Life History and Tolerance and Resistance against Herbivores in Natural Populations of Arabidopsis thaliana

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Abstract


In this thesis, I combined observational studies with field and greenhouse experiments to examine selection on life history traits and variation in tolerance and resistance against herbivores in natural populations of the annual herb Arabidopsis thaliana in its native range. I investigated (1) phenotypic selection on flowering time and plant size, (2) the effects of timing of germination on plant fitness, (3) the effect of leaf damage on seed production, and (4) correlations between resistance against a specialist and a generalist insect herbivore.

In all three study populations, flowering time was negatively related to plant fitness, but in only one of the populations, significant selection on flowering time was detected when controlling for size prior to the flowering season. The results show that correlations between flowering time and plant fecundity may be confounded by variation in plant size prior to the reproductive season.

A field experiment detected conflicting selection on germination time: Early germination was associated with low seedling survival, but also with large leaf rosette before winter and high survival and fecundity among established plants. The results suggest that low survival among early germinating seeds is the main force opposing the evolution of earlier germination, and that the optimal timing of germination should vary in space and time as a function of the relative strength of selection acting during different life-history stages.

Experimental leaf damage demonstrated that tolerance to damage was lowest among vegetative plants early in the season, and highest among flowering plants later in the season. Given similar damage levels, leaf herbivores feeding on plants before flowering should thus exert stronger selection on defence traits than those feeding on plants during flowering.

Resistance against larval feeding by the specialist Plutella xylostella was negatively correlated with resistance against larval feeding by the generalist Mamestra brassicae and with resistance against oviposition by P. xylostella when variation in resistance was examined within and among two Swedish and two Italian A. thaliana populations. The results suggest that negative correlations between resistance against different herbivores and different life-history stages of herbivores may contribute to the maintenance of genetic variation in resistance.

Keywords: Arabidopsis thaliana, life history evolution, flowering time, germination, phenology, plant-herbivore interactions, natural selection, tolerance to leaf damage, resistance to herbivory, Plutella xylostella, Mamestra brassicae

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To all who believed in, stood by, and encouraged me
----- with respect and consideration
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I  Akiyama, R. and Å gren, J. Selection on flowering time in three natural populations of *Arabidopsis thaliana*. (Manuscript)

II  Akiyama, R. and Å gren, J. Conflicting selection on the timing of germination in a natural population of *Arabidopsis thaliana*. (Manuscript)

III  Akiyama, R. and Å gren, J. Magnitude and timing of leaf damage affect seed production in a natural population of *Arabidopsis thaliana* (Brassicaceae). (Submitted manuscript)

IV  Akiyama, R., Noack, S., and Å gren, J. Genetic variation in leaf morphology and resistance against specialist and generalist insect herbivores in natural populations of *Arabidopsis thaliana*. (Manuscript)
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Introduction

Living organisms show an extraordinary diversity of life histories. To understand the evolution of this diversity requires that the functional and adaptive significance of traits influencing allocation patterns, phenology, and interspecific interactions are examined. In this thesis, I explore the consequences of variation in the timing of flowering and germination, and the effects of interactions with herbivores in natural populations of the annual herb *Arabidopsis thaliana*.

Life history theory has been used to predict the evolution of life-histories under different environmental conditions (Stearns 1992, Roff 2002). Central in life-history theory is the ideas of trade-offs. Because resources are limited, organisms cannot maximise all functions. As a result, high allocation to one function, say reproduction, is expected to reduce resources for other functions such as maintenance and growth. Increased allocation to one function is expected only to the extent that the benefit in terms of increased fitness exceeds the costs, and realised life-histories are expected to reflect compromises.

Expected trade-offs are not always observed when allocation to different functions have been estimated in natural populations. There can be at least a couple of reasons for this. First, more than two functions may often be involved in trade-offs and negative correlations are not necessarily observed between all pairs of functions. Second, if variation in resource availability is sufficiently large within a population, it may overwhelm an underlying negative correlation between allocations to different functions.

A major challenge in evolutionary ecology is to identify the important trade-offs shaping the evolution of life-histories, and to determine under which conditions trade-offs are apparent as negative correlations between allocations to different functions and when they are hidden by variation in overall resource availability among members of the population.

Life history evolution in plants

Flowering time is expected to be under stabilising selection because of the trade-off between size and age of reproduction (Stearns 1992, Roff 2002). The longer the pre-reproductive period, the more resources can be accumulated that subsequently can be allocated to seed production, but the greater
the risk of mortality before reproduction (Mitchell-Olds 1986, Metcalf and Mitchell-Olds 2009). In annual plants growing in seasonal environments, the duration of the season favourable for flowering and fruit production constrains the flowering schedule because of its effect on plant survival, and should therefore influence selection on flowering time. Stabilising selection on flowering time is generally expected, but it has been documented in rather few studies (e.g., Franke et al. 2006). A recent meta-analysis indicated that selection for earlier flowering is common (Munguía-Rosas et al. 2011). However, because within populations plant size tends to be correlated with flowering start with large plants beginning to flower earlier than small plants (Munguía-Rosas et al. 2011), it is not clear to what extent the reported trend reflects direct selection on flowering time or effects of variation in plant size and directional selection for larger plants.

A positive correlation between flowering date and size at flowering is expected from considerations of resource allocation (Mitchell-Olds 1996), but if variation in resource acquisition is sufficiently large the direction of this correlation may be reversed, as expected for resource allocation trade-offs in general (Reznick 1992, King et al. 2010). Variation in size at the onset of the season favourable for reproduction may result in within-population differences in optimal flowering time both because size influences mortality risk (Burd et al. 2006), and because limited time available for reproduction may represent a stronger constraint on flowering time for large than for small plants. If the risk of mortality decreases with plant size, optimal flowering time may be positively related to size at the beginning of the reproductive period. On the other hand, if it takes longer for large plants to transform accumulated resources to seed production than it does for small plants, this may result in an earlier optimal timing of flowering among large plants. Flowering time has been found to be phenotypically correlated with measures of plant size prior to the flowering season in several species (Rathcke and Lacey 1985, Ollerton and Lack 1998, Munguía-Rosas et al. 2011), but few studies have examined whether plant size influences selection on flowering time, i.e., whether plant size prior to the flowering season and flowering time are subject to correlational selection (but see Kelly 1992, Donohue et al. 2000).

In seasonal environments, the timing of seed germination may be subject to conflicting selection through early survival vs. survival later in life cycle and fecundity. Early germination may increase the risk of mortality during establishment, but should provide a competitive advantage and a longer period available for vegetative growth and reproduction (Verdú and Traveset 2005, Donohue et al. 2010). The latter should be advantageous because large size is often positively correlated with both survival (Regehr and Bazzaz 1979, Cook 1980, Biere 1991, Stratton 1992) and fecundity (Solbrig 1981, Kingsolver and Pfennig 2004). We can thus expect selection through surviv-
al during the establishment phase to favour later germination than selection through fecundity does.

Although an optimal intermediate germination time can be expected in many situations, stabilising selection on the timing of germination has been documented in only a few cases. Instead, most observational studies indicated selection for early germination (Verdú and Traveset 2005, Donohue et al. 2010; but see Baskin and Baskin 1972, Kelly and Levin 1997). The rarity of documented cases of stabilising selection may be due to incomplete sampling of the true variation in germination timing, but also to limited variation in timing of germination in natural populations, as would be expected if natural selection has removed genotypes with extreme values within a given site (Donohue et al. 2010). Phenotypic (Boquet and Clawson 2009) or genetic (Donohue et al. 2005, Huang et al. 2010) manipulation can be used to increase the variance in timing of germination and thus provide an opportunity to characterize the fitness function more fully.

Plant-herbivore interactions

Interactions between plants and herbivores are among the most dominant species interactions in nature in that herbivores annually consume 10-15% of the plant biomass (Carmona et al. 2011). Herbivores can strongly influence plant fitness and act as agents of selection on plant traits (Simms and Rausher 1987, Simms 1992, Strauss and Agrawal 1999, Heil and Baldwin 2002, Whittstock and Gershenzon 2002, review by Geber and Griffen 2003). Herbivory may result in selection on plant traits reducing damage and increasing the ability to cope with the damage inflicted. Since herbivory is spatially and temporally variable (Marquis 1992, Thompson 1994, 1997), selection exerted by herbivores can be highly variable in space and time (Geber and Griffen 2003).

Plant defence against herbivory can be categorised into tolerance and resistance. Tolerance is the ability to maintain fitness in the face of damage (Núñez-Farfán et al. 2007) and can be quantified by the slope of the relationship between damage and plant fitness (Tiffin and Rausher 1999). Resistance can be constitutive or induced in response to damage (Núñez-Farfán et al. 2007, Johnson 2011). Examples of traits considered to confer resistance include secondary metabolites such as glucosinolates and alkaloids, and morphological structures such as trichomes (hairs) on the leaf surface. Common indices of resistance used in surveys of variation in resistance include proportion of leaf area damaged by herbivores (where less damage is interpreted as high resistance) and the number of eggs oviposited on plants (where fewer eggs is interpreted as high resistance). Tolerance and resistance are not mutually-exclusive, i.e., a plant can express both strategies (e.g. Mauricio et al. 1997, Baucom and Mauricio 2008).
Tolerance against herbivores

Tolerance to leaf damage should depend on the timing of damage and several hypotheses have been formulated to predict how tolerance changes seasonally. Some emphasise the importance of time available for recovery from damage and suggest that herbivory early in the season and early during development should be easier to compensate than leaf damage late in the season and during reproduction (Maschinski and Whitham 1981, Strauss and Agrawal 1999). Other hypotheses suggest that changes in tolerance reflect differences in available resources and the extent to which plant fitness is limited by photosynthate relative to other resources (Stowe et al. 2000, Trumble et al. 1993, Tiffin 2002, Boege and Marquis 2005). Following this reasoning, it has been predicted that tolerance to leaf herbivory in annual plants should increase from the seedling stage until flowering as a result of resource accumulation before flowering (Trumble et al. 1993, Boege and Marquis 2005). When evaluating seasonal changes in the effects of leaf damage on plant fitness, it may thus be important to consider not only the timing of damage but also the life-history stage at which plants are defoliated.

When in the season leaf damage occurs may influence not only the magnitude of the fitness reduction but also the components of fitness influenced. In annual plants, the number of seeds is typically determined earlier than seed size (Marshall et al. 2005). Damage occurring early in the season is therefore predicted to affect the number of seeds more than seed size while the opposite is expected for damage late in the season when the number of seeds is already determined.

Most studies of seasonal changes in tolerance to leaf damage have been conducted in the greenhouse rather than in the field (e.g. Marshall et al. 2005, Boege et al. 2007). Tolerance to damage may vary considerably among environments and determining how leaf damage influences fitness across the season in natural plant populations remains a challenge (e.g. Maschinski and Whitham 1989, Strauss and Agrawal 1999, Hochwender et al. 2000, del-Val and Crawley 2005, Wise and Abrahamson 2007). To achieve this, manipulative field experiments combined with documentation of seasonality in herbivory are particularly useful.

Resistance against herbivores

Many plant traits may confer resistance against herbivores. Secondary metabolites such as glucosinolates have been well studied and shown to confer resistance, while studies on mechanical defence traits have been relatively few (Hanley et al. 2007). A recent meta-analysis showed that morphological and life-history traits, rather than secondary metabolites, are genetically correlated with resistance against herbivores (Carmona et al. 2011). Leaf
trichome density and leaf toughness have been found to be negatively correlated with oviposition (Handley et al. 2005, Hanley et al. 2007), larval feeding (e.g. Levin 1973, Ågren and Schemske 1993, Mauricio and Rausher 1997, Agrawal and Fishbein 2006, Hanley et al. 2007), and larval growth (Agrawal and Fishbein 2006). Increased trichome density and leaf toughness may thus be favoured by selection because they reduce damage from herbivores. However, substantial variation has been observed in trichome density and leaf toughness among and within populations. Costs of resistance have been invoked to explain the maintenance of variation in resistance traits, i.e., plants should evolve high resistance only to the extent that the benefits outweigh the costs (Whittaker and Feeny 1971, Agrawal et al. 2010). One way the cost of resistance could be expressed is through genetically based negative correlations between resistance to different herbivores and to herbivores of different life-history stages. Such negative correlations may reflect resource allocation trade-off between resistance traits effective against different herbivores (Strauss et al. 2002, Schoonhoven et al. 2005). The evolution of negative correlations between resistance to oviposition and larval feeding may be promoted by epistatic selection on traits that reduce the risk of herbivore attack and traits that reduce the quality of plant tissue to feeding herbivores (Wise et al. 2008), but may, on the other hand, be counteracted by selection on females to oviposit on plants suitable for larval development (Thompson and Pellmyr 1991, Gripenberg et al. 2010). The frequency at which negative correlations between preferences to oviposition and larval feeding occurs is an open question.

Arabidopsis thaliana

*Arabidopsis thaliana* began to be used in developmental studies in the early 20th century and was selected as a model plant for research in genetics in mid 20th century. Its short life-cycle (a few months to up to one year, depending on growing conditions and plant genotypes), ability to produce seeds through self-fertilisation, and small size made it a convenient study organism in the lab. It grew into one of the most popular model organism in many fields of plant biology including physiology, developmental biology, and molecular genetics. In the year 2000, its genome became the first plant genome to be completely sequenced by the Arabidopsis Genome Initiative (The Arabidopsis Genome Initiative 2000). Since then, much work has been done to assign functions to its 27,000 genes and the 35,000 proteins they encode. Today, in addition to scientific papers, numerous online resources are available on *A. thaliana* including the Arabidopsis Information Resource (TAIR) which maintains the most up-to-date version of its genome (http://www.arabidopsis.org/index.jsp) and the Arabidopsis Book which
compiles chapters on the various aspects of the biology of *A. thaliana* (http://www.aspb.org/publications/arabidopsis/). However, studies of the ecology of natural populations in the native range are still rare (cf. Arany et al. 2005, 2008, 2009a,b, Montesinos et al. 2009, Montesinos-Navarro et al. 2011), which limits our ability to interpret patterns of variation in traits of putative adaptive significance.

**Aims of this thesis**

The overall objective of this thesis was to explore processes influencing the evolution of life-history traits, and variation in tolerance and resistance against herbivores in natural populations of the highly selfing annual herb *Arabidopsis thaliana*.

I addressed the following questions:

1. Is flowering time subject to stabilising selection, and does optimal flowering start vary with plant size prior to the reproductive season? (I)
2. Is timing of germination subject to conflicting selection? More specifically, is early germination associated with low survival during establishment, but also with high survival later in life, and high fecundity? (II)
3. Does leaf damage early in the season reduce plant fitness more strongly than leaf damage during flowering, and do the fitness components affected by leaf damage shift across the season? (III)
4. Are there negative correlations between resistance against different insect herbivores and against different life-history stages of herbivores? Is resistance against insect herbivores related to leaf morphology? (IV)
Materials and Methods

Study species and their distributions

*The plant*

The study was performed on the annual herb *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). *Arabidopsis thaliana* is highly selfing (Abbott and Gomes 1989) and is native to Eurasia (Al-Shehbaz and O’Kane 2002). It has a wide latitudinal range, from 68°N in northern Scandinavia to 0° (Koornneef et al. 2004), and is typically found in disturbed habitats (Ratcliffe 1961, Engelmann and Purugganan 2006). The species is subject to damage from slugs and snails feeding on leaves (Harvey et al. 2007) and from insect herbivores feeding on leaves (Mauricio and Rausher 1997, Mauricio et al. 1997) and fruits (Arany et al. 2008). After the last glaciation, *A. thaliana* is thought to have colonised Scandinavia from Asia and Mediterranean Pleistocene refugia (Sharbel et al. 2000).

The study was conducted on populations from Sweden and Italy. The Swedish populations were located in the High Coast region of the province Ångermanland in central Sweden and the distance between the populations ranged from 8 to 25 km (Rödåsen 62°48´N, 18°12´E, Eden 62°53´N, 18°11´E, Fäberget 63°01´N, 18°19´E, Skuleberget 63°05´N, 18°22´E, Fig. 1). The Rödåsen population is located on a slope facing south-east, approximately 175 m above the sea level, the Eden population is in a scree slope on a south-facing cliff, the Fäberget population is on a steep slope facing south-west, and the Skuleberget population is in a scree slope on an east-facing cliff. The two Italian populations, Castelnuovo (42°07´N, 12°29´E) and Bolsena (42°39´N, 12°00´E), grow in dry meadow vegetation on steep slopes (Fig. 1). In all populations, the plants germinate in autumn, overwinter as leaf rosettes, and flower and set seeds the following spring. Observational studies were conducted in the Rödåsen, Eden, and Fäberget populations (I), and field experiments in the Rödåsen population (II, III). Greenhouse experiments were conducted at Uppsala University and included the Rödåsen and Skuleberget populations from Sweden and the Castelnuovo and Bolsena populations from Italy (IV). For the experiments in II and IV, eight maternal lines from each of four populations were used. The lines had gone through two generations of selfing in the lab (each line originating from a separate
maternal line sampled in the field) to reduce environmentally induced differences among lines and populations.

Figure 1. The locations of study populations of *Arabidopsis thaliana* in Sweden and Italy. The High Coast area in Sweden is enlarged. Field observations were conducted in the Rödåsen, Eden, and Fäberget populations (paper I) and field experiments were conducted in the Rödåsen population (papers II and III). Maternal lines originally collected from the Rödåsen, Skuleberget, Castelnuovo, and Bolsena populations were used in greenhouse experiments (paper IV).

The insect herbivores

The diamondback moth (*Plutella xylostella*, hereafter *Plutella*) is an oligophagous specialist herbivore feeding on crucifer species (*Brassicaceae*) (Sarfraz et al. 2006). Its ability to develop resistance to insecticides and to migrate great distances within short time spans has made it a pest of crucifer crops in many parts of the world (Chapman et al. 2002, Talekar and Shelton 1993). Its distribution (Hill 1987) includes the sites of the studied *A. thaliana* populations (IV), and it has been observed feeding on *A. thaliana* in the two source populations that have been most thoroughly studied (Rödåsen in Sweden and Castelnuovo in Italy; J. Ågren pers. comm.). Under optimal conditions, *Plutella* has a life cycle of about two weeks. It lays eggs singly (Akhtar and Isman 2003).

The cabbage moth (*Mamestra brassicae*, hereafter *Mamestra*) is a polyphagous generalist herbivore that has been observed to feed on more than 70
species from 22 families including both crop and non-crop species (Masaki 1980, Rojas 1999). Common hosts include species of the families Brassicaceae and Chenopodiaceae (Popova 1993). The studied A. thaliana populations (IV) are located within the range of Mamestra (Hill 1987). In the field, Mamestra mortality is high in early larval stages (Johansen 1997).

Studied traits

The functional and adaptive significance of morphological, phenological, and life history traits, and tolerance and resistance to herbivory were examined in observational and experimental studies in the field and in the greenhouse. Rosette area estimated from the rosette diameter was used as a measure of plant size in all studies. Flowering start was recorded as the day when the first flower had opened (I, II). Plant fitness was divided into two components, survival and fecundity, i.e., the number of seeds produced by reproducing plants (II, III). Fecundity was estimated as a product of the mean number of seeds per fruit multiplied with the number of fruits (I, II, III). In one experiment, also mean seed mass was determined (III). Leaf damage was scored as the proportion of leaf area removed by herbivores which was estimated by eye to the nearest 1% when 10% or less of the leaf area had been removed and to the nearest 5% for plants that had lost more than 10% of their leaf area. Three different measures of resistance against herbivores were recorded in the greenhouse experiments (IV). Resistance against oviposition by Plutella was assessed by scoring the number of eggs laid on each plant in one week, while larval performance of Plutella and Mamestra on different lines was scored as weight increase of newly hatched larvae after one week. Resistance to larval feeding was assessed by quantifying damage caused by Plutella and Mamestra. Leaf damage caused by Plutella was quantified as described above, while damage from Mamestra, which was less intense, was quantified as the proportion of leaves that had been damaged. Trichome density was quantified as the number of trichomes within a 25 mm² area of the distal central portion of the upper leaf surface, and for each plant a mean was calculated based on examination of three fully developed leaves. Leaf toughness at four and six weeks were quantified as dry mass per area of one fully developed leaf per plant (IV). Since the measurement of leaf toughness required leaf sampling of an intact plant, it was not compatible with the measurements of resistance. For this reason, separate sets of plants were grown to measure leaf toughness.
Selection on flowering start and plant size (I)

I quantified the direction and magnitude of phenotypic selection on flowering start and plant size in three populations (Rödåsen, Eden, and Fäberget) in Sweden \((N = 200-212 \text{ plants per population})\). The effects of standardised flowering start and plant size on relative fitness (seed output) were estimated with multiple regression analyses following Lande and Arnold (1983).

Effects of timing of germination on plant performance (II)

To examine how the timing of germination affects survival, growth, fecundity and overall fitness in the field, I conducted an experiment manipulating the timing of germination. Newly-germinated seedlings were transplanted to the site of the natural population in August, September, and October. In the field, three blocks were established prior to the first transplantation in August by removing the vegetation and soil in the area and replacing the top soil with sand collected locally but outside the population. Within each block, the positions of the seedlings from the eight lines with ten replicates per line per treatment (timing of germination; hereafter cohort) were completely randomised. At transplantation, the seedlings had produced only a pair of cotyledons except for few plants that had also one pair of true leaves.

Tolerance against leaf damage (III)

To examine how plant fitness is affected by the magnitude and timing of defoliation, I performed two separate field experiments. In the first experiment, plants were marked in groups of five, and within groups \((= \text{blocks})\) individual plants were randomly allocated to one of five treatments: 0\% (control), 10\%, 25\%, 50\%, or 75\% of the area of each rosette leaf removed with scissors. Damage was inflicted when most plants were about to start flowering (some had begun flowering). In the second experiment, I defoliated plants early in spring before flowering (18 April) or during flowering (19 May). On 18 April, approximately 60\% of the plants in the population had reached the bolting stage, while on 19 May, the great majority of plants had begun flowering. For this experiment, I therefore identified three plant categories: plants defoliated early in spring at the vegetative rosette stage, plants defoliated early in spring at the bolting stage, and plants defoliated a month later at the flowering stage. For each category, 40 triplets of plants were selected one to three days before the experimental defoliation. Triplets were arranged in blocks, with one triplet of each plant category forming a block. Each plant within a triplet was randomly assigned to one of three de-
foliation treatments: 0% (control), 25%, and 50% of the area of each rosette leaf removed with scissors.

Resistance against herbivores (IV)

*Oviposition preference of Plutella*

Variation in resistance against oviposition by *Plutella* among and within *A. thaliana* populations was quantified by exposing plants to mated female moths. The moths were released into 11 net cages each of which contained 32 four-week-old plants (one of each maternal line). Before releasing the moths, we recorded rosette size and trichome density of the experimental plants. On the sixth day after the release of the moths, the plants were removed from the cages and the number of eggs laid on each plant was counted.

*Larval feeding*

Variation within and among the *A. thaliana* populations in resistance to larval feeding was quantified by exposing plants to first instar *Plutella* or *Mamestra* larvae in two separate experiments. In each experiment, 30 larvae were introduced to 32 six-week-old plants (one of each maternal line) in each of 12 net cages. We recorded rosette size and trichome density of plants prior to the release of the larvae. The larvae were then allowed to feed for three weeks, after which the plants were removed from the cages and damage was scored. Neither *Plutella* nor *Mamestra* reached pupation during the experiment.

*Larval performance*

Variation in resistance to larval feeding among the 32 maternal lines of *A. thaliana* was further quantified by examining the performance of *Plutella* and *Mamestra* on six-week-old plants. We recorded plant size and trichome density prior to releasing the insects on plants. Newly hatched larvae were weighed before being transferred to experimental plants. Each larva was placed on a separate undamaged plant, which was then put into a cylindrical net cage (30 cm height × 12 cm diameter). The maternal lines were completely randomised within replicates consisting of 32 plants (= block). Because the number of cages was limited, a set consisting of four blocks was performed before starting the next. We had three such sets for *Plutella* and two for *Mamestra*. Each set lasted for seven days.
Results and Discussion

Correlational selection on flowering start and plant size prior to reproduction (I)

In all three populations, flowering time was negatively related to plant fitness, but in only one of the populations, significant selection on flowering time was detected when controlling for size prior to the flowering season. In this population, i.e., Rödåsen, there was selection for earlier flowering and the effect of flowering time on plant fitness was particularly strong among large plants (significant correlational selection, Fig. 2). The results suggest that correlations between flowering start and plant fecundity may often be confounded by variation in plant size prior to the reproductive season.

Figure 2. Contour plots depicting the relationship between relative fitness and standardised start of flowering and standardised rosette size in spring in the Rödåsen, Eden, and Fäberget populations of Arabidopsis thaliana.

Contrary to expectation, there was no evidence of stabilising selection on flowering time whether or not variation in initial size was controlled for. Several factors may contribute to an apparent lack of stabilising selection on traits expected to have an intermediate optimal value. First, relative fitness is typically quantified based on variation in a component of fitness rather than overall fitness. The fitness estimate in the present study did not include early survival and this could be problematic if there is a trade-off between fecundity and survival (cf. Metcalf and Mitchell-Ol 2009, Mojica and Kelly 2010). In the present study, survival from mid April to fruit maturation was
very high in the Eden and Fäberget populations (≥ 98%) and in the Rödåsen population, survival was 84% and the likelihood of survival increased with plant size in April. There was thus no evidence of conflicting selection on size in spring through survival and fecundity. This does not rule out a negative association between initial size in spring and survival earlier in the life cycle and plants should be followed from germination to explore the possibility of such conflicting selection. Second, stabilising selection may be difficult to detect if there is insufficient phenotypic variation in the trait of interest. Third, directional selection for earlier flowering may reflect a recent change in environmental factors influencing selection on flowering time.

Conflicting selection on the timing of germination (II)

There was a conflicting selection on the timing of germination through survival during establishment and through other components of fitness. As expected, early germination was associated with low survival during establishment, but with high survival later in the life-cycle (Fig. 3a) and high fecundity (Fig. 3b). In the year of study, the advantage of early germination outweighed the disadvantage and selection favoured early germination (Fig. 3c).

Size before winter varied among cohorts, and the associated differences in winter and spring survival, flowering time, and fecundity are consistent with the common observation of survival and fecundity being positively related to plant size (e.g., Regehr and Bazzaz 1979, Solbrig 1981, Stratton 1992, Donohue 2002). In the study population (paper I), and in many other natural populations of annual plants (Rathcke and Lacey 1985, Munguía-Rosas et al. 2011), size at flowering is negatively correlated with day of first flower, which apparently contradicts the expectation of a trade-off between size and age at reproduction (cf. Mitchell-Olds 1996). The results suggest that differences in the timing of germination contribute to the development of size hierarchies, variation in flowering time, and the apparent absence of a trade-off between age and size at reproduction.
Figure 3. The effects of the timing of germination on survival, fecundity, and total fitness of Arabidopsis thaliana. (a) Least-square means for the proportion of total number of plants survived per line at different timings of germination are shown for autumn, winter, spring, and the entire life-cycle (total). (b) Least-square means for ln (number of seeds per reproductive plant) ± S.E. (c) Least-square means for ln (number of seeds per seedling) ± S.E. Different letters in the figures indicate statistically significant differences in means based on Tukey’s HSD test.

Magnitude and timing of damage influence plant fitness (III)

Both the extent and timing of leaf damage influenced the fitness of A. thaliana under field conditions. The detrimental effects of defoliation on the number of seeds produced and seed size tended to increase with increasing damage, and defoliation of vegetative plants early in the season reduced seed production more strongly than did defoliation of bolting plants at the same time and defoliation of flowering plants a month later (Figs. 4a and b).
This suggests that the plants defoliated at the vegetative rosette stage were more limited by photosynthates at the time of damage than the other plant categories (cf. Wise and Abrahamson 2007). Differences among stages in tolerance to damage were not correlated with seasonal shifts in the risk of herbivory in the study population. Instead, the seasonal shift in tolerance may be related to changes in resource status (cf. Stowe et al. 2000, Hochwender et al. 2000). Plants may be particularly vulnerable to damage early in the season because their stored resources are limited and leaf damage may affect the production of new leaves and meristems in the rosette, and thus future productivity.

![Graph](image)

**Figure 4.** Effects of defoliation and plant category on the number and size of seeds. (a) log (number of seeds) and (b) log (mean seed mass [mg]) of *Arabidopsis thaliana* (least square means ± S.E.). Different letters indicate statistically significant differences in means based on Tukey’s HSD test performed separately by plant category (vegetative rosette defoliated early in the season, bolting plant defoliated early in the season, or flowering plant defoliated a month later).

The results only partly supported the hypothesis that fitness components which respond to damage shift along ontogeny from the number of seeds to seed mass. The reduction in seed production caused by defoliation in mid April was larger than the reduction following defoliation a month later, but the effect of defoliation on seed mass did not increase seasonally. Similarly, in a growth-room experiment, defoliation of the annual herb *Plantago arista-
ta before flowering reduced the number of seeds more strongly than defoliation after flowering, while no such ontogenetic shift in the negative effect of defoliation on mean seed mass was observed (Horton and Lacey 1994). In contrast, little evidence of shifts in the relative magnitude of effects on different components of reproduction was detected in a greenhouse study of *Sesbania macrocarpa* and *S. vesicaria* (Marshall et al. 2005). Taken together, seasonal changes in the relative importance of effects of defoliation on different components of reproductive output have not been detected in all species studied, and when they have been observed, they have not involved changes in the magnitude of effects on all individual components.

Genetic variation in leaf morphology and resistance to herbivory (IV)

Density of leaf trichomes, rosette area and resistance against oviposition and larval feeding of *Plutella* and *Mamestra* varied among population and among maternal lines within populations. Significant among-line variation in resistance against larval feeding by *Plutella* and *Mamestra*, and oviposition by *Plutella* indicated that at least some of the study populations have the ability to respond evolutionary to selection on traits that influence damage from herbivores, while the negative correlations between resistance against larval feeding by the two herbivores (Fig. 5), and between *Plutella* oviposition and *Plutella* larval feeding suggest that traits conferring resistance do not evolve independently.

Oviposition by *Plutella* was negatively correlated with larval feeding preference by the same species, suggesting that genotypes attractive to ovipositing females are less attractive to feeding larvae. The results suggest that ovipositing females and feeding larvae respond differently to variation in the same cue or use different cues for identifying suitable host plants.

Leaf trichome density could explain some of the variation in larval feeding by *Mamestra*. The results are consistent with previous reports indicating that leaf trichomes contribute to resistance against insect herbivory in *A. thaliana* (Mauricio 1998, Handley et al. 2005), and other species in the Brassicaceae (Ågren and Schemske 1993, Løe et al. 2007, Sletvold et al. 2010).
Figure 5. Maternal-line mean damage by *Plutella* larvae in one experiment plotted against mean damage by *Mamestra* larvae in another experiment.

Conclusions

In this thesis, I examined the functional and adaptive significance of variation in life history, and tolerance and resistance to herbivory in natural populations of *A. thaliana* in the native range. The studies revealed strong correlations between germination time, flowering time, and plant fitness. They also documented the fitness consequences of leaf damage in the field, and shed light on opportunities for the evolution of increased resistance to a specialist and a generalist insect herbivore.

A field study conducted in three populations indicated that the onset of flowering was subject to natural selection, but also that correlations between flowering time and plant fecundity may often be confounded by variation in plant size prior to reproduction.

A field experiment revealed conflicting selection on the timing of germination. The net selection on timing of germination is likely to vary temporally and spatially depending on environmental factors influencing survival and growth.

Defoliation experiments conducted in the field showed that leaf damage may significantly reduce the fitness of *A. thaliana* and suggests that given similar damage levels, leaf herbivores feeding on plants early in the season should exert stronger selection on resistance traits than leaf herbivores feeding on plants later in the season. The fitness consequences of herbivory critically depend on the seasonal timing of damage also in perennial plant species (e.g., del-Val and Crawley 2005, Garcia and Ehrlén 2002, Knight 2003). There is thus ample evidence that a comprehensive understanding of the effects of leaf herbivory on the numerical dynamics and evolutionary trajec-
ories of natural plant populations requires that both the timing and magnitude of damage are considered.

The greenhouse experiments indicated that there was genetic variation in the resistance to a specialist and a generalist insect herbivore in the studied populations of *A. thaliana*, and that leaf trichomes can contribute to resistance against insect herbivores. The negative correlations between resistance against larval feeding by the two herbivores, and between *Plutella* oviposition and *Plutella* larval feeding suggest that traits conferring resistance do not evolve independently.
Summary in Swedish

Backtravens ekologi och evolution

Varför är det viktigt att studera backtravens ekologi och evolution?


Omfattande forskning har utförts kring backtravens genetik och fysiologi, men tämligen lite är känt om de ekologiska processer som formar naturliga backtravspopulationers dynamik och evolution. Det är ett problem därför den kunskapen är viktig för att kunna tolka den genetiska variation som med dagens teknik kan dokumenteras ner till DNA-nivå. I min avhandling har jag därför studerat livshistorievariation och interaktioner med växtätare i naturliga populationer av backtrav inom dess ursprungliga utbredningsområde i Europa, och då särskilt i svenska populationer nära artens nordgräns.

Hur mycket varierar blomstart och groningstidpunkt? Spelar denna variation någon roll för växtens fortplantningsförmåga?

För att kvantifiera variationen i blomstart och dess koppling till växtens fortplantningsförmåga, genomförde jag observationer i tre backtravspopulationer på Höga Kusten i Ångermanland. Jag dokumenterade när växterna började blomma och hur många frön de bildade. Jag mätte också växternas storlek i början av våren innan blomningen kommit igång, eftersom växtens storlek ofta påverkar fröproduktionen. Tidig blomstart var kopplad till hög fröproduktion i alla tre populationerna. I två av populationerna kunde det helt förklaras av att stora plantor började blomma först. I den tredje populationen, kunde effekter av både storlek och blomstart påvisas.

Hur pass väl klarar växterna bladskador?  

Varierar växternas smaklighet för växtätare? Har olika växtätande insekter samma preferenser?  

Vad vet vi nu om backtrav som vi inte visste tidigare?  
Avhandlingen visar att blomstart och groningsstidpunkt varierar och att denna variation är kopplad till skillnader i reproduktiv framgång i naturliga populationer av backtrav. Den har vidare visat att resistens mot växtätare varierar inom och mellan populationer, och att bladskador märkbart reducerar växtens reproduktiva framgång i naturliga populationer.
Avhandlingens resultat bidrar till förståelsen av de processer som format variation i adaptiva egenskaper hos backtrav, världens genetiskt mest välstuderade växt.
シロイヌナズナ野生個体群の生活史と
植食者との生物間相互作用

一年生の自殖草本シロイヌナズナ（Arabidopsis thaliana）は、20世紀を通じてモデル植物として主に実験室において遺伝学・生理学の研究対象となってきた。そして、前紀末に全ゲノム情報が解明されて以降は各遺伝子の機能の特定が進められてきた。その中で、本種の生活史、とりわけ開花時期と、昆虫や病原菌に対する抵抗性が適応的形質として特定され、その遺伝的基盤が解明されてきた。しかしながら、安定的な実験環境下ではこれらの生物の個体群の生育条件とはかけ離れている。このため、個体群の適応進化によって重要であると実験室で特定された形質が野外においてどのような生態学的意義をもつか、という点については検証が必要である。さらに、これまで遺伝学的・生理学的研究では伝統的に少数の特定の系統が用いられてきたが、他の生物種同様にシロイヌナズナ個体間には遺伝的変異が存在する。したがって、野生個体群の研究に際しては個体群間・個体群内個体間の変異を把握することが重要である。

本学位論文では、本種の自然分布域であるスウェーデンの野生個体群を主な対象に、生活史特性と、植食者との生物間相互作用についての知見を得ることを目的とした。生活史特性と植食性昆虫との生物間相互作用は、共に進化生態学的研究における主要課題でありながら、シロイヌナズナの野生分布域であるヨーロッパの野生個体群においては未解明であった。そこで、適応的形質と目される開花時期と発芽時期、植食者による食害への耐性と抵抗性に着目し、個体群内および個体群内のこれら形質の変異の程度を明らかにした。また、これら変異が生じる要因および自然選択がこれら変異に果たす役割を定量的に評価した。

生活史特性

開花時期に対する自然選択

スウェーデンの3つの野生個体群（Rödåsen、Eden、Fäberget）を対象とした観察により、開花時期・個体サイズおよびこれら2形質の相互作用がどの程度の変異を有し、またどのような自然選択を受けてるのかを明らかにした。資源と時間に対する制約という観点から最適な繁殖開始時期を議論する生活史理論（Sterans1992, Roff 2002）によると、限
された生育期間の下では、早い時期に開花する個体は繁殖終了まで十分な時間を確保できる一方、開花までに蓄積された繁殖に充てられる資源量が少ない。逆に、遅く開花する個体は繁殖に使われる資源蓄積が大きい反面、繁殖に充てられる期間が短くなるというトレード・オフの関係が成り立つ。このトレード・オフ関係を仮定すると、中間の時期に開花する個体の適応度が最大になると考えられる。つまり、開花時期に対して安定化選択（stabilising selection）が働くと予想される。一方で、シロイヌナズナ以外の一年生草本では、個体サイズと開花時期の相互作用に対して自然選択が働くことが報告されているが、この相互作用を考慮して野外で自然選択を評価した例は少ない。そこで、ここでは個体サイズ・開花時期と、適応度（fitness）の指標として種子生産数を記録し、3群の関係を検証した。その結果、予想に反して中間の開花時期に対する自然選択はいずれの個体群についても見られなかった。自然選択は、Rödåsen個体群において早い開花時期と大きい個体サイズに、他の2個体群については大きい個体サイズに対して働くしていた。これらの結果より、スウェーデンのシロイヌナズナ野生個体群では自然選択は個体サイズと開花時期の相互作用に対して働く場合があるため、野生個体群において自然選択を吟味する際は開花時期と個体サイズの両方を評価することが重要であることがわかった。また、春先以前の資源蓄積が、獲得できる適応度を左右することが示唆された。

発芽時期に対してかかる対立する選択圧

スウェーデンのシロイヌナズナは晩夏に発芽した後ロゼットの状態で越冬し、春先以降に開花し、種子生産を経て夏に枯死するという生活史をもち、発芽期間には約2ヶ月の幅がある。早くに発芽した場合は、遅くに発芽した場合と比べ、発芽直前の晩夏の乾燥により生存率が低くなる反面、定着に成功すると越冬前に資源を獲得する期間を長く確保できるため、冬以降の生存率および種子生産力ともに高く、結果として高い適応度に結びつくと予想される。つまり、発芽直後の生存率と、定着以降の生存率と種子生産力には対立する選択圧（conflicting selection）がかかるので、適応度はそれら選択圧の総和によって決定される。最大の適応度に結びつく最適な発芽時期が発芽期間の半ばであれば、発芽時期に対して安定化選択（stabilising selection）が働く可能性がある。発芽時期、選択圧、適応度の関係を検証するため、Rödåsen個体群由来の8家系由来の自殖第2世の発芽直後の苗を、発芽期間全体にまたがる形で3回にわたってスウェーデンのRödåsen個体群に移植し、個体の生存・成長・種子生産を記録した。その結果、発芽時期は予想通り対立する選択圧を受けていたが、安定化選択は受けておらず、適応度は発芽時期が早いほど高かった。生存率、種子生産力、適応度のいずれの形質においても系統間の差、すなわち遺伝的変異は検出されなかった。発芽時期に対してかかる各選択圧の相対的な寄与は、各生育段階の生存率や成長がどの程度適応度に影響するかに左右されるため、発芽中の乾燥の厳しさや冬季の冷え込みの厳しさといった環境条件に応じて変動し、それに伴って最
も高い適応度に結びつく最適な発芽時期も個体群内・個体群間・年により変動するものと考えられる。

植食者との生物間相互作用

植物と植食者の間には、植物が食害に対して耐性（tolerance、食害を受けた場合に獲得する適応度）と抵抗性（resistance、食害の受けにくさ）の2つの戦略を進化させ、植食者がその自然選択の作用因子として働くという関係が成り立つ。多くの場合、耐性と抵抗性は排他的ではない。

食害の程度と時期が耐性に与える影響

植食者による食害に対する耐性について、適応度に影響を与える要因として食害の程度と発生時期が挙げられる。一般に、食害の程度が大きいほど適応度に対する負の影響は大きくなると考えられる。一方、食害の時期については、次に述べる2説が提唱されている。すなわち、生育期間の早い時期に起こる食害ほど種子生産（適応度の指標）までの回復期間が長くなるために負の影響は小さいとする説と、逆に早い時期の食害ほど被食による損害を補うだけの資源を獲得できていない個体が多いため、負の影響は大きいとする説である。そこで、スウェーデンのRödåsen 個体群を対象に、春先と生育期間半ばの2時点において複数のレベルでロゼット葉を切除する操作実験を行い、各処理の種子生産を比較した。その結果、切除レベルが高いほど生産される種子の数は減少する傾向にあり、この傾向はとりわけ生育期間初期の食害で顕著であった。春先の個体はロゼットのみ、もしくは主茎の伸長が始まった段階であったのに対し、生育期間半ばの個体は開花後の段階であった。これらのことより、一年草のシロイヌナズナでは、生育段階の初期の個体には損害を補う資源も、新たに資源を蓄積して繁殖に充てる時間もないために相対的な食害の影響が大きくなることと考えられる。また、春先に食害する植食者の方が生育期間半ばに食害する植食者よりも強い選択圧を与えることが示唆された。

植食性昆虫に対する抵抗性と防御形質における遺伝的変異

植食性昆虫による食害が植物の適応度に影響し得ることは、食害に対する抵抗性とそれに寄与する形質に対して自然選択が働く可能性があることを示唆する。このことは、自然選択が働いた後に、抵抗性（例えば昆虫による食害の受けにくさ）と抵抗性に関与するだろう防御形質（例えば葉の表面にある毛状突起トライコームの密度や葉の厚さ）の値が一様に高くなると予想されることを意味する。しかし実際には、抵抗性、防御形質ともに個体群間・個体群内に広く変異がみられることが様々な植物について報告されている。こうした変異が維持される背景には、野生植物は多くの場合、有効な防御方法が異なる複数種の昆虫に食害されるために、限られた資源の下では総ての昆虫に対する抵抗性を獲得できないことがあろうと考えられる。異なる植食性昆虫に対する抵抗性の間に負の相関があれば、この仮説が裏付けられる。そこで、スウェーデンの
Rödäsen と Skuleberget、そしてイタリアの Castelnuovo と Bolsena 個体群について、各個体群の 8 家系由来の自殖第 2 世代を用いて植物の抵抗性と防御形質、アブラナ科の植物の代表的な害虫であるコナガ（Plutella xylostella）とヨトウガ（Mamestra brassicae）の 2 種類の植食性昆虫の嗜好性の関係を調べる温室実験を行った。その結果、葉のトライコームの密度と葉の厚さ、昆虫の嗜好性の指標である食害の程度、幼虫の成長量、産卵数のほとんどについて個体群間・個体群内に有意な変異がみられた。また、ヨトウガによる食害とコナガによる食害の程度が負の相関を、コナガによる産卵数とコナガによる食害の程度が負の相関を、それぞれ示した。さらに、葉のトライコーム密度が高いほどヨトウガの食害は少なかった。したがって、変異がみられた項目は自然選択を受ける余地があること、植食性昆虫に・異なる発達段階間の負の相関関係が食害への抵抗性的進化を制約して変異の維持に寄与していること、そして葉のトライコームが抵抗性に寄与していることが示唆された。

本研究により、主にスウェーデンのシロイヌナズナの野生個体群に関して、その生態史特性と植食者との生物間相互作用が明らかとなった。開花時期と発芽時期、植食者による食害への耐性と抵抗性には変異が存在し、開花時期と発芽時期が自然選択の対象となりうることが示された。また、対立する選択圧が発芽時に、食害の程度と時期が耐性に、植食性昆虫の種と発芽段階と葉のトライコーム密度が抵抗性に、それぞれ関与していた。これらの結果は、これまで特定系統を対象にした実験室における研究において適応進化に関与すると特定されてきた形質が、実際に野生個体群においても生態学的意義をもつことを裏付けた新たな知見である。今回野性個体群の個体をそのまま用いた観察・実験に関しては、今後、各個体がどの程度表現型可塑性を示し、また形質の変異に遺伝的要因と環境要因がどう寄与しているのかを検証することが求められる。本研究の成果は、これまで遺伝学的・生理学といったいわゆるミクロ生物学と、進化・生態学といったいわゆるマクロ生物学に分かれて研究されてきた植物の適応進化について、ミクロ・マクロを融合して総合的に研究する基盤となる情報を提供するという形で、研究分野の今後の発展に貢献するものと考えられる。
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