

Päivi Leinonen

LOCAL ADAPTATION
AND ITS GENETIC BASIS
IN *ARABIDOPSIS LYRATA*

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PÄIVI LEINONEN

**LOCAL ADAPTATION AND
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Supervised by
Professor Outi Savolainen
Professor Katri Kärkkäinen
Associate Professor David Remington

Reviewed by
Docent Irma Saloniemi
Doctor Katja Räsänen

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University of Oulu, Faculty of Science, Department of Biology, P.O. Box 3000, FI-90014

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Abstract

Local adaptation is important evolutionary process leading to adaptive population differentiation. Currently, examining its genetic basis is a major goal of evolutionary and ecological genetics. In my thesis I studied local adaptation and its genetic basis in populations of a perennial outcrossing model plant *Arabidopsis lyrata* by combining common garden experiments at the native field sites and in controlled conditions with quantitative trait locus mapping.

Estimates of fitness in the field – both at the level of multiple components as well as hierarchical total fitness – showed that populations of *A. lyrata* were locally adapted. The studied populations were also phenotypically differentiated in ecologically relevant traits. Different components of fitness were important for the advantage of the locals depending on the environment.

Local alleles were associated with high fitness in the field, suggesting that differing directional selection pressures have been involved in phenotypic differentiation. Mostly different genomic regions governed local adaptation in different environments, but the results also suggested that some of these regions could involve rarely documented fitness tradeoffs (antagonistic pleiotropy). Loci governing flowering time differentiation differed between the studied environments, highlighting the need to conduct experiments both in the wild and in controlled conditions. In contrast to most existing studies, F₂ hybrids in general had surprisingly high fitness at one study site, largely due to beneficial dominance effects at loci governing survival in that environment. In addition to nuclear genes, cytoplasmic genomes also were found to have a role in local adaptation.

Keywords: *Arabidopsis lyrata*, fitness, local adaptation, natural selection, population differentiation, quantitative traits, transplant experiment

Leinonen, Päivi, Paikallinen sopeutuminen ja sen geneettinen tausta *Arabidopsis lyrata*lla.

Oulun yliopisto, Luonnontieteellinen tiedekunta, Biologian laitos, PL 3000, 90014 Oulun yliopisto

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Tiivistelmä

Luonnonvalinta saa aikaan paikallista sopeutumista ja adaptiivista erilaistumista. Paikallisen sopeutumisen perinnöllisen taustan selvittäminen on tällä hetkellä yksi tärkeimpiä evolutiivisen ja ekologisen genetiikan tavoitteita. Tässä väitöskirjatyössä tutkin paikallista sopeutumista ja sen geneettistä taustaa monivuotisella, ristipölytteisellä mallikasvilla, idänpitkäpalolla (*Arabidopsis lyrata*). Käytin työssäni geenikartoitusta kasveilla joita kasvatettiin yhdenmukaisissa oloissa sekä populaatioiden luontaisilla kasvupaikoilla että kontrolloiduissa olosuhteissa.

Kenttäolosuhteissa arvioitu kelpoisuus osoitti idänpitkäpalkkopopulaatioiden olevan paikallisesti sopeutuneita sekä yksittäisten kelpoisuuteen vaikuttavien ominaisuuksien että hierarkkisen kokonaiskelpoisuuden tasolla. Tutkitut populaatiot olivat myös erilaistuneita ekologisesti tärkeissä ominaisuuksissa. Kelpoisuuteen vaikuttavat ominaisuudet myös poikkesivat ympäristöjen välillä.

Paikalliset alleelit olivat yhteydessä korkeaan kelpoisuuteen luonnossa, minkä perusteella voitiin päätellä erisuuntaisen luonnonvalinnan vaikuttaneen populaatioiden erilaistumiseen. Kromosomiston eri alueet olivat tärkeitä sopeutumisessa eri ympäristöihin, mutta myös joidenkin samojen genomien alueiden havaittiin mahdollisesti vaikuttavan vastakkaisesti kelpoisuuteen eri ympäristöissä. Myös kukkimisajan erilaistumiseen vaikuttavat genomien alueet poikkesivat eri ympäristöjen välillä erityisesti verrattaessa kenttäkokeita kasvatushuone- ja kasvihuonekokeisiin. Toisin kuin useimmissa tutkimuksissa on havaittu, F₂-sukupolven jälkeläistön kelpoisuus oli yllättävän korkea yhdessä kenttäkoeympäristössä. Tähän vaikuttivat kelpoisuuden kannalta suotuisat dominoivat geenivaikutukset, jotka paransivat kasvien selviytymistä kyseisessä ympäristössä. Tumassa sijaitsevien geenien lisäksi myös soluelimien perimällä havaittiin olevan yhteys paikalliseen sopeutumiseen.

Asiasanat: Arabidopsis lyrata, kelpoisuus, kvantitatiiviset ominaisuudet, luonnonvalinta, paikallinen sopeutuminen, populaatioiden erilaistuminen, siirtoistutuskoe

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Oulu, October 2011

Päivi Leinonen

Abbreviations

AFLP	amplified fragment length polymorphism
LG	linkage group
PCR	polymerase chain reaction
QTL	quantitative trait locus
SNP	single nucleotide polymorphism

List of original articles

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

- I Leinonen PH, Sandring S, Quilot B, Clauss MJ, Mitchell-Olds T, Ågren J & Savolainen O (2009) Local adaptation in European populations of *Arabidopsis lyrata* (Brassicaceae). *American Journal of Botany* 96: 1129–1137.
- II Leinonen PH, Remington DL & Savolainen O (2011) Local adaptation, phenotypic differentiation and hybrid fitness in diverged natural populations of *Arabidopsis lyrata*. *Evolution* 65: 90–107.
- III Leinonen PH, Leppälä J, Remington DL & Savolainen O (2011) Genetic basis of local adaptation and flowering time variation in *Arabidopsis lyrata*. Manuscript.
- IV Leinonen PH, Kuittinen H, Kemi U, Quilot-Turion B, Okuloff A, Baker AM, Mitchell-Olds T, Remington DL & Savolainen O (2011) Adaptation to growing season length in a perennial plant *Arabidopsis lyrata*. Manuscript.

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1 Introduction

1.1 Local adaptation

Adaptation of populations to their environments involves natural selection (Darwin 1859). When populations inhabit heterogeneous environments, selection pressures may differ spatially across the distribution range (Hedrick 2006). As a response, populations may become locally adapted or adaptive phenotypic plasticity may evolve. The conditions for the evolution of genetic differentiation depend on the selection regimes and migration rates (Dobzhansky 1937, Linhart & Grant 1996). If these conditions are not fulfilled, phenotypic plasticity may evolve in some conditions (Nicotra *et al.* 2010). However, the presence of phenotypic differentiation does not necessarily prove that it is caused by adaptation due to natural selection (Gould & Lewontin 1979). Evidence for selection has been found for many phenotypic traits (Endler 1986, Hoekstra *et al.* 2001), but population differentiation can also result from random processes such as genetic drift (Wright 1931), demographic history (Francois *et al.* 2008) or other forms of selection. The roles of genetic drift and selection in shaping phenotypic differentiation have been under much debate, as evidenced for example by the case of flower color morphs of *Linanthus parryae* (Wright 1943, Turelli *et al.* 2001, Schemske & Bierzychudek 2007).

Local adaptation can be studied using reciprocal transplant experiments, where the fitness of the studied populations is estimated and compared in native environments in the field. Highest relative fitness of the local population in each environment (Figure 1) has been used as a definition for local adaptation (Kawecki & Ebert 2004). A meta-analysis by Leimu and Fischer (2008) suggests that local adaptation satisfying this criterion has been documented only in 45% of the reported cases in plants. Lack of evidence for local adaptation in their study could not be explained by habitat characteristics, differences in life history or geographical distance, but was instead explained by small population sizes. Hereford (2009) found that close relatedness among populations was associated with a lower degree of local adaptation. Local adaptation has also been found to be stronger at greater geographic distances in salmonid fishes (Fraser *et al.* 2011). Gene flow between populations can counteract selection for local adaptation (Holt & Gomulkiewicz 1997, Lenormand 2002), but adaptive differentiation can occur even in the face of gene flow (Endler 1977). Local adaptation can yield ecological

speciation as a by-product (Sobel *et al.* 2010), and can thus, in fact, also result in decreased gene flow (Räsänen & Hendry 2008).

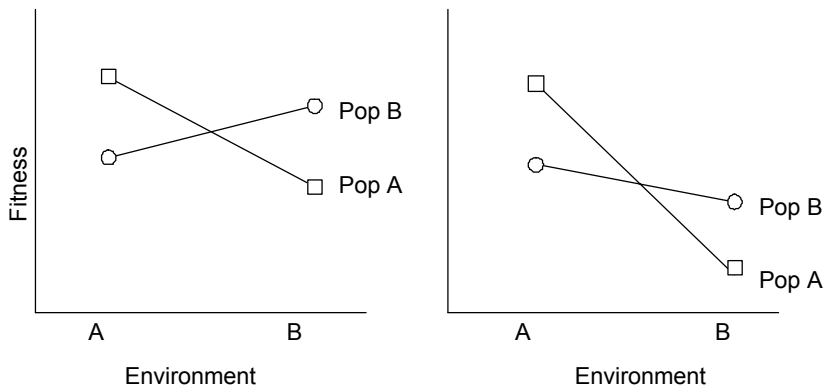


Fig. 1. Fitness differences in locally adapted populations. Hypothetical populations A and B originate from environments A and B, respectively. Figure modified from Kawecki & Ebert 2004.

1.2 The role of environmental factors in local adaptation

Environmental heterogeneity offers the opportunity for different selection pressures to lead to adaptive phenotypic differentiation. Such differentiation may occur at a large climatic scale, as for example in wild tomatoes (Nakazato *et al.* 2008) or at smaller geographical scale (Linhart & Grant 1996). For example, latitudinal clines are evidence of adaptive differentiation along climatic gradients (Savolainen *et al.* 2007). Trait correlations have been found both along latitudinal (e.g. Olsson & Ågren 2002), and longitudinal gradients, such as those in *Drosophila americana* (Wittkopp *et al.* 2011).

Differences in environments in abiotic climatic factors give rise to differential selection among plant populations. Environmental signals are important in timing of germination, for example (reviewed by Donohue *et al.* 2010). Flowering needs to occur at a favorable time for successful pollination and seed maturation (Munguía-Rosas *et al.* 2011). Plants adapt to the length of the growing season by timing the active phases of their life history to coincide with sufficiently warm parts of the season, often using photoperiod as an environmental cue (Chouard 1960, Simpson & Dean 2002). Timing of

reproduction is also important in animals (e.g. Ejsmond *et al.* 2010). Drought regimes can also favor different flowering times, as in *Brassica rapa* (Franks 2011). Evidence for the role of flowering time in local adaptation has been documented in for example monkeyflowers (Hall & Willis 2006).

1.3 Life-history evolution

Grime (1977) classified habitats of plants into different categories depending on limitations in resource availability (stress) and the amount of disturbance. In environments with low productivity and disturbance, stress-tolerant plants may be more common than in areas with high disturbance, where more resources are available. In environments with high productivity and low disturbance, competitive ability is more important. Based on this categorization, in environments where resources are scarce, such as alpine environments, perenniality is favored, whereas in environments with drought regimes, for example, a more annual life-history strategy can confer higher fitness. Further, life-history theory predicts that environments where the risk of mortality at the juvenile stage is high favor evolution of perenniality (Charnov & Schaffer 1973, Bulmer 1985, reviewed by Roff 2002). Such environments may include those of alpine plants where the growing season is short. In these areas, environmental conditions may be very harsh for successful seed development and seedling recruitment. Vegetative reproduction may then be important for the persistence of populations (Körner 2003). Populations may also diverge in their life history strategies as a result of trade-offs in allocation of resources (reviewed by Obeso 2002).

1.4 Genetic basis of adaptation

Adaptation can be based on individual genes of major effect, as in some cases of plant disease resistance (Stahl *et al.* 1999), loss of armor plates in fresh water sticklebacks (Cresko *et al.* 2004) and adaptive coloration in beach mice (Hoekstra *et al.* 2006). However, in most cases the traits conferring adaptations are typical quantitative traits: normally distributed with many genes and environment showing an influence on the phenotypic variation. Fisher (1918) first showed that the genetic basis of quantitative traits could be accounted for by the combined effects of multiple mendelian loci. He assumed that a large number of loci, each

with small effects, controlled this variation. Since then, others have shown that directional selection during adaptation to a new optimum may result in the fixation of largest effects first (Orr 1998a). Recent theoretical studies suggested that under some conditions differentiation between environments may be based on large effects at individual loci (Yeaman & Whitlock 2011). Other work has suggested that genetic differentiation between environments could be due mainly to disequilibria between loci, while differences in allelic frequencies of individual loci would have less effect (Latta 1998, LeCorre & Kremer 2003). In spite of the extensive theoretical work, the genetic architecture of local adaptation in natural populations is still poorly known. So far, for instance, there are few studies where the distribution of effect sizes for genes underlying local adaptation has been well characterized.

1.4.1 Experimental approaches: common gardens for showing genetic differences

The genetic basis of fitness differences and phenotypic differentiation can be studied with reciprocal transplant studies, which have a long tradition in plant population biology (Turesson 1922, Clausen *et al.* 1948). Plants are grown in a uniform environment at each site (in a 'common garden') to reduce environmental variation within sites, and differences between populations consist mainly of genetically-based differences. Common garden experiments in plants in the natural sites where the studied populations originate have demonstrated local adaptation (van Tienderen & van der Toorn 1991, Jordan 1992, Oyama 1994, Nagy & Rice 1997, Galloway & Fenster 2000, Geber & Eckhart 2005, Hall & Willis 2006, Sambatti & Rice 2006, Savolainen *et al.* 2007).

Experimental hybrids from controlled crosses can be used to unravel the genetic basis of adaptive differentiation (Lexer *et al.* 2003). By performing experimental crosses between populations it becomes possible to examine the genetic basis of fitness and ecological traits by comparing hybrid phenotypes to the parents and between different hybrid generations. Comparisons of hybrid fitness allow making inferences about the degree of differentiation between populations by examining the consequences of hybridization: hybrid breakdown or alternatively hybrid vigor. If population differentiation has involved additive gene action, the combination of alleles from the two populations or species should yield intermediate phenotypes compared with the parental means in later-generation hybrids. F₁ generation hybrids in particular often show evidence for

hybrid vigor, while it is more often characteristic for later-generation hybrids to show hybrid breakdown (Lynch & Walsh 1998).

Hybrid breakdown means that hybrid fitness is lower than expected and hybridization has negative consequences. Hybrid breakdown requires that non-additive gene action, dominance, underdominance and possibly epistasis also are involved. It has been suggested to be common in hybrids that have not been subjected to natural selection in the wild (Barton 2001). As a result, hybrid breakdown is expected when coevolution of nuclear genes has been important in adaptation. When beneficial epistatic interactions between nuclear and cytoplasmic genes are involved, hybrid breakdown should be seen when nuclear and cytoplasmic alleles from different populations are combined, as in the case of reproductive isolation resulting from cytonuclear incompatibilities in plants (Levin 2003, Lowry *et al.* 2008a) and in animals (e.g. Ellison & Burton 2006). Hybrid vigor instead indicates that there are beneficial effects from hybridization, also due to dominance and/or epistasis. Later-generation hybrids can also show hybrid vigor through beneficial effects of hybridization on fitness (reviewed by Arnold & Hodges 1995).

Growing the hybrids in reciprocal transplant experiments at the native field sites allows comparing fitness of the hybrids in natural conditions of each parent. If the experimental hybrids originate from a reciprocal cross between populations, it is also possible to study the roles of cytoplasmic genomes.

1.4.2 Mapping the genes

Molecular markers are needed for finding the genomic regions responsible for the adaptive differences between populations or subspecies (Paterson *et al.* 1988). The genomic regions involved in local adaptation should show evidence for fitness benefit from local alleles, and can be examined by genetic mapping methods (reviewed by Bergelson & Roux 2010). Quantitative trait locus mapping associates differences at marker loci with variation in the traits in a crossing progeny. Different crossing designs can be used depending on the aims of the mapping study. These include F_2 designs, backcrosses, and more complex crossing schemes (Lynch & Walsh 1998). When the studied species is self-compatible, recombinant inbred lines can be used, where it is possible to grow the same genotype in multiple environments.

QTL mapping is efficient for finding the differences between a given pair of parents. When the parents originate from different populations, QTL mapping can

reveal genomic areas related to phenotypic between-population differences. The accuracy of QTL mapping depends largely on recombination frequency and is limited by segregation of marker loci in the parents (Mackay *et al.* 2009). It may be difficult to distinguish closely linked loci with opposite effects (Doerge 2002). There is also a tendency of bias towards finding QTL with large effects, as closely-linked QTL in the same direction can be detected as a single large-effect QTL. When sample sizes are small, estimated effect sizes are upwardly biased due to the so called 'Beavis effect' (Barton & Keightley 2002). QTL mapping has been applied in natural environments of the populations in field studies in *Hordeum vulgare* (Verhoeven *et al.* 2004), *Avena barbata* (Gardner & Latta 2006), *Mimulus* (Hall *et al.* 2010) and in *Boechera* (Anderson *et al.* 2011a). In field conditions, detecting QTL can be more difficult as a result of increased environmental variation (Slate 2005).

Another approach is to use association mapping, where associations at the population or species level between phenotypic and genetic variation at marker loci are searched. The accuracy of association mapping depends on the level of linkage disequilibrium (LD). The pattern of LD in the genome and possible genetic structure of the mapping population may complicate the analyses, even if methods to overcome those problems have been developed (e.g. Yu *et al.* 2006, Zhao *et al.* 2007, Kang *et al.* 2008). In some cases, it may be most efficient to conduct the association analysis in individual populations, if the overall populations are highly differentiated, as for example in *Arabidopsis lyrata* (Kuittinen *et al.* 2008). Recent development of genomic tools is rapidly increasing the availability of genotypic data for many species and future efforts will involve more detailed phenotyping in different environmental conditions (Ingvarsson & Street 2011).

1.4.3 Role of cytoplasmic genomes

Several studies in plants to date have found that in addition to the nuclear genes, the cytoplasmic genomes also can have a role in local adaptation (Galloway & Fenster 2001, Campbell *et al.* 2008, Kimball *et al.* 2008, Sambatti *et al.* 2008). In fact, as many genes have moved to nucleus from the organellar genomes, there is much potential also for cytonuclear coevolution (Rand *et al.* 2004). There is also considerable coordination of gene expression between the cytoplasmic and nuclear genomes (Woodson & Chory 2008).

Evidence for the role of cytoplasmic genomes in local adaptation can be demonstrated by finding association of high fitness with the local cytoplasm. When nuclear and cytoplasmic genomes have coevolved, QTL underlying local adaptation should show high fitness from local alleles when combined with local cytoplasm.

1.4.4 Loci underlying local adaptation

In *Arabidopsis thaliana* sets of accessions have been characterized for genomewide polymorphisms (Nordborg *et al.* 2005), for phenotypic associations (Atwell *et al.* 2010) and for e.g. expression variation (Gan *et al.* 2011). Still, less is known about variation in local adaptation and their effects on fitness in nature. The few existing studies that have examined genomic areas related to local adaptation in natural field conditions, have found that different (conditionally neutral) loci are important at different sites (Verhoeven *et al.* 2004, Gardner & Latta 2006, Hall *et al.* 2010). There has been less evidence for fitness trade-offs at the same locus between sites (Hall *et al.* 2010). Similar findings have been suggested recently in a genome-wide study of *Arabidopsis thaliana* (Fournier-Level *et al.* 2011).

In plants, genes related to local adaptation can include loci involved in flowering time variation (Verhoeven *et al.* 2008, Wilczek *et al.* 2009, Salomé *et al.* 2011). In *Mimulus*, an inversion polymorphism associated with local adaptation also contributed to flowering time variation (Lowry & Willis 2010). It has been shown that the same loci that govern differentiation in flowering time – or their paralogs – can also be involved in growth cessation in perennials, which is important for survival in seasonal environments (Böhlenius *et al.* 2006, Hsu *et al.* 2011).

In perennials, adaptation to short growing season may involve differentiation in photoperiodic responses that induce formation of overwintering inflorescence meristems in the fall, which cease further development until exposed to long day length in the spring (Heide 1994). This kind of adaptation in inflorescence development after inflorescence bud formation would likely involve photoperiodic genes. Rapid development in general without differentiation in photoperiodic responses through differences in cell division rates can also be favored in environments with short growing season. In these cases, the candidate genes would not be photoperiod-related, but instead governing growth rates of organs (Johnson & Lenhard 2011).

Another way to identify loci putatively involved in adaptation is to search for signatures of selection in sequence data. As a result of recent advancement in molecular techniques, the number of such studies has been increasing (reviewed by Stapley *et al.* 2010). In *Populus*, signs of selection at photoperiod pathway genes have been reported, indicating adaptation at different latitudes (Hall *et al.* 2011). In *Arabidopsis lyrata*, sequence polymorphisms were associated with adaptation to serpentine soil (Turner *et al.* 2010). Studies including phenotypic measurements are still a valuable addition to these studies – especially when conducted both in controlled conditions and in natural field conditions (Anderson *et al.* 2011b). Studies that combine these different approaches are likely to yield a more complete understanding of genetic basis of local adaptation. Results from genetic mapping studies can be combined with functional studies for finding the causal genes that are involved (Weigel & Nordborg 2005).

1.5 *Arabidopsis lyrata* as a model for evolutionary biology

Close relatives of genetically well-known model species have emerged as important study organisms for evolutionary and ecological genetics (Stapley *et al.* 2010). *Arabidopsis lyrata* (L.) O'Kane & Al-Shehbaz (Brassicaceae) is a close relative of the annual model plant *Arabidopsis thaliana* (Mitchell-Olds 2001, Kuittinen *et al.* 2004). Molecular resources developed for *A. thaliana* have greatly facilitated genetic studies in *A. lyrata* (Clauss & Koch 2006). At the moment, a genome-wide sequence is also available (Hu *et al.* 2011).

A. lyrata is an outcrossing, herbaceous perennial plant with a disjunct distribution in the northern hemisphere in Eurasia and North America (Jalas & Suominen 1994, O'Kane & Al-Shehbaz 1997). It is currently divided as two subspecies: Eurasian *A. lyrata* ssp. *petraea* and North American *A. lyrata* ssp. *lyrata* (Al-Shehbaz & O'Kane 2002). *A. lyrata* grows in open areas with little competition with other species (Jonsell *et al.* 1995), such as in sandy riverbanks and rocky outcrops or boulders. Plants form an overwintering leaf rosette and inflorescences with white or pink flowers. Clonal reproduction has been found to be common in some populations (Gaudeul *et al.* 2007, Lundemo *et al.* 2010).

Populations of *A. lyrata* are differentiated in flowering phenology (Riihimäki & Savolainen 2004, Riihimäki *et al.* 2005, Kuittinen *et al.* 2008), response to nitrogen deposition (Vergeer *et al.* 2008), leaf morphology (Jonsell *et al.* 1995, Kärkkäinen *et al.* 2004) and trichomes (Kärkkäinen *et al.* 2004, Kivimäki *et al.* 2007). There has also been evidence for selection on flowering time and floral

display traits, and life history evolution in natural populations of *A. lyrata*, mediated by herbivores and pollinators (Sandring *et al.* 2007, Sandring & Ågren 2009, Vergeer & Kunin 2011). Recently, evidence of incipient speciation – cytoplasmic male sterility – between diverged populations of *A. lyrata* has been reported (Leppälä & Savolainen 2011). The populations are also genetically differentiated at the level of isozymes (Jonsell *et al.* 1995), microsatellites (van Treuren *et al.* 1997, Muller *et al.* 2008) and sequence variation (Balaña-Alcaide *et al.* 2006, Ross-Ibarra *et al.* 2008). Effects of post-glacial colonization have also been examined (Koch & Matschinger 2007, Muller *et al.* 2008, Ansell *et al.* 2010, Schmickl *et al.* 2010). As a result of limited gene flow between the populations occurring in a range of different climatic conditions, there is much potential for local adaptation in *A. lyrata*. Studies of natural populations that have not been subjected to growing in laboratory conditions over multiple generations are also especially valuable. *A. lyrata* is thus an attractive study organism for local adaptation and its genetic basis.

1.6 Aims of the study

This thesis aimed at answering the following questions involving populations of *A. lyrata*. First, are the studied populations locally adapted, by examining the fitness advantage of the local population in reciprocal transplant experiments both at the level of estimates of multiple fitness components as well as hierarchical total fitness? Second, are the populations differentiated in ecologically important traits such as flowering time and floral display traits and are the populations also adapted to growing season length? Third, what is the genetic basis of local adaptation? Here, I focus on three aspects of the genetics of adaptation: is local adaptation governed by conditionally neutral loci, or is there also evidence for antagonistic pleiotropy? Is there coadaptation between nuclear genes, as indicated by hybrid breakdown? Do cytoplasmic genomes contribute to local adaptation?

2 Material and methods

The material and methods are described here only briefly and in more detail in the original papers (I-IV).

2.1 Plant material

The experiments focused especially on three populations representing the northern and southern range limits of the European subspecies *A. lyrata* ssp. *petraea* and a more distantly-related population representing the North American subspecies *A. lyrata* ssp. *lyrata*. Locations of the populations are presented in Table 1. In paper I, populations from the Swedish coast were also included. To examine local adaptation and phenotypic differentiation, seeds primarily from full-sib families from controlled crosses were used to establish common garden experiments in the field, in the growth chamber and in the greenhouse. In some cases open-pollinated seeds collected in the field were also used (Papers I and IV). Each population was represented by multiple unrelated families. The common garden experiments included plants from each population and F₂ generation plants (I-IV), and also F₁ individuals (II).

Table 1. Locations of the studied *Arabidopsis lyrata* populations.

Location	Subspecies	Abbreviation	Latitude	Longitude	Altitude (m.a.s.l.)
Spiterstulen, Norway	<i>A. lyrata</i> ssp. <i>petraea</i>	'Sp'	61°38'N	8°24' E	1106
Plech, Germany	<i>A. lyrata</i> ssp. <i>petraea</i>	'Pl'	49°39'N	11°29' E	400
Mayodan, North Carolina, USA	<i>A. lyrata</i> ssp. <i>lyrata</i>	'Ma'	36°25'N	79°58' W	225

Plants from the Pl and Ma populations were crossed with the Sp population mainly for genetic mapping purposes. In the case of the between-subspecies pair Sp and Ma, the between-population F₁ and F₂ plants (between-subspecies hybrids) were also used to examine fitness effects resulting from experimental hybridization and its genetic basis.

To obtain F₂ plants for examining the genetic basis of adaptation and mapping genomic areas involved, two plants from each of the two populations Pl and Ma were crossed each with a pair of plants from the Sp population to obtain between-population F₁ plants (Figure 2). Two F₁ plants were then reciprocally crossed to obtain F₂ progeny seeds. In the cross between Sp and Ma, the parental

populations were crossed reciprocally to obtain F₁ and F₂ plants with different cytoplasmic backgrounds, enabling us to examine cytonuclear effects and interactions with the nuclear genome.

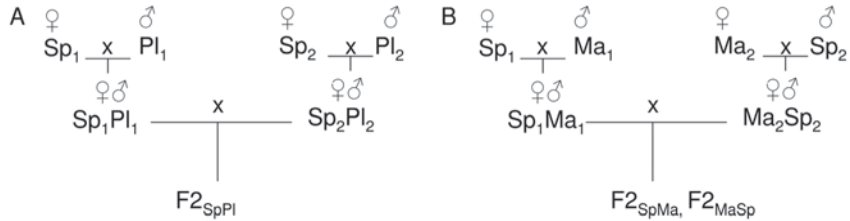


Fig. 2. Crossing design for *A. lyrata* between-population F₂ progeny for a cross between the (a) Norwegian and German and (b) Norwegian and North American population.

2.2 Study environments and experimental setup

Common garden experiments were established in the field (I-IV), in the growth chamber (IV) and in the greenhouse (III). Field experiments included reciprocal transplantations at sites representing native climatic conditions of the populations in Spiterstulen Norway (I-IV), Jena Germany (I, IV), Greensboro NC (II, III) and in Storsanden Sweden (I). There was also a common garden experiment at the experimental field at the University of Oulu in Finland (IV). Plants for all the field experiments were pre-grown in a greenhouse to improve establishment success.

Common garden experiments were established in the growth chambers and in the greenhouse at the University of Oulu in Finland between the years 2002 and 2007. Plants were grown in pots with a 1:1 mixture of peat and gravel and watered when needed. To induce flowering, the plants received a cold treatment (vernalization) in +4 °C for 5–8 weeks. In the growth chamber experiments, the plants were grown in two photoperiods: LD14:10 (light:dark) and LD20:4. In the greenhouse, the plants experienced the natural photoperiod (~22 hours of light).

In each experiment, the plants from different populations were randomized in blocks to reduce unwanted environmental effects within sites. Plants from different families were distributed evenly across blocks.

2.3 Phenotypic measurements

In the field, many phenotypic traits were measured to estimate fitness at the level of multiple components and to quantify differentiation in putatively ecologically important traits. In the beginning of the growing season at each field site, survival status of all plants was determined. Flowering start date was recorded as the day when the first flower opened. When fruits were ripening, mature fruits were collected to estimate seed production (II, III). At the end of the growing season, flowering status of each plant was recorded (flowered/did not flower), and number of inflorescences and fruits were counted, or their number was estimated at sites where large numbers of inflorescences were produced. Data was collected during 1 to 5 years depending on the site.

In the growth chamber study, the date of macroscopic appearance of inflorescence buds and open flowers was determined and the number of inflorescence shoots was counted (IV). In the greenhouse experiment, flowering start date was recorded and the number of inflorescence shoots was counted (III).

2.4 DNA isolation, genotyping and linkage maps

DNA was isolated from leaves using either FastDNA® kit (Qbiogene), DNEasy kit (Qiagen) or NucleoSpin (Macherey-Nagel). Genotyping was done using combinations of previously designed SNP, CAPS, dCAPS and microsatellite markers. All markers, PCR reactions and linkage map construction are described in Leppälä & Savolainen (2011) and in Quilot-Turion *et al.* (*submitted*).

2.5 Statistical methods

2.5.1 Fitness comparisons and ecological differentiation

Phenotypic comparisons between populations were done using likelihood ratio tests between generalized linear models (*glm* function) (I, IV), or generalized linear mixed models (*lmer* function in *lme4* package) in *R* (R Development Core Team 2009). Planting blocks were taken into account in the analyses. Binary variables were modelled as binomial traits, and count traits were modelled either as Poisson distributed traits or as normally-distributed traits with appropriate transformations. In other cases, a normal distribution was used. Normality of the residuals was visually inspected. A square root transformation was performed if it

improved normality of the residuals. If transformation did not improve normality, a nonparametric Kruskal-Wallis test or Mann-Whitney's U test was used.

To estimate hierarchical total fitness in addition to comparing separate fitness components, we also performed analyses using aster life history models in *R* (II, Geyer 2007, Shaw *et al.* 2008). Total fitness consisted of flowering conditioned on survival, and number of seeds per plant that flowered. This analysis took into account that the plants that did not survive, were not able to flower and those that did not flower were not able to produce seeds. Fitness comparisons were done using likelihood ratio tests between these models.

Fitness of the between-subspecies hybrids was compared to the parents to examine whether the hybrids show evidence for hybrid breakdown or heterosis (II). To get a robust estimate on hybrid breakdown due to epistatic gene action in the between-subspecies hybrids, additional *lmer* models with orthogonal contrasts of trait values in the parental populations and hybrids were used. Significance of cytoplasmic effects on fitness were also tested using *lmer*.

2.5.2 QTL mapping

QTL mapping was used to find genomic areas related to phenotypic between-population differentiation by examining associations between phenotypes and genetic markers. This was done using genome-wide interval mapping scans in *R/qtl* in *R* (<http://www.rqtl.org>, Broman *et al.* 2003), that allows for different distributions for phenotypes (binomial or normally distributed) as well as the use of a nonparametric method for skewed distributions (Broman & Sen 2009).

QTL mapping runs were done separately on each trait measured in each environment. To take into account the effects of cytoplasmic origin, photoperiod or block, these variables were included as additive covariates in each analysis where appropriate. To determine genomewide LOD significance threshold, we used 1000 permutations of $P = 0.05$ for each trait (Churchill & Doerge 1994). Mapping runs were done using the *scanone* function.

To find QTL showing interaction with cytoplasm or photoperiod, additional scans were performed using *scanone* by including the variable of interest both as an additive and interactive covariate. The LOD score for these interactions was obtained by subtracting LOD scores of the additive model from the additive + interactive model. Significance at the genomewide $P = 0.05$ level for interaction LOD scores was calculated using the differences between the peak LOD scores for 1000 pairs of permuted data sets obtained under both models, using the same

starting point (seed number) for both sets of runs. This was done to obtain comparable permutations under the two models. Genomic locations with significant interaction LOD scores were interpreted as QTL for significant nuclear x cytoplasm or nuclear x photoperiod interactions, depending on which covariate was used.

To test for significance of additive and dominance effects at the QTL, a custom script with *glm* in *R* was used that partitions QTL effects from outcross F_2 into additive ($2a$) and dominance (d) effects, and differences between the two heterozygous classes (i). Additional single-locus tests at genome-wide significant LOD peak locations were performed for the same trait at the other study sites or different years, and for functionally related traits in the same environment using the *fitqtl* function if there was a putative QTL peak in the corresponding region.

3 Results and discussion

3.1 Evidence for local adaptation

Results indicated evidence for local adaptation (fitness advantage of the local population in each environment) in European Sp and Pl populations from Norway and Germany (I). Local adaptation was also found between more diverged populations Sp and Ma at different continents (II). Moreover, the importance of separate components of fitness varied between environments (I, II), as documented also in *Gilia*, for example (Nagy 1997). Evidence for local adaptation was also found at the level of hierarchical total fitness between the Sp and Ma populations with aster life-history models (II).

Survival of the nonlocal populations was low compared with the local population at each site (Figure 3). The genetically more diverged population pair (Sp and Ma) showed even larger differences in survival than the more closely related pair Sp and Pl at the site in Norway (II). Number of fruits per inflorescence also showed advantage of the local population at the field sites in Norway and NC, as did number of inflorescences in NC (II). Differences in survival are consistent with expectation that increasing divergence increases the magnitude of local adaptation (Hereford 2009). Examining a larger number of populations would have enabled this to be studied in more detail.

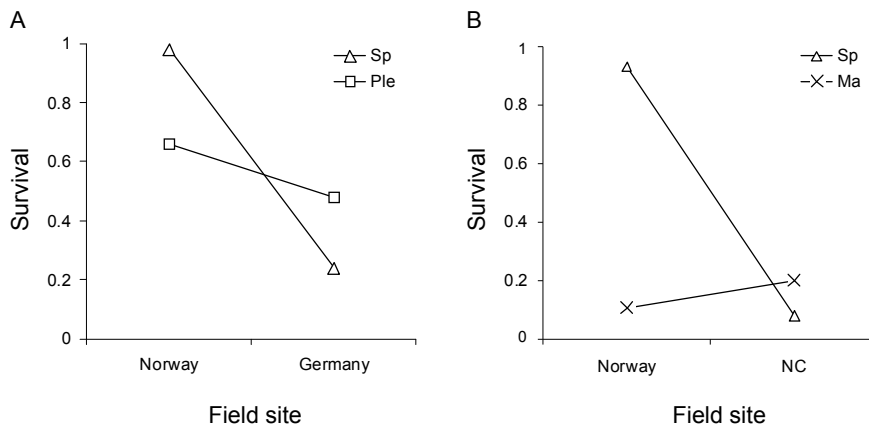


Fig. 3. Evidence for local adaptation in *Arabidopsis lyrata* in survival between populations of (a) 'Sp' from Norway and 'Pl' from Germany (I) and (b) 'Sp' from Norway and 'Ma' from NC USA (II). Survival over two years is presented from the Norway site and over one year in NC and Germany. See Figure 1 for predictions.

3.2 Ecological differentiation and life-history evolution

There were genetically-based differences between the studied populations in traits related to flowering time (II, IV). The local Sp population started flowering earlier than the nonlocal Ma and Pl populations at the site in Norway and in NC the local plants from the Ma population had early flowering start (II, IV). Selection for early flowering has been documented in the Sp natural population in the wild (Sandring *et al.* 2007) and also in other plants, according to a meta-analysis by Munguía-Rosas *et al.* (2011).

Growth chamber experiment in two day length treatments revealed that photoperiodic responses have a role in inflorescence development after bud formation (IV), as has been predicted for perennial plants (Heide 1994). Inflorescence development of plants where inflorescence buds had been formed was delayed in short day length, which was consistent with a lack of second induction in nature preventing elongation of inflorescences in the fall but enabling flowering in the spring. Corresponding effects have been found also in earlier experiments in the growth chamber (Riihimäki & Savolainen 2004, Riihimäki *et al.* 2005). These results suggest that the Sp population has adapted to the short growing season.

The Sp population from Norway showed relatively low investment in sexual reproduction and floral display, as has been documented also in earlier studies in *A. lyrata* (Sandring *et al.* 2007, Quilot-Turion *et al.* *submitted*). The plants in the Sp population produced fewer inflorescences than the plants from the Pl or Ma population in the growth chamber or greenhouse conditions (III, II). In environments where mortality is high especially at the juvenile stage, perenniality and especially iteroparity (reproduction over multiple years) are favored (reviewed by Roff 2002). In alpine environments – such as those where the *A. lyrata* Sp population originates – the cost of investment in sexual reproduction and risk of failure of seedling recruitment might be higher. In these conditions, persistence of vegetative tissues and securing persistence by vegetative propagation may be favored (Körner 2003). There might also be differences in germination behavior between the populations, but they remained undetected in this study as seedlings were pregrown in greenhouse conditions. In the field, the Sp plants were found to have smaller and fewer flowers and shorter inflorescences than the Ma plants when grown in common garden conditions, providing more support to the smaller relative investment on flowering (II). As a result, the Sp population also seems to have less phenotypic plasticity.

3.3 Genetic basis of local adaptation

Performing between-population crosses allowed examination of some questions related to the genetic basis of adaptation. In general, interesting aspects include the number, locations, effect sizes, and directions of effects of loci contributing to differences between populations in different environments. By comparing QTL results from different sites, it is possible to examine whether the same genomic areas have been important for adaptation between environments, thus finding evidence for conditional neutrality (fitness effects in only some environments) vs. antagonistic pleiotropy (single-locus trade-offs). It is also possible to examine whether adaptation has involved epistatic interactions between genes (possibly coadaptation), by finding evidence for hybrid breakdown. Coadaptation could also occur between nuclear and cytoplasmic genomes, when there is a fitness benefit from a combination of local nuclear alleles and cytoplasm. Cytoplasmic genomes could also be involved in adaptation by themselves. With fine-scale mapping and transformation studies it becomes possible to narrow down the QTL region and to infer what the underlying loci might be.

Here the design and the sample sizes allowed addressing some of these issues.

3.3.1 Conclusions based on phenotypes

Comparisons of phenotypes between the parental populations in common garden conditions provided evidence for phenotypic differentiation that has a genetic basis in the studied populations (I, II, IV). Comparisons of hybrid fitness in the Sp and Ma populations representing the two subspecies indicated that patterns of hybrid fitness depended on the environment (II). At the Norway site, both F₁ and F₂ hybrid fitness was surprisingly high, but hybrid fitness was intermediate in the NC environment (II). Different patterns of hybrid fitness across sites have also been found in *Mimulus* (Lowry *et al.* 2008b). Lack of hybrid breakdown and epistasis indicated that local adaptation in these diverged population is unlikely to have involved coadapted interactions between nuclear genes. These results were surprising, as the F₂ generation hybrids often show hybrid breakdown while the F₁ generation commonly shows hybrid vigor (Barton 2001). Recombination has been found to have beneficial effects on fitness in some instances (Verhoeven *et al.* 2004, Erickson & Fenster 2006). F₁ generation can also show hybrid breakdown in cases of genetic hybrid incompatibilities (Lowry *et al.* 2008a).

3.3.2 QTL for local adaptation: conditional neutrality and antagonistic pleiotropy

Local adaptation in diverged populations of *A. lyrata* was found to be mostly governed by conditionally neutral QTL, which had fitness effects in only some environments (III). Such findings have also been reported in the few reported studies that have examined QTL effects in natural climatic conditions of the populations (Verhoeven *et al.* 2004, Gardner & Latta 2006, Hall *et al.* 2010). Importantly, the QTL effects reported here show that local alleles were associated with high fitness both at the field site in Norway and in NC (III). This suggests that differential directional selection, rather than genetic drift or other forms of selection are involved (Orr 1998b). Some of these QTL had pleiotropic effects in more than one fitness component, but some only had an effect on a single component. This emphasizes the need to estimate fitness at multiple life history stages.

In addition to the conditionally neutral loci, also putative cases of more rarely documented antagonistic pleiotropy were also found (III). In *Mimulus*, it has been suggested that such single-locus trade-offs can contribute to local adaptation (Hall *et al.* 2010).

Different genomic regions were found to govern differentiation in inflorescence number and flowering start date in the field vs. in benign conditions in the growth chamber and in the greenhouse (III, IV). Different genomic regions have also been found between field experiments and controlled conditions also in *A. thaliana* (Weinig *et al.* 2002, Wilczek *et al.* 2009, Brachi *et al.* 2010) and in *Boechera* (Anderson *et al.* 2011).

3.3.3 QTL explaining different fitness patterns hybrids between subspecies

F₂ hybrids between Sp and Ma showed evidence for hybrid vigor at the site in Norway but not in NC, where hybrid fitness was intermediate (II). The heterotic effects in Norway were seen in survival, flowering propensity and in the number of inflorescences produced. QTL results were examined in the light of these findings (III). Interestingly at the site in Norway, there were several QTL that showed significant dominance effects, but in NC almost all QTL were additive and only one QTL with dominance was found. In Norway, the QTL showing complete dominance or overdominance were found for the same traits as hybrid

vigor was found at the phenotypic level. The positive effects of hybridization at the Norway site were due to a beneficial combination of dominance effects at QTL for fitness, especially on survival (III).

3.3.4 Role of cytoplasmic genomes in local adaptation

We found that hybrids with cytoplasmic background from the local population had relatively high fitness (II). Such fitness advantage of the local cytoplasm has been documented also in other species (Galloway & Fenster 2001, Campbell *et al.* 2008, Sambatti *et al.* 2008,). It has remained unknown whether these differences arise as a result of cytoplasmic adaptation or through coevolution the nuclear and cytoplasmic genomes. In the studied *A. lyrata* populations, we did not find evidence for cytonuclear coadaptation, as only some of the QTL with interaction with the cytoplasm indicated an advantage of local alleles when combined with the local cytoplasm (III). It has been suggested that cytonuclear coevolution could evolve because many genes from plastids have been moved to the nucleus, and there is thus need for coordinated metabolism between nuclear and cytoplasmic genomes (Rand *et al.* 2004). Cytonuclear coevolution in plants can involve protein complexes where separate subunits are coded by both nuclear and organellar genes, such as the important enzyme Rubisco (Miziorko & Lorimer 1983). Further, organellar gene expression is controlled by the nucleus to large extent (Nott *et al.* 2006, Woodson & Chory 2008). Cytoplasmic variation can have a significant adaptive role; cytoplasmic differentiation has been found to affect ecologically important traits, such as water use efficiency (McKay *et al.* 2008).

3.3.5 Possible limitations and prospects of the QTL results

Detecting QTL in field conditions can be especially difficult (Slate 2005). This can partly explain why there were relatively few QTL found in the field in the cross between Sp and Pl (IV), for example. As a result of nonuniform sample sizes across sites and experiments, no comparisons of the relative effect sizes in crosses between the two population pairs could be made. However, the QTL with largest effects were likely to have been detected in all environments. Further, additional QTL might underlie local adaptation related to more earlier parts of life history, such as germination behavior (Donohue *et al.* 2010).

QTL intervals were wide, and multiple nearby peaks underlying these intervals may have been detected as a single peak. This makes it difficult to prove that antagonistic pleiotropy is involved at QTL with putative fitness trade-offs. However, QTL involved in the timing of flowering overlapped with locations of known candidate genes for flowering time, as for example *GI* (*GIGANTEA*) and *FT* (*FLOWERING LOCUS T*) (III,IV), and can be studied further with functional or association studies (Weigel & Nordborg 2005).

4 Conclusions

These studies provided evidence for local adaptation in an emerging model species for evolutionary and ecological genetics, *Arabidopsis lyrata*. Local adaptation in the strictest sense – fitness advantage of the local population in each environment – has only been documented in less than half of the reported cases. Differentiation in ecologically important traits such as flowering time was also found. Evidence for adaptation to the short growing season was also documented, especially in the context of perennial flowering.

Fitness comparisons at multiple levels allowed concluding that different components of fitness were important at the different sites. Based on these results, future studies should emphasize estimating many components of fitness. As the comparison of hierarchical total fitness also showed evidence for local adaptation, the results suggested that the relative importance of the different components could in fact result in differentiation in life history strategies between the populations. These results were also supported by differences in investment in floral display traits.

QTL mapping results indicated that different genomic areas have likely been important for local adaptation in different environments, as has also been documented in other species. However, here also putative cases of more rarely documented fitness trade-offs at single QTL peaks were detected. Nearly all QTL for fitness indicated association of high fitness with local alleles, providing evidence for differential selection pressures as a cause of population differentiation. QTL for differentiation in flowering-related traits overlapped with known candidate genes.

These studies also revealed unexpected patterns of between-subspecies hybrid fitness, as beneficial effects of hybridization were seen in the F₂ generation in one environment. No evidence for the role of coevolved epistatic effects in local adaptation were found. Beneficial effects of hybridization were mostly explained by relatively large effects at QTL, especially for survival. In fact, local adaptation was driven by the importance of survival in the environment with low productivity and that of reproductive output in the high-productivity environment. The results also indicated that cytoplasmic adaptation has likely occurred but is not likely due to coadaptation between nuclear and cytoplasmic genomes.

These results can be used in future studies examining the genetic basis of local adaptation in more detail, by narrowing down the genomic regions found in this study by association mapping and analyses of full genome sequence data.

Examining also cytoplasmic genomes can reveal more about the role of those factors in local adaptation.

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Original articles

- I Leinonen PH, Sandring S, Quilot B, Clauss MJ, Mitchell-Olds T, Ågren J & Savolainen O (2009) Local adaptation in European populations of *Arabidopsis lyrata* (Brassicaceae). *American Journal of Botany* 96: 1129–1137.
- II Leinonen PH, Remington DL & Savolainen O (2011) Local adaptation, phenotypic differentiation and hybrid fitness in diverged natural populations of *Arabidopsis lyrata*. *Evolution* 65: 90–107.
- III Leinonen PH, Leppälä J, Remington DL & Savolainen O (2011) Genetic basis of local adaptation and flowering time variation in *Arabidopsis lyrata*. Manuscript.
- IV Leinonen PH, Kuittinen H, Kemi U, Quilot-Turion B, Okuloff A, Baker AM, Mitchell-Olds T, Remington DL & Savolainen O (2011) Adaptation to growing season length in a perennial plant *Arabidopsis lyrata*. Manuscript.

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