0.1 volume of 5M NaCl solution, deposited in 4℃ for more than 1 hour and centrifuged at 13,000rpm for 15min. Pellet was resuspended with TE buffer(1/10 diluted) 100㎕.

3) Crude protein analysis

Total nitrogen contents was determined by the micro-Kjeldahl method and the amount of crude protein was calculated by multiplying nitrogen by 6.25(Chang and Yoshida, 1973a).

RESULTS and DISCUSSION

1. Decomposition of DNA of the litters

In the decay of the litter, let the amount of DNA contained in the litter per square meter of the ground surface be D, the annual increment of DNA be L, and the varied amount of D per unit time, dt, be dD. If dt and dD approach zero, then according to Olson (1963), and Chang and Yoshida(1973),

$$\frac{dD}{dt} = L - kD \quad \cdots\cdots(1)$$

In the equation (1), k is a decay constant of DNA.

If the annual accumulation of the litter shows no variation and reaches a steady state level, Dss, then the rate of annual change in the equation (1) is zero. Therefore,

$$L = kDss \quad \cdots\cdots(2)$$

In this equation (2), the decay constant k can be estimated by the following equation

$$k = \frac{L}{Dss} \quad \cdots\cdots(3)$$

L is an annual DNA production of fallen leaves, dead twigs, barks, flowers and fruits.

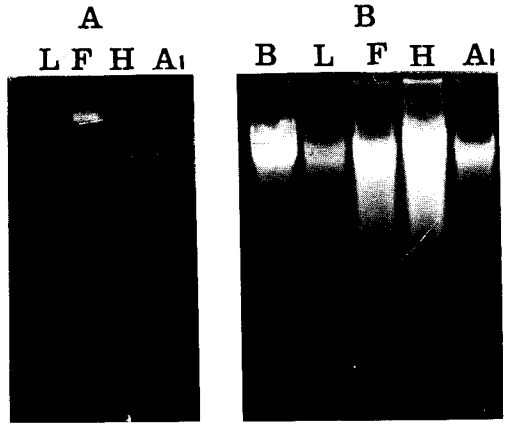

Fig. 1. Agarose gel electrophoresis patterns of free(A) and total(B) DNA extracted from B(leaves) and soils of L, F, H and A_1 horizons in a *Z. japonica* grassland on Mt. Kwanak.

Table 1. The estimates of the production and accumulation of dry weight, total and free DNA for the surface soils under the *Z. japonica* grassland ecosystem on Mt. Kwanak

Horizons	Dry weight soil(g /m²)	Total DNA(μg /g.soil)	Free DNA(ng /g.soil)
L	780.5	58.30	50.0
F	292.5	84.17	327.9
H	553.9	76.67	272.0
A_1	8,344.9	19.17	30.0

Dss is the total amounts of accumulation of DNA of L, F, H and A_1 layers on the mineral soil. If the annual accumulation of the *Z. japonica* litters on Mt. Kwanak reaches a steady state level, the decay constant k can be calculated by the equation (3).

The isolation results of DNA from soils of L, F, H and A_1 horizons has become a useful tool with which to study the ecological functions of certain characterized genes that encode important metabolic pathways(Walia *et al.*, 1990), allow tracking of genetically engineered organisms(Holben *et al.*, 1988 ; Jansson *et al.*, 1989), and reveal bacterial DNA diversity(Torsvik *et al.*, 1990 ; Torsvik *et al.*, 1990) in microbial ecosystem(Tsai and Olson, 1991).

The modified application of extraction methods of free and total DNA to soil samples of L, F, H and A_1 horizons can help researchers to understand the occurrence of natural transformation of free DNA in the humus soils. Fig. 1A and B illustrate the free and total DNA extracted from soil samples of L, F, H and A_1 horizons. Production and accumulation of dry weight of surface soils, total and free DNA are shown in Table 1.

According to the equation(3), the decay constant k of DNA is 0.167 for the *Z. japonica* litters. This decay constant of DNA is smaller than 0.181 for crude protein of the *Z. japonica* litters(Chang, 1996).

If initial and special case in which there is no litter fallen, $L=0$, the DNA accumulated on the top of mineral soil would be gradually decreased by the lapse of time. Therefore, the equation (1) can be rewritten as follows;

$$\frac{dD}{dt} = -kD \quad \cdots\cdots (4)$$

Multiplying by dt/D, the above equation becomes

$$\frac{dD}{D} = -kt \quad \cdots\cdots (5)$$

The decay represented by the equation (5) is a mere fraction of the DNA currently remaining.

Let the initial amount of DNA at $t=0$ be Do, and the amount remaining after a certain period of time t be D, the equation (5) can be rearranged as follow in estimating the decay amount.

$$Ln\left(\frac{D}{Do}\right) = -kt \quad \cdots\cdots (6)$$

Antilogarithms of both sides of the equation (6) give the fraction remaining as a negative exponential function,

$$\frac{D}{Do} = \exp(-kt) \quad \cdots\cdots (7)$$

This curve is shown in Fig. 1.

The time t which is required for the deoxyribonucleic acid D to decrease up to half (50%) of Do can be calculated from the equation (7).

$$D = \frac{Do}{2}$$

$$\frac{\frac{Do}{2}}{Do} = -\exp(-kt)$$

$$\frac{1}{2} = -\exp(-kt)$$

Taking logarithms of both sides, the above equation becomes

$$Ln\frac{1}{2} = -kt$$

$$t = \frac{-Ln\frac{1}{2}}{k} = \frac{Ln2}{k} = \frac{0.693}{k}$$

Let $t_{0.50}$ be the value of t in the above equation, then

$$t_{0.50} = \frac{0.693}{k} \quad \cdots\cdots(8)$$

When the D decreases to 95% and 99% of Do, the values of $t_{0.95}$ and $t_{0.99}$ are as following respectively:

$$t_{0.695} = \frac{3}{k} \quad \cdots\cdots(9)$$

$$t_{0.99} = \frac{5}{k} \quad \cdots\cdots(10)$$

The decay time, $0.693/k = t_{0.50}$, may be viewed as a half-time in accumulation or decomposition of DNA of litter. This half-time has an analogy with radioactive half-time. The time period $3/k$ means the time required for attaining 95% of the final level of accumulation or elimination of litter, while $5/k$ should approximate the time needed to required 99% of the final level. This reciprocal can be viewed as the time schedule for the circuit of DNA in a *Z. japonica* grassland ecosystem.

According to the equations(8) to (10), the time needed to reach half elimination of DNA is 4.1 years while for 95% and 99% elimination, 18.0 and 29.9 years, respectively. The steady-state level, $Dss = L/k$, should vary with the increase or decrease of values of k. Therefore, the time needed to reach the steady-state is different not only among different plant species, but also according to the levels of nutrient, moisture and temperature of the forest and grassland stands.

If litter fall with a steady-state constant level, the equation (1) can be rewritten after dividing all terms by k;

$$\frac{dD}{kdt} = \frac{L}{k} - D$$

This has been integrated;

$$Ln\left(\frac{L}{k} - D\right) = -kt - \text{constant} \quad \cdots\cdots(11)$$

The initial quantity of the litter on the grassland floor at $t = 0$, D is zero, then

$$Ln\frac{L}{k} = -\text{constant} \quad \cdots\cdots(12)$$

Therefore, the antilog of the equation (11) gives the solution a rising curve like that shown in Fig. 2a.

$$D = \frac{L}{k}(1-\exp(-kt))$$
$$= Dss[1-\exp(-kt)] \quad \cdots\cdots(13)$$

Litter falls at the end of the growing season, hence the decay rate kD is determined by the below equation.

$$kD = L[1-\exp(-kt)] \quad \cdots\cdots(14)$$

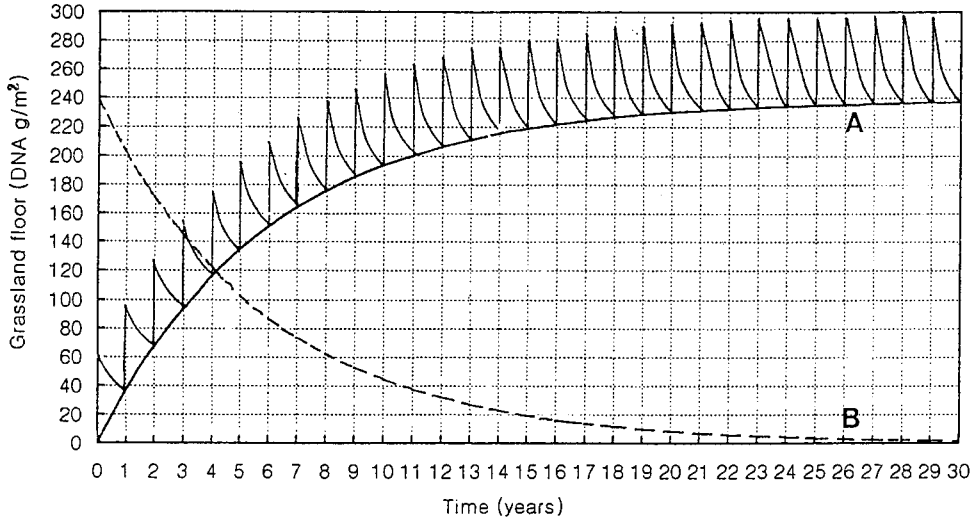

Fig. 2A. Gradually rising exponential curve for the accumulation under conditions of fall and loss of litter DNA in a *Z. japonica* grassland on Mt. Kwanak.

B. Gradually decreasing negative exponential curve for *Z. japonica* litter DNA decay assuming weight loss proportional to the amount remaining at any one time.

The equation (13) is the mirror image of the curve for the decay shown in Fig. 2b, which, as the time lapses, still is rising up to the steady-state with a zigzag path caused

Table 2. Crude protein for the litters under a *Z. japonica* grassland on Mt. Kwanak

Horizon	Soil dry weight (g/m²)	Total N (%)	Crude protein (%)	Yield of crude protein(g/m²)
L	780.6	0.69	4.31	3,364.39
F	292.5	1.53	9.56	2,796.30
H	553.9	0.26	1.63	902.86
A_1	8,344.9	0.22	1.38	11,515.96

by the accumulation and the decomposition of litter DNA.

As shown in Figs. 1 and 2, the amount of free and total DNA in the soils of L, F, H and A_1 horizons are analysed by the modified method of the authors. The times needed to reach 50, 95 and 99% of the steady state of production and elimination of litter DNA were latest of organic matter(Chang *et al.*, 1995a), N(Chang *et al.*, 1995b), P(Chang *et al.*, 1995c) and K(Chang *et al.*, 1995d) and crude protein, respectively. According to Table 2, the decay constant k of crude protein of *Z. japonica* litters on the grassland floor was 0.181, and the time periods for litter decomposition of 50, 95 and 99% were 3.8, 16.6 and 27.6 years, respectively. This result suggests that DNA is more stable, transformable and transductable than crude protein. It is an important fact of molecular ecological changes for the interaction among the species in the natural environments.

적 요

본 연구는 관악산의 한 잔디 초지 생태계에 있어서 낙엽에 의해 생산되는 DNA의 축적과 분해를 수학적으로 model화하여 분해상수를 구하고 반감기, 95%감기 및 99%감기를 계산하였다. 그 결과 관악산의 잔디 초지 생태계 내에서 낙엽의 DNA가 분해할 때의 분해상수는 0.167이었고, 50, 95 및 99% 분해되거나 DNA의 축적이 평형상태에 도달하는데 필요한 시간은 각각 4.1, 18.0 및 29.9년이었다. 조단백질의 경우는 분해상수가 0.181로 DNA보다 컸으며 50, 95 및 99% 분해되거나 축적이 평형상태에 이르기까지 요구되는 시간은 DNA에 비교하여 각각 3.8, 16.6 및 27.6년으로 빨랐다.

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