

Figure S2. Two extra GISH/FISH metaphase plates of *L. multiflorum* x *F. arundinacea* F2 3-18 donor ( $2n=6x=42$ ) plant. Images of the chromosome metaphase spreads, probed in two ways: (a,c) *L. multiflorum* in red and *F. pratensis* in mauve, *F. glaucescens* is blue (DAPI); (b,d) metaphase spreads reprobed detecting *F. glaucescens* in orange, and 45S rDNA cluster sites in green; images are merged for red (rhodamine) and green (fluorescein). 45S rDNA signals are abundant in b,d; in b, several sites of 45S rDNA are broken, resulting in split chromosomes and a derivative microstructure containing 45S rDNA is indicated by an arrow; in d, 45S rDNA sites and chromosomes remain intact; in c sticking chromosomes involving *F. pratensis* chromatin marked by an arrow. Scale bar = 10  $\mu$ m.

