Sexual Differentiation in Population of Prothallia in *Lygodium japonicum*

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In *Lygodium japonicum*, the archegonium was formed when the prothallium grew to approximately 1.5 mm in width irrespective of age, photoperiod, temperature or concentration of sucrose in the medium. Surgically cut prothallia produced archegonia only when the fragments regenerated to ca. 1 mm in width. Prothallia of smaller dimensions formed antheridia rather than archegonium, but only if archegoniated prothallia of the larger size coexisted in the population.

Antheridiogens and inhibitors of archegonial differentiation became detectable by bioassay in conditioned media of 14- and 16-day-old prothallia, respectively, and continued to accumulate in the medium during culture in the light at 25°C. Twelve-day-old or younger prothallia are very sensitive to exogenously applied hormonal substances, whereas by day 14 the response had diminished.

Key words: Antheridium — Archegonium — Sexual differentiation — Fern gametophyte — Antheridiogen — *Lygodium*.

Individual prothallia of homosporous ferns are supposed to form both antheridia and archegonia, but unisexual male, unisexual female and bisexual prothallia are often observed in one population (Miller, 1968). Such a sexual differentiation of individual prothallia in a population may be favourable to avoid intragametophytic selfing, which would increase genetic risk (Voeller, 1971; Klekowski, 1973). It is generally concluded that most prothallia grow into female individuals under conditions favouring vigorous vegetative growth, while under less favourable regimes, the majority become male (Miller, 1968; Duckett, 1970). However, the reasons for such behaviour remain unknown (Hauke, 1977).

Differentiation of reproductive organs in fern gametophytes is known to be regulated by hormonal substances produced by prothallia. Namely, antheridia are induced by antheridiogen in many fern species (Döpp, 1950; Näf et al., 1975). An antheridiogen of *Anemia phyllitidis* was characterized as a gibberellin-related diterpenoid (Nakanishi et al., 1971), and another antheridiogen obtained from the conditioned medium in *Lygodium japonicum* was identified as gibberellin A9 methyl ester (GA9-Me) (Yamane et al., 1979). Archegonial differentiation was inhibited by hormonal substances.

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secreted from prothallia in *L. japonicum* (Takeno et al. 1979), one of which was determined as GA₄-Me (Yamane et al., 1979).

The aim of the present study was to clarify the correlation between prothallial size and sexual differentiation of prothallia in a population of *Lygodium* and to find the role of antheridiogens and the inhibitors of archegonial differentiation in population, if any.

**Material and Methods**

**Plant Material**

Spores of *Lygodium japonicum* (Thunb.) Sw. were collected from pinnae with sporangia harvested at the Botanic Gardens, the University of Tokyo, Koishikawa, Tokyo, in the autumn of 1974 to 1978 and stored in glass tubes at 3 °C.

**Culture of prothallia**

Spores were sterilized and inoculated on a piece of cellophane (2 x 2 cm²) put on surface of 7 ml of 1/10 strength modified Murashige and Skoog’s (1962) mineral salts solution, solidified with 0.3% agar in a 3 cm in diameter and 18 mm in height Petri dish (Takeno and Furuya, 1975). The inoculated spores were aseptically cultured at 25 °C under continuous irradiation with fluorescent white light (5 W m⁻²) (Toshiba 40S D/NL, Tokyo Shibaura Electric Co., Ltd., Kawasaki). Resulted prothallial population density was 1494 ± 46 in a dish. When the culture conditions were modified, it is described in the text.

**Surgical treatment**

Prothallia were aseptically placed on a slide glass with tweezers and cut by a razor under a binocular microscope. Treated fragments were transplanted to fresh medium and cultured as above.

**Assay for antheridial and archegonial formation**

Formation of antheridia and archegonia was detected in both ventral and dorsal sides of the prothallium under a microscope, and was noted in terms of development of cap cell for the former and the four neck cells for the latter, respectively (Nayar and Kaur, 1971). In this work, the width of prothallium was also measured with an ocular micrometer.

**Methanol extraction of prothallia**

Prothallia (1 g fresh weight (f.w.)) grown on the surface of the medium for 3 weeks were soaked in 10 ml of methanol at room temperature for 1 hr. After removal of the prothallia, the methanol extract was evaporated to dryness in vacuo at 45 °C, and the residue was considered to contain antheridiogens and inhibitors of archegonial differentiation (Takeno et al., 1979).

**Bioassay of antheridiogens and inhibitors of archegonial differentiation**

Prothallia grown for 10 days were carefully selected under aseptic conditions so as to be uniform in size and shape. About 40 prothallia thus selected were cultured for 2