It is here suggested that the disharmonies in the sexual behavior of the hybrid females may serve as a very efficient isolating mechanism between the incipient species. It is unfortunately not known whether the Amazonian and the Andean-South Brazilian subspecies occur anywhere sympatrically, but such sympatric occurrence has been recorded for other pairs of *D. paulistorum* subspecies (*1*). If such sympatric occurrence does exist, the possibility of hybridization cannot be excluded. However, the hybrid females, though potentially fertile, in the sense that their ovaries may be full of normal and mature eggs, would probably never mate. This would make the appearance of backcross progenies impossible.

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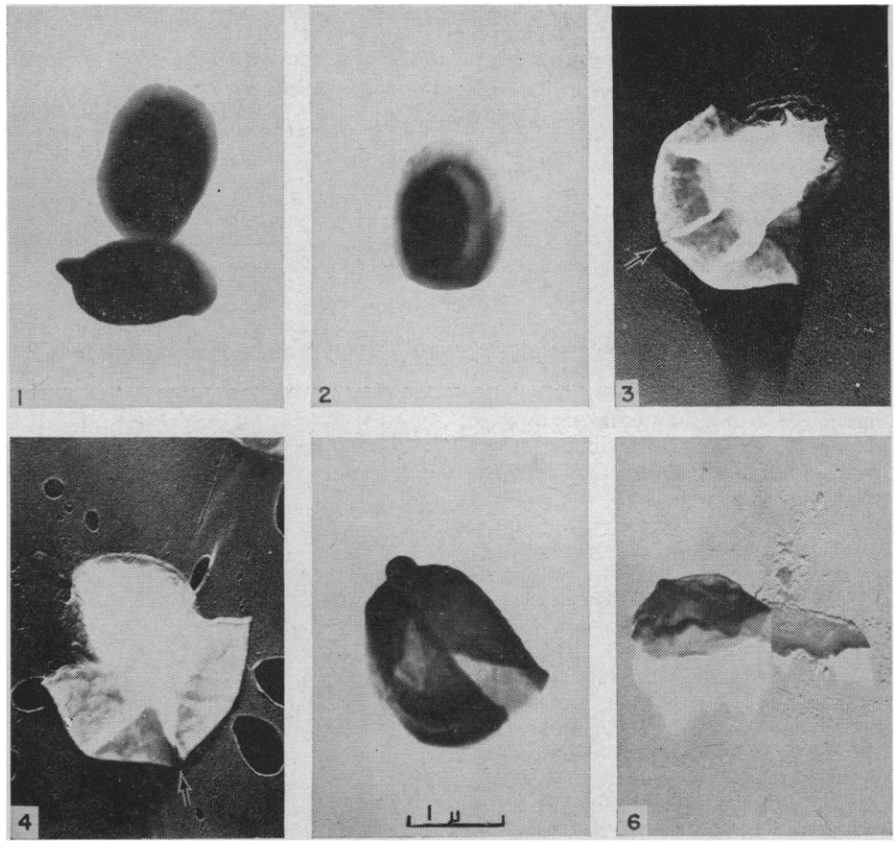

Figs. 1–6. Electron micrographs of *Bacillus megaterium*, taken with an R.C.A. electron microscope (EMU-2A) at an original magnification of 7400. Figures 3 and 4 are positive images; the others are negative. Fig. 1. Resting spores. Fig. 2. Spores incubated for 10 minutes—germination. Figs. 3 and 4. Spores incubated for 50 minutes—emergence of new cell and some elongation. The arrows indicate the split in the spore coat. Fig. 5. Empty spore case of an emerged cell, 60 minutes. Fig. 6. Empty spore case of a resting spore, obtained by grinding with glass beads. (× 12,500)

Spore Germination and Emergence of Bacillus megaterium

Abstract. Bacillus megaterium spores have a characteristic polar knob and equatorial ridge, or groove. During germination, the spore case appears to split along this ridge, and thus allows the new cell to emerge. Mechanically ground spores also split along this ridge, one part of the spore case being hinged to the other, the ridge being evident along a free edge. The equatorial ridge appears to be an area of susceptibility to mechanical pressures and, perhaps, in normal germination, to enzymic action as well.

We are primarily interested in the initial stages (*1*) of spore germination, when the spore loses its heat resistance, becomes stainable, and begins to consume oxygen. The study of later stages such as emergence may, however, help to illuminate early changes in spore germination. Electron micrographs of resting and germinating spores of *Bacillus megaterium* were prepared. The spores were germinated at 30°C in a medium buffered with 0.05*M* phosphate at *p*H 6.9 and containing 0.5 percent peptone, 0.02 percent yeast extract, and 0.025*M* glucose. Respiration in this medium has been described by Mandels *et al.* (*2*). The resting spores are opaque to electrons (Fig. 1). After 10 minutes in the germination medium, the spores become somewhat swollen, and dense material moves toward the periphery (Fig. 2). The spore case splits after 50 minutes of incubation, and a new cell protrudes from the case and begins to elongate (Figs. 3, 4).

The spore very often has what appears to be a polar knob and a ridge or groove circumscribing the major equator of the spore case. During the transition from spore to vegetative cell the spore case is often freed of the emerging cell (not adherent as in Figs. 3 and 4). The polar knob and equatorial ridge or groove are particularly pronounced in the discarded spore case shown in Fig. 5. We conceive of the equatorial ridge, or groove, as a line of weakness in the spore case more susceptible than the rest of the case both to physical and to enzymic attack. It is along this ridge, weakened perhaps by enzymic action (*3*) or by mechanical pressure of the swelling spore, that the case splits and allows the new cell to emerge. The cracks indicated by arrows in Figs. 3 and 4 support this idea. Furthermore, spores ground with glass beads show the same sort of split, one part of this physically damaged coat being hinged to the other with the ridgelike appearance evident along a free edge (Fig. 6) (*4*).

Note added in proof. Since we submitted this report, P. C. Fitz-James and I. E. Young have published electron micrographs which also show an equatorial ridge in the outer coat of *B. megaterium* spores (*5*).

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