# Genome-wide association study reveals escape by delayed growth from aphid herbivory on field-grown *Arabidopsis thaliana*

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# Abstract

Growing number of ecological studies have shown that plant developmental and phenological traits greatly contribute to shaping field herbivory. To reveal genetic bases underlying aphid herbivory, we conducted a genome-wide association study (GWAS) of aphid abundance in a field population of *Arabidopsis thaliana*. Out of a single significant peak on the third chromosome, we isolated *NAME OF THE GENE* (*NOG*) that encoded a ribosomal gene (AT3G13882). Knockout mutants of *nog* exhibited slower growth and ca. x-days later flowering than Col-0. Our laboratory bioassay further showed that a colony of the mustard aphid *Lipaphis erysimi* was difficult to establish on *nog* due to its small rosette size. These findings suggest that side effects of life-history traits on biotic interaction play a more critical role *in natura* than currently appreciated.

# Introduction

Plants are attacked by herbivores across their life cycles in natural environments. While chemical and physical traits have long been a main focus of anti-herbivore defense [1], plant life-history traits also account for herbivory in field environments [2, 3]. For example, phenological shifts can allow plants to escape from seasonal herbivory [4, 5]. Plants’ visibility for herbivores, namely plant apparency [6], often changes from vegetative to reproductive phase, which alters the risk of herbivore attacks across plant ontogeny [3, 7]. By focusing on intraspecific variation within a plant species, several studies have shown that plant apparency shapes heritable variation in herbivory among plant genotypes [8–10], though key genes remain unknown.

Genome-wide association study (GWAS) is increasingly recognized as a powerful approach to dissect the genetic architecture of ecologically important traits [11, 12] and identify novel genes from natural phenotypic variation [13, 14]. However, field studies have suggested that controlled laboratory conditions unlikely reflect outdoor environments where interspecific interactions typically occur [15, 16], highlighting the importance of *in natura* study on gene functions [17–20]. For *in natura* understanding of interspecific interactions, it is necessary to conduct GWAS under field conditions.

*Arabidopsis thaliana* is the model plant species distributed in Europe and naturalized around the world. While *A. thaliana* usually bloom in spring after over-wintering, some cohorts have overlapped life cycles from spring to autumn [20]. When plants emerge from late spring to early summer, they are threatened by various herbivores, such as aphids and beetles [10, 23]. Of the diverse insect herbivores, aphids are a major herbivore that can exert selection across a natural distribution range of *A. thaliana* [24]. Because aphids often suck phloem saps from flowering stems, we hypothesized that plant life-history traits may play a key role in aphid colonization in the field.

In this study, we discovered a new locus *NAME OF THE GENE* (*NOG*) by using GWAS of aphid herbivory on field-grown *A. thaliana*. To conduct GWAS, we first recorded aphid abundance on 196 *A. thaliana* accessions grown in a field site of Zurich, Switzerland. To validate candidate genes, we then cultivated and released the mustard aphid *Lipaphis erysimi* on *A. thaliana* mutants. The field-based discovery of *NOG* will gain genetic insights into plant apparency on insect herbivory *in natura*.

# Materials & Methods

## Field GWAS

### Plants

We selected 196 accessions from RegMap [25] and 1001 Genomes [26] projects, most of which were overlapped with previous GWAS of biotic interaction [27]. The list of accessions are available in Table S1 alongside their phenotypes recorded in this study.

### Field experiments

Our field survey was conducted in the Irchel-Campus of the University of Zurich….

### Data analysis

GWAS was performed using the GWA-portal website (<https://gwas.gmi.oeaw.ac.at>) [28]. Target phenotypes were the total number of *Lipaphis erysimi* and *Brevicoryne brassicae* per plant; or presence (1) or absense (0) of bolting. The imputed fullsequence dataset were chosen as SNP data for the 196 accessions. Pseudo-heritability was calculated for each target phenotype before association mapping. Accelerated mixed models were used for association mapping with a correction of kinship structure. The genome-wide significance level was given at with Bonferroni correction of multiple testing. The number of aphids was log()-transformed to improve normality. Input phenotype data are available as a supplementary material (Table S1). After the association mapping, candidate genes were searched within ca. 10 kb near a focal SNP. To estimate gene functions from gene expression information, we used the Arabidopsis eFP browser [29], 1001 transcriptome data [30], and ATTED-II coexpression database [31] (<https://atted.jp/>).

Gene ontology enrichment analysis was performed for the results of GWA-portal. xxxx. To deal with statistical non-independence of SNPs, we employed an unbiased GO analysis implemented in the Gowinda program. The cut-off value of minor allele frequency (MAF) was set at 0.025 for the entire SNP set, and SNPs having the top 1% value of -log10() were tested against the entire set. The latest gene feature format (GFF) and GO slim annotation of *A. thaliana* were downloaded from The Arabidopsis Information Resource (TAIR) (<https://www.arabidopsis.org/>). The Gowinda program was run with the key arguments of –simulations 1000000 –min-significance 1 –gene-definition updownstream1000 –mode gene –min-genes 2.

## Mutant analysis

### Plants

T-DNA insertion lines of *A. thaliana* were obtained from the Nottingham Arabidopsis Stock Centre (NASC) (<https://arabidopsis.info/>).

### Bioassay

# Results & Discussion

Bolting: pseudo h2 = near to 1.0  
log\_BbLe\_max: pseudo h2 = 0.7  
log\_BbLe\_sum: pseudo h2 = 0.71

Indirect association with herbivory through growth rate Although xxx, the difficulty in plant GWAS (pseudo-correlation, population structure). To solve this issue, xxx.

This study dealt with virtual data and thereby provided no biological significance. Citation Style Language (.csl) is usually available at journal’s page or other repository (<https://github.com/citation-style-language/styles>). If you need a more complex format, HTML or even LaTeX may be required. Try to minimize manual handling until you give up, and export to .docx lastly.

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