

## Analysis of the ability of Gamone1 to induce conjugation among four species in the genus *Blepharisma*

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### SUMMARY

In the genus *Blepharisma*, 21 morphologically distinct species are classified into four groups according to the macronuclear shape. Conjugation usually begins after mating type I secretes mating pheromone Gamone1 under a food-deprived condition. Gamone1 is a glycoprotein in *B. japonicum*; it reportedly functions as species-specific signaling molecule. Therefore, Gamone1 can be a barrier to interspecies conjugation. However, our previous report suggested the possibility of interspecies conjugation in the genus *Blepharisma*. In this study, we examined the ability of Gamone1s of *B. undulans* (binodal macronuclei, group II), *B. americanum* (multinodal macronuclei, group III) and *B. japonicum* (filiform macronuclei, group IV) to induce pair formation in *B. stoltei* (group IV), and attempted to determine the extent of effect of Gamone1 as barriers. Results showed that Gamone1 of *B. japonicum*, but not the other two species, induced pair formation in type II cells of *B. stoltei*. We examined *gamone1* genes in these four species. The amino-acid sequence homology of Gamone1 was approximately 90% in the same macronuclear group and 75% in the different groups. Each Gamone1 has four presumptive N-glycosylation sites, and our results suggest that Gamone1 of these species have N-linked oligosaccharide with binding affinity to ConA beads. Furthermore, Gamone1 molecular weights were suggested as differing among the three species, which might be attributable to the difference of sugar residues attached to the Gamone1. Consequently, barriers to conjugation in the genus *Blepharisma* might have been established among macronuclear groups by difference in conformation or/and sugar arrangement of Gamone1.