Viewer's Notes

"The Kingdom Protista:
The Dazzling World of Living Cells"

Released Feb. 2006

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Scope of these notes: Since there are many excellent texts on the algae and protozoa and much information on the web, here I will simply give some background, anecdotal information that might prove interesting and useful in teaching. The Journal *Protist* has a section entitled "*Protist Web Alert*" which is a valuable resource (Simpson, 1999) and "*Protists on the Web*" can be accessed through Larry Simpson's home page:
http://dna.kdna.ucla.edu/simpsonlab/
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Phylogeny of Protists: Organization of the Chapters in the DVD. The phylogeny of the protists and their relationship to the other four Kingdoms are lively and often contentious topics (e.g., Baldauf et al. 2000, 2003). This DVD is not a platform from which to enter these discussions. To emphasise a neutral position, I use informal names for the protistal groups. A few groups (e.g., the forams) are not represented, a situation to be remedied as material becomes available. Numbers in square brackets, thus [10], refer to chapters in the DVD.

Identifications. While most species' identification has been checked by experts (to whom I am very grateful), I would appreciate being informed of mistakes.

Time-lapse data. Time-lapse recording can give misleading impressions of cellular activity. The data given on the sequences is approximate and serves to remind viewers of this artefact.

Satellite Images of Phytoplankton Blooms. Two related sites that offer superb downloadable images are: http://modis.gsfc.nasa.gov/gallery/index.php and
http://rapidfire.sci.gsfc.nasa.gov/gallery/

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Endosymbiosis

The phenomenon of endosymbiosis is central to understanding protistal evolution. While some endosymbioses are unimaginably ancient, this type of evolutionary experimentation by cells, particularly dinoflagellates, is still actively going on, and has presumably been doing so continuously since the origin of eucaryotes. Keeling (2004) reviews the evolutionary history of symbiotic plastids and their hosts.

The green algae [29-32], red algae [28] and the glaucophytes [27] arose from ancestral cells that took up and entered into endosymbiosis with cyanobacteria. Electron

microscopy shows that these chloroplasts are generally enclosed by two membranes. This simple one-step process is called ***primary endosymbiosis***. Over immense periods of time, much of the symbionts' genetic complement has become transferred to the nucleus and incorporated into the host DNA. Detailed analysis of the modified host and "chloroplast" genome has provided extraordinary insights into the identities involved in endosymbiosis and its subsequent evolution.

Later, ancestral green algae (including their endosymbiont, now a chloroplast), were taken up by other hosts in two separate, secondary symbiotic events that gave rise to the euglenids [25] and the chlorarachinopyhtes [9]. This process is called ***secondary endosymbiosis*** (McFadden, 2001). The parallel uptake of red algae into other secondary endosymbioses led to the evolution of all the golden-brown pigmented phytoplankton [10-18, 20-22]. These chloroplasts are enclosed by 3 or 4 membranes. There is much debate whether this endosymbiosis occurred once or up to seven different times. An unexpected discovery is that apicomplexans [23] arose from a parasitic alga of either red or green algal ancestry; most of the photosynthetic abilities of the ancient symbiont have been lost, but the presence of a few distinctive genes unequivocally demonstrates this ancestry (McFadden & Wallr, 1997). The host cell-type appears to have been close to dinoflagellates .

Electron microscopy shows that in the cryptophytes [1, 10] and chlorarachniophytes [9], the symbiont is still a separate entity identifiable as a reduced cell contained within its own membrane inside the host. Its nucleus is represented by the **nucleomorph** which, in both groups, contains three highly reduced chromosomes. These are the smallest chromosomes of all eukaryotes and the most compact in terms of gene content (Douglas et al., 2001; Gilson & McFadden, 2001). Since there are no microtubules or spindle apparatus, one wonders what mechanism distributes the chromosomes during cell and chloroplast division.

The dinoflagellates are the most adept exponents of symbiosis. *Lepidodinium viride* [22] seems to have taken up a green alga comparatively recently (Watanabe et al., 1987). *Peridinium balticum* and *P. foliaceum* [22] contain endosymbionts derived from diatoms (Inagaki et al., 2000) whose entire cytoplast (including its nucleus but minus the cell wall) is distinguishable within the host, also with its dinoflagellate nucleus. During cell division, the dinoflagellate nucleus divides as expected (see below), but the "diatom " nucleus, instead of forming the distinctive spindle of diatoms [17] (Pickett-Heaps, 1991), merely pinches in two like the macronucleus of ciliates (Tippit & Pickett-Heaps, 1976). A species of *Dinophysis* appears to have a cryptomonad as its endosymbiont, while in *Karlodinium nucrum* and *Karenia* sp.*,* the endosymbiont appears to be a haptophyte (Yoon et al., 2002). When the new symbiont is itself the result of a secondary endosymbiosis (e.g., if it is a haptophyte, diatom or cyptomonad), the new combination displays ***tertiary endosymbiosis***. *Gymnodinium acidotum* may also have taken up a cryptomonad, but such organisms ideally require culturing to confirm that they have achieved a prolonged, stable symbiotic relationship.

Molecular detective work indicates further remarkable complexities. For example, there is a report that an extant dinoflagellate has two differently pigmented "chloroplasts". Another dinoflagellate, *Karlodinium micrum*, currently has a chloroplast derived from a haptophyte. Its nuclear genome, however, also has some plastid-derived genes that could not have a haptophyte origin. Apparently, the cell was host to an earlier symbiont which provided these genes and which was subsequently replaced. In addition, these primordial genes have been incorporated into the functioning repertoire of the current symbiont (Patron et al., 2006), demonstrating the complexity of multiple cross-transfers of genetic material between several genomes. *Hatena arenicola* is a green cryptophyte (Katablepharid) containing a green endosymbiont (Okamoto & Inouye, 2005); when it divides, one daughter

cell inherits the whole "chloroplast"; the other daughter is colorless. The latter then develops a feeding apparatus and it ingests a single cell of the green alga *Nephroselmis* which swells and becomes transformed into the new chloroplast; it enlarges considerably while its eye-spot becomes functionally adapted to controlling the host cell's flagellar apparatus. Apparently this endosymbiosis has not yet evolved into a truly interdependent relationship.

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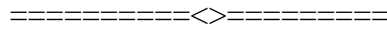
"Heterokont" and "Isokont" Flagella. The number and morphology of the flagella are highly conserved features of major taxonomic importance. The green algae and prymnesiophytes are "***isokont***': their flagella are of equal length and equivalent function. This is, however, not strictly true (at least in the greens) since in subtle but significant ways, even these flagella are different. This difference is of importance in phototaxis and the phenomenon of flagellar maturation (below). The majority of algae have "***heterokont***" flagella which are conspicuously different in length and function. Typically, one flagellum is longer and decorated by fine hairs (***mastigonemes***); these change the hydrodynamics of flagellar beat with the result that the cell moves in the direction the flagellum is pointing.

Melkonian and co-workers (Beech *et al.*, 1991) made an extraordinary discovery, now termed "***flagellar maturation***" or "***flagellar transformation***". One flagellum (usually the longer, depending on the species) is invariably the mature form. During cell division, the shorter flagellum elongates and transforms into the mature form; meanwhile, two new short flagellar arise and each daughter cell gets one long, mature flagellum and one short, immature flagellum. Thus, each flagellum takes two cell cycles to achieve the mature state. This phenomenon has since been shown to occur in animal cells, and may be a universal property of flagella.

Alveolates. A recently recognized structural feature characteristic of certain protistal groups (dinoflagellates, ciliates, euglenoids and apicomplexans) is the presence of flattened vesicles, "***cortical alveoli***", deployed around the cell just under the cell membrane (electron microscopy is, of course, needed to discern them). In the dinoflagellates and euglenoids, the characteristic wall components (cellulosic plates and proteinaceous strips, respectively) are always inside the alveoli and thus inside the cell. There is little doubt that these are important taxonomic indicators, a conclusion supported by DNA sequence data.

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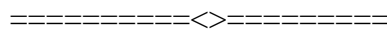
Comments on Individual Chapters

Chap. 2. The **Choanoflagellates** have long exercised the imagination of microscopists (e.g., the classic illustrations by Kent (1880-1882). They are very similar to collar cells (choanocytes) of sponges and DNA data shows they share a common ancestor. Studies of signal transduction genes confirm that choanoflagellates are on early line of evolution that also led to animals and thus, they can be considered our closest relatives in the protists. Structurally, they have a very complex flagellar apparatus, unlike anything anywhere else in the protists. For examples of their remarkable thecae and loricae and how they assemble them, see Leadbeater, (1994a, b) and Thomsen et al., (1990).

Chap. 3 **Diplomonads** are usually single-celled , anaerobic parasites found in the digestive tracts of many animals. Unusual in many respects, they can be visualized as "double" cells whose two nuclei and two sets of (typically) four flagella , are arranged in

mirror-image symmetry. They have no mitochondria or golgi bodies and are often regarded as being primitive.

Chap. 4 **Parabasilids** are also unusual, distinctive single cells. Of the two main groups, the trichomonads are usually parasites, and are simpler than the hypermastigotes, extraordinarily complex cells with a massive flagellar apparatus. The latter occupy a very specific ecological niche, the gut of termites and cockroaches . The various species are symbionts, major components of an intestinal flora involved in the digestion of cellulose fragments which they take up into digestive vacuoles. It is worth remembering here that this symbiosis-dependent ability to digest cellulose makes termites one of the most successful and widespread organisms on the land where they recycle up to a third of the annual production of wood, grass and leaves.

Chap. 5 The **Cellular Slime Molds** were formerly considered members of the fungi, related to cellular slime molds [6] and the oomycetes [19], but these groups are now known to be unrelated. At different stages in their life cycle, cellular slime molds are unicellular, colonial or differentiated into several types of cells. The colonial stage is a slime covered slug-like organism that gives this group its popular name. These organisms have provided cell and developmental biologists with classic material for studying the interaction of individual cells, including a central role in cellular aggregation played by cyclic-AMP, a signaling molecule subsequently found to be used in animal tissues.

Chap. 6 **Acellular Slime Molds** are excellent examples of *coenocytes*, giant cells containing numerous nuclei. They are commonly found in damp forests on rotting debris and may grow quite large. Their streaming cytoplasm provided early biochemists with an excellent source of actin and myosin in a filamentous complex of actomyosin which could be reactivated and investigated *in vitro*.

Chaps. 7, 8 The splitting of **Amoebae** into the two groups, **Rhizopods** and **Actinopods**, follows the proposal of Levine et al., (1980), with the axopod being a highly specialized, needle-like form of pseudopodium. They have a single or multiple nuclei, depending on the species. Rhizopods have no fixed shape but polarity in movement and organization is often apparent. They push out pseudopods of varying morphology for movement and prey capture. Their cytoplasm undergoes the characteristic phenomenon of "actin cycling", as shown in the DVD. Some species of both rhizopods and actinopods secrete mineralized scales of various shapes which form protective coverings or "*tests*", while certain actinopods, the radiolarians (not illustrated in the DVD), make extraordinarily complex and beautiful silica skeletons. The actinopods are planktonic, and important participants in the food web of open oceans.

Chap. 9 In their flagellate stage, **Chlorarachniophytes** are difficult to tell apart from green algae, but the symbiotic origin of their chloroplasts is obvious from electron microscopy (see earlier) and molecular biology.

10. **Cryptomonads** are very common in marine and fresh water habitats but they never seem to form blooms. They show great diversity in color and are classic organisms for studying endosymbiosis. Their chloroplast came from a red alga; some species have two chloroplasts, each with its own nucleomorph. Colourless species have no chloroplast genes, so these probably never had a chloroplast. Molecular data shows that the colourless *Goniomonas* (shown live in the DVD) is a close relative and probably resembles the ancient host cell. Their thin cell wall, the periplast, is revealed by freeze-etch techniques to consist of a complex system of crystalline plates under the cell membrane, with pores for ejectosomes Brett *et al*., 1994). A few species have minute, delicate rosette scales.

11. **Raphidophytes** were formerly thought to be uncommon. However, they can form toxic blooms. The cells are usually large, always heterokont, and contain numerous chloroplasts. They are truly naked cells, devoid of any wall; this is exceptional in the

phytoplankton.

12.   **Silicoflagellates** are very difficult to culture and are not well known. Modern species belong to only one genus, *Dictyocha*.   They are abundant in the fossil record, but they appear to have been in a slow decline over many millions of years and possibly heading for extinction.

13.   **Pedinellids** are common and distinctive algae which, from molecular data, are close to silicoflagellates with which they share various structural features. The long anterior stalk has a core of three microtubules attached to the nuclear envelope. *Apedinella* has unusual hinging organic (i.e., non-siliceous) spines. Their movement is controlled by an extraordinarily complex cytoskeleton of actin,and microtubuls (Koutoulis & Wetherbee, 1993). The function of these erectile spines is not known.

14.   The **Pelagomonads** are a new class described by Andersen et al. (1993). *Pelagomonas* is tiny (1-2 uM) and is included on the DVD as a representative of the "***picoplankton***", an assemblage largely ignored in the past because they are so difficult to work with. Recent work suggests that the picoplankton may comprise an immense biological resource dispersed in the open oceans. As more of these tiny cells are cloned and analysed, they seem to be diverse in origins and affinities; thus, many major groups may have pico representatives.

The paper by Andersen et al. demonstrates how such minute organisms are now characterized using a combination of electron microscopy and molecular sequence data. *Pelagomonas* has one flagellum plus mastigonemes and no additional, second basal body - the only known protist to be this way even when dividing.   Its closest relatives seem to be the silicoflagellates and pedinellids.

15, 16. The **Chrysophytes** and **Snurophytes** share several important taxonomic characteristics, so some authorities prefer that they not be split apart. However, other features are different, most importantly the morphology of the flagellar apparatus and its system of structural microtubules. In the chrysophytes, the basal bodies are angled at about 120° (Andersen, 1987) and certain of these microtubules can slide to form the feeding lasso shown in the DVD. In contrast, the synurophytes do not feed; their basal bodies are parallel and the morphology of their flagellar cytoskeleton may preclude feeding in this fashion. The direct participation of the heterokont flagella in both prey capture and selection is analysed by Wetherbee & Andersen (1992) and the mechanism creating the feeding loop using microtubules of the flagellar apparatus, is described in Andersen & Wetherbee (1992).

The scale cases are often very tough. The scales are precisely positioned and during division, the molecular "Velcro" that holds them together, loosens to form a suture line along the cleavage furrow (e.g., see Lavau & Wetherbee, 1994).

17.   **Diatoms.** Cytographics offers a DVD (*Diatoms: Life in Glass Houses*) that covers most aspects of the biology of living diatoms (details from the Cytographics website).   In addition, a Teacher's Guide can be downloaded which gives background information on these organisms.

Chap. 18   The **Brown algae,** a major constituent of what are commonly lumped together as "sea weeds", are actually closely related to the diatoms. In larger forms, their plant body or "" are quite differentiated, and some possess transport cells like the sieve-tubes of green plants. Many species that live in the shoreline are subjected to never-ending abrasion and battering by wave action and they can be remarkably tough, with holdfasts that are tenacious.

Chap. 19   The **Oomycetes** include such familiar organisms as downy mildew and water molds. Their filamentous, coenocytic hyphae at first indicated an affinity with fungi (see Chap. 5 above); however, the walls of these cells are cellulosic instead of chitinous, as in

fungi. Their heterokont zoospore soon indicated that they are related to the golden-brown algae, and close to brown algae, as has since been confirmed by molecular techniques.

Chap. 20   The **Xanthophytes**, often now called the Tribophytes, are a small group of greenish golden-brown algae. Although their flagella are heterokont, they are usually difficult to separate from green algae. However, the green algae have starch while the xanthopytes do not so the simple starch-iodide test can distinguish them. The unusual large multiflagellated zoospore of *Vaucheria* arises as a result of incomplete cleavage of cytoplasm in the multinucleate zoosporangium.

Chap. 21.   The **Prymnesiophytes** are generally phytoplankton of the open oceans where they can bloom in immense numbers. They are sometimes associated with oil deposits and chalky sediments. Their mineralized scales ("***coccoliths***") are of major interest to geologists as stratigraphic indicators. Phylogenetically, they are separate from other golden-brown algae, as indicated by their flagella and possession of the characteristic appendage, the haptonema. The flagella are different, being isokont and undecorated by hairs (except in the Pavlovales). The haptonema has a core of 6-7 mMTs ensheathed by a tube of endoplasmic reticulum, all within the cell membrane. These microtubules originate near the basal bodies. The haptonema was thought to function mainly in cell attachment. However, Inouye's group (e.g., Inouye & Kawachi, 1994) showed that it can have a major function in feeding/prey capture. Some species use phagocytosis alone and the role of their haptonema is not known. But other species catch particles on the haptonema, and then transport them to an aggregation site; the haptonema periodically sweeps down over the cell surface, depositing these particles on the anterior surface where they are phagocytosed.

Cooccolith formation provides a classic example of the function of the golgi apparatus which is unusually large and comprised of many cisternae containing scales in sequential stages of assembly. Brown and colleagues showed different stages in the delicately controlled assembly of the polysaccharide components, demonstrating the existence of celluose in golgi cisternae. Mineralization of scales with calcium carbonate to transform these organic scales into coccoliths has been widely documented and reviewed (e.g., Faber & Preisig, 1994). Interestingly, some species of tintinnids make their tests (outer covering) entirely from coccoliths.

Chap. 22.   The **Dinoflagellates** are extraordinary cells and also one of the most successful, constituting a significant proportion of the earth's living biomass. Most species have two heterokont flagella, one forming a tight spiral around the cell, with the second trailing. Many are mixotrophs with many ways of capturing food organisms. In predatory armoured forms, cytoplasm emerges from the wall as a pseudopod which envelopes the prey, secretes digestive enzymes and then absorbs nutrients while the food vacuole remains outside the cells. Some unarmoured forms develop a sucker which attaches to the prey and sucks the cellular contents out, so that the predator swells considerably. These predatory habits contribute to dinoflagellates' facility at endosymbiosis.

Their cell walls consist of cellulosic plates which actually lie within membranous vesicles inside the cell membrane. In so-called "naked" forms, these vesicles are still present. Thus, dinoflagellates are alveolates (see above).

Until about 30 years ago, little was known about dinoflagellate life cycles and sexual reproduction, now known to be widespread in the group.   These topics have become much more important as the role of dinoflagellates in toxic blooms ("red tides") became clear, with resistant spore stages providing the source of widespread and quick growing blooms.   Some hypnozygotes are viable after 80 years. *Alexandrium catenatum* and *Noctiluca scintillans* were formerly unknown in Australian waters but their common appearance is now ascribed to transport in ships' ballast water and the resultant widespread distribution of their spores in

sediments.

The dinoflagellate nucleus is unusual; the chromosomes are "***permanently condensed***": they are visible during interphase and have a striking appearance under the electron microscope. Their genomes are enormously large and they lack normal histones and nucleosomes (Hackett et al., 2004). During endosymbiosis (above), large numbers of genes are imported into the nucleus from their symbionts.

Their mitotic spindle is unusual; spindle microtubules never actually penetrate the nuclear envelope. Instead, microtubules connect to kinetochores through the nuclear envelope which remains intact throughout mitosis. An early claim (Kubai & Ris, 1969) that chromosomes were moved by the nuclear membrane – a traction mechanism resembling that which separates replicated genomes in bacteria – initiated the concept that their spindle represents an early intermediate stage in the evolution of the eukaryotic spindle, and thus that they were an intermediate in the evolution of eukaryotic cells. Their interpretation of mitosis was soon (e.g., Oakley & Dodge, 1974) proved incorrect. However, so attractive is this myth that it has continued to reappear for over 30 years in cell biology textbooks (e.g., p. 1059 in "*Molecular Biology of the Cell*", B. Alberts et al., Garland Science, publ.; 2002).

Chap. 23   The **Apicomplexans** are all small parasitic cells, some dangerous pathogens. Their life cycles are often complex involving being passed on to several different hosts. They are devoid of flagella and contractile vacuoles and move in a limited fashion by wriggling/gliding. Their name is derived from th*e "apical complex"* of organelles that enables them to penetrate the membrane of host cells.

Chap. 24   The **Ciliates** are the most complex of all cells. They are voracious predators and many have a mouth and gullet (***cytostome***) for ingesting prey; these are lined by cilia which sweep the food into digestive vacuoles at the base of the gullet. They commonly have ejectile organelles (***trichocysts***) in their pellicle, whose role is uncertain.

One distinctive feature is their invariable possession of two types of nuclei: one or more large "*macronuclei*" which contain multiple copies of each gene and thereby provide the "working copies" of genes and genetic information used in everyday metabolism and cell function; and one or more "*micronuclei*" which act the master copies of the cell's genetic information, duplicated carefully and transmitted accurately each cell division by mitosis. During sexual reproduction, the micronuclei of compatible mating types fuse and undergo recombination and meiosis, while the macronuclei degenerate. Later new macronuclei arise from great enlargement and gene replication in one or more of the micronuclei.

Chap. 25   The **Euglenids** are widespread and distinctive unicells. The chloroplasts of green species are identical to those of green algae, while the cells are completely different to all greens, clearly indicating a secondary acquisition of their chloroplasts. Furthermore, some colorless forms have no chloroplasts, other reduced plastids. Paramylon, the storage material, is very similar chemically to starch, but unlike in green algae where starch is located within the chloroplasts, paramylon grains are free in the cytoplasm. The phagotrophic forms have complex feeding apparatuses and often specialized diets. A few species appear to have prokaryotic (i.e., bacterial) symbionts. The host cell of euglenids appear close to the kinetoplastids or kinetoplasts [26], and more specifically, a group called the bodonids.

The wall or pellicle consist of long interlocked proteinaceous strips each of which is contained within a membrane and, like the wall components of other alveolates, always within the cell membrane.

Chap. 26   The **Kinetoplasts** include some of the world's most dangerous parasites, causing diseases like sleeping sickness, Leishmaniasis and Chagas' disease. They have evolved the ability to survive in the host's bloodstream by circumventing the immune

response. One of their distinctive features is their mitochondrial DNA which is condensed into a large mass comprised of densely stained fibrils. Simpson et al., (2002) describe this DNA as a network of network of interlocked circular molecules of two classes, maxicircles and minicircles.

Chap. 27   The **Glaucophytes** are uncommon, and of immediate interest because their chloroplasts are clearly derived from endosymbiotic cyanobacteria. These "*cyanelles*" retain a vestige of their original cell wall containing a peptidoglycan like those of bacterial cell walls. Flagellated stages are heterokont.

Chap. 28   The **Red Algae** (Rhodophytes) are a widespread, ancient group of distinctive plants whose cells tend to have thick gelatinous walls and which in some groups are interconnected by distinctive complex pores called "*pit connections*". Their photosynthetic pigments allows some species to grow at depths where only blue light penetrates. Some impregnate their walls with calcium carbonate and these "*coralline algae*" can be important reef builders.

   The red algae are all devoid of centrioles and flagella. Because the structure of the eukaryotic flagellum is so pervasive and so precisely conserved across the Four eukaryotic Kingdoms, the earliest ancestral reds may have evolved separately away from the ancestral eukaryotes that evolved the flagellum. Their lack of flagella has led to the widespread assumption that they have no motile stages. However, this is incorrect, since different types of spores in diverse species display a variety of gliding or amoeboid movements (Pickett-Heaps et al., 2001). During sexual reproduction, fertilization requires small male cells called "*spermatia*" to adhere to specialized female hairs called "*trichogynes* " and transfer their nucei into it. This unique process is illustrated in living cells by Pickett-Heaps & West (1998). They also have unusually complicated, three-stage life cycles; both sex and motile spores are illustrated in the DVD and Cytographics offers a video ("*Reproduction in Red Algae*") showing these events in greater detail

Chaps. 29-32. The **Green Algae** are the most diverse and widespread of all algae and they are comprehensively covered in all phycology text books. The various forms are unicellular, colonial, multicellular (including filamentous and branched forms) or siphonous. Some groups such as the Volvocales and Chlorococcales, provide beautiful examples of the evolution of different and increasingly complex morphological species from simple flagellated ancestors. They have widely different modes of sexual reproduction, and life cycles. There is no doubt that certain ancient forms [32] gave rise to the land plants ([33]; see Lewis & McCourt, 2004, for review) .

   Cytographics has published two old style laser discs (soon to be reissued in DVD format) on the Volvocales and Chlorococcales, plus another DVD on the Oedogoniales (see Cytographics website).

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