

# Genetics tests of ecological roles of ergot alkaloids produced by an epichloë endophyte of ryegrass

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Ergot alkaloids are produced by ergot fungi (*Claviceps* spp.) that parasitise ears of grain, and related epichloë endophytes (*Epichloë* and *Neotyphodium* spp.) that systemically persist in grasses as protective mutualists. These alkaloids can serve as useful pharmaceuticals or can contaminate food, pasture or feed and thereby poison humans or livestock. Current understanding of the ergot alkaloid biosynthetic pathway is shown in Scheme 1, with labelled arrows indicating the roles of characterised genes. We sequenced ergot alkaloid gene clusters from *Claviceps fusiformis*, which produces elymoclavine (6) as an end product, and from the endophytes *Epichloë festucae* and *Neotyphodium coenophialum*, which produce ergovaline (8). Including *dmaW*, eight shared genes were identified, which may be sufficient

to direct the biosynthesis of 6. In addition, we have produced perennial ryegrass-*Neotyphodium* sp. symbiota in which the endophyte was disrupted in *dmaW* (and produced no ergot alkaloids), or in *lpsA* (and produced clavines), along with controls with the wild-type endophyte (producing ergovaline and clavines) and no endophyte (no ergot alkaloids). These symbiota were tested in preference and satiety tests with rabbits, and in preference and no-choice rearing tests with black cutworm (*Agrotis ipsilon*). Ergovaline deterred feeding by black cutworm in preference tests, and reduced growth and survival rates of cutworm in rearing tests. In rabbits, clavines deterred feeding and ergovaline suppressed appetite.

