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Nucleotide Composition and Kinetic Complexity of the Genomic DNA of an Intracellular Symbiont in the Pea Aphid *Acyrthosiphon pisum*

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Summary. An intracellular symbiont was isolated from the mycetocyte of the pea aphid *Acyrthosiphon pisum*, and its genomic DNA was compared with those of *Escherichia coli* and *Mycoplasma capricolum* with respect to nucleotide composition and kinetic complexity. Thermal dissociation, CsCl density equilibrium centrifugation, and high-performance liquid chromatography of the nuclease P1 digest all indicated that the G+C content of the endosymbiont DNA is as low as 30%. In this respect, the endosymbiont resembled *Mycoplasma* species. The reassociation kinetics of genomic DNA labeled by nick translation suggested that the endosymbiont genome is 1.4×10^{10} daltons in size, about 5 and 18 times as large as those of *E. coli* and *M. capricolum*, respectively. The results were confirmed by reassociation of endosymbiont DNA labeled by incubation with [^{3}H]thymidine in Grace's medium. The endosymbiont genome of the aphid was about 500 times larger than those of leafhopper endosymbionts previously analyzed by ultracentrifugation. These characteristic properties of the aphid endosymbiont genome are discussed in connection with the evolution of cell organelles, and with reference to a previous finding that most of the genes of the aphid endosymbiont are not expressed when present intracellularly.

Key words: Endosymbiont — Aphid — Genome size — Nucleotide composition — Cell organelle — *Mycoplasma* — *Escherichia coli*

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Introduction

Structural and functional analogies exist between intracellular symbionts of the aphid and DNA-containing organelles such as mitochondria and chloroplasts (Ishikawa 1984a). Both appear to be surrounded with a host-derived membrane and firmly integrated into the developmental cycle of the host (Hinde 1971). Neither the endosymbionts nor the organelles can successfully replicate themselves extracellularly. Both have their own systems of DNA, RNA, and protein synthesis, distinct from those of the host cell. They are not attacked by the host cell's defense mechanisms. Moreover, both seem to possess properties inherent in the prokaryotic cell. In fact, the intracellular symbionts in the mycetocytes of aphid species are typically prokaryotic in terms of their capabilities for DNA, RNA, and protein synthesis (Ishikawa 1982a). Under in vitro conditions, the endosymbionts are able to synthesize at least several hundred protein species. In contrast with this, the endosymbiont in situ in the host insect seems to be under stringent control, synthesizing only one protein species, symbionin (Ishikawa 1984b).

For all these reasons, the aphid endosymbiont can be regarded as an evolutionary intermediate between the cell organelles, on the one hand, and inter- and intracellular parasites such as mycoplasmas and rickettsiae, on the other. In this respect, it is interesting to compare the basic properties of the aphid endosymbiont DNA with those of DNA from bacteria and true organelles.

Materials and Methods

Materials. A long-established parthenogenetic clone of pea aphids, *Acyrthosiphon pisum* (Harris), was maintained on young broad bean plants, *Vicia faba* (L.), at 15°C under a long-day regime of 18 h light–6 h dark. *Escherichia coli* HB101 was cultured in L broth. Purified DNA of *Mycoplasma capricolum* was supplied by Dr. A. Muto (Nagoya University).

Isolation of Intracellular Symbionts. Mycetocytes and endosymbionts were isolated using published procedures (Ishikawa 1982b). Aphids were lightly crushed in 30 volumes (w/v) of buffer A [0.035 M Tris-HCl (pH 7.6), 0.25 M sucrose, 0.025 M KCl, 0.01 M $MgCl_2$, 1 mM dithiothreitol, 5 mM phenylthiourea] and passed through four layers of gauze. The mycetocytes were collected by centrifugation at 1000 × g at 4°C for 25 min, followed by homogenization in buffer A containing 0.3% (v/v) Nonidet P-40. Aliquots of the homogenate were layered over linear gradients of 10–90% (v/v) Percoll solution and centrifuged at 6000 × g for 30 min in a Hitachi RPS 25-2 rotor. For the formation of Percoll density gradients, the methods of Takabe et al. (1979) for the isolation of chloroplasts from spinach leaves were followed with modifications. Five grams of polyethylene glycol 6000, 1 g bovine serum albumin (fraction V), 1 g Ficoll, and 8.6 g sucrose were dissolved in 100 ml Percoll. The mixture was diluted with buffer A and 10–90% linear gradients of Percoll were obtained. After the centrifugation, the banded fraction of endosymbionts was collected by aspiration into a centrifuge tube, diluted threefold with buffer A, and sedimented by centrifugation at 8000 × g at 4°C for 15 min.

Labeling of Endosymbiont DNA with [^3H]Thymidine. The isolated endosymbionts were suspended in Grace's medium supplemented with [methyl-^{3}H]thymidine (50 Ci/mmol) at 10 μCi/ml, and then incubated at 25°C with shaking for 1 h (Ishikawa 1982a). The labeled DNA was extracted and purified as described below.

Extraction and Purification of DNA. The isolated endosymbionts were washed once in 0.1 M Tris-HCl (pH 8.0), 0.01 M ethylenediaminetetraacetate (EDTA), and resuspended in the same buffer. Lysozyme was added to 100 μg/ml, and the suspension was incubated at 37°C for 5 min. The suspension was made 0.5% with respect to sodium dodecyl sulfate and incubated again at 37°C for 5 min. An equal volume of water-saturated, neutralized phenol was added, and the mixture was stirred slowly on ice for 30 min. The mixture was centrifuged at 3000 × g for 10 min and the upper phase, mixed with 0.1 volume of 10% NaCl and 2 volumes of 100% ethanol, was allowed to stand at −20°C for 1 h. The resulting fibrous DNA was recovered with a glass rod and resuspended in 1× SSC (0.15 M NaCl, 0.015 M sodium citrate) (Smith 1967). The suspension was washed several times with equal volumes of 96% chloroform–4% isoamyl alcohol. The washed suspension was made 50 μg/ml with respect to pancreatic RNase A that had been heated; the suspension was then incubated at 37°C for 30 min. The RNase-treated sample was washed with chloroform–isoamyl alcohol until the ratio of its absorbance at 260 nm (A_{260}) to that at 280 nm became greater than 1.8. The purified DNA was precipitated in ethanol. The *E. coli* DNA was extracted and purified in a similar manner. The *M. capricolum* DNA was treated with RNase A and washed in the same way.

Thermal Melting Experiments. Purified DNA was suspended in 1× SSC at 1–2 A_{260} units at 25°C. Increases in the A_{260} values of the DNA samples on raising the temperature were recorded automatically using a Gilford 250 spectrophotometer equipped with a reference compensator and a thermosensor for continuous temperature monitoring of the cell housing. The rate of heating was 0.5°C/min. The A_{260} values were not corrected for dilution of the DNA due to thermal expansion of the solvent.

Buoyant-Density Analysis of DNA. Three grams of CsCl were poured into 2.3 ml 0.013 M Tris-HCl (pH 8.0) containing nick-translated DNA (see below) from either *E. coli* or the aphid endosymbionts to make up a solution with a specific gravity of 1.710 g cm^{-3} (Ikeda and Tomizawa 1965). The solution was centrifuged at 35,000 rpm at 15°C for 45 h in a Hitachi RPS 50 rotor, and fractionated into 45 fractions by dropping from the bottom of the tube. The specific gravities of these fractions were determined through measurement of their refractive indices. Five microliters of each fraction was adsorbed onto a glass filter and the radioactivity was determined in a toluene-based scintillation fluid in a Beckman liquid scintillator spectrophotometer.

High-Performance Liquid Chromatography (HPLC) Analysis of Nucleotide Composition. Purified DNA was suspended in deionized water and denatured by heating at 100°C for 5 min followed by a rapid cooling. The denatured DNA was incubated with nuclease P1 (Yamasa; 10 μg DNA/μg enzyme) in 0.014 M veronal acetate (pH 5.3) containing 0.1 mM $ZnCl_2$ at 50°C for 1 h (Fujimoto et al. 1974). Separation of the four 5'-nucleoside monophosphates was performed with a Shimadzu LC-5A liquid chromatograph fitted with a 6-mm × 150-mm Shim-pack CLC-ODS column and equipped with a UV detector operating at 260 nm. Elution was perfomed at room temperature with 0.01 M potassium phosphate (pH 7.0) containing 1% CH_3CN at 1.5 ml/min. The sizes of the UV peaks were determined with a computer-aided analyzer, and the relative amounts of the four nucleotides were calculated on the basis of the values obtained for an equimolar mixture of the authentic nucleotides that was resolved and analyzed in the same way. The molar percentage of each nucleotide was calculated from the equation

$$\%N = \frac{N_x/N_s}{C_x/C_s + T_x/T_s + G_x/G_s + A_x/A_s}$$

where N_x is the peak area of dNMP in the nuclease P1 digest of DNA, and N_s is the peak area of dNMP in the standard solution.

Reassociation Kinetics of DNA. Purified DNA was suspended in 0.1× SSC and subjected to sonication for 3 min at an output of 150 W. DNA fragment sizes were determined by flatbed electrophoresis in 1% agarose using 0.1 M Tris-phosphate buffer, pH 8.0, and the fragments of 600–1000 bp were used for further analysis. Nick translation of the DNA fragments was performed based on the method of Rigby et al. (1977) using [^{35}S]dATPαS (Amersham; 410 Ci/mmol) as a substrate. The protocol supplied with the nick-translation kit (Amersham) was followed. The specific activity of the labeled DNA was adjusted to 5 × 10^5 dpm/μg or less for convenience. The DNA in 0.1× SSC was heated at 100°C for 10 min, cooled rapidly on ice, and diluted with an equal volume of 0.02 M Tris-HCl (pH 7.0) containing 2.4 M NaCl (Sullivan et al. 1973). Twenty microliters of the solution, containing 5–10 μg single-stranded DNA, was sealed into a glass capillary and incubated at 65°C (*E. coli*) or 58°C (endosymbionts and *M. capricolum*) for up to 5 days. After the incubation, single-stranded fragments that had not reassociated were digested with nuclease S1 (Takara; 320 units/ml) in 0.05 M sodium acetate (pH 4.6), 4.5 mM $ZnSO_4$, 0.3 M NaCl, containing 20 μg/ml denatured DNA from salmon sperm at 37°C for 30 min (Maniatis et al. 1982). Yeast tRNA was added at 300 μg/ml and the reaction was stopped by adding cold 10% trichloroacetic acid. The precipitate was collected onto a glass-microfiber filter and washed extensively with cold 10% trichloroacetic acid. The filter was dried and the radioactivity determined in a toluene-based scin-

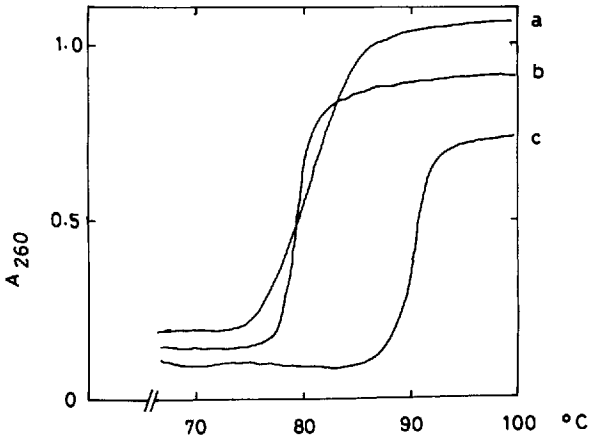

Fig. 1. Thermal denaturation of DNA. Purified DNA was suspended in 1 × SSC. Increases in the absorbances at 260 nm (A_{260}) of the DNA samples on raising the temperature by 0.5°C/min were recorded automatically using a Gilford 250 spectrophotometer. The amounts of aphid endosymbiont (a), *Mycoplasma capricolum* (b), and *Escherichia coli* (c) DNA used were 2.15, 2.04, and 1.55 A_{260} units at 25°C, respectively. The base lines are positioned arbitrarily and do not represent the actual absorbances of the samples before denaturation. The melting temperatures of endosymbiont, *M. capricolum*, and *E. coli* DNA were 82.0°C, 79.5°C, and 90.0°C, respectively.

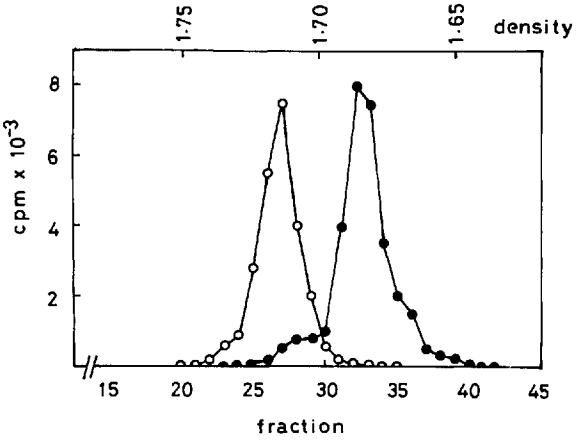

Fig. 2. CsCl density equilibrium centrifugation of DNA. Genomic DNA purified from *E. coli* (○) and the aphid endosymbionts (●) was labeled with [^{35}S]dATPαS by nick translation. DNA solution containing CsCl with a specific gravity of 1.710 g cm^{-3} was centrifuged at 35,000 rpm at 15°C for 45 h in a Hitachi RPS 50 rotor and divided into 45 fractions. The specific gravities and radioactivities of these fractions were determined. For convenience, results obtained in parallel on *E. coli* and endosymbiont DNA were combined.

tillation fluid in a scintillation spectrophotometer. The extent of reassociation was determined by the ratio of the observed radioactivity to that of the undenatured sample.

Results

Thermal Melting Profile of DNA

Measurement of the melting temperature (Tm) is the easiest wasy to estimate the guanine plus cytosine (G+C) content of double-stranded DNA. For this reason, we compared the thermal melting profile of DNA from the aphid endosymbiont in 1 × SSC with the profiles for the DNAs from *E. coli* and *M. capricolum*, whose G+C contents are known (Fig. 1). Under the conditions used, the apparent Tm values of the *E. coli* and *M. capricolum* genomic DNAs were 90.0°C and 79.5°C, respectively. According to the empirical formula %(G+C) = 2.44(Tm − 69.3) (Marmur and Doty 1962), these correspond to 50.5% and 24.9% G+C, respectively. Both values agree well with the known G+C contents for the two bacterial DNAs (Tully and Razin 1977). The apparent Tm of the endosymbiont DNA under the same conditions was 82.0°C, suggesting that its G+C content is around 31%.

Buoyant Density of DNA

Genomic DNA purified from *E. coli* and the pea aphid endosymbionts was labeled with [^{35}S]dATPαS by nick translation as described by Rigby et al. (1977). The two DNA samples were analyzed in parallel by CsCl density equilibrium centrifugation as described in Materials and Methods. For the sake of convenience, results are summarized in Fig. 2. The *E. coli* genomic DNA was determined to have a buoyant density close to 1.710, while that of the endosymbiont DNA appeared to be about 1.690. According to the empirical formula ρ = 1.660 + 0.00098[%(G+C)] (Mandel et al. 1968), the G+C contents of *E. coli* and endosymbiont DNA are about 50% and 30%, respectively. The values coincided well with those obtained based on the Tm values. The peak for the endosymbiont DNA had a minor shoulder at the heavier side, which was reproducibly observed. It is not rigorously excluded that the shoulder represents DNA from the other sources (host, mitochondria, and secondary endosymbionts) contaminating the endosymbiont fraction used. It is also possible that the shoulder is due to satellite DNA in the endosymbiont genome.

HPLC Analysis of Nucleotide Composition

Nuclease P1 from *Penicillium citrinum* specifically hydrolyzes 3′–5′ phosphodiester bonds along single-stranded nucleic acid to produce 5′-nucleotide quantitatively (Fujimoto et al. 1974). Thus, the enzyme may be used in combination with HPLC to analyze the nucleotide composition of heat-denatured DNA.

To confirm the low G+C content of the endosymbiont DNA that was suggested by the two meth-

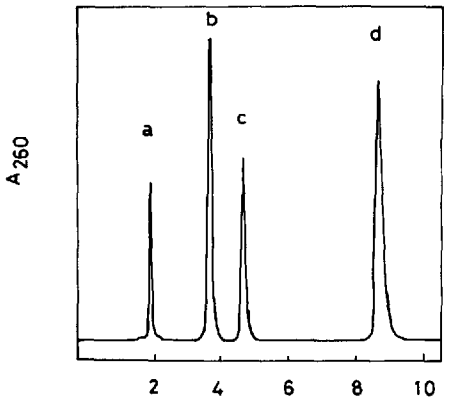

Fig. 3. HPLC analysis of the nucleotide composition of the endosymbiont DNA. Genomic DNA purified from the aphid endosymbionts was suspended in deionized water and denatured by heating at 100°C for 5 min followed by rapid cooling. The denatured DNA was incubated with nuclease P1 (10μg DNA/μg enzyme) in 0.014 M veronal acetate (pH 5.3) containing 0.1 mM $ZnCl_2$ at 50°C for 1 h (Fujimoto et al. 1974). Separation of the four 5'-nucleoside monophosphates was performed with a Shimadzu LC-5A liquid chromatograph fitted with a 6-mm × 150-mm Shim-pack CLC-ODS column and equipped with a UV detector operating at 260 nm. Elution was performed at room temperature with 0.01 M potassium phosphate (pH 7.0) containing 1% CH_3CN at 1.5 ml/min. a, dCMP; b, dTMP; c, dGMP; d, dAMP

ods discussed above, we made a direct determination of the nucleotide composition using nuclease P1 and HPLC (Fig. 3). In the system employed, dCMP, dTMP, dGMP, and dAMP were completely separated and eluted from the column, in that order. The molar percentages of each nucleotide in the nuclease P1 digests of the endosymbiont and *E. coli* DNAs were calculated based on the equation shown in Materials and Methods. The results are shown in Table 1. The G+C content of the endosymbiont DNA was estimated to be 30.3%, while that of *E. coli* DNA was 50.5%, which is compatible with reported values (Normore 1976). Thus, the G+C content of the aphid endosymbiont DNA was shown clearly to be as low as 30%.

Reassociation Kinetics of DNA

Large-sized genomic DNA is liable to be cut into small pieces in the process of extraction and purification. Accordingly, for the present the most reliable method of estimating the size of a genome without reiterated sequences is determining its complexity by means of the reassociation kinetics of the DNA (Britten and Kohne 1968).

DNA from either the aphid endosymbiont, *E. coli*, or *M. capricolum* was fragmented by sonication, labeled by nick translation, and heat denatured, and the reassociation of the single-stranded DNA fragments at 100–500 μg/ml was determined.

Table 1. Nucleotide composition of nuclease P1 digest as determined by HPLC

DNA source	Molar %			
	C	T	G	A
Endosymbiont	15.5	34.9	14.8	34.8
E. coli	25.5	24.8	25.0	24.7

The sizes of the four UV peaks (Fig. 3) were determined with a computer-aided analyzer, and the relative amounts of the four nucleotides were calculated on the basis of the values obtained for an equimolar mixture of the authentic nucleotides that was resolved and analyzed in the same way. The molar percentage of each nucleotide was calculated as

$$\%N = \frac{N_x/N_s}{C_x/C_s + T_x/T_s + G_x/G_s + A_x/A_s}$$

where N_x is the peak area of dNMP in the nuclease P1 digest of DNA, and N_s is the peak area of dNMP in the standard solution

In consideration of their low G+C content, the fragments from the endosymbiont and *M. capricolum* DNA were incubated at 58°C, while those from *E. coli* DNA were incubated at 65°C, 25°C below the Tm value (Britten and Kohne 1968). The relative amounts of double-stranded DNA formed by reassociation during the incubation were estimated by determining the acid-precipitable radioactivity after nuclease S1 treatment. The genome size of *E. coli* has been reported to be 2.8×10^9 daltons (Tully and Razin 1977), and under the conditions employed the fragments of the *E. coli* DNA reassociated with a Cot (mol sec/l)$_{1/2}$ of 2.2. The genome size of *M. capricolum* is 6.8×10^8 daltons (Tully and Razin 1977), and the fragments from the DNA reassociated with a $Cot_{1/2}$ of 0.60 (Fig. 4). Under similar conditions, the DNA fragments from the aphid endosymbiont reassociated with a $Cot_{1/2}$ of 11 (Fig. 4).

What should be kept in mind is that there is an important difference between the bacterial DNAs and the endosymbiont DNA. The former are from cells that replicated many times in a medium free of any foreign cell. The endosymbiont DNA, on the other hand, was extracted from cells isolated directly from other cells, namely the mycetocytes. Thus, it is possible that the endosymbiont DNA was somewhat contaminated by the foreign DNA, which would lead to an overestimation of the Cot value for the endosymbiont DNA. The possibility was tested by using DNA from the endosymbionts that had been incubated with [^{3}H]thymidine extracellularly. It has been shown that, even extracellularly, the endosymbiont can synthesize DNA using thymidine as a substrate. Moreover, the DNA labeled by this method is, without doubt, that of the endosymbiont because the labeling was inhibited very sensitively by nalidixic acid added into the incu-

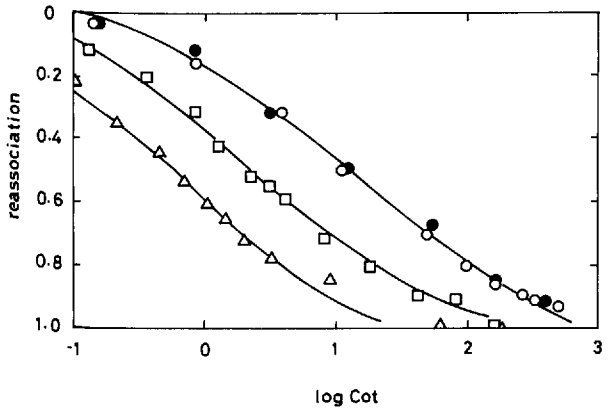

Fig. 4. Reassociation of DNA. Purified DNA was suspended in 0.1 × SSC and subjected to sonication for 3 min at an output of 150 W. DNA fragments of 600–1000 bp were used for further analysis. Nick translation of the DNA fragments was performed based on the method of Rigby et al. (1977) using [^{35}S]dATPαS as a substrate. For labeling of the endosymbiont DNA with [^{3}H]thymidine, the isolated endosymbionts were incubated in Grace's medium at 25°C with shaking for 1 h. The DNA in 0.1 × SSC was heated at 100°C for 10 min, cooled rapidly on ice, and diluted with an equal volume of 0.02 M Tris-HCl (pH 7.0) containing 2.4 M NaCl. Twenty microliters of the solution, containing 5–10 μg single-stranded DNA, was sealed into a glass capillary and incubated at 65°C (*E. coli*) or 58°C (endosymbionts and *M. capricolum*). After the incubation, single-stranded fragments were mixed with carrier DNA and digested with nuclease S1. The trichloroacetic acid precipitate was collected onto a glass-microfiber filter and counted for radioactivity. The extent of reassociation was shown by the ratio of the observed radioactivity to that of the undenatured sample. ○, Endosymbionts; ●, ^{3}H-labeled endosymbionts; □, *E. coli*; △, *M. capricolum*

bation medium (Ishikawa 1984c). The ^{3}H-labeled DNA fragments reassociated at much the same rate as those that were labeled in vitro by nick translation (Fig. 4), suggesting that the endosymbiont DNA used was acceptably pure. The $Cot_{1/2}$ value of the endosymbiont DNA was compared with that of the *M. capricolum* DNA determined at the same temperature, and the genome size of the endosymbiont was estimated to be 1.4×10^{10} daltons, about 5 times that of *E. coli*.

Discussion

This is the first report on the kinetic complexity and nucleotide composition of the genome of an intracellular symbiont in an animal cell. Three different methods all indicated that the G+C content of the aphid endosymbiont DNA is as low as 30%. With the exception of the genomic DNA of *Mycoplasma* species, this value is the lowest one found for the genomic DNA of a prokaryote (Normore 1976). Several species of the Rickettsiaceae contain DNAs with similarly low G+C contents (Normore 1976). It is likely that obligate endosymbiosis and parasitism bring about a lowering of the G+C content of genomic DNA.

To explain this, reference to the characteristic evolution of pseudogenes may be helpful. Because pseudogenes are apparently subject to no functional constraints, their pattern of nucleotide substitution should reflect the pattern of intrinsic mutation (Li 1983). Their pattern suggests that a sequence under no functional constraint will become rich in A and T (Gojobori et al. 1982). Most of the aphid endosymbiont genes encoding proteins resemble the pseudogenes in that they are scarcely expressed intracellularly, and thus may be subject to little functional constraint (Ishikawa 1984b). In fact, it has been suggested that the endosymbiont genes are changing much more rapidly than those of the host insects in two closely interrelated aphid species (Ishikawa and Yamaji 1985).

It was somewhat unexpected that the $Cot_{1/2}$ value of the aphid endosymbiont DNA would be so high. Based on the value obtained, its genome size was estimated to be several times that of *E. coli*. The estimate is based on the assumption that in the endosymbiont genome, nucleotide sequences are no more repetitive than in the *E. coli* and *M. capricolum* genomes. Otherwise, the true size of the genome is larger than that estimated based on the $Cot_{1/2}$ value (Britten and Kohn 1968). Thus, the estimated size of 1.4×10^{10} daltons for the endosymbiont genome should be taken to represent the minimum. This result forms a sharp contrast to those obtained for two endosymbionts of the leafhopper *Euscelis incisus* (Schwemmler et al. 1975). According to Schwemmler et al. (1975), the genome sizes of the t and a endosymbionts are barely 22×10^{6} and 26×10^{6} daltons, respectively. These sizes are even smaller than the average size of chloroplast genomes and are comparable to the size of mitochondrial genomes in lower eukaryotes (Kirk 1976; Schwemmler 1983). The size estimated for the aphid endosymbiont genome is at least 500 times those values. That the genome of the aphid endosymbiont cannot be very small has also been suggested by the number of protein species it potentially encodes. When the endosymbiont is incubated extracellularly, it is capable of synthesis of at least several hundred proteins, which is completely inhibited by rifampicin or chloramphenicol (Ishikawa 1982c, 1984b). Supposing the average molecular weight of the proteins to be 25,000, a simple calculation indicates that the aphid endosymbiont genome cannot be smaller than 5×10^{8} daltons. Since phylogenetically the leafhopper and aphid are closely interrelated, it is surprising that their respective endosymbionts differ so much from each other.

A plausible explanation for this difference is possible if one assumes that molecular evolution of the

endosymbiont consists of three stages: (1) repression of many genes and selective expression of certain genes as a result of intracellular life (Ishikawa 1984b), (2) accumulation of A and T in the repressed genes due to their pseudogene-like character (Gojobori et al. 1982), and (3) reduction in genome size (Schwemmler et al. 1975). Scrutiny of *Rhizobium*–legume symbiosis suggests that stage 1 is attained almost instantaneously at the onset of intracellular life (Verma and Long 1983). In contrast, stage 2 will, naturally, cover a long period of evolution because in this stage many nucleotide substitutions have to accumulate in the genome. It is possible that the aphid endosymbiont is now at the end of stage 2 while that of the leafhopper is already at stage 3.

The linkage of reduction in genome size to accumulation of A and T is actually evident in the evolution of mitochondria. Though the mitochondrial genomes in lower and higher eukaryotes have almost the same number of active genes, the former is up to 7 times the size of the latter in the animal kingdom (Mahler 1983). It should be pointed out that such larger mitochondrial genomes are in general extremely rich in A and T, while smaller mitochondrial genomes in the higher eukaryotes are no richer in the two bases than are the genomic DNAs in the respective nuclei (Kirk 1976).

Judging from the genome size estimated in the present study and from the morphology (Ishikawa et al. 1985), it is likely that the aphid endosymbiont is a specialized descendant of a *Pseudomonas* species (Tully and Razin 1977). However, there is a striking distinction between the two, since the *Pseudomonas* species, as Gram-negative bacteria, are generally characterized by a high G+C content in their genome (Normore 1976). Taking all the evidence together, it is conceivable that a *Pseudomonas* species has been undergoing rapid evolution within the aphid mycetocyte, which might eventually lead to the creation of a cell organelle (Margulis 1981).

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