The discrete regulation of supercoiling, catenation and knotting by DNA topoisomerases is well documented both in vitro and in vivo, but the relationship and putative interplay between them are still poorly understood. Here we studied DNA catenanes of bacterial plasmids arising as a result of DNA replication in Escherichia coli cells whose topoisomerase IV activity was preferentially inhibited. We combined high-resolution two-dimensional agarose gel electrophoresis with numerical simulation in order to better understand the interplay between DNA supercoiling generated by DNA gyrase and the interlinking that results from replication of circular DNA molecules. We showed that right-handed catenation and negative supercoiling compete with each other. In interlinked molecules with low catenation numbers the sister rings are supercoiled to the same extent as noncatenated monomeric plasmids. As interlinking increases, though, the negative supercoiling of catenated rings progressively decreases. A notable structural transition takes place that reflects a general change of appearance of catenated rings that differ in their degree of interlinking. At low catenation numbers interlinking between the two rings tend to be localized while for high catenation numbers interlinking redistributes along the entire length of both rings. This mutual exclusion of interlinking and supercoiling suggests that in vivo the negative supercoiling introduced by DNA gyrase creates the appropriate conditions favorable for topoisomerase IV-mediated final decatenation of freshly replicated sister duplexes.