Title	Studies on ploidy-dependent temperature responses of male gametophytes in Lilium leichtlinii
Author(s)	呂，依丹
Description	この博士論文の全文は利用可能日以降に公開されます。
Degree Grantor	北海道大学
Degree Name	博士(環境科学)
Dissertation Number	甲第16734号
Issue Date	2026-03-25
DOI	https://doi.org/10.14943/doctoral.k16734
Doc URL	https://hdl.handle.net/2115/99579
Type	doctoral thesis
File Information	LYU_Yidan_abstract.pdf, 論文内容の要旨 https://creativecommons.org/licenses/by/4.0/

学　位　論　文　内　容　の　要　旨/Dissertation Abstract

博士（環境科学）　　　　氏　名/Applicant　　呂　依丹

学　位　論　文　題　名/Title of Dissertation

Studies on ploidy-dependent temperature responses of male gametophytes in *Lilium leichtlinii*
（*Lilium leichtlinii* の雄性配偶体における倍数性依存的な温度応答に関する研究）

This doctoral dissertation aims to investigate the relationship between ploidy level and the development of the male gametophyte in *Lilium leichtlinii* 'Hakugin', and to further elucidate how temperature stress influences pollen morphology, pollen tube behavior, and cell-cycle progression within pollen tubes under different ploidy backgrounds. By integrating pollen morphological measurements, reproductive performance parameters, and flow cytometry (FCM) analysis, this study systematically evaluates how ploidy affects pollen storage stability, pollen germination dynamics, and generative cell division. This study also explores the relevance of ploidy-dependent temperature responses to breeding applications and to improving reproductive and thermal resilience in *L. leichtlinii*. The plant materials used in this study included naturally diploid and colchicine-induced autotetraploid *L. leichtlinii*. Comparative analyses were performed to assess differences in pollen physiology, pollen tube structural characteristics, and regulatory mechanisms of cell-cycle progression.

In angiosperms, pollen tube elongation and generative cell division are critical steps for successful double fertilization. After landing and adhering to the stigma, mature pollen grains germinate under suitable conditions and produce a pollen tube that grows toward the ovule. During this elongation process, the generative cell undergoes one mitotic division to form two sperm cells, one of which fuses with the egg cell to form the embryo, while the other fuses with the polar nuclei to form the endosperm. Thus, the stability and timing of these processes directly determine fertilization efficiency and seed set, and are essential for reproductive success in crop species.

In recent years, temperature stress resulting from global climate change has increasingly disrupted plant reproductive processes, which are generally more temperature-sensitive than vegetative growth. Previous studies have demonstrated that pollen viability, pollen germination, pollen tube guidance, and gametophytic mitosis exhibit strong temperature dependence. High-temperature stress typically inhibits pollen development and reduces pollen tube growth rate, while low temperatures suppress metabolic activity, causing delayed pollen germination and postponed sperm cell formation. Even short periods of temperature fluctuation during the reproductive window can markedly reduce fertilization success, underscoring the importance of improving reproductive stability in breeding and cultivation systems.

Polyploidy, defined as whole-genome duplication, is widely recognized for enhancing organ size, metabolic capacity, and stress tolerance, and is therefore commonly applied in crop improvement. However, reproductive performance does not necessarily increase in proportion to ploidy level. In some cases, polyploids exhibit reduced pollination success and lower seed set, which may be associated with compromised pollen germination, abnormal pollen tube growth, or instability of generative cell division. Consequently, whether the advantages conferred by genome duplication extend to reproductive processes remains an important open question.

Although attention to the interaction between ploidy and temperature stress has increased, most existing studies focus on whole-plant performance or yield traits rather than cellular-level reproductive responses. Moreover, whether polyploid plants outperform diploids under temperature stress during reproduction remains inconclusive. This study addresses this knowledge gap by analyzing

how ploidy level interacts with optimal, low-temperature, and high-temperature conditions to influence pollen germination, pollen tube elongation, and generative cell mitosis, and by clarifying the physiological and regulatory mechanisms underlying any observed differences.

This study first evaluated long-term pollen storage strategies to secure material consistency for subsequent analyses. Four storage temperatures (room temperature, 4°C, −30°C, and −80°C) and two humidity conditions (dried vs. non-desiccated) were compared. The results demonstrated that storage at −80°C under non-desiccated conditions best maintained pollen viability in both diploid and tetraploid plants, preserving stable pollen germination capacity for up to 140 days. These findings provide a standardized storage reference and indicate that ploidy does not alter the physiological response of pollen to long-term low-temperature preservation.

This study also revealed that ploidy significantly affected pollen functional traits, based on comparative analysis. Pollen from tetraploid plants displayed a lower maximum pollen germination rate than pollen from diploid plants; however, pollen tubes from diploid plants elongated more rapidly during the early growth phase, whereas pollen tubes from diploid plants showed higher elongation rates during the later phase. With respect to cell-cycle dynamics, generative cell division initiated at approximately 9 h after germination in pollen tubes from diploid plants, whereas division was delayed until approximately 18 h in tetraploid samples; nevertheless, both ploidy levels completed mitosis by approximately 24 h. These results indicate that genome dosage and karyotype effects jointly shape ploidy-dependent reproductive characteristics.

This study further characterized ploidy-dependent reproductive responses under six temperature conditions (12°C, 17°C, 22°C, 27°C, 32°C, and 37°C), and flow cytometry-based quantification of nuclear DNA content provided supporting evidence for temperature-induced changes in generative cell division progression. Low temperatures strongly inhibited reproductive processes in both diploid and tetraploid material. Yet pollen tubes from tetraploid plants were more likely to initiate generative cell division, indicating a potential cold-tolerance advantage. Under high-temperature stress, mitosis of generative cells was markedly suppressed in pollen tubes from diploid plants, leading to cell-cycle arrest. In contrast, pollen from tetraploid plants completed mitosis even at 37°C, demonstrating a significantly greater degree of thermotolerance in the cell cycle. This enhanced tolerance may be associated with increased genome dosage, improved protein homeostasis, or modified checkpoint regulation during mitosis. These findings provide new cytological evidence supporting enhanced reproductive adaptability of polyploids under thermal extremes.

This study established a ploidy comparison system under a uniform genetic background and revealed a clear association between ploidy level and temperature response. The results demonstrate that ploidy affects not only morphological and growth traits but also the regulatory patterns of the cell cycle during reproduction, providing a verifiable basis for understanding the reproductive advantages observed in polyploid plants under environmental stress.

This conceptual shift moves the research focus beyond phenotypic comparison and toward mechanistic interpretation, offering a new theoretical direction for understanding the temperature sensitivity of reproductive processes under global climate change. As climate variability intensifies, the stability of reproductive systems will become increasingly important for food production and the horticultural industry.

The insights gained from pollen storage behavior, reproductive cell dynamics, and temperature-response patterns may serve as references for screening reproductive resilience and optimizing breeding strategies, particularly in identifying temperature-tolerant polyploid germplasm. Further in vivo and multi-omics investigations across species will help refine the regulatory framework revealed in this study.

In conclusion, this study integrates ploidy regulation with temperature-response mechanisms, providing both theoretical support and methodological foundations for temperature-resilient breeding in *Lilium* and related crops. It also offers a research direction for further understanding reproductive adaptation and evolutionary strategies in polyploid plants.