Organisms belonging to the class Mollicutes are among the simplest self-replicating organisms known. These wall-less eubacteria are characterized by small genomes that have low DNA guanine-plus-cytosine (G+C) contents. The class Mollicutes is currently divided into eight genera: Asteroleplasma, Anaeroplasma, Achroplasma, Entomoplasm, Mesoplasm, Spiroplasma, Mycoplasma, and Ureaplasma.

Measurement of the genome sizes of bacterial species has been greatly facilitated by the development of pulsed-field gel electrophoresis (PFGE) (32). This technique, associated with restriction enzyme digestion and Southern blot hybridization, has made it possible to obtain physical and genomic maps of numerous bacterial species (33, 34). PFGE has been used to determine the genome sizes of several mollicute species (9, 13, 14, 19, 21, 24, 26, 28, 29, 35, 39, 41, 49, 52, 53). A continuum of sizes has been found, ranging from 580 kbp for the smallest genome, the Mycoplasma genitalium genome (9, 35), to 1,820 kbp for the largest genome, the Spiroplasma citri genome (53).

Spiroplasma citri, the causative agent of citrus stubborn disease, was the first Spiroplasma species to be described (30). Today, numerous spiroplasmas have been isolated from plants and arthropods, mainly insects (50). An approach to spiroplasma classification based on serologic relatedness data, electrophoretic patterns of proteins, G+C DNA contents, and levels of DNA-DNA homology was first proposed in 1980 (18). Up to 1993, 24 groups were recognized (3, 15, 37) but recently, the group XVII spiroplasmas have been shown to be members of group VIII (12); thus, group XVII contains no organisms at the present time. Groups may be divided into subgroups on the basis of genome size, replication and to prevent the initiation of new rounds. The cells were then collected by centrifugation for 30 min at 20,000 x g.

The pelleted cells were embedded in LMP agarose (Gene-line; Beckman, Palo Alto, Calif.) and were treated as described previously (27), except that the cellular pellet was resuspended in STE (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) instead of phosphate-buffered saline (53). Genome sizes were determined by using full-length (nonrestricted) chromosomes that were linearized by random breakage during the extraction procedure (14). In addition, restriction enzyme NotI was used as recommended by the manufacturer (New England Biolabs, Beverly, Mass.). Electrophoresis was performed with a GeneLine II apparatus (Beckman) by using a transverse alternating field electrophoresis system and Tris-borate-EDTA as the running buffer. With the exception of nonrestricted genomic DNA from Spiroplasma isodeitis Y32T (T = type strain), the genomic linear molecules and NotI restriction fragments were separated on a 1% LE agarose gel (Beckman) under the following electrophoresis conditions: 370 mA; three steps of 12 h each, with pulse times of 1, 2, and 3 min; 8°C. The sizes of the resulting fragments were evaluated by comparing them with Saccharomyces cerevisiae chromosomal DNA fragments ranging in size from 215 to 2,200 kbp (Bio-Rad, Hercules, Calif.) and lambda phage DNA concatemers (New England Biolabs). For Spiroplasma isodeitis Y32T, nonrestricted genomic DNA was subjected to PFGE on a 0.6% LE agarose gel by using the conditions described above, except that there was an additional step of 9 h with a pulse time of 4 min. In this case, the size of the genomic DNA was determined by comparing its mobility with the mobilities of chromosomes obtained from Candida albicans (1,010 to 3,000 kbp) and Saccharomyces cerevisiae (215 to 2,200 kbp).

The genome sizes of the spiroplasmas which we examined are shown in Table 2. For most of the spiroplasma genomes, a single band was obtained after digestion with NotI, indicating either that there were no sites for this enzyme or that a single site was present. For two spiroplasmas, Spiroplasma sp. strain EA1 and Spiroplasma sabaudiaense AR-1343, three bands were obtained after digestion with NotI, indicating that there were three restriction sites for NotI. In every case, the genome size estimated from NotI digestion...
products was the same as the size determined with nondigested linear molecules.

The sizes of spiroplasma chromosomes range from 940 kbp for the smallest chromosome, the Spiroplasma monobiae MO1-T (group VII) chromosome, to 2,220 kbp for the largest chromosome, the Spiroplasma moioides Y32-T (group VI) chromosome. Thus, the genome of Spiroplasma moioides Y32-T appears to be more than twice as large as the genome of the spiroplasma with the smallest genome and is the largest genome described so far for a mollicute species. The genome of this organism is four times larger than the genome of the mollicute with the smallest genome, Mycoplasma genitalium (9, 35).

Figure 1 shows the distribution of genome sizes in different genera of the class Mollicutes. Genus sizes determined by PFGE are not available for members of the genera Asterole-
that are twice as large as the genomes of other species. Genome size heterogeneity also occurs at the species level; in *Ureaplasma urealyticum*, *Mycoplasma mycoides*, *Mycoplasma hominis*, and *Spiroplasma citri*, variations of up to 30% in genome size have been observed for different strains of the same species (21, 29, 49, 51).

### REFERENCES


