sents failure to respond to environmental change by species selection. Because most lineages are short-lived, exhibiting little phyletic change and containing few successional species, speciation is essential for long-term survival of higher taxa. An asexual clone may diversify, but will not speciate in the normal sense and will be much more susceptible to extinction than the entire clade formed from a successfully speciating sexual species. The clade typically will include a variety of species occupying an adaptive zone much broader and more heterogeneous than the niche of the clone. Species selection, which determines the course of large-scale evolution, must rapidly eliminate most asexual taxa (see Fig. 1).

The foregoing arguments provide an explanation for the success of the prokaryotes without extensive genetic recombination. It is not simply that prokaryotes have short generation times and, hence, can evolve phyletically to adjust to changing conditions. Even without phyletic change it is hard to envision the extinction of a typical bacterium or blue-green alga having enormous population size, worldwide distribution, and a broad niche. Extinction rates are so low, with or without sexuality, that rapid diversification is not needed to offset them. It is also evident why asexual taxa of higher plants (*3, 13*) and animals (*7*) tend to occur only sporadically, having close sexual relatives. Such taxa tend not to persist on an evolutionary time scale (*3, 7*).

The major exception (*7*) is readily explained. This is the rotifer class Bdelloida. The fact that all 300 or so recognized "species" of the Bdelloida are parthenogenetic suggests a monophyletic origin for this condition (*14, 15*). Bdelloids typically inhabit minute bodies of water, such as those found at the base of the leaflets of damp mosses, and individual types have extraordinarily broad niches. In general, they are capable of extreme anabiosis (*15*). Many can remain frozen or in states of extreme dessication for months or even years, and most types are cosmopolitan (*16*). Thus, it is easy to see how, even in the absence of normal quantum speciation, the bdelloids, which are very simple creatures and may well have originated in Paleozoic times, have been able to survive by virtue of low rates of extinction. Freshwater members of the Chaetonotoidea (Gastrotricha) are also exclusively parthenogenetic (*15*), and similar arguments would seem to apply to this group of minute, abundant, and commonly cosmopolitan forms. Existing "species" in such groups are discrete simply because they represent scattered adaptive peaks surviving from a continuum of forms (*14, 15*).

In summary, recombination permitted by sexual modes of reproduction may have some value among eukaryotes for survival of lineages in the face of environmental change, but phyletic evolution is much more sluggish and less significant than has generally been recognized and there is no evidence that asexual species are appreciably shorter-lived than sexual species. The overwhelming dominance of sexual reproduction among eukaryotes must result from the fact that for most taxa, normal extinction rates can only be balanced by high rates of diversification provided by the frequent formation of divergent new species.

STEVEN M. STANLEY

Department of Earth and Planetary Sciences, Johns Hopkins University, Baltimore, Maryland 21218

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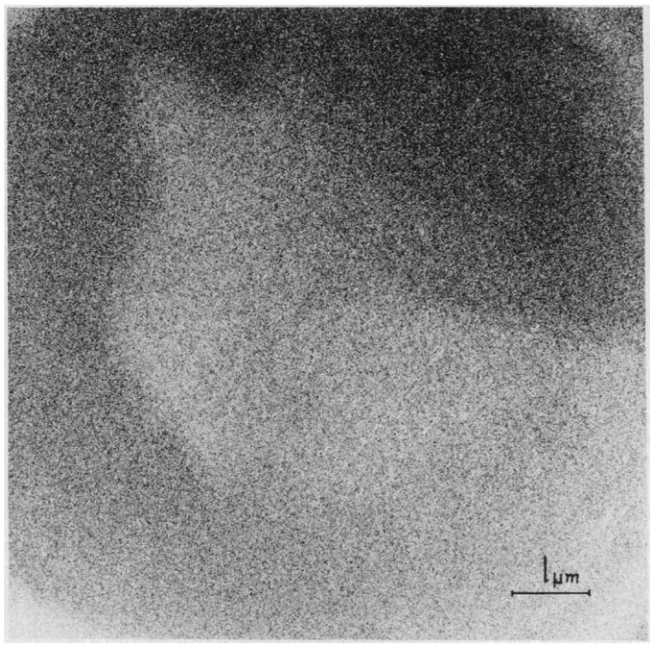
Direct Observation of Domains in Wet Lipid Bilayers

Abstract. *Domain structure and phase separation in hydrated lipid bilayers have been imaged directly by selected reflection dark-field electron microscopy. Domains in multicomponent bilayers are much smaller than those in single component bilayers, in agreement with results obtained by selected area electron diffraction.*

The hexagonal packing of acyl chains in phospholipid bilayers has been known for some time (*1*), yet the extent of the coherently packed areas (domains) in a bilayer is still uncertain. Little information has been obtained from x-ray diffraction because of the large diameter of the x-ray beam. If the areas of continuously striated surface seen in freeze-fracture electron micrographs are regarded as domains, the domains would measure from a fraction of a micrometer to several micrometers across, depending on the material, the cooling rate, and the quenching temperature of the membranes (*2, 3*). Separation of multicomponent bilayers into small, single phase domains has also been indirectly indicated by calorimetric (*4*) and spin-label electron paramagnetic resonance studies (*2*). By using an environmental stage in an electron microscope and applying techniques of electron diffraction and dark-field electron microscopy, we have been able to directly observe the sizes and shapes of domains of different compositions, as well as different orientations in molecular packing in bilayer membranes under physiological conditions.

Bilayers of pure dipalmitoylphosphatidylcholine (DPPC), of mixed DPPC and dilauroylphosphatidylcholine (DLPC), and of mixed DPPC and cholesterol were formed without support on an electron microscope grid by a previously described method (*5*). By using an environmental stage for a Siemens Elmiskop IA electron microscope (*6*), the specimen may be viewed in a fully hydrated state in the temperature range between −10° and 50°C. Results were recorded on sensitive Kodak No-Screen x-ray films, although the patterns were normally invisible on the phosphor screen of the electron microscope because of the extremely low beam current in the experiment (10^{-6} amp/cm^2 at the specimen level).

Selected area electron diffraction was done by restricting the illumination area to a few micrometers in diameter, using a pointed filament and a 10 μm second condenser aperture. Below the transition temperature of DPPC bilayers, three orders of a hexagonal pattern were recorded, whereas diffuse rings only were seen above the transition temperature (*5, 7*). Differently oriented diffraction patterns were distinguishable between adjacent membrane areas (domains) 5 μm apart. Occasionally,



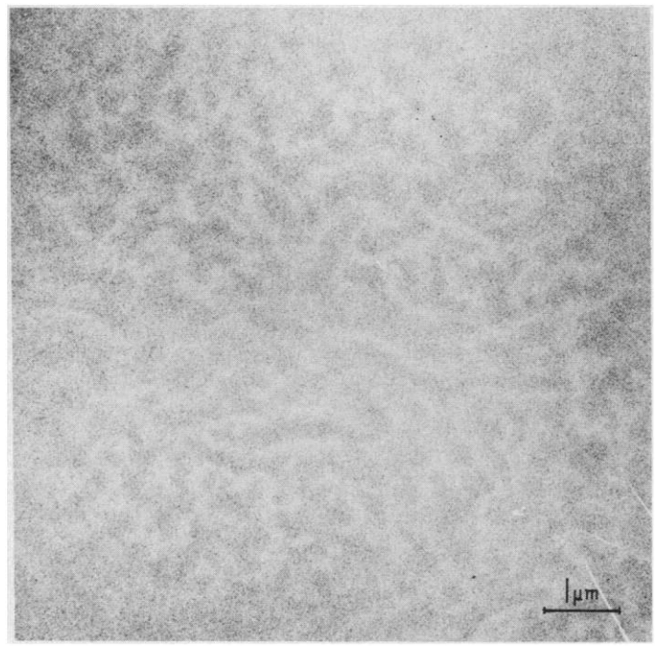


Fig. 1 (left). Selected reflection dark-field micrograph of a hydrated DPPC single bilayer at 17°C showing two neighboring domains of different orientations in molecular packing. Fig. 2 (right). Selected reflection dark-field micrograph of a hydrated single bilayer in a mixture of cholesterol and DPPC with a molar ratio cholesterol/DPPC = 0.65 at 11°C.

two or more superimposed patterns were recorded from the same area, which indicated that the observation area covered several domains. In a two-component system such as mixed DPPC and DLPC, or mixed DPPC and cholesterol, no single domain pattern was seen. Instead, there were "powder" diffraction rings, showing the domain size to be much smaller than the selected observation area.

Selected reflection dark-field electron microscopy was done by placing a selection aperture in the back focal plane of the objective lens. To enhance domain contrast, the selection aperture was made such that only the reflections of certain solid domains were transmitted. The dark-field image formed by the transmitted pattern showed remarkable contrast between differently oriented domains. The domains of DPPC bilayers measured by this method were typically several micrometers wide, as shown in Fig. 1. The domain structure corresponds well with our model derived solely from electron diffraction results (*5*). No domain structure was observed at temperatures above the phase transition (41.5°C).

In bilayers of an equimolar mixture of DPPC and DLPC, low-contrast domains typically 300 nm wide were observed at temperatures between the higher and lower endothermic peaks in the calorimetric scan of the mixture (*8, 9*). The low contrast is probably due to imperfect masking (by the selection aperture) of the diffuse diffraction pattern produced by some liquid-crystalline domains. Since the selection aperture is designed to mask spot patterns, better contrast may be obtained if all the domains are in the solid state. Contrast between domains was generally improved at temperatures below the lower endothermic peak, indicating that all domains were, in fact, solid at these temperatures. Again, no domain structure was observed at temperatures above the higher endothermic peak. The domain sizes we measured agree with those of the striated areas observed in freeze-fracture experiments (*3*). In mixtures of DPPC and cholesterol, a sharp diffraction ring corresponding to the packing of pure palmitic acyl chains remained visible until the molar cholesterol content reached 1 : 1. It was therefore possible to segregate the pure DPPC reflections from the total diffraction pattern. A dark-field electron micrograph thus formed showed ribbonlike structures in mixed DPPC-cholesterol bilayers (Fig. 2), the ribbons being less than 100 nm wide. The ribbons could not be interpreted as the loci of domains that moved during the exposure time of 5 seconds since the tracks were never crossed. The ribbon structure was similar to the lowest-energy configuration of systems of two immiscible components [such as eutectic solids (*10*) or magnetic domains in superconductors (*11*)]. The proportions of light and dark domains varied with the composition of the mixture. It is possible that these domains represent areas of pure components or particular eutectic complexes. Both the ribbon pattern and the DPPC diffraction ring disappeared as the temperature was raised beyond the transition temperature of the corresponding mixture.

Although the domain size may be related to the striated areas observed from freeze-fracture electron microscopy, or may be deduced within certain limits from line widths of diffraction patterns, our electron optical observation provides direct evidence of a long-suspected phenomenon at the temperatures of biological interest. Our observations of erythrocyte ghost membranes and hepatocyte plasma membranes showed that their domains were much smaller than those in lipid bilayers.

S. W. Hui, D. F. Parsons
Electron Optics Laboratory, Biophysics Department, Roswell Park Memorial Institute, Buffalo, New York 14263

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