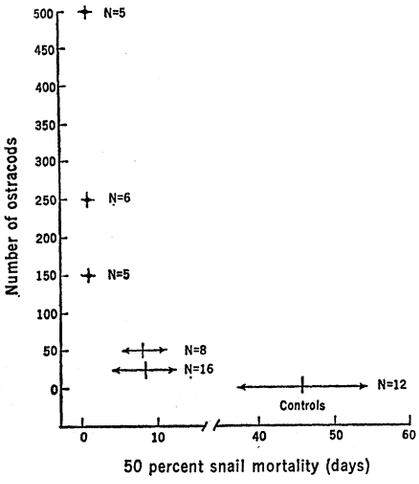

Fig. 1. Number of days for 50 percent of the snails to die; *N* is the number of experiments; the mean is at the midpoint of each line, and the arrow extends 1 standard error in each direction.

cods, 50 percent of the snails died in an average of approximately 9 days. In experiments involving 500 ostracods, 50 percent of the snails died in an average of less than 1 day.

Table 1 is a summary of additional experiments with 10 to 50 snails 1 to 3 days old in dishes 80, 105, and 190 mm in diameter. These data indicate that 50 percent of the snails in the experimental groups died significantly before the same percentage of those in the control groups (shown in Fig. 1) and that snail abundance has relatively little effect on the rate of snail predation by *C. kawatai*. They also suggest that the rate of predation is controlled by the density of ostracods.

Experiments were conducted at temperatures lower and higher than room temperature. *Cypretta kawatai* is an effective predator at temperatures between 15° and 30°C, and 12°C is near the lower range of its temperature tolerance.

The ostracods *Cypridopsis vidua* and *Cypricercus reticulatus* (Zaddach, 1844) are known to eat snail feces (*2*). We added snail feces to the lettuce in three experiments with 250 ostracods and 5 snails. In these experiments, 50 percent of the snails died in 0.6 to 3.9 days. In five additional experiments with 250 ostracods and 5 snails, we added a small amount of feces daily. We had to terminate this set in 5 days. During this observation period, 50 percent of the snails had died in only two of the experimental groups. These data indicate that additional feces decrease the rate of ostracod predation.

Our experiments indicate that *C. kawatai* is a more effective predator than the species used by Lo (*2*). We established that *C. kawatai* is parthenogenetic and lays eggs 14 days after hatching; these eggs hatch in 4 days. Adult individuals were observed to lay as many as 60 eggs during an 8-day period. This species can be raised in quantity for experimentation in nature.

We collected *C. kawatai* in aquariums containing *B. glabrata* both in Baltimore, Maryland, and Washington, D.C. (*5*). The geographic habitat of this species is probably Brazil, the same as that of the red mutant strain of *B. glabrata* (*6*). The ostracods could have been transported with the snails in the water, or as eggs either attached to the snails' shells or in their digestive tracts (*7*).

The growing interest in the biological control of trematode diseases makes information on all enemies of vector snails significant (*8*). Field tests are necessary to determine the efficacy of ostracods as a biological control.

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4. Deliberate *nomen nudum* [see G. G. Simpson, *Science* **161**, 75 (1968)]. We will validate this species in a specialty journal.
5. We thank Dr. K. Kawata, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, for giving us breeding specimens of *B. glabrata* and *C. kawatai*; Dr. P. Chiang and J. Bourgeois, Johns Hopkins University Department of Pathobiology, for allowing us to collect specimens of *C. kawatai* from aquariums in their laboratory; Dr. J. I. Bruce, Schistosomiasis Research Unit, Department of Medical Zoology, Walter Reed Army Institute of Research, Washington, D.C., for specimens of *C. Kawatai* in his aquariums.
6. After this report was completed we received an unpublished progress report, "Notes on laboratory and field observations regarding planorbides' competitors and predators: protozoans, crustaceans and mollusks," presented by R. M. de Andrate on 30 April 1971 at the Sociedade de Biologia de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. He reported that a species of *Cypretta* was seen to attack and kill in laboratory aquariums snail vectors of schistosomiasis mansoni.
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9. The salary of a research assistant was allotted by Dr. I. E. Wallen, Office of Environmental Sciences, Smithsonian Institution, Fund 7213. The capable assistance during July and August 1971 of R. M. Snead is gratefully acknowledged. Publication authorized by the director, U.S. Geological Survey.

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Homology between Nucleic Acids of Blue-Green Algae and Chloroplasts of Euglena gracilis

Abstract. *Ribosomal RNA from several blue-green algae was hybridized to DNA from* Euglena gracilis *chloroplasts by the membrane filter procedure. This hydridization was competitive with chloroplast ribosomal RNA and indicates significant genetic homology between blue-green algae and plastids from* Euglena gracilis.

The measurement of nucleic acid homologies and hence genetic relatedness by the degree of hybridization of single-stranded DNA from two species has been applied to several prokaryotic taxonomic questions (*1*), and the extension of the technique to DNA-RNA hybridization makes use of the highly conserved sequences thought to occur in ribosomal RNA (*2*). We describe here some experiments in which *Euglena gracilis* chloroplast DNA is hybridized with ribosomal RNA from several species of blue-green algae and bacteria. The degrees of similarity between the fine structure and chemical composition of chloroplasts and blue-green algae have been described. For many years the theory initially propounded by Mereschkowsky (*3*) that plant plastids were derived from an endosymbiont comparable to a blue-green algae received little attention and less experimental support. However, increasing information on the morphology and function of nucleic acids from both chloroplasts and blue-green algae has provided evidence both in support of and contrary to their suggested evolutionary origin (*4*). Our data indicates a significant degree of homology between nucleic acids of *E. gracilis* chloroplasts

Table 1. Hybridization between *Euglena gracilis* chloroplast DNA and rRNA from *E. gracilis* and from *Anabaena variabilis*. Values are corrected for nonspecific absorption of RNA onto the filter.

E. gracilis rRNA		*A. variabilis* rRNA	
DNA/RNA	Hybridization of RNA to DNA (%)	DNA/RNA	Hybridization of RNA to DNA (%)
6/1	4.5	15/1	2.85
10/1	6.0	22/1	3.18
15/1	11.5	25/1	4.05
21/1	16.5	30/1	4.65
26/1	20.0	40/1	6.15
32/1	24.0	45/1	7.3
41/1	31.0	52/1	8.55

and blue-green algae and, we suggest, provides the clearest indication thus far of a relation between free-living prokaryotes and the plastids of eukaryotes.

Euglena gracilis (strain Z) was maintained on nutrient agar slopes and grown autotrophically in the light at 30°C on the medium of Eisenstadt and Brawerman (*5*), supplemented with [8-^{3}H]adenine (1 μc/ml) when labeled DNA was required. When isotopically labeled RNA was to be prepared from *E. gracilis*, the phosphate salts were omitted from the growth medium, and $(NH_4)_2SO_4$ (0.8 g/liter) and 0.1*M* tris buffer, *p*H 7.4, were included together with [^{32}P]orthophosphate (2 μc/ml). Aeration with a mixture of air and CO_2 (95 : 5 by volume) assisted in keeping the organism in suspension. Heterotrophically grown *E. gracilis* were used as a source of 80*S* ribosomes and were cultured on the medium of Bach (*6*) modified by reduction of inorganic phosphate concentration by 90 percent and the inclusion of sodium citrate (19 g/liter) and $CaCl_2$ (0.2 g/liter). [^{32}P]-Orthophosphate (5 μc/ml) was included, and the culture shaken at 30°C in the dark. Blue-green algae were maintained and cultured as described (*7*). When these organisms were grown for the preparation of labeled RNA, the mineral salt medium was modified by omission of phosphate salts and the substitution of equimolar tris base and supplemented with [^{32}P]orthophosphate (4 μc/ml). More specific labeling of RNA from blue-green algae was handicapped by the impermeability of these microorganisms to uracil and other RNA precursors (*8*). Photosynthetic bacteria were cultured as described (*9*), cultures containing 2 μc of [^{32}P]-orthophosphate per millimeter had their normal phosphate salts replaced by an equimolar concentration of citric acid, and the *p*H was adjusted to 6.8 with sodium hydroxide. Heterotrophic bacteria were grown on a minimal medium (*10*) which was modified by the omission of phosphate; 0.1*M* tris, *p*H 7.3, and KCl (0.1 g/liter) were added when [^{32}P]orthophosphate was supplied (2 μc/ml). Cultures of *Staphylococcus aureus* were routinely supplemented with 0.1 percent yeast extract. In all cases in which the medium was supplemented with [^{32}P]orthophosphate the final phosphate concentration of the various media was 5 percent of the normal.

Chloroplasts were isolated (*5*), and DNA was prepared by the method of Pigott and Midgley (*11*). Ribosomes were released from chloroplasts that had been lysed by the addition of Triton X-100 to a final concentration of 5 percent, and from the prokaryotic cells that had been disrupted by extrusion at 20,000 pounds per square inch through a French pressure cell and were purified by the method of Rawsen and Stutz (*12*). RNA was prepared from isolated ribosomes by the method of Pigott and Midgley (*11*).

Hybridization was carried out according to the method of Gillespie and Spiegelman (*13*). ^{3}H-Labeled DNA (50 to 100 μg) from chloroplasts was denatured with alkali and bound to cellulose nitrate filters (Sartorius type MF 50, 2.5 mm in diameter) and incubated at 65°C for 16 hours with ^{32}P-labeled ribosomal DNA dissolved in 6× SSC (sixfold strength saline sodium citrate). The filters were removed and placed in a solution of 2× SSC containing ribonuclease (20 μg/ml) [ribonuclease (British Drug Houses) from bovine pancrease was heated at 80°C for 10 minutes to destroy deoxyribonuclease prior to use] and held at room temperature for 1 hour. Both sides of the filter were then washed with 50 ml of 2× SSC, dried in a vacuum for 1 hour at 80°C and placed in scintillation vials; radioactivity was then measured (*14*). This procedure enabled us to estimate the [^{3}H]DNA bound to the filter and the amount of ^{32}P-labeled ribosomal RNA (rRNA) hybridized to it.

The percentage of RNA bound was directly proportional to the ratio of DNA to RNA for (i) chloroplast DNA and chloroplast rRNA and (ii) for blue-green algal DNA and rRNA (Table 1). All determinations of hybridization used in assessing relatedness were carried out at ratios of between 20/1 and 40/1 and were usually within the range 25/1 to 35/1. The data in Table 2 express the corrected hybridization occurring at ratios of DNA to RNA of 100/1, all 15 cases of cross hybridization being thus directly comparable at this common arbitrary ratio. The hybridization at 50/1 between chloroplast DNA and chloroplast rRNA of 40 percent indicates a reasonably efficient process of

Table 2. Hybridization of *Euglena gracilis* chloroplast DNA and rRNA from blue-green algae and bacteria. Figures are averages of the number of determinations (*N*) shown in the final column.

Sources of rRNA	Hybridizaiton at 100/1 (%)	Homology with chloroplast DNA (%)	*N*
Euglena gracilis chloroplast	75	100	7
Euglena gracilis cytoplasm	1.0	1.0	4
Unicellular blue-green algae			
*Gloeocapsa alpicola**	35	47	2
Anacystis nidulans	9.7	13	6
Chlorogloea fritschii	25	32	5
Filamentous blue-green algae			
Nostoc muscorum	25	32	5
Anabaena variabilis	16	21	8
*Mastigocladus laminosus**	9.3	12	4
Anabaena cylindrica	8.7	11	2
Photosynthetic bacteria			
Rhodomicrobium vannielii	4.5	5.9	2
Rhodospirillum rubrum	3.0	3.9	2
Heterotrophic bacteria			
Klebsiella aerogenes	3.8	5	2
Serratia marcescens	2.8	3.7	1
Staphylococcus aureus	2.7	3.6	2
Escherichia coli†	2.6	3.5	2
Pseudomonas sp.	1.1	1.5	2

* Indicates that the specific radioactivity of the rRNA was low, leading to less accuracy in counting.
† This result was impaired by a nonspecific retention of rRNA, as indicated on a blank filter.

hybridization and is taken to indicate 100 percent homology; that is, if we assume that the chloroplast rRNA is coded for by plastid, not nuclear, DNA. The blue-green algae tested provide a graded degree of hybridization from 30 percent to 9 percent. The percentage hybridization obtained with chloroplast DNA and *Gloeocapsa alpicola* rRNA indicated homology of 47 percent. *Chlorogloea fritschii* and *Nostoc muscorum* rRNA possessed identical homology to chloroplast DNA of 32 percent. The rRNA from seven species of bacteria all showed a significantly lower degree of homology to chloroplast DNA than did blue-green algae rRNA. Of the bacteria examined, the RNA of *Rhodomicrobium vannielii* exhibits the highest affinity to chloroplast DNA. This organism is a photosynthetic bacterium whose fine structure is similar to certain blue-green algae (*15*). When a 50-fold excess of choroplast rRNA was included with the blue-green algae rRNA, the hybridization of all species of blue-green algae RNA to chloroplast DNA was reduced to about the background level due to random nonspecific RNA attached to a washed blank filter. This result indicated that the rRNA from blue-green algae was hybridized to specific sites on the chloroplast DNA.

Chloroplast DNA has been shown to hybridize with chloroplast rRNA but not with cytoplasmic rRNA (*16*), and our results confirm this. An examination of the interaction of cytoplasmic and chloroplastic nucleic acids has been presented by Ingle *et al.* (*17*), who have shown that higher plant chloroplast rRNA will bind to both chloroplast and nuclear DNA, whereas cytoplasmic RNA does not bind effectively to chloroplast DNA. The interspecies hybridization of rRNA and DNA in *Myxobacter* (*2*) and *Bacillus* species (*18*) indicate that considerable interspecies homology exists, presumably a reflection of the conserved sequences of the ribosomal cistrons (*19*).

Our data indicate a significant degree of homology between rRNA from species of blue-green algae and chloroplast DNA of *E. gracilis*. The species exhibiting the greatest degree of hybridization, *Gloeocapsa alpicola*, has a polyunsaturated fatty acid distribution most similar to that of chloroplasts from *E. gracilis* (*20*). Examination of other chloroplast DNA for homology with blue-green algal rRNA is desirable, as well as measurements on interplastid hybridization. An implication of our hybridization data is that the cistrons for rRNA synthesis in certain blue-green algae and *Euglena* chloroplasts are similar, and, with respect to this macromolecule at least, a meaningful degree of relatedness may be observed.

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Activities of Sodium and Potassium Ions in Epithelial Cells of Small Intestine

Abstract. *Intracellular molar Na^+ activity (a_{Na}), measured with cation-selective glass microelectrodes, in epithelial cells of isolated bullfrog small intestine immersed at 26°C in a sodium sulfate Ringer solution containing mannitol was 0.0144 ± 0.0031 (average value plus or minus standard deviation). The corresponding K^+ activity (a_K) was 0.0854 ± 0.0060. Combination of these values with previous estimates of intracellular Na^+ and K^+ concentrations under identical conditions indicated that a substantial fraction of the cellular Na^+ is in an osmotically inactive state. When the cells were exposed to a Ringer solution in which 26 millimoles of mannitol per liter were replaced by 3-O-methyl glucose, highly significant decreases in a_K and a_{Na} were observed. The decrease in a_K was proportionately larger than the corresponding decrease in a_{Na}.*

The Na^+ gradient hypothesis (*1*) is an attractive model for coupled transport of Na^+ and organic solutes in small intestine and other tissues (*2*). For the interactions observed between Na^+ transport and that of sugars and neutral amino acids in epithelial cells of small intestine, this hypothesis and its experimental basis may be summarized as follows (*2*). When isolated small intestine is immersed in normal Ringer solutions, net movement of sugars and amino acids from lumen to cell against a concentration gradient depends on the presence of Na^+ in the mucosal medium. Conversely, net mucosal to serosal Na^+ transport across the tissue and its electrical correlates, transmural potential difference (PD) and short circuit current, are dramatically increased when actively transported sugars or amino acids are added to the luminal medium. At constant mucosal Na^+ concentration, these increases are saturable functions of the luminal concentration of sugar or amino acid. According to the Na^+ gradient hypothesis, the energy needed for net accumulation of sugars or amino acids across the brush border of the cells is derived from concomitant movement of Na^+ ions into the cells in the direction of their activity gradient. This gradient is conserved by the operation of a Na^+ pump in the lateral-serosal cell membrane which depends directly on metabolic energy and which can effect net movement of Na^+, against an electrochemical gradient, from the cell interior to the serosal medium. Current models for this process postulate that simultaneous translocation of Na^+ and