# Genome-wide association study highlights escape by delayed growth from aphid herbivory on in 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*. Focusing on a single significant peak on the third chromosome, we discovered several growth-related genes and a new locus *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 [20–22]. 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 occurring 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.

To reveal the genetic architecture of aphid herbivory, we combined GWAS and mutant analysis in *A. thaliana*. We first conducted GWAS of aphid abundance on 196 *A. thaliana* accessions grown in a field site of Zurich, Switzerland. Near a GWAS peak, we discovered a new locus *NAME OF THE GENE* (*NOG*) as well as several known genes involved in delayed growth. To further validate the 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 (*Yasu will fill in this section*).

### 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 a supplementary material.

### 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. 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 (*Please fill in this section*)

### Plants

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

Please describe  
- which lines did you order?  
- how did you confirm the knockout mutations?  
- how many times did you backcross?

### Bioassay

Please describe  
- what species of aphids did you use?  
- how did you cultivate plants?  
- how did you construct the experimental arena?  
- how did you record the number of aphids?

# Results

## Field GWAS of the aphid abundance (*Yasu will fill in this section*)

Bolting: pseudo h2 = near to 1.0; log\_BbLe\_max: pseudo h2 = 0.7; log\_BbLe\_sum: pseudo h2 = 0.71. We found a significant peak for the aphid abundance (Fig. [1](#fig:ManPlot)).

## Mutant analysis in a laboratory (*Please fill in this section*)

### Growth and flowering of *A. thaliana* mutants

### Aphid attachment experiments

# Discussion (not yet written, combined Results & Discussion possible)

possible topics  
- ecological discussion on the genetic basis of phenological escape from herbivory  
- side effects of ribosomal genes on plant growth (Takashi & Hiro’s papers)  
- caveats on other candidates near the GWAS peak  
- summary or concluding paragraph

# Tables & Figures

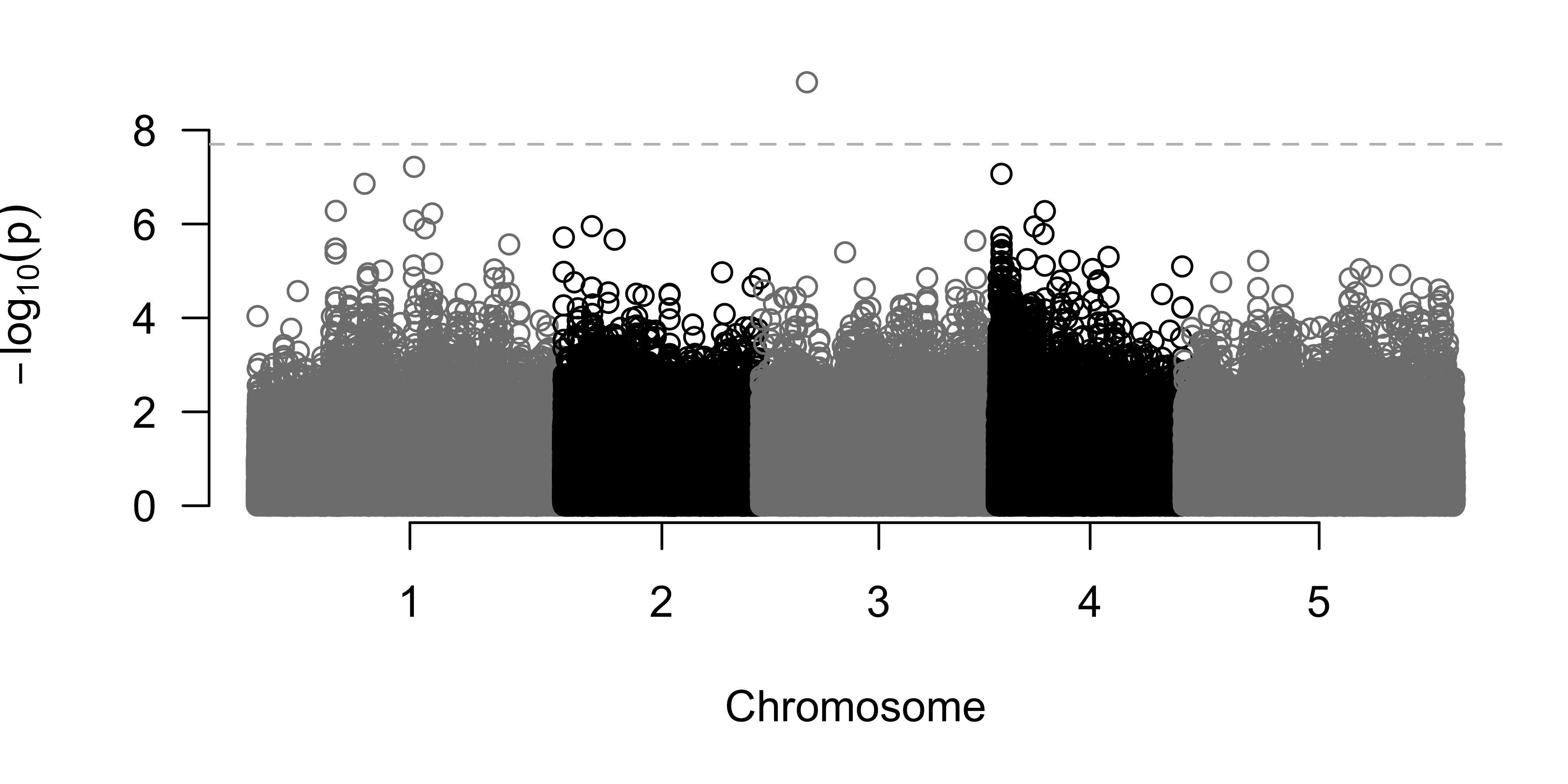


Figure 1. GWAS Manhattan plot of the aphid abundance. Horizontal dashed line indicates the genome-wide Bonferroni threshold at

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