Speciation and Symbiotic Dinoflagellates

Abstract. *Morphometric analyses based on three-dimensional reconstruction of the nuclei of four different strains of the symbiotic dinoflagellate* Symbiodinium microadriaticum, *the algae that inhabit corals, giant clams, and other marine invertebrates, revealed marked differences in chromosome numbers and chromosome volumes. The differences are not consistent with different ploidy states within the same species, but can most easily be interpreted as indicating different species.*

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The endosymbiotic dinoflagellates that occur as coccoid cells when inside their invertebrate hosts and produce gymnodiniod motile cells periodically when in culture (*1–3*) have been regarded as representing a single species (*2*). Freudenthal (*1*) originally described the symbiotic dinoflagellates from the Caribbean jellyfish *Cassiopeia* sp. as *Symbiodinium microadriaticum*. Taylor (*2, 4*) subsequently modified the generic epithet to *Gymnodinium*, and Loeblich and Sherley (*5*) further changed the binomial to *Zooxanthella microadriatica*.

The tendency among investigators of symbioses involving these algae has been to regard them as conspecific. However, fundamental differences among the algae isolated from different hosts of different geographical origins have been found: for example, differences in isoenzymes (*6*), sterols (*7, 8*), and the isoelectric forms of the peridinin–chlorophyll-a proteins (*9, 10*), and differences in morphology (*11, 12*), infectivity (*13–15*), and photoadaptive physiology (*3, 9, 16*) have been reported. Taxonomically it is important that these characteristics are stable and are not altered with changing environments (*6, 9*). The observed differences, although strongly suggestive, did not provide conclusive evidence that the different populations represented distinct species, and hence they have been regarded as different strains of the same species (*6*). Resolution of the species problem has been hampered by a lack of genetic evidence. Analysis of the genetics of the species complex called *S. microadriaticum*, and in particular of the chromosome number in four strains of algae, shows that the number of chromosomes is different in each strain, suggesting that the different strains are distinct genetic entities.

Morphometric data on nuclei and chromosomes in the four strains of *S. microadriaticum* analyzed (*17*) are shown in Table 1. In reconstructing the nuclei and counting the chromosomes, we assumed the simplest chromosome morphology. This procedure resulted in the most reproducible numbers within a given strain, as indicated by the small standard deviations. It is clear that the number of chromosomes is distinct in each strain. The chromosome numbers that we observed are well within the range recorded previously among dinoflagellates (*18*).

The algae obtained from *Cassiopeia xamachana* have the highest number of chromosomes, but the lowest total chromosome volume. There is no relation between chromosome numbers and chromosome volumes among the different strains. Whereas the algae from *Montipora verrucosa* had a few rather large chromosomes, those from *C. xamachana* had many small chromosomes. This difference is not readily discerned by examination of single profiles of nuclei (Fig. 1) but becomes evident on three-dimensional reconstruction of nuclei from serial sections. The nuclear volumes observed in the different strains were also different. Such differences may be related to the differences in algal cell size; the algae from *Heteractis lucida* are the largest and had the largest nuclei.

It has been suggested that chromosome numbers may increase when some dinoflagellates are maintained in culture (*19*), but we have not observed this phenomenon. Some of our cell populations have been in culture for 10 years; other cell populations originating from the same host species (*10*) and characterized as the same strain have only recently been isolated. The chromosome numbers were the same for both groups. In addition, in previous biochemical analy-

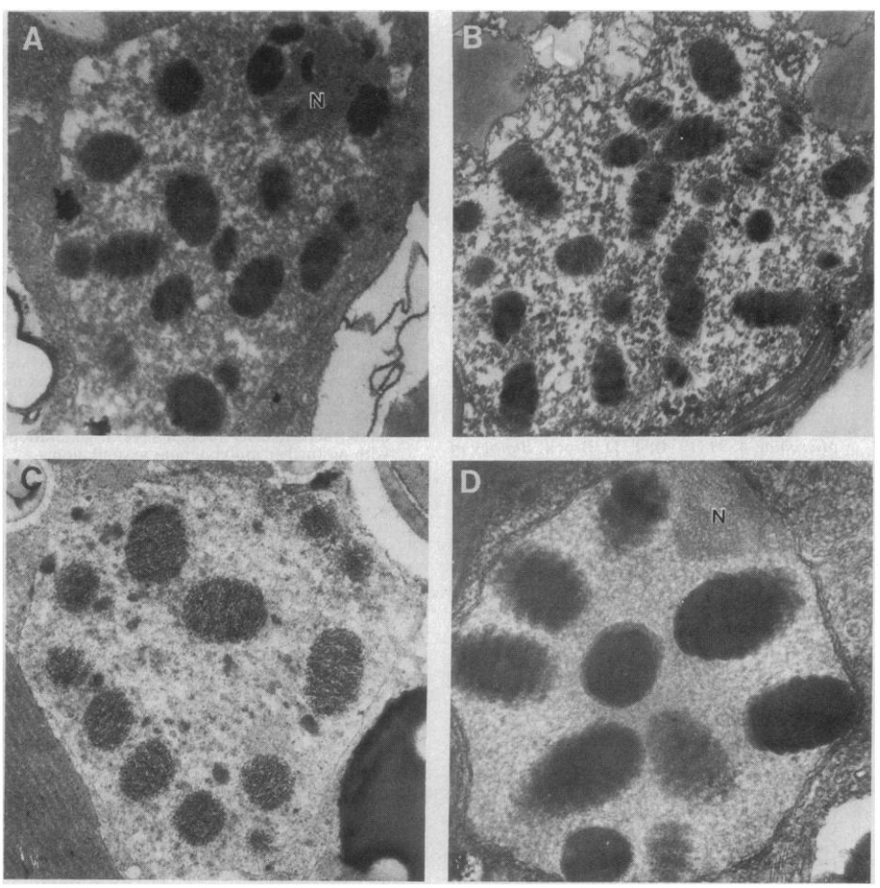

Fig. 1. Transmission electron micrographs of nuclei of the symbiotic alga *S. microadriaticum* derived from four different invertebrate host species. These photographs were selected to illustrate the maximum number of chromosomes that could be seen in any single profile. (A) Nucleus of the cultured type species, *S. microadriaticum*, isolated from *C. xamachana*; magnification, ×35,000. (B) Nucleus of the cultured symbiont isolated from *H. lucida*; magnification, ×23,600. (C) Nucleus of the symbiont freshly isolated from *A. elegantissima*; magnification, ×23,000. (D) Nucleus of the cultured symbiont isolated from *M. verrucosa*; magnification, ×38,000. N indicates the nucleolus.

ses, algae from *C. xamachana* could not readily be distinguished from those of *C. frondosa* (*6, 9, 10*). Our chromosome numbers and volumes were identical for the algae from the two jellyfish species.

The relations between chromosome volumes and nuclear volumes within any given strain appear to be a function of the stage in the cell cycle at which the cells are fixed in preparation for microscopic analysis. The cell cycle of *S. microadriaticum* is rather poorly understood (*3*), mainly because investigators have not been able to achieve the synchronous growth of cell populations. However, some observations can be made about aspects of the cell cycle of these symbiotic algae.

Reconstruction of a recently divided algal cell from *C. xamachana* (that is, cytokinesis completed but daughter cells not separated) indicated that each daughter cell was undergoing karyokinesis (Fig. 2A). Since karyokinesis was essentially complete, each nucleus could be considered as distinct, thereby establishing an M phase. Morphometric analysis of the reconstructed nuclei showed that each nucleus had the same number of chromosomes, but half the chromosome and nuclear volumes when compared to a nondividing nucleus. We did not observe karyokinesis in the other strains, but cells were found in each case that had half the nuclear and chromosome volumes of the nondividing (G_2) cells. In the algae from *Anthopleura elegantissima*, we also found instances where a typical nuclear volume was associated with a halved chromosome volume. Figure 2B shows the appearance of the chromosomes in algae from *A. elegantissima* at a stage interpreted as the S phase by Spector *et al.* (*20*) in *Peridinium cinctum f. ovoplanum*. Within the nuclei of algae from *A. elegantissima*, we often observed spheres (Fig. 2C) to which chromosomes were attached. These spheres were digestible with ribonuclease, suggesting that they may represent nucleolar organizing centers. In these instances, the rather large peripheral nucleolus with associated chromosomes was still evident.

On the basis of these observations, we propose that karyokinesis (Fig. 2A), during which chromosome and nuclear volumes are halved (M phase), is followed by a G_1 phase, when the nuclear volume returns to normal. The following S phase (Fig. 2B) increases the chromosome volume to that characteristic of the G_2 phase. The duration of the different phases of the cell cycle are not yet known.

The data on chromosome numbers might be interpreted as representing different ploidy states within the same species. This interpretation implies that different ploidy states of the same algal species specifically inhabit different hosts (*14*). Different ploidy states would be inconsistent with the data on chromosome volumes. A simpler explanation is that

Table 1. Morphometric data on chromosomes and nuclei in *S. microadriaticum*. Values are mean ± standard deviation; *n* is the number of cells assayed.

Cell-cycle phase	Chromosome number	Chromosome volume (μm^3)	Nuclear volume (μm^3)	Chromosome volume: nuclear volume
		Cassiopeia xamachana and *C. frondosa*		
G_2	97 ± 2 (n = 6)	1.6 ± 0.1 (n = 4)	11.3 ± 2.0 (n = 4)	0.14
M		0.8 ± 0 (n = 4)	5.5 ± 0 (n = 4)	0.15
		Heteractis lucida		
G_2	74 ± 2 (n = 3)	7.8 (n = 1)	27.2 (n = 1)	0.29
M		4.6 ± 0.1 (n = 2)	15.4 ± 1.6 (n = 2)	0.30
		Anthopleura elegantissima		
G_2	50 ± 1 (n = 4)	3.7 ± 0.3 (n = 4)	13.9 ± 1.3 (n = 5)	0.27
M		1.6 ± 0 (n = 2)	5.9 (n = 1)	0.27
		Montipora verrucosa		
G_2	26 (n = 3)	3.2 (n = 1)	7.6 (n = 1)	0.42
M		1.7 ± 0.1 (n = 5)	3.9 ± 0.4 (n = 5)	0.44

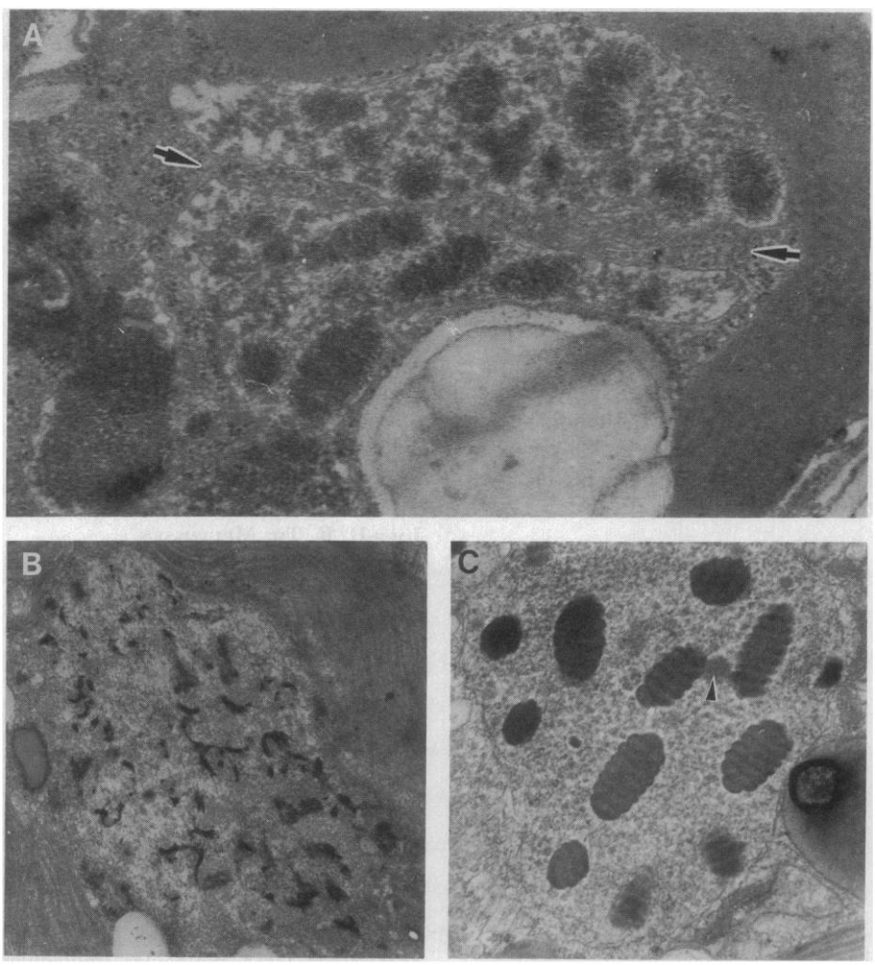

Fig. 2. Transmission electron micrographs of nuclei of *S. microadriaticum* in different stages of the cell cycle. (A) A nucleus in *S. microadriaticum*, from *C. xamachana*, undergoing karyokinesis (M phase). Arrows indicate the division furrow. No microtubules were observed in association with the furrow; magnification, ×49,000. (B) A nucleus of the alga isolated from *A. elegantissima* interpreted as the S phase; magnification, ×21,400. (C) A nucleus, of the alga isolated from *A. elegantissima* illustrating the sphere (arrow) associated with the chromosomes; magnification, ×26,000. Serial sections showed several spheres within a single nucleus.

the algae from *C. xamachana* and *C. frondosa* are *S. microadriaticum*, but that the others that we studied are distinct species.

As has been discussed (*6, 21–23*), there are several considerations that make the concept of a single species *S. microadriaticum* unreasonable. Although superficially these symbionts appear similar when freshly isolated from their hosts, they show stable and consistent differences in chemistry, physiology, behavior, and details of morphology when maintained in culture under uniform conditions. In addition, different hosts accept certain strains of *S. microadriaticum* as symbionts and repeatedly reject others. Altogether, the available evidence supports the concept that the binomial *S. microadriaticum* encompasses a large species complex. It appears that the systematic problems involving these algae are not unlike those pertaining to *Tetrahymena* (*24*), *Paramecium* (*25*), or *Crypthecodinium* (*26*), but unlike these organisms data on sexual recombination in *S. microadriaticum* are lacking. It is therefore not yet possible to test directly for speciation in these symbiotic algae in the context of the biological species concept, but the differences in chromosome organization of the various strains described here suggest a strong likelihood of severe cytogenetic problems if these strains could exchange genetic material in nature. We therefore suggest in the context of the evolutionary species concept (*27*), that the different strains are different species. Furthermore, we suggest that entirely different taxa of dinoflagellates, superficially resembling *S. microadriaticum*, may be involved in symbioses with marine invertebrates.

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17. The symbiotic algae used in this study were derived from *Cassiopeia xamachana* (and *C. frondosa*), the Jamaican sea anemone *Heteractis lucida*, the Californian sea anemone *Anthopleura elegantissima*, and the Hawaiian stony coral *Montipora verrucosa*. With the exception of the algae from *A. elegantissima*, all the cells analyzed were maintained under uniform conditions of culture (*6*). Cultured cells were harvested by centrifugation; algal cells from *A. elegantissima* were fixed immediately after isolation from the host. Fixation methods used included OsO_4 (20 mg/ml) in sea water or 50 m*M* cacodylate buffer (*p*H 8.1); 3 percent (by volume) glutaraldehyde in cacodylate buffer and then fixation in OsO_4; 3 percent (by volume) acrolein in 200 m*M* phosphate buffer (*p*H 8.1) and then fixation in OsO_4; or acrolein-glutaraldehyde fixation followed by OsO_4 fixation. Cells were stained en bloc with uranyl acetate (saturated in 70 percent ethanol by volume) and infiltrated and embedded in Spurr's resin [A. R. Spurr, *J. Ultrastruct. Res.* **26**, 31 (1969)]. Serial sections approximately 65 nm thick were prepared on an LKB Ultratome V and were viewed and photographed with a Philips-300 electron microscope. Morphometric analyses were conducted with a Zeiss MOP-3 using photographs printed at magnifications between ×20,000 and ×40,000 [R. Blank, E. Hauptmann, C. G. Arnold, *Planta* **150**, 236 (1980)].
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Chromosome-Sized DNA Molecules of *Plasmodium falciparum*

Abstract. *At least seven chromosome-sized DNA molecules (750 to 2000 kilobases in length and one fraction of undetermined molecular weight) from cultured clones and isolates of* Plasmodium falciparum *have been separated by pulsed-field gradient gel electrophoresis. Whereas asexual blood stages and sexual stages of the same line have identical molecular karyotypes, the length of chromosome-sized DNA molecules among different geographical isolates and several clones derived from a single patient is different. These length alterations of chromosomes are the result of DNA rearrangements that must occur unrelated to sexual differentiation.*

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The protozoan *Plasmodium falciparum*, the causative agent of one form of human malaria, is transmitted by an insect vector (*Anopheles* sp.) and undergoes extensive differentiation in its life cycle (*1*). The comparison of chromosome-sized DNA molecules (molecular karyotypes) of *P. falciparum* from different stages of its life cycle and from isolates from different geographical areas may give insight into the flexibility of the genome and the contribution of chromosomal recombination events to differentiation and the development of drug resistance.

Pulsed-field gradient (PFG) gel electrophoresis separates chromosome-sized DNA molecules up to at least 2000 kb in length (Figs. 1 to 3) (*2–6*). Degradation of DNA has been shown not to occur during the process, indicating that the separated molecules represent the DNA of the full-length chromosomes; they are therefore referred to as chromosome-sized DNA molecules (*2–6*). Molecules larger than 2000 kb are difficult to recover quantitatively. PFG electrophoresis relies on the variable capacity of DNA molecules of different lengths to reorient in electrical fields that are perpendicular to each other. Separation is dependent on the frequency of field switching, which critically affects the different molecular weight classes (*2–6*). Since the two electrical fields are applied alternatingly in the north-south and west-east direction, the molecules migrate at the diagonal of the electrical fields.

Gentle lysis of a mixture of asexual stages (ring forms, trophozoites, and schizonts) of *P. falciparum*, followed by separation of the chromosome-sized DNA molecules by PFG electrophoresis (*2–6*), allowed the visualization of at least seven chromosome-sized DNA molecules [Fig. 1a, samples 1, 2, and 3, six