

Chapter 1

Significance of Single-Pollen Genotyping in Ecological Research

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1.1 Introduction

Pollination – the transfer of pollen from the anther to the stigma – results in genetic exchange, recombination, and successful reproduction in plants; therefore, it is one of the most important processes that facilitate sexual reproduction in angiosperms and gymnosperms. Because pollen grains are extremely small, direct observation of pollination is impossible, and this limitation imposes constraints on the conduct of pollination studies. However, significant advances in molecular biology techniques in the past two decades have facilitated the analysis of even small amounts of DNA, such as that from a single pollen grain. In this volume, we study the significance and application of single-pollen genotyping in ecological research.

1.2 Pollination Methods Have Diversified During the Past 130 Million Years

Pollen grains develop from microspores, which are formed through meiosis in the stamen (male reproductive organ) and are involved in sexual reproduction in gymnosperms and angiosperms. In animals, mosses, ferns, and several gymnosperms, the sperms transfer genes to the next generation, whereas in angiosperms and most gymnosperms, the sperm nuclei pass on the genes during the process of fertilization. This difference may be attributed to plant adaptation to dry environments, as the male gametes of angiosperms have lost the ability to move using flagella. Therefore, plants were required to develop methods to transfer pollen grains from the anther to the stigma. Pollen grains were transferred by wind in most gymnosperms, which dominated and flourished in terrestrial ecosystems more than 100 million years ago. However, various methods of transfer of microspores or pollen grains in plants have evolved over the years. Various kinds of organisms are known to enhance pollination or fertilization efficiency in organisms other than angiosperms, such as gymnosperms (e.g., Norstog and Nicholls 1997) and mosses (Cronberg et al. 2006). Angiosperms appeared around 130 million years ago (Soltis et al. 2005), and they rapidly spread to various environments and evolved tremendous adaptations to fulfill pollination. Therefore, we can observe a variety of pollination modes in nature, including pollination by wind (anemophily), water (hydrophily), birds (ornithophily), mammals (zoophily), and insects (entomophily). Among these, entomophily may be one of the most diverse methods of pollination, and the ecological and evolutionary success of angiosperms is largely attributable to their coevolution with advanced orders of insects, such as Diptera, Lepidoptera, and Hymenoptera, over the past 100 million years (Schoonhoven et al. 2005). In the first stage of entomophily, insects feed on tissues, secretions, and pollen grains of plants. When the insects that are carrying pollen grains move from one flower to another, they incidentally transfer the pollen grains to the other flower, thereby facilitating cross-pollination and promoting plant fitness. The more attractive the flowers and rewards of plants became, the more frequently they were visited by pollinators and thus enjoyed greater fitness.

Presently, we can observe a wide range of specialization in the relationships between the pollinator and flowering plants. Many plants attract a variety of pollinators, whereas some plants attract only specific pollinators. Each type of relationship has its advantages and disadvantages. The former plants have the advantage of frequent visits from pollinators, but the disadvantage is that their pollen grains will not necessarily be transferred to the stigma of the same plant species. The latter plants attract a narrow range of pollinators, thereby ensuring pollen transfer to the stigma of the same plant species. However, the stronger dependence on these pollinators might cause some vulnerabilities to environmental variation.

1.3 Importance of Studying Pollen Movement

Several studies have been conducted to elucidate the relationships among flower characteristics (morphology, flowering time, duration, etc.), pollinator attraction, and plant fitness. In addition to the findings of these studies, information on the genetic traits of each pollen grain will facilitate better understanding of ecology and evolution of angiosperms. However, to date, few studies have been performed on this aspect. Gene flow is a spatial movement of alleles, and a large proportion of gene flow in plants is achieved by pollen transfer. Therefore, analysis of the patterns and amount of pollen transfer is important for studying the many fundamental aspects needed to understand the regeneration process of a plant population, the amount of gene exchange between individuals and communities, and the degree of genetic isolation in a population. Human impact (habitat loss or degradation, overexploitation, usage of agrochemicals, global warming, etc.) will result in reduction or changes in the number of pollinators. The consequent changes in pollen movement can be used as an indicator to evaluate the effects of human activities, such as forest degradation or fragmentation, on plant communities.

Pollination is also important in applied aspects because it is a requisite for development of fruit- and seed-sets, thereby ensuring an optimal and a sustainable crop yield. Some crop plants are self-incompatible; therefore, their yield is highly dependent on pollen grains from other plant individuals. Moreover, even self-compatible plants require adequate agents to bring a sufficient amount of self-pollen. Knowledge of the pattern and amount of pollen movement is also essential for evaluating one of the risks associated with transgenic crops – escape of the transgenes into related wild species through pollen movement.

1.4 Traditional and Novel Methods to Infer Pollen Movement

Direct determination of pollen movement is complicated, and pollen transfer from the anther to the stigma has been evaluated indirectly by measuring various parameters at the several stages of pollination, such as visitation frequency of pollinators,

amounts of pollen removed from the anthers and/or deposited on the stigma, and number of fruit- or seed-sets. Gene flow through pollen movement has been estimated indirectly by comparing the genotypes of putative parents and the offspring (Devlin and Ellstrand 1990; Smouse et al. 2001; Isagi et al. 2004, 2007), observing the frequency of the visitations of pollinators (Galen and Newport 1987; Herrera 1989), counting the number of pollen grains removed/deposited by pollinators (e.g., Herrera 1989; Conner et al. 1995; Muchhala and Potts 2007), and by using a fluorescent dye as a pollen analogue (Murawski and Gilbert 1986; Adler and Irwin 2006). Most of these methods provide no information on the genetic traits or diversity of the transferred pollen grains. Genetic composition will differ among pollen grains that are (1) transferred by pollinators, (2) deposited on the stigma, and (3) inherited to the cohorts of seedlings under the influence of self-incompatibility, pollen-tube competition, pollen viability, and inbreeding depression. Therefore, the pattern and amount of effective gene flow may differ from those of pollen flow.

The single-pollen genotyping technique resolves some of the aforementioned difficulties in pollination studies. This method was developed on the basis of a study conducted by Suyama et al. (1996), who sequenced the DNA of a single fossil pollen grain from Pleistocene peat. Matsuki et al. (2007) modified the method of Suyama et al. (1996) and succeeded in conducting multilocus genotyping of a single pollen grain in a polymerase chain reaction (PCR) tube (the method is described in Chap. 2). This method enabled various analyses, such as analysis of parentage, discrimination of self-pollen, and evaluation of the genetic diversity of pollen grains collected from a pollinator. In this book, we have provided the following examples of ecological analyses conducted with this novel method: pollen transfer in a plant population (Chaps. 3–6), evaluation of the functions of different pollinators (Chap. 3), analysis of the genetic traits of pollen grains obtained from pollinators (Chaps. 3, 5, and 6) and styles (Chap. 4), comparison of genetic traits between pollen grains and seeds (Chaps. 4 and 6), and effect of biological invasion during competition for pollination (Chap. 7). Single-pollen genotyping provides useful information about not only pollen grains of the biota of this age but also about those belonging to ancient times. Information from fossil samples can be used to study the processes of migration, microevolution, and the demographic histories of plants. The method for DNA analysis from fossil pollen is described in detail in Chap. 8.

By taking advantage of the haploid nature of pollen grains and the large numbers of pollen grains produced, single-pollen genotyping would enable us to do recombination analysis and construct a linkage map more easily than with conventional pedigree analysis of inbred lines from experimental crossing. The haploid genome of the pollen grains also facilitates DNA sequencing of nuclear loci. The possible applications of single-pollen genotyping in evolutionary genetic and genomic research are explained in Chap. 9.

Readers will find the examples described in each chapter interesting, and single-pollen genotyping will be useful for studies in pollen biology, reproduction biology, plant breeding, forestry, agriculture, horticulture, forensic science, conservation ecology, and other disciplines.

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