Population differentiation in *Lythrum salicaria* along a latitudinal gradient

**Katarina Olsson**

2004

Department of Ecology and Environmental Science
Umeå University
SE-901 87 Umeå
Sweden

*Akademisk avhandling*

som med vederbörligt tillstånd av rektorsämbetet vid Umeå universitet för erhållande av filosofie doktorsexamen i ekologi kommer att offentligen försvaras lördagen den 11 december 2004, kl. 10.00 i Stora hörsalen, KBC.

Examinator: Professor Lars Ericson, Umeå universitet

Opponent: Dr. Susan Kalisz, Department of Biological Sciences, University of Pittsburgh, Pennsylvania, USA

© Katarina Olsson 2004

Printed by: Solfjädern Offset AB
In this thesis, quantitative genetic approaches, common-garden experiments, and field studies were combined to examine patterns of population differentiation and the genetic architecture of characters of putative adaptive significance in the widely distributed perennial herb *Lythrum salicaria*. In this work, I (1) documented patterns of population differentiation in phenology, life-history, and morphology along latitudinal gradients at different geographical scales, (2) investigated the genetic architecture of phenology, flower morphology, and inflorescence size, and (3) combined estimates of phenotypic selection in the field with information on the genetic variance-covariance matrix (G) to examine potential constraints to adaptive evolution.

A common-garden experiment demonstrated latitudinal variation in life-history, and phenology of growth and reproduction among *L. salicaria* populations sampled across Sweden. Flower morphology varied significantly among populations, but was, with the exception of calyx length, not related to latitude of origin. A second experiment, which included two Swedish, two Dutch, and two Spanish populations, indicated that the latitudinal gradient in reproductive and vegetative phenology might extend throughout Europe.

A quantitative-genetic study of two Swedish populations revealed significant additive genetic variation for all phenological and morphological traits investigated. The G matrices of the populations differed significantly according to common principal component analysis, and genetic correlations within the study populations did not strictly correspond to trait correlations observed among populations.

In a field study, I detected directional selection through female function for larger inflorescences in two consecutive years. Relative fitness increased disproportionately with inflorescence size in the year when supplemental hand-pollination indicated that pollen limitation was severe. Genetic correlations with inflorescence size considerably influenced predicted response to selection in other characters.

Taken together, the results suggest that among-population differences in phenology and life-history in *L. salicaria* have evolved in response to latitudinal variation in length of the growing season. They demonstrate that the evolutionary potential of local populations may be considerable. The genetic covariance structure substantially influences predicted short-term evolutionary trajectories. However, the weak correspondence between genetic correlations documented within populations and trait correlations among populations, suggest that the G matrix has not imposed strict constraints on patterns of among-population differentiation.

**KEY WORDS**: additive genetic variance, clinal variation, evolutionary constraints, G matrix, genetic correlation, natural selection, pollen limitation, population differentiation
TILL INGRID & AGNES
LIST OF PAPERS

This thesis is a summary and discussion of the following papers, which will be referred to in the text by their Roman numerals.


II. Olsson, K. & Ågren, J. Adaptive population differentiation across Europe in the perennial herb Lythrum salicaria. Submitted manuscript.


Paper I is published with the kind permission of the publishers.
### TABLE OF CONTENTS

**INTRODUCTION**
- Population differentiation and natural selection 7
- Evolutionary response to selection 7
- Genetic constraints to evolution 8
- Phenotypic selection 9

**AIMS OF THE THESIS**

**MATERIAL AND METHODS**
- Studied species and populations 10
- Description of the studies 12
  - Population differentiation in *Lythrum salicaria* (Paper I, II) 12
  - Genetic architecture (Paper III) 13
  - Phenotypic selection and genetic response (Paper IV) 13

**MAJOR RESULTS AND DISCUSSION**
- Population differentiation in *Lythrum salicaria* (Paper I, II) 14
- Genetic architecture (Paper III) 15
- Phenotypic selection and genetic response (Paper IV) 17

**CONCLUSIONS** 18

**ACKNOWLEDGEMENTS** 18

**REFERENCES** 18

**TACK TILL...** 24

**APPENDICES:**  
**PAPER I – IV**
INTRODUCTION

Population differentiation and natural selection

The magnitude and geographical structuring of genetic variation in natural populations depends on the relative importance of natural selection, genetic drift, and gene flow among populations (Slatkin 1987, 1994). Whereas natural selection and genetic drift promote population differentiation, gene flow counteracts these factors. The degree and pattern of population differentiation may also differ considerably depending on which characters that are considered. Among-population variation in neutral characters is expected to be the result of history and stochastic processes, whereas characters subject to selection should be subject also to differential selection along environmental gradients (Spitze 1993, Podolsky & Holtsford 1995, Karhu et al. 1996, Waldmann & Andersson 1998, McKay & Latta 2002).

To elucidate how populations have diverged in response to variation in climatic conditions is of fundamental importance for an understanding of local adaptation in many species (Clausen et al. 1940, Linhart & Grant 1996), but also for predicting the consequences of climate change and for an understanding of the dynamics of range expansion in invasive species (Etterson & Shaw 2001, Lee 2002). In plants with a wide distribution, both phenology and life-history can be expected to vary regularly along latitudinal gradients, where climate is one of the main sources of environmental variation. For a given altitude, temperature, solar irradiation, and the length of the growing season decrease with increasing latitude, while day-length during summer increases. In perennial herbs, populations growing in environments with a short growing season should be subject to selection for rapid growth early in the season and early initiation and completion of reproduction. Consistent with this prediction, phenology of growth and flowering has been found to vary latitudinally in several plant species (e.g. Turesson 1930, Clausen et al. 1940, Kalisz & Wardle 1994, Li et al. 1998, Weber & Schmid 1998, Kollmann & Bañuelos 2004). In contrast, characters important for adaptation to local soil conditions, e.g. heavy-metal tolerance (Antonovics et al. 1971, Macnair 1993) can be expected to vary in a more mosaic fashion, depending on the distribution of soil types across the landscape. Similarly, spatial variation in biotic interactions can potentially result in mosaic variation in plant characters important for interactions with pollinators, pathogens, and herbivores (e.g. Thompson 1994, Brody 1997, Burdon et al. 1999, Pilson 2000).

Evolutionary response to selection

To predict the evolutionary trajectories of natural populations require information both about the strength and shape of selection and about the genetic architecture of traits under selection (Lande 1979, Falconer & MacKay 1996). The multivariate regression techniques proposed by Lande and Arnold (1983) provides us with estimates of selection gradients, which describe the direct selection on a trait after accounting for selection on other traits included in the model. Lande and Arnold (1983, see also Arnold & Wade
1984) demonstrated that evolutionary response to selection ($\Delta z$) within a generation could be predicted as $G\beta$, where $G$ is the additive genetic variance-covariance matrix and $\beta$ is the vector of selection gradients. Hence, predicted short-term response to selection can be modified by genetic correlations. Strong positive genetic correlations will slow adaptation if the correlated traits are experiencing selection in opposite directions, while strong negative genetic correlations could limit response to selection if both traits are selected to increase or decrease (Conner & Via 1993). A non-adaptive trait may experience evolutionary change if it is genetically correlated with an adaptive trait subject to selection (Conner & Via 1992). Therefore, evidence for phenotypic selection may not accurately predict the magnitude, or even the sign, of the expected response (Mitchell et al. 1998, Caruso 2004). If the $G$ matrix is stable over time it may also shape character correlations among populations (Lande 1979, 1982, Arnold 1992) so that patterns of among-population divergence should reflect genetic covariances observed within populations.

**Genetic constraints to evolution**

Lack of genetic variation and trade-offs among life-history characters may constrain evolutionary response to selection. The magnitude of genetic variation in fitness-related traits present in a local population should be a function of the form and strength of selection acting on those characters, gene flow, population history and stochastic processes (e.g. Falconer & Mackay 1996, Roff 1997). Genetic correlations among characters subject to selection may constrain adaptive evolution, but may also contribute to the maintenance of genetic variation in individual components of fitness (e.g. Lande 1979, Arnold 1992).

Resource allocation trade-offs form the basis of models of life-history evolution, and should be reflected as negative genetic correlations among traits, provided there is genetic variation for allocation to different functions (Roff 1992, Stearns 1992). Resource allocation trade-offs may occur at different levels (de Laguerie et al. 1991, de Jong 1993). For example, in plants there may be a trade-off between allocation to reproduction and to vegetative growth, and survival (Obeso 2002), but also among different components of reproductive allocation, such as between flower size and number (Cohen & Dukas 1990, Morgan 1993, Worley & Barrett 2000). At which level trade-offs can be observed should depend on the relative magnitude of variation in overall resource acquisition and in proportional allocation to different functions (van Noordwijk & de Jong 1986, Houle 1991), and the relative magnitude of variation at different levels of the allocation hierarchy (de Laguerie et al. 1991, de Jong 1993). Correlations among different life-history traits should depend on the timing of allocation to different functions. Stronger correlations are expected among functions that overlap in time than among functions that are separated in time. Delayed flowering may allow the plant to build up more resources than can be used for reproduction later in the season, and therefore result in both higher vegetative and reproductive allocation if the growing season is sufficiently long (Ågren & Schemske 1994). Conversely, if flowering continues late into the season, allocation to reproduction may compete with allocation to storage and survival.
Phenotypic selection

Plants are often under strong selection to produce more flowers (e.g. Johnston 1991, Conner & Rush 1997, Caruso 2000, 2001, but see Andersson 1996), which is expected since flower number is a component of total reproductive output and usually related to plant size. Selection for larger flowers have been detected in some species (e.g. Galen 1989, Totland et al. 1998) and can be expected both because flower size may be positively correlated with ovule number (Fenster & Carr 1997), and because flower size contributes to floral display, and may thus influence attractiveness to pollinators (Schemske & Ågren 1995). There is evidence of negative selection on flowering time in plants (e.g. Andersson 1996, reviewed in Geber & Griffen 2003), which can be expected from rapid development due to short growing season (Geber & Griffen 2003, Stinson 2004). However, if the ability to attract pollinators is density-dependent, flowering start may be subject to stabilising selection to promote synchronous flowering within a population (O’Neil 1997). Further, a long flowering period could reduce the uncertainty of pollination (Wilson & Rathcke 1974, Ackerman 1989, Preston 1991) and directional selection on flowering duration may therefore be positive.

The magnitude and direction of selection may also vary considerably within or between years (e.g. Kalisz 1986, Schemske & Horvitz 1989, Maad & Alexandersson 2004). Selection on traits involved in pollen acquisition should be particularly strong through female function when seed production is pollen-limited rather than resource-limited (Haig & Westoby 1988, Ashman & Morgan 2004). Temporal variation in pollen limitation could thus contribute to temporally variable selection on reproductive characters.

Fig. 1 Tristyly in *Lythrum salicaria*. The short-styled (S), the mid-styled (M), and the long-styled (L) morphs differ in the relative positioning of stigma and anthers in the flowers.
AIMS OF THE THESIS

The purpose of this thesis was to gain a greater understanding of how natural selection affect the structuring of phenotypic variation in phenological, life-history, and morphological characters, both within and among natural populations of *Lythrum salicaria*. The main questions addressed in this thesis are:

- What is the magnitude and geographical structuring of among-population differentiation in phenology, life-history, morphology, and ability to flower and survive at the northern range margin? (Papers I, II)

- What is the genetic architecture of phenology, flower morphology and inflorescence size? (Paper III)

- What is the strength and mode of phenotypic selection on flowering phenology and floral morphology, and does selection vary temporally? (Paper IV)

- How may the genetic architecture influence the evolution of phenology and reproductive morphology among and within populations? (Papers III, IV)

MATERIAL AND METHODS

Studied species and populations

Structuring of genetic variation is most easily studied in plant species with wide distribution because such species are likely to be exposed to considerable variation in environmental conditions, and hence exhibit phenotypic variation in many biologically important characters. Purple loosestrife, *Lythrum salicaria* L. (Lythraceae), is therefore well suited for studies of large-scale population differentiation due to its wide south-north distribution in Europe. Further, studies involving crossing designs and transplantations are simplified by using a species that can be easily hand-pollinated, germinated and kept in the greenhouse, and transplanted to other sites.

*L. salicaria* is a tristylous, self-incompatible perennial herb, found in a variety of wetland habitats such as lake- and seashores, riversides, and fens. The aboveground shoots develop from winter buds, formed on the rootstock in autumn previous year. It may produce one to several flowering shoots where flower buds develop in leaf nodes (commonly 2-5 flowers per leaf node) in the upper part of the floral shoot. In Sweden, *L. salicaria* flowers for six to eight weeks in July to August, flowering is acropetal (base to tip), and the inflorescence increases in size throughout the flowering period. The flowers are visited primarily by bumblebees, but also by honeybees, syrphid flies, and lepidopterans (Waites & Ågren 2004, K. Olsson personal observation). The seeds mature six to eight weeks after flowering, and regeneration is almost exclusively from seeds.

Tristylos plants are characterised by the presence of three morphologically distinct mating types that can be easily distinguished on the basis of their floral morphology. The
long-styled (L), the mid-styled (M), and the short-styled (S) morphs differ in the relative positioning of stigma and anthers in the flowers (Fig. 1). This is accompanied by an incompatibility system where full seed set is only achieved if pollen is transferred between morphs and from an anther level that corresponds to the position of the receiving stigma (Darwin 1877, O’Neil 1994). Thus, the self-incompatibility system enforces phenotypic disassortative mating among morphs. Possible effects of style morph need to be evaluated before making inferences on phenotypic variation (I, II), additive genetic variation (III), and phenotypic selection (IV). In all papers (I-IV), style morph was shown to have no significant effect on any of the investigated relationships, and was excluded from final analyses.

**Fig. 2** Maps of Europe and Sweden showing the locations of the *Lythrum salicaria* populations used in the thesis. Populations SE1-SE15 were used in Paper I, populations ESP1, ESP2, NL1, NL2, SE8, and SE11 were used in Paper II, populations SE8 and SE16 were used in Paper III, and population SE16 was used in Paper IV.

*L. salicaria* is native to Europe and Asia, but has also been introduced to North America and Australia (Hultén & Fries 1986). In Europe, it extends from southern and central Europe to northern Scandinavia and east through Siberia; however, it is rarely found at latitudes above 67°N, in the Scandinavian mountain range, or along the east coast of Norway (Hultén & Fries 1986). It is an invasive species in North America, where it has become geographically widespread during the last 100 years (Thompson et al. 1987). The populations used in this thesis are located along a gradient covering most of the latitudinal distribution of *L. salicaria* in Europe (latitude 37°N - 66°N, Fig. 2).
**Description of the studies**

*Population differentiation in Lythrum salicaria (Papers I, II)*

The magnitude and geographical structuring of genetic variation in natural populations may differ considerably depending on which characters are considered and also on the geographical range studied. To compare the degree and amount of genetic population structure in phenology, life-history, flower morphology, and ability to flower and survive close to the northern range margin, we used plants originating from populations distributed along latitudinal gradients at two different geographical scales. In a common garden in northern Sweden, we grew seeds from 15 populations distributed along a latitudinal gradient across Sweden (latitude 57°N - 66°N, I) and from six populations covering most of the European latitudinal distribution (two from Sweden, two from the Netherlands, and two from Spain, latitude 37°N - 64°N, Fig. 2, II). To examine whether the patterns of variation documented were likely to be the result of maternal environmental effects, we took two approaches (I). First, we recorded phenological, life-history, and morphological characters of the plants grown in the common garden during two to three growing seasons. Second, we compared the patterns of population differentiation observed among progenies from controlled crosses performed in a common environment to those observed among progenies raised from seeds collected in the field. During the course of the studies, we scored plant height (I, II), recorded flowering start (I, II) and duration (I), node of first flower (II) and plant height at flowering start (II), age at first reproduction (I), flowering frequency (I), flowering propensity in the first year (II), and survival (I, II), counted the number of winter buds (I), and measured the length of the longest winter bud (I), corolla width (I), petal length (I), and calyx length (I, for illustration of floral traits see Fig. 3).

![Fig. 3 Schematic diagram of a Lythrum salicaria flower.](image)

Floral characters analysed in Papers I, III, and IV included in this thesis were (a) corolla width, (b) petal length, and (c) calyx length.
Genetic architecture (Paper III)

Lack of additive genetic variance and genetic correlations may constrain evolutionary response to selection on phenological and morphological characters, thus affecting patterns of among-population differentiation. Our results in Papers I and II suggest that there exists genetic variation in a number of traits. To determine how the genetic architecture may influence the evolution of phenological, morphological, and reproductive characters we performed a crossing experiment (North Carolina I, Mather & Jinks 1982) to estimate the additive genetic variance and covariance, the narrow-sense heritability, the coefficient of additive genetic variation, and the genetic correlations for 11 traits in two *L. salicaria* populations. Plants were grown from seeds collected in two populations originating from two geographically separated regions in Sweden (SE8 and SE16) in a common garden in northern Sweden (Fig. 2). The traits included in the analyses were plant height in early summer and at the end of the growing season, flowering start, flowering end, and flowering duration, node of first flower, inflorescence size, length of the longest winter bud, corolla width, petal length, and calyx length (for illustration of floral traits see Fig. 3).

Phenotypic selection and genetic response (Paper IV)

To understand how selection influences the evolutionary trajectories of natural populations remains a central problem in evolutionary biology and requires information both about the strength and shape of selection and about the genetic architecture of traits under selection. The results in Papers I-III showed that a number of phenological and morphological characters are differentiated among populations, are genetically determined, and have significant heritabilities. They further suggested that population differentiation is not seriously constrained by genetic correlations and that the scope for adaptive evolution of many characters therefore is considerable in *L. salicaria*. The purpose of Paper IV was to determine the strength and mode of phenotypic selection acting on phenology and morphology through female function and whether any temporal variation in selection can be attributed to between-year variation in pollen limitation of seed production in a natural population of *L. salicaria*. Further, the influence of the genetic architecture on evolution of floral morphology and flowering phenology was investigated.

The investigated population was located at Vitskärsudden on the shore of the Gulf of Bothnia south of Umeå (63°39' N, 20°25' E). We estimated the strength and direction of selection through female function on inflorescence size, flowering start and duration, corolla width, and petal length in two consecutive years. We used total seed production per plant as fitness measure, and the regression techniques outlined by Lande & Arnold (1983) to quantify selection gradients. To test whether seed production per flower was limited by insufficient pollination, we performed supplemental hand-pollinations in each year. To examine how the genetic architecture may influence the evolution of floral morphology and flowering phenology, the estimates of phenotypic selection were combined with data on additive genetic variances and covariances obtained in a previous study (III).
MAJOR RESULTS AND DISCUSSION

Population differentiation in *Lythrum salicaria* (Papers I, II)

In common-garden experiments, we demonstrated significant among-population differentiation in phenology, life-history, and characters influencing flowering propensity and survival at the northern range margin and that much of this variation can be related to latitude of origin. Much of the documented among-population variation in investigated traits is likely to be due to genetic differentiation rather than to maternal environmental effects (I).

Both among populations across Sweden and among populations covering the north-south European distribution of *L. salicaria*, we found that populations originating from higher latitudes initiated and finished aboveground growth and flowering earlier, were shorter, and had a higher survival in the common garden than populations from lower latitudes (I, II). In addition, plants from northern Sweden were older at first reproduction than plants from southern Sweden (I). All measures of flower size (corolla width, petal length, and calyx length) varied significantly among populations within regions, but not among regions, and only calyx length was related to latitude of origin (I).

High latitude is associated with low temperature and short growing season, which constrains the period available for growth. This should select for early completion of growth and thereby, indirectly, for smaller overall size (I, II). Selection for early completion of aboveground growth may partly be driven by a trade-off between above- and belowground growth (e.g., production of winter buds). A negative relationship between latitude of origin and phenology of growth and adult plant size may be common in plants and has been suggested to reflect adaptation to differences in climate (e.g. Kalisz & Wardle 1994, Li et al. 1998, Weber & Schmid 1998, Bastlová et al. 2004, Kollmann & Bañuelos 2004).

A short growing season also constrains the time available for fruit maturation and should therefore also select for early initiation and completion of flowering. Consistent with this prediction, phenology of flowering has been found to vary latitudinally in several plant species (e.g. Rathcke & Lacey 1985, Winn & Gross 1993, Weber & Schmid 1998, Bastlová et al. 2004, Kollmann & Bañuelos 2004). The results of Papers I and II suggest that the latitudinal variation in flowering time documented among populations in northern Europe (I) extends to populations from southern Europe (II), at least when tested under the long-day conditions of northern Sweden. The among-population differences in flowering phenology may reflect inherent differences in the developmental stage at which flowering is initiated, but may also mirror among-population differentiation in the environmental cues needed for flower initiation (II).

Flower morphology has become differentiated among populations, but does not vary with latitude (I). Variation in flower morphology may lack a clear geographic structure because most of the variation observed is selectively neutral, because selection on flower morphology varies considerably on a more local scale, or because of stochastic processes.

Northern populations reached reproductive maturity later than southern populations in Sweden (I). The latitudinal gradient was apparently a function of among-population
differences in aboveground growth, rather than of differences in the size at which the plants begin to reproduce. Common-garden experiments have revealed latitudinal variation in the length of the juvenile period also in other plant species, e.g. *Aster tripolium* (Gray 1997), *Verbasum thapsus* (Reinartz 1984), and *Daucus carota* (Lacey 1988).

Variation in survival among Swedish populations was associated with latitudinal variation in allocation patterns and phenology of growth (I). The poor survival of Dutch and Spanish populations in the common garden in northern Sweden is likely to be the consequence of poor timing of growth and flowering in relation to seasonal changes in temperature, but may also reflect low allocation to belowground parts (Chapin & Chapin 1981, Reinartz 1984, Li *et al.* 1998, but see Clevering *et al.* 2001), and low frost tolerance (Junttila *et al.* 1990, II).

**Genetic architecture (Paper III)**

The results suggest that the evolutionary potential of the two *L. salicaria* populations investigated is considerable. There was significant additive genetic variation for all characters examined. Moreover, the weak correspondence between genetic correlations within populations, and trait correlations among populations suggest that the genetic covariance structure does not impose strict constraints to the long-term response to selection. The results suggest that the considerable among-population differentiation in phenology and morphology previously documented among *L. salicaria* populations across Sweden and Europe (I, II) may have evolved locally.

There was significant additive genetic variation in all investigated characters. Many studies have suggested that selection should reduce additive genetic variation in characters closely related to fitness (e.g. Mousseau & Roff 1987, Roff & Mousseau 1987, Houle 1992, Roff 1997, but see Houle *et al.* 1996, Merilä & Sheldon 1999). Plant size at the end of the season has been shown to be related to survival and flowering propensity in *L. salicaria* in the field (J. Alcantara & J. Ågren unpublished data), and had relatively low heritability and coefficient of additive genetic variation in the two study populations. However, the heritabilities and coefficients of additive genetic variation were not lower for inflorescence size, a component of reproductive output, than for other characters. Both antagonistic pleiotropy, i.e., negative genetic correlations among components of fitness, and spatial and temporal variation in selection combined with gene flow may contribute to the maintenance of genetic variation in traits closely related to fitness (e.g. Falconer & Mackay 1996, Roff 1997).

Our results suggest that the structure of the $G$ matrix may evolve. There was a significant difference in $G$ matrix between the two study populations, as well as a weak correspondence between genetic correlations estimated within populations and correlations among population trait means. To compare $G$ matrices, we used common principal component analysis, which is rather sensitive to differences in matrix structure (Steppan *et al.* 2002). The results of previous studies using this approach indicate that intraspecific variation in $G$ matrix structure may vary considerably among species (e.g. Arnold & Phillips 1999, Waldmann & Andersson 2000, Widén *et al.* 2002, Ashman
Similar to our results, within-population character covariances did not correspond to patterns of character divergence among populations in two bird species (Merilä & Björklund 1999, Badyaev & Hill 2000). Such differences indicate that divergence among populations may have an adaptive basis rather than being dictated by genetic constraints.

Several trait correlations observed among *L. salicaria* populations are consistent with adaptive differentiation in response to latitudinal, climatic variation across Sweden, and may have been shaped by selection rather than genetic constraints. The short growing season at high latitudes should select for early start and completion of growth and flowering (I, II, Etterson 2004). This may explain why plants in northern populations are relatively tall early in the season, relatively short at the end of the season, begin and finish flowering early, and produce large winter buds compared to southern populations (I, II). Restricted gene flow may contribute to clinal variation among semi-isolated populations and may thus also have contributed to these trait correlations among populations. However, not all quantitative characters vary clinally in *L. salicaria*. As mentioned above, flower morphology has diverged significantly among populations, but variation in flower morphology does not display any clear geographic pattern, suggesting that selection on flower morphology varies on a more local scale, or that the variation in flower morphology observed is selectively neutral and is governed by stochastic processes (I).

There was no evidence of trade-offs between flower production (inflorescence size) and vegetative growth and survival, and flowering performance the following year. Negative correlations between inflorescence size and other characters of putative adaptive significance may be lacking because genetic variation in overall resource acquisition is large relative to variation in proportional allocation to different life-history functions (cf. van Noordwijk & de Jong 1986, Houle 1991), or alternatively, because trade-offs involve more characters than those considered in these correlations (Charlesworth 1990, Ågren & Schemske 1994).

A negative correlation between flower size and number is an assumption underlying many models of floral evolution (Cohen & Dukas 1990, Morgan 1993, Sakai 1995, Schoen & Ashman 1995, Harder & Barrett 1996), and is expected if a constant total amount of resources is allocated to flower production. We did not detect a significant negative genetic correlation between inflorescence size and measures of flower size (petal length and calyx length) in our study. This suggests that genetic variation in the total amount of resources allocated to flowering is large relative to variation in flower size in *L. salicaria*, a situation that may not be uncommon in plants (Worley & Barrett 2000, but see Caruso 2004). In the Vitskärsudden population, calyx length was positively genetically correlated with inflorescence size, while in the Utvalnäs population the corresponding correlation was weak, negative and statistically non-significant. This among-population difference in additive genetic covariance implies that directional selection on inflorescence size could by itself result in population divergence in flower morphology.
Phenotypic selection and genetic response (Paper IV)

In a study of a natural *L. salicaria* population in northern Sweden, we detected directional phenotypic selection through female function for increased inflorescence size, and found that among-year variation in the shape of selection was associated with differences in the degree of pollen limitation. Most of the directional selection on flowering phenology occurred through phenotypic correlations with inflorescence size, and genetic correlations between inflorescence size and other traits had a substantial effect on predicted response to selection on these other traits, highlighting the importance of the **G** matrix for short-term evolutionary trajectories.

The selection analysis indicated that inflorescence size was subject to direct selection, while selection on flowering phenology and flower morphology could be attributed to phenotypic correlations with inflorescence size. Strong selection for more flowers is expected and has been documented in several other studies (e.g. Johnston 1991, Conner & Rush 1997, O’Neil 1997, Maad 2000, but see Andersson 1996). Traits that are phenotypically correlated with flower number should therefore commonly be subject to indirect selection in plants (cf. Johnston 1991, O’Neil 1997).

In the year when hand-pollination indicated that pollen limitation was most severe, relative fitness increased disproportionately with inflorescence size. This is consistent with the prediction that selection through female function on characters contributing to pollinator attraction should be particularly strong when seed production is pollen-limited (Haig & Westoby 1988, Ashman & Morgan 2004).

This and a previous study conducted in the introduced range in eastern North America, indicate that selection on flowering phenology may vary among *L. salicaria* populations. O’Neil (1997) documented a combination of positive directional and stabilising selection on flowering start in a large *L. salicaria* population in Maine, USA. In contrast, we found no evidence for either directional or stabilising selection on flowering start in the *L. salicaria* population at Vitskärsudden. The contrasting results may be related to differences in how pollination intensity and pollen limitation vary seasonally in the two environments. However, because estimates of selection gradients are sensitive to which variables are included in the analysis and potential problems with multicollinearity (Mitchell-Olds & Shaw 1987), differences in which traits were examined and in trait correlations in the two populations may also have contributed to the different results. However, they are not likely to be simply the results of differences in sampling effort and statistical power. In the first year, almost twice as many plants were sampled in the Vitskärsudden population as in the population studied by O’Neil (1997).

The predicted short-term response to selection on inflorescence size was positive, and essentially the result of response to direct selection. In contrast, predicted evolutionary responses in flowering phenology were to a large extent a function of correlated responses due to selection on genetically correlated characters, in particular selection on inflorescence size. Predicted responses to selection on corolla width and petal length were small as a result of weak selection, moderate heritabilities, and weak genetic correlations with other measured traits.
CONCLUSIONS

In this thesis I have demonstrated considerable among-population differentiation in phenology and life-history among *L. salicaria* populations along a latitudinal gradient across Sweden and that the patterns of differentiation were correlated with latitude of origin. In contrast, variation in flower morphology did not show a clear latitudinal pattern. The latitudinal gradient in reproductive and vegetative phenology might also extend throughout Europe. I found significant amounts of additive genetic variance in two Swedish populations, suggesting potential for further evolution. The additive genetic variance-covariance matrices differed between the populations, and genetic correlations within populations did not strictly correspond to trait correlations among populations. Further, I detected directional selection through female function for larger inflorescences in two consecutive years, and relative fitness increased disproportionately with inflorescence size in the year when supplemental hand-pollination indicated that pollen limitation was severe. Genetic correlations with inflorescence size were found to affect the short-term response to selection in other characters.

ACKNOWLEDGEMENTS

I would like to thank Pär K. Ingvarsson and Jon Ågren for providing helpful comments and discussions on earlier versions of this thesis. Financial support for the studies in this thesis was kindly provided by the Gunnar and Ruth Björkman Foundation, the J.C. Kempe Foundation, the Swedish Council for Forestry and Agricultural Research (SJFR), and the Swedish Natural Sciences Research Council (NFR).

REFERENCES


TACK TILL…

Jon. Som handledare har du utan tvekan varit absolut viktigast för tillkomsten av den här avhandlingen. Ditt intresse, kunnande och oerhörda fokus har hjälpt mig komma dit jag är idag.

Forskargrupp-gänget. Anita, Anna, Julio och Ronny, ett litet gäng som med tiden likt vindspridda från har spriddits över världen men som ändå liksom funnits kvar.

Fältarbetrar-gänget. Jan-Erik och Tomas, för allt fältarbete, men framför allt för bulgarer, fältmat, abborrfiske, havsbad, campingstolen när mina ben höll på att gå av, Oddset, alla sångstunder. Nu har jag passerat Sävar...


Genetiken-gänget, som gav mig min första kontakt med forskning.

Uppsala-gänget. Mia, Arvid och Frida, Cissi, Sofia, Karin och Edvin, Peter och Anki, som härbergerat mig under mina många Uppsalabesök.

Växtbio-gänget. Christer, Emil, Geir, Håkan, Saskia, Ulla och Willy, där Christer försynt frågade om jag möjliga skrev en monografi eftersom jag vid varje besök i Uppsala under två års tid skrev på samma artikel.

Innehandy-gänget, som har bestått av så många olika människor genom åren. Det har varit ett verkligt andningshål för mig.

Festlig Fredags-gänget. Anders, Jan-Erik, Frank, Katarina, Ronny, Tomas och Åsa, som vet hur man festar till det en fredagslunch (på senare tid även mitt i veckan!).

Mexiko-gänget I och Mexiko-gänget II, som jag fick uppleva harpya, skorpiongifödda, vrälapor, tortillas och svart bönor, chiggers, Oaxaca, pyramider, korrupta poliser, vulcanisadora, skalbaggar som kan räkna, Mexikos näst mest romantiska stad, och mindre begåvade parkeringsvakter tillsammans med.
Gänget! Anna B, Anna S, Anna-Karin, Bent och Pernilla, Bettan, Kajsa, Katarina och Anders, Kicki, Maja-Lena, Maria, Maria i Öjebyn, Sussi och Ulle och Jocke, som har funnits där när jag har behövt det under årens lopp.

Sportfåne-gänget. Elisabet, Jan-Erik, Maria, Ronny, Tomas och Åsa, tillsammans med vilka jag alltid har känt mig hemma.

Stefan, som följde med mig en bit på vägen.


Ingrid och Agnes, som är det bästa som någonsin har hänt mig.