A laboratory rearing of the Antarctic midge, *Belgica antarctica*: effect of the temperature and diet and possible seasonal adaptation.

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The Antarctic midge, *Belgica antarctica*, is a terrestrial insect endemic to the Antarctic Peninsula and offshore islands. Its habitat is under damp soil including dead plant, algae, moss and vertebrate feces. The midge has a two-year life cycle and spends most of the period as a larva. Larvae are tolerant to low temperature, freezing, severe desiccation and other environmental stresses. The physiological mechanisms underlying acquisition of the stress tolerance has been extensively investigated. However, the method for laboratory propagation of the midge has not been established, and therefore, these studies have used insects collected in the Antarctica. This largely limits the research progress. To dissect out the physiological system of this extremophile further, it is very important to propagate the midge in the laboratory. Here we aim to establish the effective rearing method of the larvae of this species with special attention to temperature conditions and diet. During this study, we found a possible strategy to adapt to Antarctic environmental conditions, i.e., a developmental arrest, and thus, we investigated the environmental conditions that break the arrest.

We first focus on the temperature conditions optimal for laboratory rearing of the newly hatched 1st instar larvae. Among 0, 4, 10 and 15 °C temperature conditions, 4 °C was found to be optimal. Otherwise, larvae could not survive long. We next focus on diet. Milk agar frequently used for rearing of various midge species was effective for rearing the larvae, whereas milk agar with a commercial diet for phytophagous fish could shorten the larval period in some midge. We found the mixed diet is very effective and larvae can develop to the final (4th) instar larvae. However, all the larvae arrested their development at the early phase of the 4th instar and none could develop to pupae, i.e., spontaneous developmental arrest. This might be a strategy to adapt the Antarctic seasons, and some environmental stimulus may be necessary to break the arrest. Since larvae experience winter twice during their life cycle and pupae and adults are only found in early summer in the fields, winter environmental conditions may play an important role. Under this assumption, we put soil including some final-instar larvae into a winter condition at -5 °C under constant darkness for 3 months and transferred it to a summer condition at 4 °C under a 18-h light and 6-h darkness photoperiod, to mimic the Antarctic seasonal conditions. A few weeks after the transfer we observed emergence of some adults, whereas only a few adults appeared in the soil continuously maintained in a summer condition.

In conclusion, the newly 1st instar larvae can develop to the final instar larvae with a diet containing milk agar and a diet for phytophagous fish at 4 °C. The final instar larvae arrest their development spontaneously. By exposing the larvae to a winter condition, the developmental arrest can be terminated, and pupae and adults appear. The previous study revealed that adults immediately copulate, and females lay eggs within a few days. Eggs hatch within 3-4 weeks at 4 °C. Thus, we here verified the effective environmental conditions for laboratory rearing of the Antarctic midge.

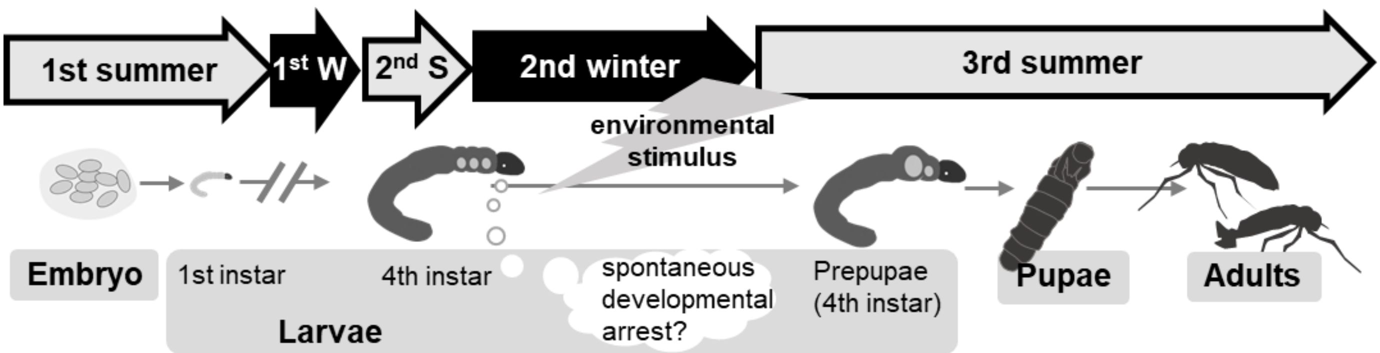

Figure.
The life cycle of the Antarctic midge *Belgica antarctica*.