

# Host size matters for reproduction: evolution of spawning preference and female reproductive phenotypes in mussel-symbiotic freshwater bitterling fishes

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## Abstract

Bitterling fishes evolve an idiosyncratic symbiosis with freshwater mussels, in which they are obligated to spawn in the gills of mussels for reproduction. In recent years, freshwater mussel populations have been drastically diminishing, due to accelerating anthropogenic impacts, which can be large threats to the risk of bitterling's extinction cascade (i.e. 'coextinction'). The host mussel size may be an important factor driving the adaptation and evolution of bitterling's reproductive phenotypes. Here we examined the host size preference and morphological adaptation of female bitterling to the host size from 17 localities at the Han River in Korea. Using our developed molecular-based species identification for bitterling's eggs/larvae inside the mussels, we further determined the spawning patterns of seven bitterling species. Mean length of spawned mussels (N=453) was significantly larger than that of unspawned mussels (N=1,814), suggesting that bitterling prefers to use larger hosts as a spawning ground. Spawning probability was clearly greater as mussel size increases. Results of our reciprocal transplant experiments do provide some evidence supporting the 'bitterling's larger host preference' hypothesis. Interspecific competition appeared to be intense as two fish species often spawned eggs in the same mussel individuals simultaneously. Longer ovipositor and more elongated egg may evolve in females of *Tanakia signifier* in response to larger host environments. The observed bitterling's spawning preference for large-sized mussels may evolve perhaps because of the fitness advantage in relation to the offspring survival. Our findings further inform on the development of effective conservation and management strategy for the endangered bitterling fishes.

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