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Population divergence at different spatial scales in a wide-spread amphibian

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ACTA
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UPPSALA
2019

ISSN 1651-6214
ISBN 978-91-513-0723-7
urn:nbn:se:uu:diva-391039

Dissertation presented at Uppsala University to be publicly examined in Zootissalen, EBC, Villavägen 9, Uppsala, Friday, 20 September 2019 at 13:15 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: Professor Bengt Hansson (Department of Biology, Lund University).

Abstract
Mörch, P. R. 2019. Population divergence at different spatial scales in a wide-spread amphibian. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1841. 53 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0723-7.

To study the distribution of genetic and phenotypic variation in different environments and at different spatial scales is important in order to understand the process of local adaptation and how populations will respond to future climate change. In my thesis I study populations of moor frogs (*Rana arvalis*) at different spatial scales, first along a 1700 km latitudinal gradient (**Paper I, II, IV**) and, second, in a system of inter-connected wetlands (**III, IV**). In **Paper I,** I present evidence for a major latitudinal break-point in larval life-history traits which is linked to a post glacial contact zone between two lineages that colonized Scandinavia after the last ice age. Using Q_{ST}-F_{ST} comparisons I found divergent selection acting on life-history traits, where a major source of differentiation comes from the two colonization routes. In **Paper II** I focus on genomic variation, demographic history and selection along the gradient. Using demographic modeling I confirm the proposed demographic history and show historical signatures of gene flow between regions and over the contact zone. In terms of genetic variation showing extreme differentiation as well as associations with growing season length I identify numerous variants under putative divergent selection, some of which have functions relating to immunity and development. I further show that differentiation outlier variation is higher in the north, as compared to neutral variation and variation associated with growing season length, which both decrease with latitude. These patterns are shaped by gene flow over the contact zone and the increased strength of drift at higher latitudes. I reduce the spatial scale in **Paper III** and characterize larval environments, landscape and geographical distance, to partition their influence on genetic variation. I show that environment explained more of the genetic variation than landscape and geographic distance, indicating that adaptive divergence can persist under high gene flow. Using the environmental variables, I identify genetic variants under putative divergent selection with functions associated with development and immunity. Using data from both scales, Q_{ST}-F_{ST} comparisons and gene-phenotype associations I show in **Paper IV** that selection on both larval traits aligns across scales, whereas selection on plasticity only aligns in size at metamorphosis. This further connects to the influence of temperature and seasonal time constraints in colder environments. Finally, I find several genetic variants associated with the traits and plasticity at both spatial scales with functions relating to immunity and metamorphosis.

Keywords: Adaptive divergence, environmental gradients, genomics, life-history, amphibians

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ISSN 1651-6214
ISBN 978-91-513-0723-7
urn:nbn:se:uu:diva-391039 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-391039)

To all the frogs

List of Papers

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

I Luquet, E., **Rödin-Mörch, P.,** Cortazar-Chinarro, M., Meyer-Lucht, Y., Höglund, J., Laurila, A. (2019) Post-glacial colonization routes coincide with a life-history breakpoint along a latitudinal gradient. *Journal of Evolutionary Biology*, 32: 356–368

II **Rödin-Mörch, P.,** Luquet, E., Meyer-Lucht, Y., Richter-Boix, A., Höglund, J., Laurila, A. (2019) Latitudinal divergence in a widespread amphibian: Contrasting patterns of neutral and adaptive genomic variation. *Molecular Ecology*, 28: 2996-3011

III **Rödin-Mörch, P.,** Palejowski, H., Cortazar-Chinarro, M., Kärvemo, S., Richter-Boix, A., Höglund, J., Laurila, A. Small scale population divergence is driven by local larval environment in a temperate amphibian. *Manuscript*

IV **Rödin-Mörch, P.,** Luquet, E., Palejowski, H., Cortazar-Chinarro, M., Richter-Boix, A., Höglund, J., Laurila, A. Divergence and plasticity of larval life-history align at different spatial scales in a high-latitude amphibian. *Manuscript*

The following papers were published during the course of my PhD but are not part of my dissertation:

Kozma, R., **Rödin-Mörch, P**., Höglund, J. (2019) Genomic regions of speciation and adaptation among three species of grouse. *Scientific Re ports,* 9: 2045-2322

Meyer-Lucht, Y., Luquet, E., Johannesdottir, F., **Rödin-Mörch, P**., Quintela, M., Richter-Boix, A., Höglund, J., Laurila, A. (2019) Genetic basis of amphibian larval development along a latitudinal gradient: Gene diversity, selection and links with phenotypic variation in transcription factor C/EBP-1. *Molecular Ecology*, 28: 2786-2801

Contents

Abbreviations

SNP	Single nucleotide polymorphism
ddRAD-seq	Double digest restriction association DNA sequencing
PCR	Polymerase chain reaction
LGE	Large scale experiment
SSE	Small scale experiment

Introduction

Populations inhabiting heterogeneous environments are influenced by an interplay between spatially varying divergent selection pressures, gene flow and genetic drift. This interplay occurs over large spatial scales (Endler 1977), as well as over smaller or micro-geographic scales (Richardson et al. 2014), and if divergent selection is strong enough this may eventually lead to adaptation to the local environmental conditions (Kawecki & Ebert 2004). Understanding the genomic and phenotypic basis of this process is important as adapting to new or changing environments is one of the three main outcomes for populations and species facing global environmental change, the other two being migration to more favorable environments or extinction (Williams et al. 2008).

Small scale divergence

Selection acts to maximize individual fitness in a given environment. If the environments are different enough, divergent selection will push the populations inhabiting them towards different phenotypic and genetic optima, ultimately resulting in adaptive divergence (Kawecki & Ebert 2004). Over small geographical scales, the ability of populations to adapt to the local environment depends on the relative influence of divergent selection pressures, gene flow and genetic drift (Kawecki & Ebert 2004; Blanquart et al. 2013; Richardson et al. 2014). In systems like these, where heritable variation is not affected by dispersal limitation, i.e. there is no major influence of isolation-by-distance (Wright 1943), adaptive divergence can become constrained, or even hindered by genetic drift and in particular by gene flow between populations (Räsänen & Hendry 2008). Unless selection is very strong, gene flow has traditionally been viewed as a force constraining adaptive divergence through gene swamping, where detrimental alleles are introduced into the population (Haldane 1930). Furthermore, when the amount of gene flow is high adaptive divergence can also be constrained by phenotypic plasticity, i.e. an organism's ability to modify the phenotype in response to changes in the environment (Schmid & Guillaume 2017). Recently, a more multifaceted view of the influence of gene flow on adaptive divergence has started to emerge (reviewed in Tigano & Friesen 2016), emphasizing that unless complete gene swamping occurs, gene flow may increase adaptive divergence. For example, introduced novel alleles may yield a higher fitness benefit in the new environment or just

add to the pool of standing genetic variation upon which selection can act on if the environment changes (Barret & Schluter 2008; Hedrick 2013). Associations between environmental heterogeneity and population differentiation, referred to as isolation-by-environment (Rundle & Nosil 2005; Wang & Summers 2010), is usually taken as evidence of adaptive divergence. This is especially the case when environmental differences result in tight associations between environmental variation and allele frequencies (Kawecki & Ebert 2004; Sexton et al. 2014). However, these patterns can also be influenced by neutral processes such as genetic drift and biased dispersal (Wang & Bradburd 2014).

Divergence along environmental gradients

When gene flow between populations is constrained by dispersal limitation (IBD, Wright 1943), such as along large-scale environmental gradients associated with latitude, the effect of gene flow on adaptive divergence may substantially decrease. Thus, population divergence is mainly shaped by the interplay between spatially varying selection, contemporary genetic drift and the demographic history of the populations (Eckert et al. 2008; Sexton et al. 2009; Guo 2012). These processes may complicate any attempt to identify genetic variants under selection along a gradient or cline, where genetic variation and environmental variation often co-vary with geographical distance. This may result in neutral processes producing patterns that mimics our expectations of divergent selection (Vasemägi 2006). Along large climatic gradients phenotypic variation often follows a pattern of counter-gradient variation (Conover & Schultz 1995). This type of variation represents a form of non-adaptive plasticity where the environmental influence on a phenotype counteracts the genetic influence. This ultimately results in the apparent absence of phenotypic variation in spite of strong environmental differences, leading to cryptic population divergence along a gradient. For example, in many high-latitude ectotherms lower temperatures and shorter growing season favors genetically faster growth and development as compared to individuals found at lower latitudes. However, this genetic capacity for faster development is usually only realized when populations are reared in a common environment. As an opposite pattern, co-gradient variation, refers to a situation when the environmental and genetic influence on a phenotype act in the same direction, leading to adaptive divergence more easily observed in natural settings (Conover & Schultz 1995). Phenotypic plasticity in general, may facilitate adaptation along a gradient by aiding in the establishment of populations in new and changing environments, as well as by acting as a buffering mechanism until divergent selection can act on standing genetic variation (Ghalambor et al. 2007).

Latitudinal gradients are also characterized by a pattern of decreasing genetic diversity with increasing latitude, where populations at higher latitudes have lower effective population sizes and less genetic variation (Guo 2012; Adams & Hadly 2013; Miraldo et al 2016). In Europe in particular, this has been influenced by glacially mediated range contractions and expansions: when the ice cover retreated after the last ice age, organisms (re)colonized the areas previously covered in ice, re-establishing and extending their previous distribution ranges. This has most likely occurred through serial founder events and population bottlenecks, as well as in different selective environments as organisms expanded their range northwards (Hewitt 2000; 2004). Possibly, also aided by the evolution of increased phenotypic plasticity at the expansion front, which is often found in current day populations towards their range margins (Lancaster et al. 2015; Orizaola & Laurila 2016). In Europe, many species emerged from multiple glacial refugia, representing separate phylogeographic lineages. They colonized Central and Northern Europe from different directions, leading to the formation of secondary contact zones where gene flow between the lineages may occur (Hewitt 2000; 2004). Gene flow over the contact zones can be of both a neutral and adaptive nature (Linnen et al. 2008; Carvalho et al. 2010; Harrington et al. 2018), and may lead to the formation of evolutionary novelty at the vicinity of the contact zone.

Amphibian population divergence

Adaptive divergence has previously been shown along environmental gradients in ectotherms at different spatial scales (Hoffman & Weeks 2007; Cheng et al. 2012; Campbell-Staton et al. 2017; Brennan et al 2018), including amphibians (Berven & Gill 1983; Laugen et al. 2003; Palo et al. 2003; Lindgren & Laurila 2009; Orizaola et al. 2010; Lind et al. 2011; Richter-Boix et al. 2011, 2013, 2015). However, previous amphibian studies have contributed to our understanding of population divergence along environmental gradients by mostly focusing on phenotypic traits, using at most a handful of microsatellite markers. By taking a more genome-wide approach on adaptive divergence along environmental gradients, which has generally been lacking (but see Bonin et al. 2006; Guo et al. 2016; Yang et al. 2016; Pastenes et al 2017; Czypionka et al. 2018), we can obtain a broader view of selection throughout the genome as well as being able to take demographic history into account. Moreover, none of these previous studies has focused on genome-wide latitudinal divergence. Amphibians with their complex life cycles represent a good model system for investigating large and small-scale adaptive divergence since many species have wide geographical distributions that cover different habitat types and thermal regimes. Amphibians, like other ectotherms are at higher latitudes exposed to lower temperatures and stronger seasonal time constraints compared to their southern counterparts, and these spatially varying climatic

conditions can impose strong selection pressures (Blanckenhorn 1991; Nielsen et al. 2009; Prasad et al. 2011; Swaegers et al. 2015; Machado et al. 2016). Understanding these processes is also important because due to habitat alteration, emerging infectious disease and climate change amphibians are currently recognized as the most threatened group of vertebrates (IUCN 2019).

Research aims

In this thesis I investigate the influence of divergent selection, genetic drift and demographic history on adaptive divergence and the spatial distribution of genomic and phenotypic variation in populations of the moor frog (*Rana arvalis*) in northern Europe. I first characterize the distribution of, and selection on, variation in fitness related larval life-history traits and the influence of latitude and demographic history along a 1700km latitudinal gradient spanning from northern Germany to northern Sweden, hereafter referred to as the latitudinal gradient experiment (LGE) (**Paper I**). Then, using genomic data I characterize large-scale population structure and the distribution of neutral and putatively adaptive genetic variation, and reconstruct post-glacial demographic history of the populations along the latitudinal gradient (**Paper II**). I then zoom in, looking at a smaller spatial scale and the influence of differences in larval environment, landscape and spatial separation on adaptive genomic divergence in a network of wetlands in central Sweden, hereafter referred to as small-scale experiment (SSE) (**Paper III**). Finally, in **Paper IV**, I investigate differences in selection on phenotypic plasticity in the two basal life history traits investigated in **Paper I**, by comparing selection patterns using the SSE and LGE data and conducting association studies in order to identify candidate genes related to variation in these traits.

Methods

Study species (Paper I, II, II, IV)

Rana arvalis is a wide-spread amphibian found mainly in temperate environments from western Europe to western Siberia (Babik et al. 2004; Sillero et al. 2014). It is an explosive breeder inhabiting temporary and permanent ponds, marshes and lakes. At higher latitudes, *R. arvalis* populations are seasonally time constrained, with large variation in breeding time and considerably shorter growing season in the north (**Paper I, II**). After the last glacial maximum, *R. arvalis* colonized Scandinavia from two different directions, one entering from the north via Finland and the other one entering from the south via Denmark. The two lineages form a contact zone somewhere between Uppland and Västerbotten counties in central Sweden, but the exact location of the contact zone is unknown (Knopp & Merilä 2009, Cortazar-Chinarro et al. 2017; Fig. 1).

Sample collection and common garden experiments (Paper I, II, II, IV)

Eggs were collected in 13 populations for LGE (Fig. 1) in 2014, with two additional populations from Northern Germany sampled in 2015, spanning almost the entire latitudinal distribution of *R. arvalis*. Eggs were collected from 10 egg clumps in each population, each egg clump representing a separate, presumably unrelated family. All study populations bred in open-canopy permanent ponds, surrounded by mixed forests and agricultural land. The distance between populations within each region ranged between 8 and 50 km (average 20 km). Variation in the seasonal time constraint along the latitudinal gradient is indicated by the large difference in growing season length (161 to 278 days), defined as the number of days when mean temperature reaches ≥5°C, averaged over 10 years.

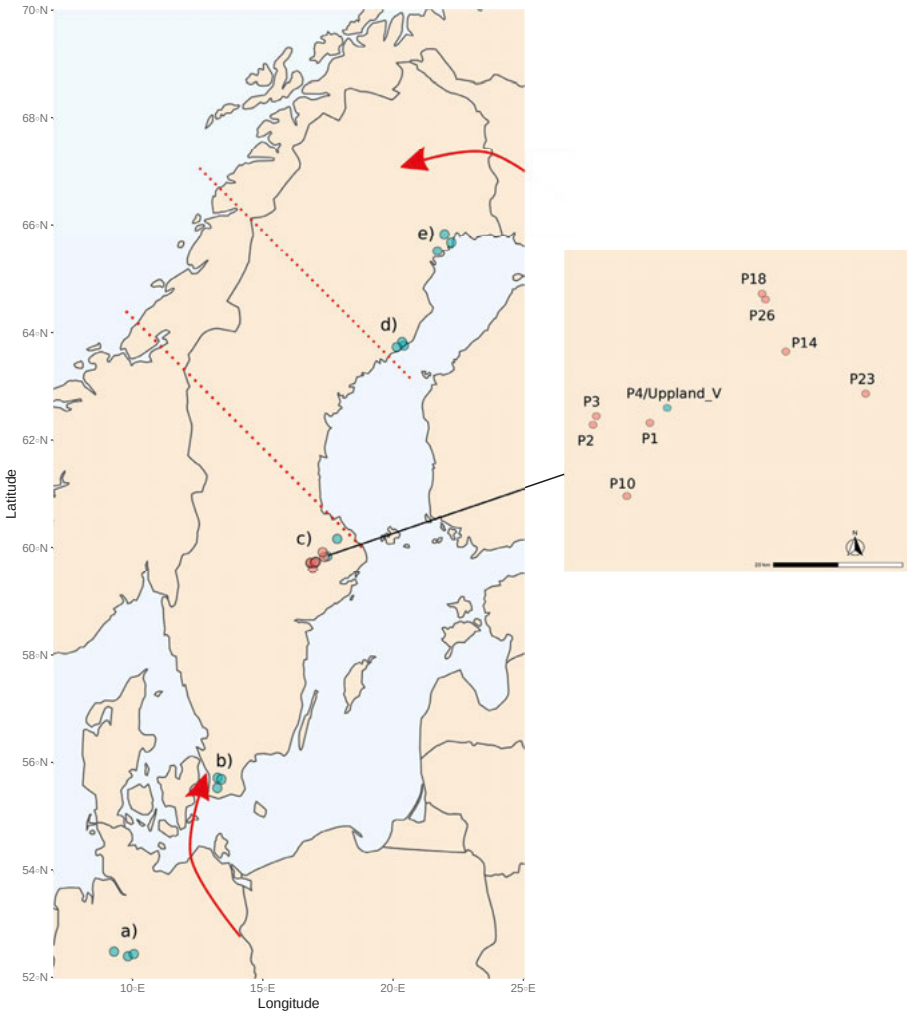

Fig. 1. Map of sampling localities in LGE in blue and SSE in red. In SSE map, the population colored blue was sampled in both LGE and SSE. The red arrows show the direction of post-glacial colonization and dashed lines the approximate location of the secondary contact zone. For LGE a) Lower Saxony, b) Skåne, c) Uppland, d) Västerbotten, d) Norrbotten.

In SSE 16-20 egg clumps were collected from each of 9 populations in 2016. These populations, located in the county of Uppland near Uppsala (Fig. 1), inhabit a mixture of forest, marsh and open field ponds that differ in terms of canopy cover, temperature, pH, predator abundance, amount of aquatic vegetation and breeding time. The distance between the ponds varied between 1.1 and 42.6 km, but other, more closely located ponds that has not been sampled here, allow for dispersal between the ponds (Richter-Boix et al. 2013). Tadpoles from populations collected in 2014 for LGE and from 7 of the 9 populations collected for SSE were raised in two common garden experiments that

were set up identically for both studies. Eggs were kept in 0.75 l plastic vials until hatchlings reached stage 25 (Gosner 1960), when they were transferred to individual 0.75 l plastic containers kept in two different temperatures (16°C & 19°C) with a photoperiod of 16 h light: 8 h dark. In LGE, six larvae from each of the 10 families were raised in each of the two temperatures. In SSE, three larvae from each of the 16-20 families were raised in each temperature. Water was changed every 3rd day in conjunction with feeding the growing larvae chopped spinach *ad libitum*. The experiment continued until larvae reached metamorphosis (emergence of the first forelimb; Gosner stage 42), at which stage the metamorphs were euthanized with an overdose of MS222 and preserved in 96% ethanol at -20°C for DNA extraction. Larval period, defined as the number of days elapsed between Gosner stages 25 and 42 and mass at metamorphosis (dry weight in grams) were scored. These life-history traits are closely linked with future fitness (Altwegg & Reyer 2003; Earl & Whiteman 2015). In **Paper I**, the composite trait growth rate was also calculated, defined as mass at metamorphosis divided by larval period (g/days). In **Paper IV**, plasticity index for larval period and mass at metamorphosis was calculated using the difference between individual trait values within families between the temperature treatments. Following Lind et al. (2011) the plasticity index was defined as the difference in trait value between a specified individual within a family in the 16°C treatment and the 19°C treatment.

Local larval environments (**Paper III**) were characterized by measuring canopy cover, mean temperature, predation risk index, aquatic vegetation, pH, and breeding time in each wetland. Canopy cover was measured in early June by visually estimating the amount of non-visible sky into 10% categories for each pond (Korhonen et al. 2006). Temperature, as the average over two months following egg-laying, was measured using data loggers (HOBO Water temp Pro v2 Data Logger) set to record every 15 minutes. Predation risk was estimated by counting macro-invertebrates and newts collected using five standardized dip net sweeps at each of five locations in each wetland and then calculating predation risk index following Michel (2011) and Carlson & Langkilde (2014). The percentage of aquatic vegetation cover was estimated along a 15m transect (Palik et al. 2001). pH was measured at the time of egg laying using a multi-parameter device (HANNA instruments). Lastly, breeding time was defined as the number of days from January first, until eggs were discovered in the ponds. *R. arvalis* is an explosive breeder with a very short egg-laying period (Richter-Boix et al. 2013), and the date when fresh eggs are first found provides a good proxy of breeding date.

The eggs were laid between 29th of March and 18th of April with a maximum breeding time difference of 20 days. In order to characterize the landscape and how that influences population differentiation (**Paper III**), four landscape variables were measured around each of the nine sites by summing up data using

circular buffer zone at a spatial scale of 2000 m, as this is the most common movement distance in amphibians (Smith & Green 2005). The amount of arable land defined as cropland and fruit farms was measured by using topographic vector maps converted into raster format. Mature forest was quantified using k-Nearest-Neighbour-raster (Reese et al. 2003) originally at 25 m x 25 m resolution and due to low volume accuracy at the original scale (Gjertsen 2007) aggregated to 100 m x 100 m by averaging. Total road length was estimated by summing up the total length of roads within the 2000 m buffer zone. Lastly, a connectivity index was measured using a kernel estimation weighted by distance (smoothing curve of 5 km from each pond), where every pond represents a pixel that is given a value based on the number of adjacent ponds. We also included the perimeter of the surrounding ponds, where longer shorelines result in higher values, as vegetated shallow shores are important habitats for *R. arvalis*. All landscape data were processed in ArcMap 10.6 (ArcGIS, ESRI, Redlands, CA, USA).

DNA extraction and ddRADseq (Paper I, II, II, IV)

Genomic DNA was extracted from the hind legs of metamorphosing individuals. One individual was used for DNA extraction from each family, resulting in a total of 150 individuals in LGE and 163 individuals in SSE. To extract DNA, a modified version of the High salt extraction protocol by Paxton et al. (1996) was follwed, with an added extra ethanol precipitation step for cleaning. Libraries for double digest restriction-site associated DNA sequencing (ddRADseq) were prepared in an identical manner in LGE and SSE following the protocol of Johansson et al. 2017 (modified from Peterson et al. 2012; Mastretta-Yanes et al. 2015). DNA was digested using the restriction enzymes *SbfI*-HF, which has a longer recognition site and thus cuts more infrequently, and *MseI* which is a more frequent cutter. Digestion lasted for 18 hours and success was confirmed by gel electrophoresis. 16 P1 adapters containing a unique 6-bp barcode and a P2 adapter were ligated to the digested DNA, and the ligation product was cleaned using AMPure XP beads. Four separate PCRs were then performed for each sample in order to alleviate any stochastic and biased amplification, using PCR primers containing 12 unique indices allowing multiplexing of a total of 192 individuals. Size selection of the pooled amplified product was then performed on agarose gel, with bands between ~400-600 bp (LGE) and ~350-600 bp (SSE) extracted. Libraries were sequenced by SciLifeLab in Uppsala on the Illumina Hiseq 2500 (2x125) in high throughput mode in two (LGE) or four (SSE) lanes.

ddRAD bioinformatics (Paper I, II, II, IV)

The software package STACKS (Catchen et al. 2013) was used for bioinformatic processing of raw ddRADseq reads. Version 1.42 was used for LGE data and version 2.1 for SSE. The workflow was similar for the two sequencing runs, where raw reads were first cleaned and de-multiplexed using the STACKS program *process_radtags.* To reconstruct loci and call SNPs, the *denovo_map* pipeline was used for LGE data, whereas each sub-program in the *denovo_map* pipeline was run separately for SSE. In order to maximize the number of variant sites obtained from both data sets a parameter optimization approach was used. The key parameters (-m, -M, -n) in version 1.42 and (-m, -n) in version 2.1 were optimized by running the program with parameter setting differing by one step multiple times in order to evaluate which parameter combination resulted in the largest number of SNPs without too large drop in coverage. After the parameter optimization was completed, the last step of the pipeline, the *populations* program, was re-run with stricter filtering to obtain the final SNP data set. Filtering options employed in this last step were a minor allele frequency cutoff of 5% in LGE and 2% in SSE. A SNP was only considered valid if it occurred in at least 13 populations and 70% of the individuals in LGE and 9 populations (7 for **Paper IV**) and 70% of the individuals in SSE. Finally, based on the filtering steps above and only obtaining the first SNP on each RAD-tag, in-files for downstream analysis, such as VCF and plink, were created and population level summary statistics, such as expected and observed heterozygosity (H_E, H_O), inbreeding coefficient (F_{IS}) and nucleotide diversity (π) were calculated.

Data analyses

Identifying differentiation outliers under putative divergent selection

The SNP data sets were scanned for signatures of divergent selection based on SNPs showing higher than usual association with the major axes describing population structure (i.e. differentiation outliers) using the R package *pcadapt* (Luu et al. 2017: **Paper II, III**). In **Paper II**, an F_{ST} based method were used based on ancestry coefficients estimated using sparse non-negative matrix factorization, implemented in the R package *LEA* v. 1.8.1 (Frichot & Francois 2015).

Genetic variation, structure, admixture and demographic history

Global and pair-wise F_{ST} were calculated for LGE (**Paper II**) and SSE (**Paper III**) with *assigner* v.0.4.1 (Gosselin et al. 2016) using the total SNP data set. Population structure were estimated using only the neutral part of the data sets, where SNPs showing a signature of putative divergent selection were filtered out. To infer population structure the programs *admixture* v.1.3 (Alexander et al. 2009: **Paper I**), the spatially informed algorithm in the R package *tess3r* (Caye et al. 2016, 2017: **Paper II, III**), principal component analysis (PCA) in the R package *adegenet* v.2.1.1 (Jombart 2008; Jombart & Ahmed 2011: **Paper II**) and discriminant analysis of principal components (DAPC) in *adegenet v.2.1.1* (**Paper III**) were used. Hybrid indices were used to estimate the amount of gene flow over the secondary contact zone between the two colonizing lineages, and comparisons were made between neutral SNPs and SNPs under putative divergent selection, estimated in the R package *gghybrid* (Bailey 2018), based on the method of Buerkle (2005) (**Paper II**). The post-glacial demographic history of the populations along the latitudinal gradient was inferred using demographic model selection in *fastsimcoal 2.6* (Excoffier et al. 2013: **Paper II**). Eight models corresponding to different post-glacial demographic histories were simulated and compared to the empirical data using differences in model likelihood values and Akaike information criteria. 95% confidence intervals around the parameter point estimates for the best fitting model were obtained by parametric bootstrap.

In order to estimate genetic variation distributed along the gradient, diversity indices such as nucleotide diversity were regressed with latitude (**Paper II**). Hierarchical F statistics (F_{ST}) were calculated for 1000 (**Paper I**) or 5000 neutral SNPs (**Paper IV**) using the R package *hierfstat* 0.04-22 (Goudet & Jombart 2015). Hierarchical estimates of quantitative trait differentiation (Q_{ST}: Chapuis et al. 2007) were calculated for larval life history traits and phenotypic plasticity using variance components estimated from nested linear mixed models in *lme4* v. 1.1-21 (Bates et al. 2015). Inference of selection was done by using these two estimates of differentiation and simulating a neutral distribution of Q_{ST} values (Whitlock & Guillaume 2009), using the method implemented in Lind et al. (2011). Linear mixed models implemented in lme4 were used to estimate the effect of temperature and population origin on phenotypic variation (**Paper I, IV**) and plasticity (**Paper IV**). Finally, redundancy analysis (**Paper III**), and mantel tests (**Paper II, III**), both implemented in the R package *vegan* 2.5-5 (Oksanen et al. 2018) were used to estimate the relative influence of local larval environment, landscape features and geographic distance on total genetic variation and differentiation.

Gene-environment and gene-phenotype association studies

As many association methods require complete data, missing genotypes were first imputed using random forest on-the-fly imputation (Tang & Ishwaran 2017) in the *randomForestSRC* package v.2.7 (Ishwaran & Kogalur 2007; Ishwaran et al. 2008) implemented through the *grur* package v.0.0.11 (Gosselin 2018: **Paper III, IV**). Latent factor mixed models implemented in the R package *LEA* (**Paper II, III**) were then used to estimate the selective influence of local larval environment and growing season length in a univariate framework. Furthermore, redundancy analysis implemented in the R package *vegan* was used to estimate divergent selection pressures in the local larval environment in a multivariate framework (**Paper III**). Finally, Bayesian sparse linear mixed models implemented in the software package *GEMMA* v.0.98 (Zhou & Stephens 2012; Zhou et al. 2013) were used to find associations between SNPs and the two larval life-history traits and their plasticity in SSE and LGE (**Paper IV**).

Results and discussion

Paper I: Post-glacial colonization routes coincide with a life-history breakpoint along a latitudinal gradient

Here I explored the influence of post-glacially mediated range expansion as well as latitudinal effects on divergence in larval life-history traits tightly associated with fitness in *R. arvalis*. I did so by comparing differentiation in quantitative traits (Q_{ST}) and neutral genetic differentiation (F_{ST}) along the gradient. The proposed phylogeographic structuring was corroborated by admixture analysis using 1000 neutral SNPs, showing a clear separation between the south and the north representing two lineages at K=2. However, I found that the optimal K was 5, corresponding to the geographic sampling regions. This means that neutral genetic variation was mostly shaped by strong isolation-by-distance (IBD) and post-glacial demographic history. The pattern of strong IBD was further supported by a highly significant mantel test. By calculating hierarchical F statistics (F_{ST}), I found strong differentiation between the colonization routes as well as among the regions within colonization routes. Differentiation among populations within regions was low and comparable to small-scale differentiation found previously (Richter-Boix et al. 2013).

Focusing on larval life-history traits, using linear mixed models I found strong regional differences in all three traits (larval period, mass at metamorphosis and growth rate), and significant region-by-temperature interactions for all three traits, indicating that variation in these traits in part depended on the thermal environment. In the common garden, larvae originating from the northern part of the gradient had a higher mass at metamorphosis and developed and grew faster than larvae from the south (Fig. 2). These results reveal counter-gradient variation along the gradient, where northern individuals developed and grew faster at high temperature (19°C) compared to southern individuals, but where lower temperature (16°C) counteracted the genetic capacity for faster development by reducing the rate of development and growth. These results agree with earlier latitudinal studies on amphibians (Laugen et al. 2003; Palo et al. 2003; Orizaola et al. 2010). Counter-gradient variation is a common latitudinal pattern in ectotherms (Conover et al. 2009). Mass at metamorphosis, however, was always higher in northern individuals and especially so at low temperatures while there was very little difference between the temperature treatments in the south.

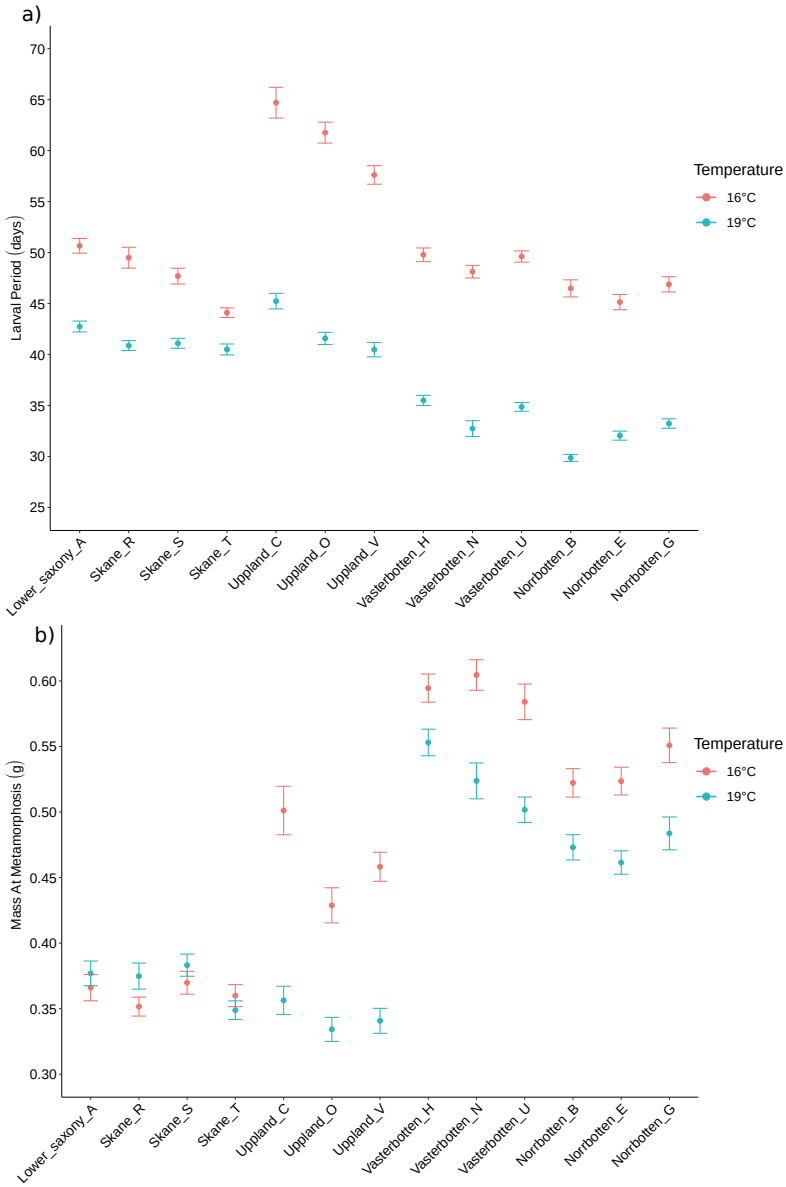

Fig. 2. Population means ± SE in LGE for a) larval period and b) mass at metamorphosis in two temperatures.

This pattern follows co-gradient variation where the environmental and genetic influences on the phenotypes goes in the same direction (Conover & Schultz 1995). These phenotypic differences are explained in terms of seasonal time constraints where selection favors a fast developing and growing phenotype at higher latitudes. However, due to the overall lower temperature in the northern region the capacity can only be utilized during windows of

opportunity, i.e. at peak temperatures (Orizaola & Laurila 2016), possibly due to physiological constraints at lower temperatures. Higher mass at metamorphosis, on the other hand, is beneficial at northern latitudes because it conveys a higher chance of survival during the extended overwintering period (Altwegg & Reyer 2003; Munch et al. 2003). I found that a large part of latitudinal larval life-history divergence was due to differences among the two colonization lineages for essentially all three traits, with a larger Q_{ST} than F_{ST} among routes. This would indicate that divergent selection has shaped phenotypic differences between the two routes, likely as a result of different selection pressures encountered during range expansion as well as during adaptation to local circumstances after colonization. With the exception of larval period at 16°C and globally, and for mass at metamorphosis at 16°C, I found little evidence that divergent selection is acting on phenotypic variation among regions within colonization routes. This could be the result of environmental similarity within the southern route and possibly relatively smaller differences in seasonal time constraints among regions within the northern route compared to between colonization routes. Interestingly, the phenotypic breakpoint (Fig. 2) separating the two routes is associated with the secondary contact zone, with the regions of Västerbotten and Uppland located at each side of the zone (Fig.1). Gene flow between the two routes could explain the large phenotypic differences between temperatures and the long larval period at 19°C in the Uppland region (Fig. 2a). Among populations within regions I only found evidence of divergent selection ($Q_{ST} > F_{ST}$) for larval period in both temperatures and globally, whereas for the other two traits divergent selection could not be differentiated from drift in any temperature, most likely due to the similarities of the environments within regions.

Paper II: Latitudinal divergence in a widespread amphibian: Contrasting patterns of neutral and adaptive genomic variation

Analyzing 27 590 SNPs using the two differentiation outlier approaches *pcadapt* and F_{ST} in *LEA* I identified a total of 812 candidate SNPs under putative divergent selection. 446 of these were identified by *pcadapt*, 129 of these were associated with principal component 1 (PC1) separating the two colonization lineages, and 85 with PC2 separating regions within the two lineages (Fig. 3).

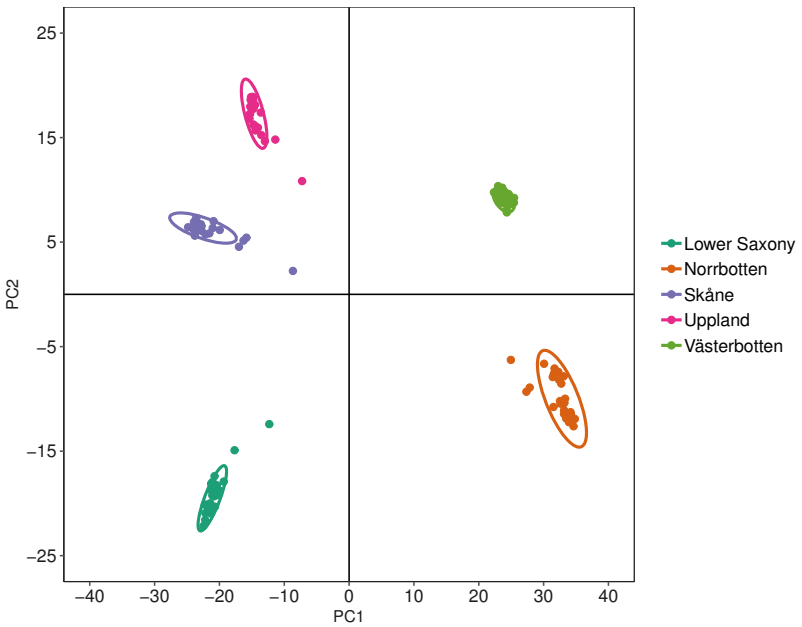

Fig. 3. PCA of the populations along the latitudinal gradient using the neutral part of the data set.

Using *LEA,* I identified additional 631 SNPs with F_{ST} values ranging between 0.603 – 0.975. However, the two methods only converged on 265 candidate SNPs. Using *BLAST,* I matched a small number of the RAD-tags containing the differentiation outlier candidate SNPs to known proteins and genes. Of particular interest were hits that matched a protein similar to bifunctional apoptosis regulator, which suppresses cell death in mammals (Zhang et al. 2000). As apoptosis is an important component of amphibian metamorphosis involving large-scale restructuring of larval morphological features into terrestrial morphology (Nakajima et al. 2005), this provides a possible developmental link in latitudinal adaptation. Furthermore, I obtained two matches to two immune system-related genes ECSIT and Brevinin-2CE. ECSIT has diverse functions, being involved in the innate immune system, (Kopp et al. 1999), bone morphogenic protein signaling pathway (Lin et al. 2017; Wang et

al. 2018), and in stress response to salinity and cold in *Arabidopsis* (Furuya et al. 2013). Brevinin 2-CE is an antimicrobial peptide that is secreted on the skin of amphibians and protects against bacteria, viruses and fungal pathogens (Conlon et al. 2004; Rollins-Smith & Conlon 2005; Rollins-Smith 2009). This finding agrees with previous studies highlighting the importance of immune gene variation along this latitudinal gradient (Cortazar-Chinarro et al. 2017, 2018), which is most likely linked to the decrease in pathogen diversity towards higher latitudes (Schemske et al. 2009).

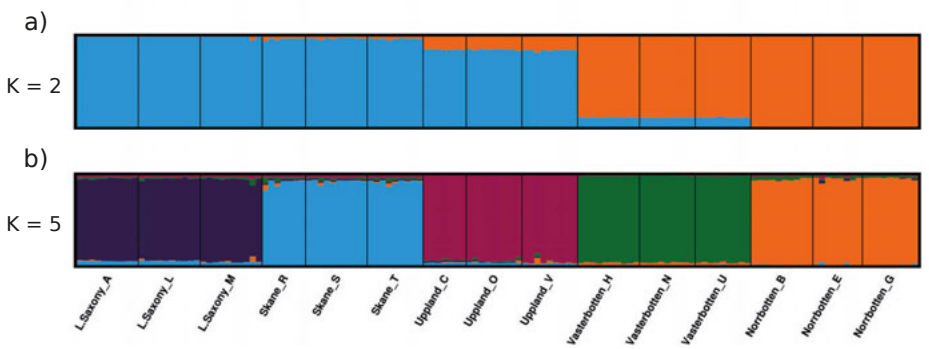

Fig. 4. Tess3 ancestry plots of populations along the latitudinal gradient for a) K=2 and b) K=5 based on the neutral part of the data set.

Using growing season length as an explanatory variable in latent factor linear mixed models I identified 290 candidate SNPs associated with growing season length. However, only 11 of these converged with the differentiation outlier candidates. Using BLAST, I identified another position within Brevinin-2CE as well as larval-specific keratin gene. The latter gene is involved in the conversion of larval to adult epidermis during metamorphosis and its expression is regulated by thyroid hormones (Suzuki et al. 2001). I then filtered out the candidates identified by all three methods in order to create a neutral data set for analysis of population structure and demographic history.

Using the spatially informed method tess3r I found evidence of strong neutral population structure using 26500 SNPs, corresponding to the same patterns of structure as in **Paper I.** However, I also found evidence of admixture between the two regions on each side of the secondary contact zone at K=2 (Fig. 4a), as well as population structure corresponding the geographic regions at K=5 (Fig. 4b). In the PCA I found five distinct clusters and the major axis of variation (PC1) separated the two colonization lineages, with PC2 separating the two latitudinal end points from the remaining three regions (Fig. 3). Using demographic model selection in the software fastsimcoal2, I found that the model best fitting the data, with the lowest delta likelihood and AIC, described the demographic scenario of two lineages colonizing the latitudinal gradient after the last glacial maximum. Furthermore, I found bidirectional gene flow

occurring between the adjacent regions as well as over the secondary contact zone after establishment (Fig. 5). Point estimates of the large number of parameters for the model had wide confidence intervals indicating a high degree of uncertainty. I observed an expected decrease in effective population size with increasing latitude and towards the limits of the latitudinal range for each lineage. For time of divergence, I found that the oldest split, the one between the southern and northern lineage had a point estimate of ~70 000 years before present, which predates the last glacial maximum. The second oldest split, between Lower Saxony and the two southern Swedish regions had a point estimate of ~39 000 years which also indicates that the split predates the range expansion after the ice receded. However, the wide confidence intervals include post LGM times as well. This means that either the second oldest split occurred in the refugia, when the latitudinal gradient was covered with ice, or after LGM when the southern lineage expanded its distribution range. Based on the point estimates, the two most recent splits, between Skåne and Uppland and between Norrbotten and Västerbotten occurred ~5400 and ~4700 years before present, i.e. well after LGM. However, the upper end of the confidence intervals overlaps somewhat with a time with the current range was covered in ice.

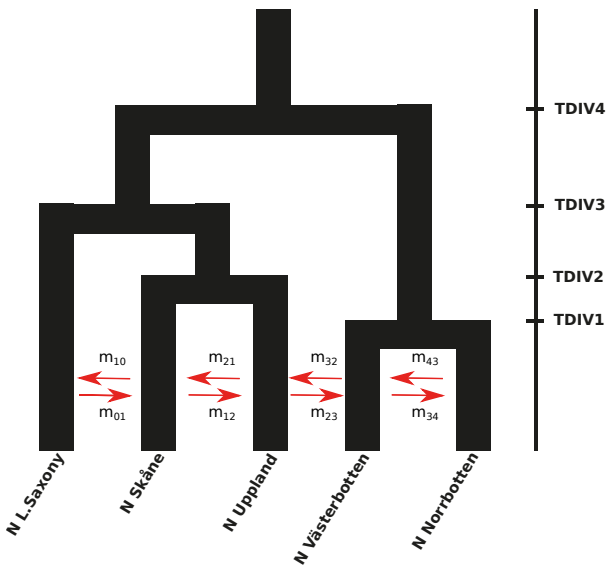

Fig. 5. Best fitting demographic model, with regional effective population sizes (N), migrations between adjacent regions (m) and divergence times (TDIV).

I found that the amount of gene flow that has occurred between regions was fairly low, which is not surprising given the philopatric nature of amphibians (Blaustein et al. 1994; Smith & Green 2005). Interestingly, the amount of gene flow over the contact zone was estimated to be considerably higher in the southward than northward direction, highlighting the possible influence of

environmental factors and increased seasonal time constraints as an obstacle for southern individuals to become established in the north. Furthermore, I place the position of the contact zone between the two lineages in between the regions Västerbotten in the north and Uppland in the south, which is several hundreds of kilometers south from the position estimated in a previous study (Knopp & Merilä 2009).

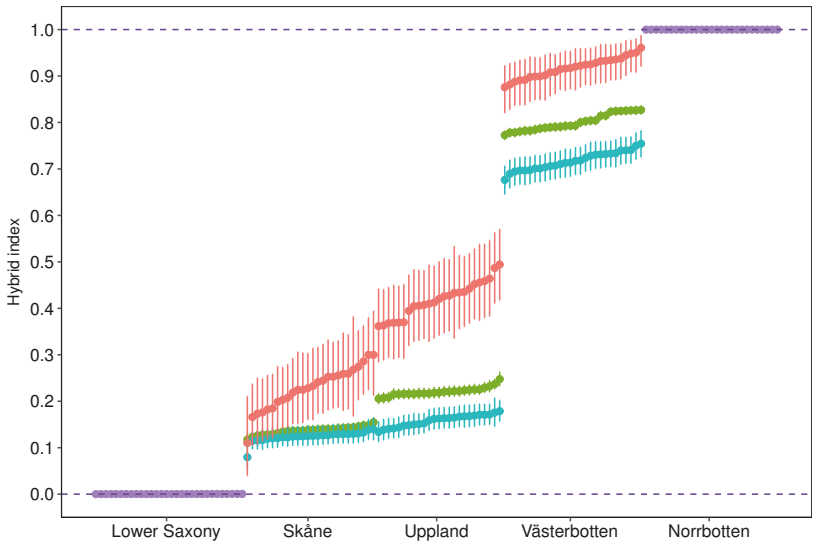

Fig.6. Hybrid index estimation with credible intervals for neutral variation (green), differentiation outliers (blue) and growing season length associated variation (red). Parental populations are colored purple.

Using hybrid index estimation, I obtained further support for gene flow based on neutral variation having occurred across the secondary contact zone (Fig. 6), with slightly higher northern ancestry in the region on the southern side of the contact zone than vice versa. Furthermore, hybrid indices for SNPs under putative divergent selection revealed contrasting patterns depending on whether the SNPs were identified as differentiation outliers, or if they were associated with growing season length. In differentiation outliers, the northern populations had a higher number of southern alleles than vice versa. For SNPs associated with growing season length, the northern populations had a very small number of shared alleles with the south, whereas the southern population has a large number of northern alleles. Taken together this would indicate a more unidirectional pattern of gene flow. Another possible explanation is that differentiation outlier alleles from the south are either more favorable or removed to a lesser extent if they arrive in the northern regions than vice versa. If the alleles are associated with growing season length however, southern alleles are expected to be detrimental in the northern regions and possibly either beneficial or neutral if they are coming from the north into the southern

regions. The pattern of shared alleles for growing season length associated SNPs fits rather well with the pattern of strong seasonal time constraints in the north. Alleles from the south have been removed from the northern populations as they could be maladaptive, whereas on the other hand northern alleles are either favorable or neutral in the south and hence persist to a greater extent.

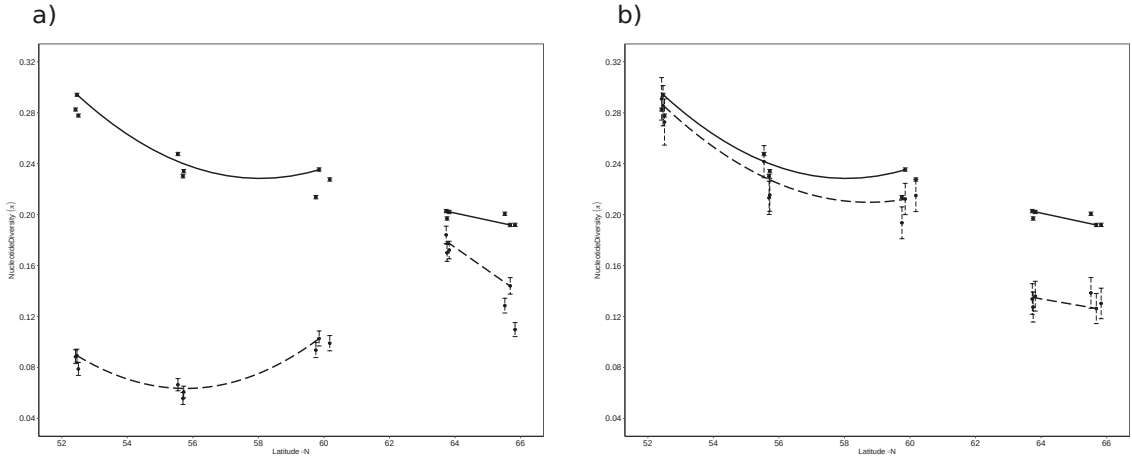

Fig. 7. Regression of nucleotide diversity with latitude ± SE, within each lineage for a) neutral (solid line) and differentiation outliers (dashed line) and b) neutral (solid line) and growing season length associated variation (dashed line).

Based on the distribution of nucleotide diversity for each population along the gradient I observed contrasting patterns of variation along the gradient by regressing nucleotide diversity with latitude (Fig. 7). As expected, within the southern lineage neutral nucleotide diversity decreases with increasing latitude (Eckert et al. 2008; Guo 2012). Within the northern lineage, nucleotide diversity was lower than within the southern lineage, but did not change significantly with latitude. For differentiation outliers however, the pattern was the opposite with higher levels of diversity within the northern lineage. In the southern linage nucleotide diversity was lower in all populations compared to the northern lineage, variation was lowest in Skåne and highest in the mid Swedish region of Uppland. Within the northern lineage, diversity significantly decreased with increasing latitude. This could be the result of adaptive gene flow from the south to the north, which is corroborated by the hybrid index estimation. This pattern could be strengthened by higher levels of genetic drift in the north counteracting the directional effect of selection, maintaining higher diversity at these sites. Nucleotide diversity for growing season associated SNPs decreased in a similar manner as neutral diversity within the southern lineage. Within the northern lineage, diversity was considerably lower compared to the south but did not decrease with latitude. As suggested above, this can be explained by stronger selection associated with seasonal

time constraints in the north reducing variation, whereas selection is more relaxed within the southern lineage.

Paper III: Small-scale divergence is driven by local larval environment in a temperate amphibian

Using the total data set, I found low levels of global population differentiation (F_{ST} = 0.0277, 95CI 0.0274-0.0282) and low pair-wise F_{ST} between all population pairs (0.0111 – 0.0417) in SSE. Using only the neutral part of the data set, where candidate SNPs from gene-environment association and differentiation outlier analysis (see below) were removed, the discriminant analysis of principal components (DAPC) revealed the presence of five to six fairly discrete clusters separated by the first two axes (Fig. 8).

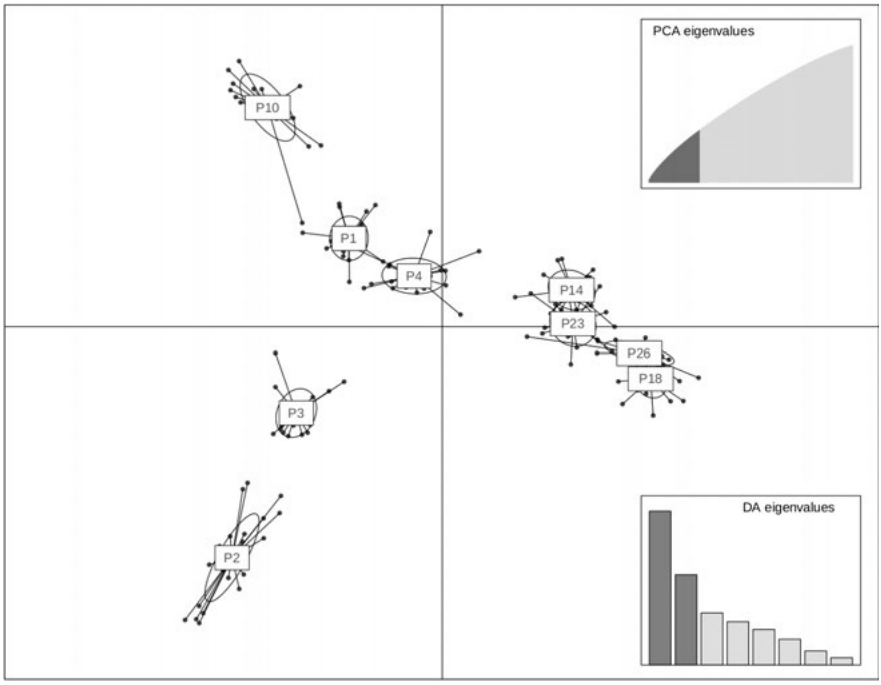

Fig. 8. Small scale neutral population structuring revealed by DAPC.

This pattern of population structure was further supported by estimation of ancestry coefficients by *tess3r* where the optimal number of populations (K) lies between 6 and 8, where each population forming a discrete cluster with the exception of the populations immediately adjacent to each other. Although I did not explicitly estimate gene flow, the low levels of differentiation combined with admixture inferred using tess3 are both indicators that relatively high gene flow is in fact occurring in this system. Even though some of the

populations are further apart then the maximum dispersal distance of *R. arvalis* (Vos et al. 2001), the study area is characterized by interconnected wetlands not sampled in the present study (Richter-Boix et al. 2013).

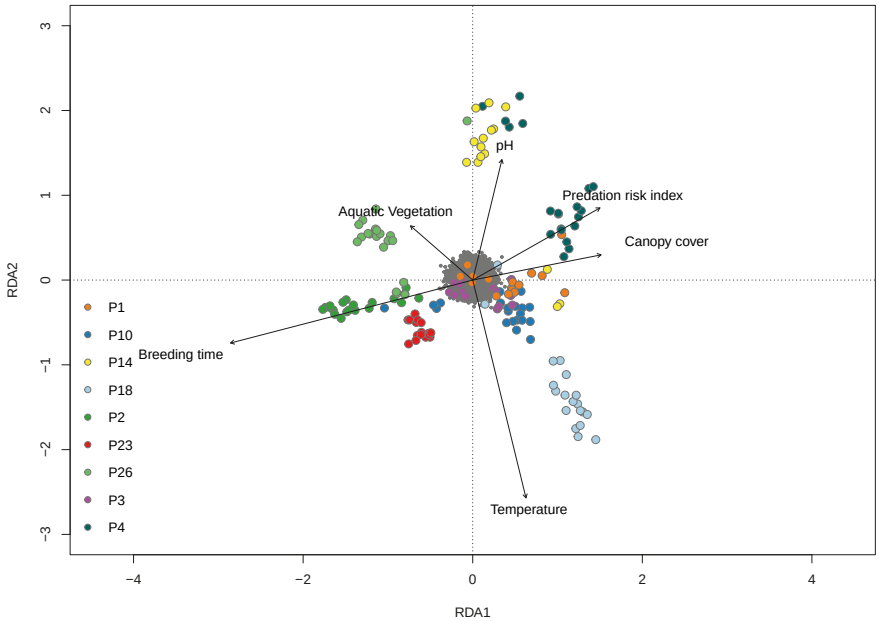

Fig. 9. RDA plot showing environmentally based ordination in the small-scale system. The relative size of the environmental vectors denotes their influence.

Using partial redundancy analysis (pRDA) to partition the relative influence of local larval environment, landscape features and geographical distance on total genetic variation, I found that local larval environment explains a significant proportion of total genetic variation (2.3%) after controlling for geographical distance, resulting in fairly clear environmental ordination of the populations in the landscape (Fig. 9). Local larval environment explains more variation than the landscape features (1.8%) after controlling for distance and when only including geographical distance (0.8%). Since all models were highly significant ($p<0.001$), these results indicate isolation-by-environment, isolation-by-distance and isolation-by-landscape-resistance, with all three factors shaping divergence in the small-scale system of wetlands. However, partial mantel tests to estimate the correlation between genetic distance and environmental, geographical and landscape distances indicated that only environmental and geographical distance matrices were significantly correlated with genetic differentiation. Using the pRDA and the univariate LFMM for gene-environment association I found that larval environmental differences were associated with variation in a fairly large number of SNPs at this small scale.

Reflecting the low levels of population differentiation in this system, using *pcadapt* I only found 29 differentiation outliers and could not annotate any of them using BLAST. Using pRDA, I identified 576 candidate SNPs associated with the local larval environment. The most important environmental variable was canopy cover having the largest number of SNPs with the highest correlation to this variable, followed by mean temperature, pH, breeding time, predation risk index and amount of aquatic vegetation in their respective order of importance. The environment explained 9.6% of the total variation in pRDA-identified candidates. Using the univariate LFMM I identified 547 candidates associated with the environment. The relative importance of the individual environmental variables was quite different compared to the pRDA, with breeding time identifying most candidates followed by predation risk index, amount of aquatic vegetation, canopy cover, pH and mean temperature. The combined effect of the environment explained less of the identified LFMM candidates (5.2%) than for pRDA. The two methods converged on 123 SNPs resulting in a total of 1000 unique candidate SNPs under putative environmentally mediated divergent selection.

Using BLAST, I matched RAD-tags containing environment-associated SNPs to 32 known genes or genomic regions and 21 proteins of which 8 matched hypothetical or unknown proteins. The matches have various biological functions, with a handful of genes and proteins related to immune system function and amphibian development. For example, I obtained a match to the same antimicrobial peptide as I identified in **Paper II**, Brevinin-2CE correlated to mean temperature, as well as an additional antimicrobial peptide Palustrin-2CE most correlated to pH. Additionally I identified the immune genes MHC class I, which is part of the adaptive immune system (Braciale et al. 1987), and here most correlated to the amount of aquatic vegetation, TRIM25 which is part of the regulating antiviral response (Martin-Vicente et al. 2017) and NLRP3 (inflammasome), which is part of the response to viral infections and cellular damage (Jin & Flavell 2010). The last two genes were most correlated with mean temperature. This is in line with previous studies showing the influence of local pond environmental characteristics on disease risk (Johnson et al. 2006; Holt & Roy 2007; Becker et al. 2012; Heard et al. 2014). Genes with potential involvement in development were ITGB1, Mab21 and DAGLB (Lau et al. 2001; Darribere et al. 2012; Oudin et al. 2011) most correlated with mean temperature, pH and breeding time, respectively. Interestingly, I obtained two matches to thyrotropin-releasing hormone receptor 1 (TRHR1) that was most correlated to breeding time and pH. This gene stimulates the release of growth hormone and prolactine, which promote growth and inhibit amphibian metamorphosis (reviewed in Galas et al. 2009). Taken together, my results show that even small scale local environmental characteristics can result in strong enough selection to counteract the effects of genetic drift and gene flow resulting in detectable genomic signatures of adaptive divergence.

Paper IV: Divergence and plasticity of larval life-history align at different spatial scales in a high-latitude amphibian

Using Q_{ST}-F_{ST} analyses I explored the patterns of selection on larval life-history trait and plasticity in *R.arvalis* at two different spatial scales. I also conducted genotype-phenotype association studies in order to identify candidate genes shaping variation in traits and plasticity across the two scales. In **Paper I**, I found strong regional, temperature and interactive effects on larval period, mass at metamorphosis and growth rate. Here, I only analyzed the two basal traits larval period and mass at metamorphosis in SSE (Fig. 10) and found strong population and temperature effects on larval period, but no population x temperature interaction. For mass at metamorphosis only the temperature effect was significant. I found a strong regional effect on plasticity index for larval period and mass at metamorphosis in LGE. In SSE, the population effect on plasticity of mass at metamorphosis was significant, but this was not the case in larval period plasticity (Fig. 11).

In SSE, differentiation in larval period was significantly different from neutral expectations in both temperatures, indicating divergent selection acting on the trait ($Q_{ST}>F_{ST}$). This was not the case in mass at metamorphosis ($Q_{ST}\sim F_{ST}$), with the exception of a slightly larger Q_{ST} at 19°C. This pattern aligns with the results of LGE in **Paper I**, where there was evidence for divergent selection among populations within regions for larval period in all temperatures, but not for mass at metamorphosis. In LGE, I found evidence of divergent selection on plasticity of both larval period and mass at metamorphosis among the regions and among populations within the regions. For the top hierarchical level among colonization routes however, I obtained a singular fit in the model yielding zero variance, and thus a $Q_{ST} = 0$. This is most likely a result of model complexity and the amount of data, and should not be taken as evidence of lack of selection on plasticity among colonization routes. In SSE, I found evidence of divergent selection on plasticity for mass at metamorphosis but not for larval period. Taken together, SSE mirror LGE with respect to selection acting on larval life-history traits and mass at metamorphosis plasticity as a result of time constraints and variation in the thermal environment. Interestingly, along the latitudinal gradient the highest plasticity is observed in Uppland followed by the two northernmost regions which represents the end points of the post glacial range expansion. This is in line with previous studies that has found that populations distributed towards the range margins of a distribution tend to evolve higher levels of plasticity (Lancaster et al. 2015; Orizaola & Laurila 2016). The higher plasticity in Uppland compared to Västerbotten further north could be explained by the patterns of gene flow through secondary contact which I showed in **Paper II**, where northern alleles

associated with seasonal time constraints in the north have been able to move southwards.

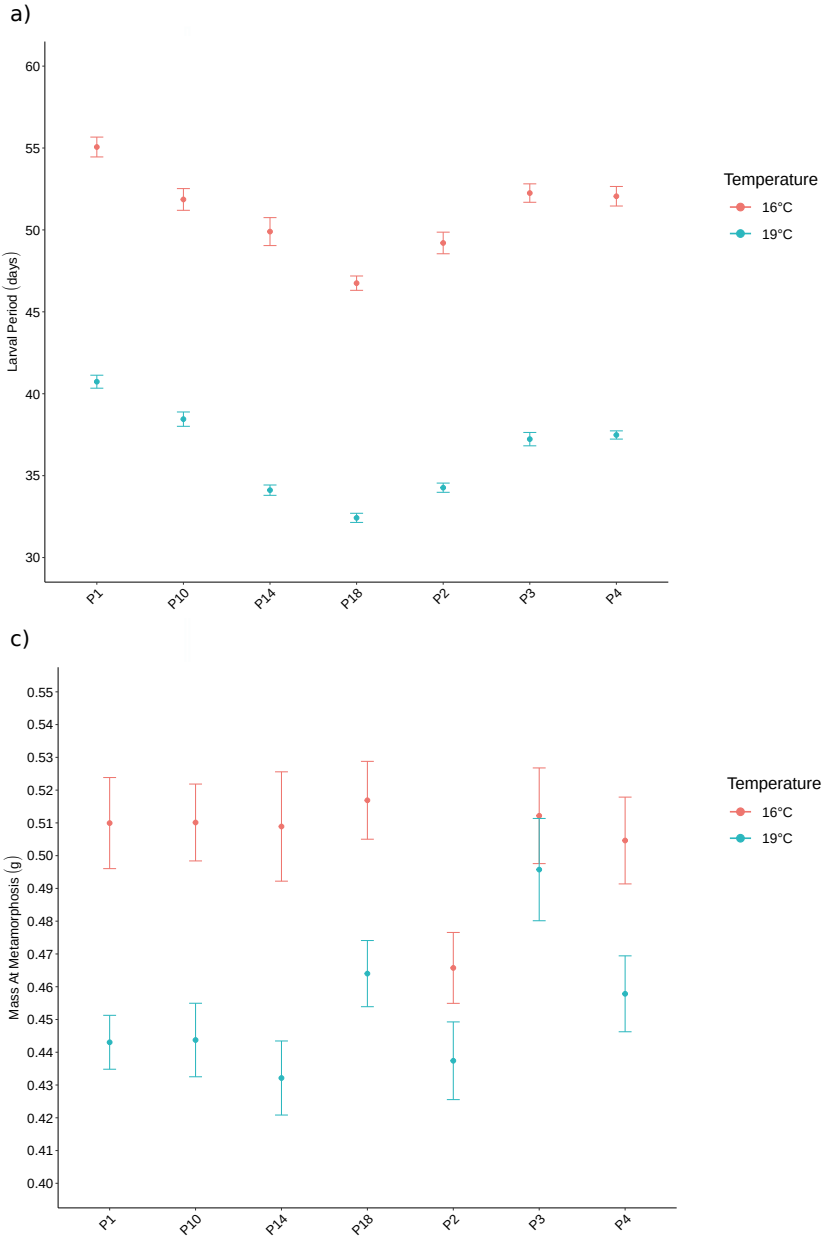

Fig. 10. Population means ± SE in SSE for a) larval period and b) mass at metamorphosis in two temperatures.

With a total of 123 individuals and 22425 SNPs for SSE and 116 individuals and 18892 SNPs for LGE, using Bayesian sparse linear mixed models and a less conservative significance threshold (PIP>0.01) I identified a number of SNPs associated with each trait and plasticity, and a smaller number passing a more conservative threshold (PIP>0.1). I also found that estimates of proportion of variance explained by all SNPs, or chip heritability, as well as the proportion of variance explained by large effect genes, were very high with wide equal tailed posterior intervals, most likely inflated due to low sample size and marker number (Pallares et al. 2014). After searching for similar genes and proteins using BLAST of the RAD-tags containing the associated SNPs I obtained a small number of matches. One of these matches, associated with mass at metamorphosis plasticity and larval period in SSE, was thyrotropin-releasing hormone receptor 1 (TRHR1), which I identified as a candidate under putative divergent selection in **Paper III**. Since this gene inhibits metamorphosis and promotes growth by hormone secretion (reviewed in Galas et al. 2009), it is interesting that I found this gene associated with variation in mass at metamorphosis plasticity and larval period. This hormone binding receptor interacts with the thyroid hormone cascade (Morley 1981), and this aligns with the previously identified candidate gene C/EBP-1 for larval period and mass at metamorphosis at both spatial scales, which is part of the same cascade (Richter-Boix et al. 2013; Meyer-Lucht et al. 2019). I also identified the protein similar to bifunctional apoptosis regulator, which showed evidence of divergent selection in **Papers II** and **III**. Here, I found it associated with mass at metamorphosis plasticity and larval period.

Furthermore, I identified the antimicrobial peptide (AMP) genes Brevinin-2CE and Palustrin-2CE, that were also found in **Paper III** to be associated with the local larval environment. Here, in LGE I found Brevinin-2CE associated with mass at metamorphosis and its plasticity, and Palustrin-2CE with mass at metamorphosis, larval period and larval period plasticity. In SSE I found that Palustrin-2CE is also associated with larval period. At first sight these associations might seem curious, but a recent study investigating the expression of AMPs in 17 species of amphibians found that fast-developing species had lower AMP expression compared to slow developers highlighting the potential trade-off between development rate and investment in immune function (Woodhams et al. 2016). Similar trade-offs have been observed in other species (Stoks et al. 2006; Gervasi & Foufopoulos 2007; Hoverman et al. 2011), including *R. arvalis* (Murillo-Rincon et al. 2017). The results of this paper highlight the influence of post-glacial demographic history, latitudinal patterns relating to seasonal time constrains and pathogens, and small-scale environmental differences in shaping trait divergence and plasticity.

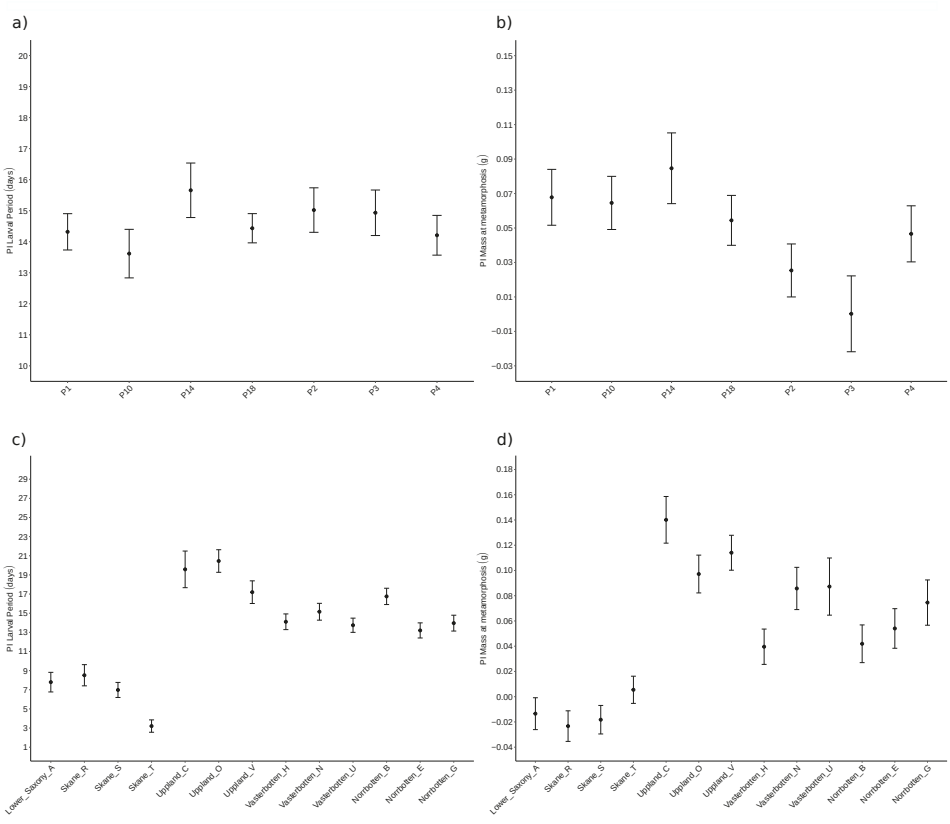

Fig. 11. Plasticity indices ± SE for SSE a) larval period, b) mass at metamorphosis and LGE c) larval period and d) mass at metamorphosis.

Conclusions and future directions

In my thesis I have focused on the interplay between divergent selection and demographic history and how this shapes genomic and phenotypic variation at different spatial scales. Overall the results show a tight connection between divergence and various time constraints imposed by seasonality and local thermal environments. I show in **Paper I** that a major source of divergence along a 1700km latitudinal gradient are the two phylogeographic lineages colonizing Scandinavia from two different directions, creating a phenotypic break-point associated with a zone of secondary contact. Thus, divergent selection pressures during post glacial colonization and after establishment, together with the seasonal time constraints experienced at higher latitudes have strongly shaped the observable divergence in three important larval life-history traits.

In **Paper II**, I utilize the same study design but focus on the latitudinal distribution of genomic variation. Here, by reconstructing the post glacial demographic history I confirm that these are in fact two lineages that diverged before the last glacial maximum, and where historically gene flow has occurred between regions and more importantly over the secondary contact zone. This was further supported by estimating admixture/hybridization of the regions on each side of the contact zone. In this paper I also find a signature of divergent selection in numerous loci showing high levels of differentiation and association with growing season length with biological functions relating to immunity and development. Comparing variation at neutral and selected sites reveals contrasting patterns along the latitudinal gradient. Neutral variation and variation associated with growing season length decreases with latitude. However, variation identified based on high levels of differentiation show elevated levels of diversity at higher latitudes. I propose that this is a combination of adaptive and/or maladaptive gene flow over the secondary contact zone, together with elevated levels of genetic drift in the north.

In **Paper III** I considerably reduce the spatial scale under investigation by looking at populations distributed in a network of connected wetlands in the mid Swedish region of Uppland. Here I characterize the relative influence of local larval environment, landscape features and geographical separation on total genetic variation. I find that the pond environment explains more variation than landscape characteristics and that geographical separation explains a relatively small yet significant part of genetic variation. This shows that even

over a small spatial scale with ample opportunity for gene flow there is isolation-by-environment, isolation-by-landscape and isolation-by-distance all contributing to adaptive and neutral divergence. I find a fairly large number of SNPs associated with the local larval environment, with a wide range of biological functions such as immunity and development. These results show that selection imposed by local, small-scale conditions is strong enough to counteract drift and gene flow.

Finally, in **Paper IV** I simultaneously consider both spatial scales and focus on the distribution of phenotypic plasticity and how it is shaped by divergent selection and demographic processes. I also use a gene-phenotype association approach to identify candidate SNPs shaping variation in larval life-history traits and plasticity. I find that plasticity for larval period and mass at metamorphosis are both shaped by divergent selection in LGE. In SSE I find, however, that mass at metamorphosis as a trait shows no signature of divergent selection, but mass at metamorphosis plasticity does, and the opposite is true for larval period and its plasticity. This pattern is also mirrored at the local scale in LGE. I find that plasticity is highest in the north, and especially around each side of the secondary contact zone which represents the end point of each lineage's latitudinal distribution. Overall both in this paper and in **Paper I**, I find evidence of counter-gradient variation in larval period at both scales and co-gradient variation in mass at metamorphosis in LGE. I find a number of SNPs associated with the larval life-history traits and plasticity, and much like in Paper II and Paper III these are involved in development and growth as well as immunity. This indicates a potential trade-off between larval development rate and investment in immune defense.

In terms of future research perspectives, there is certainly a need to focus in and around the secondary contact zone in order to better capture gene flow dynamics and distribution of adaptive genetic and phenotypic variation. With this in mind, a second replicated latitudinal gradient, preferably on the eastern side of the Baltic would yield further insights to the postglacial demographic history of *R. arvalis*. This would also greatly improve our estimation of selection acting in the genome, as well as aid in teasing apart the confounding effects of the one-dimensional environmental gradient. The 4+ years that have passed since I started working on this thesis have resulted in technical improvements and significant decrease in price of various sequencing options. In order to better understand the genomic basis of adaptive divergence and phenotypic variation both at a large and small scale, a denser genome-wide marker panel would be highly beneficial and, with current genomic techniques, highly feasible. In more general terms we still know very little about how adaptive phenotypic variation corresponds to variation at the genome level, both in terms of single nucleotide variation and structural variation, and new mutations versus standing genetic variation. Furthermore, investigations

into the regulatory mechanisms governing changes in gene expression between contrasting environments is a necessary step to get a more complete picture of how the genome shapes phenotypic variation and adaptive divergence.

Svensk sammanfattning

Populationer utspridda i heterogena miljöer utsetts för ett samspel mellan divergent selektion som varierar rumsligt, genflöde och genetisk drift. Samspelet pågår över stora geografiska distanser samt distanser som skulle karakteriseras som mikrogeografiska, där effekten av divergent selektion kan leda till lokala anpassningar. Det är viktigt att förstå den genomiska och fenotypiska bakgrunden av lokala anpassningar eftersom det är en av de möjliga utfallen av klimatförändringar. Ett kraftfullt sätt att studera effekter av olika klimat på populationers genetiska och fenotypiska variation är att använda storskaliga miljögradienter, så som latitudinella gradienter. Populationer utspridda längs sådana gradienter är ofta karaktäriserade av en historia av migration över relativt långa distanser när de koloniserade lämpliga habitat vid högre latitud som tidigare var täckt av is under senaste istiden. Latitudinella gradienter är också ofta karaktäriserade av ett mönster som kallas för kontragradient variation. Det betyder att vid högre latitud så motsätter sig miljöinfluensen på en fenotyp, exempelvis låga medeltemperaturer i norr, den genetiska riktningen för att uttrycka den fenotypen, vilket ofta leder till reducerad fenotypisk variation längs gradienten, men resulterar i kryptisk divergens. Motsatsen är medgradient variation när miljöns påverkan går i samma riktning som den genetiska påverkan på fenotypen vilket resulterar i observerbar divergens längs gradienten.

I min avhandling så fokuserar jag på hur divergent selektion och demografisk historia formar genomisk och fenotypisk variation på olika geografiska skalor. Rent generellt så visar mina resultat att det finns en tät koppling mellan populationsdivergens och diverse tidsmässiga begränsningar som ett resultat av skillnader i säsongslängd och temperatur. I **Artikel I** visar jag att längs en 1700 km latitudgradient att divergens i livshistoriekaraktärer under larvstadiet som är direkt kopplad till framtida fitness är formade av latitudprocesser och demografisk historia. En stor del av divergensen är kopplad till de två fylogeografiska linjer som återkoloniserade Skandinavien från två olika riktningar efter den senaste istiden. Det har resulterat i en fenotypisk brytpunkt kopplad till en zon av sekundär kontakt efter återkoloniseringen. Sammanslaget så innebär det att divergenta selektionstryck under processen av återkolonisering samt efter populationerna har etablerats, kombinerat med säsongsmässiga tidsbegränsningar vid högre latitud har format en stor del av den observerbara divergensen i tre viktiga livshistoriekaraktärer.

I **Artikel II** använder jag populationerna från samma latitudgradient men här fokuserar jag på fördelning av genomisk variation längs gradienten. Jag rekonstruerar den demografiska historian för populationerna längs gradienten med fokus på vad som hände efter senaste istiden. Här visar jag att två fylogeografiska linjer som återkoloniserade Skandinavien divergerade innan senaste glaciala maxima. Jag visar också hur genflöde har pågått mellan regioner och framförallt över den sekundära kontaktzonen. Det här resultatet stöds även av mina hybridestimat som visar på stor mängd delat ursprung mellan regionerna som ligger på båda sidorna av den sekundära kontaktzonen. I den här artikeln så presenterar jag även resultat som visar en signatur av divergent selektion på många loci, antingen som en konsekvens av extrem differentiering eller som en association med längden på tillväxtsäsongen längs gradienten. De identifierade loci har biologiska funktioner relaterat till immunsystemfunktion och embryouteveckling mfl. Genom att jämföra genetisk variation i neutrala och selekterade loci längs gradienten visar jag olikheter i hur de fördelas baserat på latitud. Variation som evolverar neutralt och selekterad variation som är associerad med tillväxtsäsong minskar med ökad latitud. Selekterad variation som identifierats baserat på extrem differentiering visar ökade nivåer vid högre latitud. Det här mönstret har mest troligt formats av en kombination av genflöde över kontaktzonen, där nivån beror delvis på om variationen är gynnsam eller inte på vardera sida om kontaktzonen samt, tillsammans med ökade nivåer genetisk drift i de norra regionerna.

I **Artikel III** så minskar jag den geografiska skalan avsevärt genom att studera populationer som härstammar från ett småskaligt nätverk av våtmarker i Uppland med god konnektivitet. Här undersöker jag den relativa påverkan av den lokala miljön som grodlarverna växer upp i, aspekter av landskapet samt geografisk distans på total genetisk variation. Jag visar här att den lokala miljön förklarar mer total genetisk variation än landskap och att geografisk distans förklarar en relativt liten del av variationen. Det här innebär att trots en liten geografisk skala med god möjlighet för genflöde finner jag att isolering baserat på miljön, landskap och geografisk distans alla bidrar till att forma adaptiv och neutral divergens. Jag identifierar även ett ganska stort antal genetiska markörer associerade med lokala miljön, markörerna i fråga matchar gener och protein med en biologisk funktion relaterat till immunsystem och utveckling. Det här visar på att selektionstrycket från den lokala miljön är stark nog att motsätta sig genetisk drift och genflöde.

Slutligen, i **Artikel IV** så undersöker jag båda geografiska skalor från tidigare artiklar samtidigt. Jag fokuserar här på fördelning av variation av fenotypisk plasticitet och hur det har formats av divergent selektion och demografiska processer. Jag använder mig även av fenotyp-genotypassociation analyser för att identifiera genetiska markörer som bidrar till variation I livshistoriekaraktärer och plasticitet. Jag finner att plasticitet för larvperiod och vikt vid

metamorfos är båda formade av divergent selektion längs den latitudinella gradienten. Längs gradienten är plasticitet högst inom de norra regionerna, speciellt i regionerna som ligger på båda sidorna av den sekundära kontaktzonen. På den mindre geografiska skalan i våtmarken i Uppland så observerar jag ingen signatur av divergent selektion på vikt vid metamorfos, men på plasticitet av den egenskapen. Jag observerar dock det motsatta när det kommer till larvperiod och plasticitet i den egenskapen. Rent övergripande så visar jag i både **Artikel I** och i den här artikeln resultat som tyder på kontragradient variation i larvperiod samt medgradient variation i storlek vid metamorfos längs gradienten. Slutligen så identifierar jag en rad genetiska markörer associerade med livshistoriekaraktärer och plasticitet. Precis som I **Artikel II** och **Artikel III** så är de mest intressanta markörerna involverade I immunsystemet och utveckling, vilket I den här artikeln tyder på en avvägning mellan utvecklingshastighet och tidig immunrespons.

Acknowledgements

There are many people I would like to thank who made this thesis possible. I want to start by thanking all the frogs used in this thesis, their sacrifice was a very sad but necessary part of the work.

I would like to express enormous gratitude to my main supervisor **Anssi Laurila** for hiring me as a PhD student and for letting me pursue my own ideas that may have involved a bit more population genetics than what I believe was first intended. That to me is a sign of great scientific mentorship. I would also like to thank you for all the rewarding scientific discussions, encouragement, and help during this whole time.

I would also like to thank my second supervisor **Jacob Höglund** for always being encouraging and very optimistic. Thanks for all the scientific discussions we have had in your office, that always happened to be about football whenever Anssi or Frank walks in. Funny how that happens every time.

I also want to thank both of you for being encouraging, and when needed convincing me that my results are interesting, as I sometimes have a tendency to be overly pessimistic about various things. I could never imagine a better main and second supervisor!

The current and former members of the extended Laurila/Höglund lab-group have been extremely important in creating a good scientific atmosphere, and I would like to thank all of you. Thank you, **Yvonne**, for all the support, help with lab questions and nice conversations, since my masters up until now. **Maria**, thanks for everything, we have planned projects together, done fieldwork, and discussed so many ideas.

Thanks to **Rado**, who was my first office mate and created a very welcoming office atmosphere when I first started. Many thanks to **Peter** for answering all my library prep related questions. **Emilien** for laying the foundation for my PhD work by doing harsh and extensive field work. **Alex** for the great times in the field and nice scientific discussions. Thanks also to **Magnus**, **Rike**, **Gowri**, **Mette** and **Kai** (who makes the world's best dumplings).

I would also like to thank the people of the Guschanski lab who have been part of conservation lab meetings, for all the inspiring and stimulating exchange of ideas. I would also like to further thank **Katja** as well as **Sophie** for being on my committee and giving me great advice during our committee meetings.

Many thanks for all the fun at and outside of work: **Will**, who I have known the longest in Uppsala, we started the master's program together, and we started our PhD at the same time in the same department. We are sure all going to miss you now when you go on and have yourself *a nu start*. **Jordi**, we go way back too. It was fun having you as a roommate and you're always just fine. And thanks to **Mattias** a.k.a. Chefen, whom I have also known since my masters, you are always in a shiny mood and a pleasure to talk to. Thanks to **Sara** and a pleasure to share an office with, where we nerd out about frogs, fantasy and sci-fi – in addition, of course, to the serious business of science. To **Jay**, who also participates in nerd out business when it comes to watching blockbuster genre movies at the cinema, although no frogs just yet.

To **Josefine**, **Ivain**, and **Johanna** for always livening up the place, where lunch conversations can go in whatever direction imaginable. Thanks to **Martin** for corridor discussions about epigenetics, plasticity, R programming and IPA. Thanks to **Gunilla** and **Reija** for being invaluably helpful in the lab, helping me when I come back after a 10-month lab hiatus and have forgotten where all the basic reagents are located.

Many thanks to **Ingrid** for all the help and support and thanks to the rest of the **people** at Animal Ecology for creating a very nice work environment.

Thanks to my parents **Jan-Ove** and **Anneth** for all the support, relatives who are still here or have passed; **Inez**, **Jörn**, **Gustav**, **Kerstin**, **Ronny**, **Pia**, aunts and cousins. **Hundfan**, nej! And last but definitely not least, **Paula**, thank you for all your love and support throughout these years, te amo mais, I could not have done this without you. Thanks also for the beautiful tadpole drawing used on the cover of this thesis.

References

Adams RI, Hadly EA (2013). Genetic diversity within vertebrate species is greater at lower latitudes. *Evolutionary Ecolology* **27**: 133–143.

Alexander DH, Novembre J, Lange K (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research* **19**: 1655–1664.

Altwegg R, Reyer H-U (2003). Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* **57**: 872–882.

Babik W, Branicki W, Sandera M, Litvinchuk S, Borkin LJ, Irwin JT, *et al.* (2004). Mitochondrial phylogeography of the moor frog, Rana arvalis. *Molecular Ecology* **13**: 1469–1480.

Bailey, RI (2018). gghybrid: evolutionary analysis of hybrids and hybrid zones. R package version 0.0.0.9.

Barrett RDH, Schluter D (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution* **23**: 38–44.

Bates D, Mächler M, Bolker B, Walker S (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Becker CG, Rodriguez D, Longo AV, Talaba AL, Zamudio KR (2012). Disease risk in temperate amphibian populations is higher at closed-canopy sites. *PLOS ONE* **7**: e48205.

Blanckenhorn WU (1991). Life-history differences in adjacent water strider populations: phenotypic plasticity or heritable responses to stream temperature? *Evolution* **45**: 1520–1525.

Blanquart F, Kaltz O, Nuismer SL, Gandon S (2013). A practical guide to measuring local adaptation. *Ecology Letters* **16**: 1195–1205.

Blaustein AR, Wake DB, Sousa WP (1994). Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* **8**: 60–71.

Bonin A, Taberlet P, Miaud C, Pompanon F (2006). Explorative genome scan to detect candidate loci for adaptation along a

gradient of altitude in the common frog (*Rana temporaria*). *Molecular Biology and Evolution* **23**: 773–783.
Braciale TJ, Morrison LA, Sweetser MT, Sambrook J, Gething M-J, Braciale VL (1987). Antigen presentation pathways to class I and class II MHC-restricted T lymphocytes. *Immunological Reviews* **98**: 95–114.
Brennan RS, Healy TM, Bryant HJ, La MV, Schulte PM, Whitehead A (2018). Integrative population and physiological genomics reveals mechanisms of adaptation in killifish. *Molecular Biology and Evolution* **35**: 2639–2653.
Buerkle CA (2005). Maximum-likelihood estimation of a hybrid index based on molecular markers. *Molecular Ecology Notes* **5**: 684–687.
Campbell-Staton SC, Cheviron ZA, Rochette N, Catchen J, Losos JB, Edwards SV (2017). Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. *Science* **357**: 495–498.
Carlson BE, Langkilde T (2014). Predation risk in tadpole populations shapes behavioural responses of prey but not strength of trait-mediated indirect interactions. *Oikos* **123**: 1519–1527.
Carvalho DD, Ingvarsson PK, Joseph J, Suter L, Sedivy C, Macaya-Sanz D, *et al.* (2010). Admixture facilitates adaptation from standing variation in the European aspen (*Populus tremula L.*), a widespread forest tree. *Molecular Ecology* **19**: 1638–1650.
Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013). Stacks: an analysis tool set for population genomics. *Molecular Ecology* **22**: 3124–3140.
Caye K, Deist TM, Martins H, Michel O, François O (2016). TESS3: fast inference of spatial population structure and genome scans for selection. *Molecular Ecology Resources* **16**: 540–548.
Caye K, Jay F, Michel O, Francois O (2017). Fast inference of individual admixture coefficients using geographic data. *bioRxiv*: 080291.
Chapuis E, Trouve S, Facon B, Degen L, Goudet J (2007). High quantitative and no molecular differentiation of a freshwater snail (*Galba truncatula*) between temporary and permanent water habitats. *Molecular Ecology* **16**: 3484–3496.
Cheng C, White BJ, Kamdem C, Mockaitis K, Costantini C, Hahn MW, *et al.* (2012). Ecological genomics of *Anopheles gambiae* along a latitudinal cline: a population-resequencing approach. *Genetics* **190**: 1417–1432.

Conlon JM, Sonnevend A, Patel M, Al-Dhaheri K, Nielsen PF, Kolodziejek J, *et al.* (2004). A family of brevinin-2 peptides with potent activity against *Pseudomonas aeruginosa* from the skin of the Hokkaido frog, *Rana pirica*. *Regulatory Peptides* **118**: 135–141.

Conover DO, Duffy TA, Hice LA (2009). The covariance between genetic and environmental influences across ecological gradients. *Annals of the New York Academy of Sciences* **1168**: 100–129.

Conover DO, Schultz ET (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology & Evolution* **10**: 248–252.

Cortázar-Chinarro M, Lattenkamp EZ, Meyer-Lucht Y, Luquet E, Laurila A, Höglund J (2017). Drift, selection, or migration? Processes affecting genetic differentiation and variation along a latitudinal gradient in an amphibian. *BMC Evolutionary Biology* **17**: 189.

Cortázar-Chinarro M, Meyer-Lucht Y, Laurila A, Höglund J (2018). Signatures of historical selection on MHC reveal different selection patterns in the moor frog (*Rana arvalis*). *Immunogenetics* **70**: 477–484.

Czypionka T, Goedbloed DJ, Steinfartz S, Nolte AW (2018). Plasticity and evolutionary divergence in gene expression associated with alternative habitat use in larvae of the European Fire Salamander. *Molecular Ecology* **27**: 2698–2713.

Earl JE, Whiteman HH (2015). Are commonly used fitness predictors accurate? A meta-analysis of amphibian size and age at metamorphosis. *Copeia* **103**: 297–309.

Eckert CG, Samis KE, Lougheed SC (2008). Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Molecular Ecology* **17**: 1170–1188.

Endler J (1977). Geographic variation, speciation, and clines. Princeton University Press: Princeton, NJ

Excoffier L, Dupanloup I, Huerta-Sánchez E, Sousa VC, Foll M (2013). Robust demographic inference from genomic and SNP data. *PLOS Genetics* **9**: e1003905.

Frichot E, François O (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution* **6**: 925–929.

Furuya T, Matsuoka D, Nanmori T (2013). Phosphorylation of *Arabidopsis thaliana* MEKK1 via Ca(2+) signaling as a part of the cold stress response. *Journal of Plant Research* **126**: 833–840.

Galas L, Raoult E, Tonon M-C, Okada R, Jenks BG, Castaño JP, *et al.* (2009). TRH acts as a multifunctional hypophysiotropic factor in vertebrates. *General and Comparative Endocrinology* **164**: 40–50.

Gervasi SS, Foufopoulos J (2008). Costs of plasticity: responses to desiccation decrease post-metamorphic immune function in a pond-breeding amphibian. *Functional Ecology* **22**: 100–108.

Ghalambor CK, McKAY JK, Carroll SP, Reznick DN (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* **21**: 394–407.

Gjertsen AK (2007). Accuracy of forest mapping based on Landsat TM data and a kNN-based method. *Remote Sensing of Environment* **110**: 420–430.

Gosner KL (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**: 183–190.

Gosselin T Anderson E. C., Bradbury, I. (2016). assigner: assignment analysis with GBS/RAD data using R. R package version 0.4.1.https://github.com/thierrygosselin/assigner. doi:10.5281/zenodo.51453

Gosselin T, (2018). grur: an R package tailored for RADseq data imputations. R package version 0.0.10 https://github.com/thierrygosselin/grur. doi:10.5281/zenodo.496176

Guo Q (2012). Incorporating latitudinal and central–marginal trends in assessing genetic variation across species ranges. *Molecular Ecology* **21**: 5396–5403.

Guo B, Lu D, Liao WB, Merilä J (2016). Genomewide scan for adaptive differentiation along altitudinal gradient in the Andrew's toad *Bufo andrewsi*. *Molecular Ecology* **25**: 3884–3900.

Haldane JBS (1930). A mathematical theory of natural and artificial selection. (Part VI, Isolation.). *Mathematical Proceedings of the Cambridge Philosophical Society* **26**: 220–230.

Harrington SM, Hollingsworth BD, Higham TE, Reeder TW (2018). Pleistocene climatic fluctuations drive isolation and secondary contact in the red diamond rattlesnake (*Crotalus ruber*) in Baja California. *Journal of Biogeography* **45**: 64–75.

Heard GW, Scroggie MP, Clemann N, Ramsey DSL (2014). Wetland characteristics influence disease risk for a threatened amphibian. *Ecological Applications* **24**: 650–662.

Hedrick PW (2013). Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. *Molecular Ecology* **22**: 4606–4618.
Hewitt G (2000). The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.
Hewitt GM (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences* **359**: 183–195.
Hoffmann AA, Weeks AR (2006). Climatic selection on genes and traits after a 100-year-old invasion: a critical look at the temperate-tropical clines in *Drosophila melanogaster* from eastern Australia. *Genetica* **129**: 133.
Holt RD, Roy M (2007). Predation can increase the prevalence of infectious disease. *The American Naturalist* **169**: 690–699.
Hoverman JT, Gray MJ, Haislip NA, Miller DL (2011). Phylogeny, life history, and ecology contribute to differences in amphibian susceptibility to ranaviruses. *EcoHealth* **8**: 301–319.
Ishwaran H, Kogalur UB (2007). Random survival forests for R. R News 7(2), 25–31.
Ishwaran H,Kogalur UB (2018). Fast unified random forests for survival, regression, and classification (RF-SRC), R package version 2.7.0.
IUCN 2019. The IUCN red list of threatened species. Version 2019-2. http://www.iucnredlist.org. Downloaded on 18 July 2019.
Jin C, Flavell RA (2010). Molecular mechanism of NLRP3 inflammasome activation. *Journal of Clinical Immunology* **30**: 628–631.
Johansson F, Halvarsson P, Mikolajewski DJ, Höglund J (2017). Genetic differentiation in the boreal dragonfly *Leucorrhinia dubia* in the Palearctic region. *Biological Journal of the Linnean Society* **121**: 294–304.
Johnson PTJ, Stanton DE, Preu ER, Forshay KJ, Carpenter SR (2006). Dining on disease: how interactions between infection and environment affect predation risk. *Ecology* **87**: 1973–1980.
Jombart T (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**: 1403–1405.
Jombart T, Ahmed I (2011). adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* **27**: 3070–3071.
Kawecki TJ, Ebert D (2004). Conceptual issues in local adaptation. *Ecology Letters* **7**: 1225–1241.

Knopp T, Merilä J (2009). The postglacial recolonization of Northern Europe by *Rana arvalis* as revealed by microsatellite and mitochondrial DNA analyses. *Heredity* **102**: 174–181.

Kopp E, Medzhitov R, Carothers J, Xiao C, Douglas I, Janeway CA, *et al.* (1999). ECSIT is an evolutionarily conserved intermediate in the Toll/IL-1 signal transduction pathway. *Genes & Development* **13**: 2059–2071.

Korhonen L, Korhonen K, Rautiainen M, Stenberg P (2006). Estimation of forest canopy cover: a comparison of field measurement techniques. *Silva Fennica* **40**.

Lancaster LT, Dudaniec RY, Hansson B, Svensson EI (2015). Latitudinal shift in thermal niche breadth results from thermal release during a climate-mediated range expansion. *Journal of Biogeography* **42**: 1953–1963.

Laugen AT, Laurila A, Räsänen K, Merilä J (2003). Latitudinal countergradient variation in the common frog (*Rana temporaria*) development rates – evidence for local adaptation. *Journal of Evolutionary Biology* **16**: 996–1005.

Lin YH, Zhang W, Li JW, Zhang HW, Chen DY (2017). Amphioxus ortholog of ECSIT, an evolutionarily conserved adaptor in the toll and BMP signaling pathways. *Journal of Molecular Biology* **51**: 34–40.

Lind MI, Ingvarsson PK, Johansson H, Hall D, Johansson F (2011). Gene flow and selection on phenotypic plasticity in an island system of *Rana Temporaria*. *Evolution* **65**: 684–697.

Lindgren B, Laurila A (2010). Are high-latitude individual's superior competitors? A test with *Rana temporaria* tadpoles. *Evol Ecol* **24**: 115–131.

Linnen CR, Kingsley EP, Jensen JD, Hoekstra HE (2009). On the origin and spread of an adaptive allele in deer mice. *Science* **325**: 1095–1098.

Luu K, Bazin E, Blum MGB (2017). pcadapt: an R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources* **17**: 67–77.

Machado HE, Bergland AO, O'Brien KR, Behrman EL, Schmidt PS, Petrov DA (2016). Comparative population genomics of latitudinal variation in *Drosophila simulans* and *Drosophila melanogaster*. *Molecular Ecology* **25**: 723–740.

Mastretta-Yanes A, Arrigo N, Alvarez N, Jorgensen TH, Piñero D, Emerson BC (2015). Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly

optimization for population genetic inference. *Molecular Ecology Resources* **15**: 28–41.
Meyer-Lucht Y, Luquet E, Jóhannesdóttir F, Rödin-Mörch P, Quintela M, Richter-Boix A, *et al.* (2019). Genetic basis of amphibian larval development along a latitudinal gradient: Gene diversity, selection and links with phenotypic variation in transcription factor C/EBP-1. *Molecular Ecology* **28**: 2786–2801.
Michel MJ (2011). Spatial dependence of phenotype-environment associations for tadpoles in natural ponds. *Evolutionary Ecology* **25**: 915–932.
Miraldo A, Li S, Borregaard MK, Flórez-Rodríguez A, Gopalakrishnan S, Rizvanovic M, *et al.* (2016). An Anthropocene map of genetic diversity. *Science* **353**: 1532–1535.
Morley JE (1981). Neuroendocrine control of thyrotropin secretion. *Endocrine Reviews* **2**: 396–436.
Munch SB, Mangel M, Conover DO (2003). Quantifying natural selection on body size from field data: winter mortality in *Menidia Menidia*. *Ecology* **84**: 2168–2177.
Murillo-Rincón AP, Kolter NA, Laurila A, Orizaola G (2017). Intraspecific priority effects modify compensatory responses to changes in hatching phenology in an amphibian. *Journal of Animal Ecology* **86**: 128–135.
Nakajima K, Fujimoto K, Yaoita Y (2005). Programmed cell death during amphibian metamorphosis. *Semininars in Cell & Developmental Biology* **16**: 271–280.
Nielsen EE, Hemmer-Hansen J, Poulsen NA, Loeschcke V, Moen T, Johansen T, *et al.* (2009). Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). *BMC Evolutionary Biology* **9**: 276.
Oksanen J, Guillaume-Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2019). vegan: community ecology package. R package version 2.5-5. https://CRAN.R-project.org/package=vegan
Orizaola G, Laurila A (2016). Developmental plasticity increases at the northern range margin in a warm-dependent amphibian. *Evolutionary Applications* **9**: 471–478.
Orizaola G, Quintela M, Laurila A (2010). Climatic adaptation in an isolated and genetically impoverished amphibian population. *Ecography* **33**: 730–737.

Palik B, Batzer DP, Buech R, Nichols D, Cease K, Egeland L, *et al.* (2001). Seasonal pond characteristics across a chronosequence of adjacent forest ages in northern Minnesota, USA. *Wetlands* **21**: 532–542.

Pallares LF, Harr B, Turner LM, Tautz D (2014). Use of a natural hybrid zone for genomewide association mapping of craniofacial traits in the house mouse. *Molecular Ecology* **23**: 5756–5770.

Palo JU, O'Hara RB, Laugen AT, Laurila A, Primmer CR, Merilä J (2003). Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative genetic data. *Molecular Ecology* **12**: 1963–1978.

Pastenes L, Valdivieso C, Genova AD, Travisany D, Hart A, Montecino M, *et al.* (2017). Global gene expression analysis provides insight into local adaptation to geothermal streams in tadpoles of the Andean toad *Rhinella spinulosa*. *Scientific Reports* **7**: 1966.

Paxton RJ, Thorén PA, Tengö J, Estoup A, Pamilo P (1996). Mating structure and nestmate relatedness in a communal bee, *Andrena jacobi* (*Hymenoptera, Andrenidae*), using microsatellites. *Molecular Ecology* **5**: 511–519.

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLOS ONE* **7**: e37135.

Prasad A, Croydon-Sugarman MJF, Murray RL, Cutter AD (2011). Temperature-dependent fecundity associates with latitude in *Caenorhabditis Briggsae*. *Evolution* **65**: 52–63.

Räsänen K, Hendry AP (2008). Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecology Letters* **11**: 624–636.

Reese H, Nilsson M, Pahén TG, Hagner O, Joyce S, Tingelöf U, *et al.* (2003). Countrywide estimates of forest variables using satellite data and field data from the National Forest Inventory. *Ambio* **32**: 542–548.

Richardson JL, Urban MC, Bolnick DI, Skelly DK (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology & Evolution* **29**: 165–176.

Richter-Boix A, Katzenberger M, Duarte H, Quintela M, Tejedo M, Laurila A (2015). Local divergence of thermal reaction norms

among amphibian populations is affected by pond temperature variation. *Evolution* **69**: 2210–2226.
Richter-Boix A, Quintela M, Kierczak M, Franch M, Laurila A (2013). Fine-grained adaptive divergence in an amphibian: genetic basis of phenotypic divergence and the role of nonrandom gene flow in restricting effective migration among wetlands. *Molecular Ecology* **22**: 1322–1340.
Richter-Boix A, Quintela M, Segelbacher G, Laurila A (2011). Genetic analysis of differentiation among breeding ponds reveals a candidate gene for local adaptation in *Rana arvalis*. *Molecular Ecology* **20**: 1582–1600.
Rollins-Smith LA (2009). The role of amphibian antimicrobial peptides in protection of amphibians from pathogens linked to global amphibian declines. *Biochimica et Biophysica Acta* **1788**: 1593–1599.
Rollins-Smith LA, Conlon JM (2005). Antimicrobial peptide defenses against chytridiomycosis, an emerging infectious disease of amphibian populations. *Development & Comparative Immunol* **29**: 589–598.
Rundle HD, Nosil P (2005). Ecological speciation. *Ecology Letters* **8**: 336–352.
Schemske DW, Mittelbach GG, Cornell HV, Sobel JM, Roy K (2009). Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics* **40**: 245–269.
Schmid M, Guillaume F (2017). The role of phenotypic plasticity on population differentiation. *Heredity* **119**: 214–225.
Sexton JP, Hangartner SB, Hoffmann AA (2014). Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution* **68**: 1–15.
Sexton JP, McIntyre PJ, Angert AL, Rice KJ (2009). Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics* **40**: 415–436.
Sillero N, Campos J, Bonardi A, Corti C, Creemers R, Crochet P-A, *et al.* (2014). Updated distribution and biogeography of amphibians and reptiles of Europe. *Amphibia-Reptilia* **35**: 1–31.
Smith MA, Green DM (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **28**: 110–128.

Stoks R, Block MD, Slos S, Doorslaer WV, Rolff J (2006). Time constraints mediate predator-induced plasticity in immune function, condition, and life history. *Ecology* **87**: 809–815.

Suzuki K, Sato K, Katsu K, Hayashita H, Kristensen DB, Yoshizato K (2001). Novel *Rana* keratin genes and their expression during larval to adult epidermal conversion in bullfrog tadpoles. *Differentiation* **68**: 44–54.

Swaegers J, Mergeay J, Geystelen AV, Therry L, Larmuseau MHD, Stoks R (2015). Neutral and adaptive genomic signatures of rapid poleward range expansion. *Molecular Ecology* **24**: 6163–6176.

Tang F, Ishwaran H (2017). Random forest missing data algorithms. *Statistical Analysis and Data Mining* **10**: 363–377.

Tigano A, Friesen VL (2016). Genomics of local adaptation with gene flow. *Molecular Ecology* **25**: 2144–2164.

Vasemägi A (2006). The adaptive hypothesis of clinal variation revisited: single-locus clines as a result of spatially restricted gene flow. *Genetics* **173**: 2411–2414.

Vos CC, Jong AGA, Goedhart PW, Smulders MJM (2001). Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). *Heredity* **86**: 598.

Wang H, Behrens EM, Pignolo RJ, Kaplan FS (2018). ECSIT links TLR and BMP signaling in FOP connective tissue progenitor cells. *Bone* **109**: 201–209.

Wang IJ, Bradburd GS (2014). Isolation by environment. *Molecular Ecology* **23**: 5649–5662.

Wang IJ, Summers K (2010). Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. *Molecular Ecology* **19**: 447–458.

Whitlock MC, Guillaume F (2009). Testing for spatially divergent selection: comparing QST to FST. *Genetics* **183**: 1055–1063.

Williams SE, Shoo LP, Isaac JL, Hoffmann AA, Langham G (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *PLOS Biology* **6**: e325.

Woodhams DC, Bell SC, Bigler L, Caprioli RM, Chaurand P, Lam BA, *et al.* (2016). Life history linked to immune investment in developing amphibians. *Conservation Physiology* **4**.

Wright S (1943). Isolation by Distance. *Genetics* **28**: 114–138.

Yang W, Qi Y, Fu J (2016). Genetic signals of high-altitude adaptation in amphibians: a comparative transcriptome analysis. *BMC Genetics* **17**: 134.

Zhang H, Xu Q, Krajewski S, Krajewska M, Xie Z, Fuess S, *et al.* (2000). BAR: An apoptosis regulator at the intersection of caspases and Bcl-2 family proteins. *Proceedings of the National Academy of Sciences* **97**: 2597–2602.

Zhou X, Carbonetto P, Stephens M (2013). Polygenic modeling with bayesian sparse linear mixed models. *PLOS Genetics* **9**: e1003264.

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Editor: The Dean of the Faculty of Science and Technology

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