Summary

Biocommunication between plants and honeybees through pollen fluorescence

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Anthers and pollen fluoresce under UV irradiation, which has been proposed as a visual cue for pollinating insects. Fluorescent compounds occurring in anthers and pollen may attract pollinating insects, while protecting DNA from UV-induced damage. Biocommunication between plants and insects through fluorescence has been discussed for decades, but the perceptive capability of insects for the fluorescence and the fluorescent compounds in anthers and pollen has not been investigated. This thesis describes the identification of fluorescent compounds and the behavioral response of honeybees to fluorescence. The major findings described below will open up new aspect of biocommunication between plants and insects.

Chapter II. Intact anthers with the pollen of six plant species displayed human-blue fluorescence under UV excitation. The pollen fluorescence was emitted from pollenkitt. The fluorescent compounds were identified as six hydroxycinnamoyl acid derivatives: 3,5-dicaffeoylquinic acid, chlorogenic acid, (*E*)-acteoside and its (*Z*)-isomer, and 1-*O*-(*E*)-feruloyl-β-D-glucose and its (*Z*)-isomer. These compounds may protect DNA not only by the transduction of absorbed UV energy to emission but also by antioxidant activity.

Chapter III. A behavioral assay on the western honeybee (*A. mellifera*) revealed that they perceived and preferred the fluorescence from chlorogenic acid under the sun. This result suggests that the fluorescence from anthers and pollen functions in attracting pollinators.

Chapter IV. The author found that *Pr. mume* bears two types of flowers in individuals: flowers with pollen grains that fluoresce under UV irradiation and those that do not. The fluorescent pollen abnormally developed to be sterile, likely by low temperature stress. The abnormal development and preference of honeybees for the fluorescence from sterile pollen may cause the low yield of *Pr. mume* fruit. The anthers with non-fluorescent pollen contained eight isomers of N^1,N^5,N^{10}-tri-*p*-coumaroylspermidine as major constituents; whereas the anthers with fluorescent pollen contained only a limited amount of the isomers, but relatively large amounts of the

fluorescent chlorogenic acid. These differences may be responsible for the fluorescence of the sterile pollen. The photoisomerization of N^1,N^5,N^{10}-tri-*p*-coumaroylspermidine may have occurred *in vivo* since the anthers and pollen are exposed to the sunlight.

Chapter V. N^1,N^5,N^{10}-Tri-*p*-coumaroylspermidine isomers showed an unusual photoequilibrium ratio, wherein the (*ZZZ*)-isomer predominated over the (*EEE*)-isomer. NMR spectroscopy, NOESY, and *ab initio* molecular orbital calculations indicated that multiple intramolecular CH/π, T-shaped π/π, and OH...O=C hydrogen bonding interactions can provide the unusual thermodynamic stability of the (*ZZZ*)-isomer.