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Chapter 2

Local Scale Genetic Diversity and its Role in Coping with Changing Climate

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Additional information is available at the end of the chapter

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Abstract

Climate change is thought to alter the patterns of genetic diversity within species and populations. Yet, it is not well-understood how genetic diversity influences organism's adaptation to changing climate. In this chapter, I explore how within-population genetic diversity may be affected by local environmental heterogeneity and to what extent this variation may promote adaption. I focus on mountain ecosystems since they are heterogeneous environments at a fine scale that offer a unique mosaic of highly localized environmental conditions. I start summarizing the drivers of genetic isolation at a local scale and the diversification and adaptation patterns that result from it. I continue discussing these processes in terms of populations' reactions to changing conditions using my own long-term ecological genomic studies. This allows me to demonstrate that local-scale variation, in the long term, may offer safe places for species in a warming world due to their fine-scale topographic variability, which may provide suitable habitats within only a few meters of species' current locations. Yet, such fine-scale habitat variability can also lead to locally genetically adapted populations, so that individuals and populations adapted to a narrow range of conditions may respond poorly to future environments.

Keywords: genome-wide scans, hereditability, evolutionary responses, genetic adaptation

1. Introduction: The mosaic of environmental heterogeneity at a fine scale

Understanding how organisms respond to climate change is a main research area in evolutionary genetics. Organisms, populations or species may respond to environmental change in three possible ways: by migrating, persisting in current locations or going extinct [1]. Persistence in new environments may be mediated by phenotypic plasticity, which is the range of phenotypes that a single genotype can express as a function of its environment [2] or

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by adaptation from genetic variation by increasing the frequency of existing allelic variants that can cope with the new conditions [3]. Local adaptation to heterogeneous habitats has been documented [4, 5], but the genetics of locally adapted populations are not always well understood [5].

Globally, some of the largest impacts of climate change are expected to occur in alpine environments, particularly near mountain summits, which are dominated by long-lived plant species. In these environments, snow cover and summer temperatures are the major drivers of vegetation composition [6]. The increase in temperature over the last decades has already led to patterns of upward migration in several species [7]. The alpine region is a highly heterogeneous environment that is characterized not only by strong elevational gradients in temperature but also by local topography. Microtopographical features include depressions in which the snow accumulates and disappears very late in the summer (i.e. snowbeds) and more exposed ridges with less snow and where the snow disappears several weeks to months earlier (**Figure 1**). Similar heterogeneities or gradients that may occur across the alpine zone are due to wind exposure, water availability, rockiness and neighbouring shrubs [8], among other factors. Some of these local-scale differences have been shown to cause local adaptation (e.g. in *Dryas* and in *Ranunculus* [9]). For species that occur in heterogeneous habitats, such small-scale variation can have dramatic implications for their response potential to climate change. Small-scale environmental variability may provide new locations with suitable habitats only a few meters away from present locations [10, 11]. Alternatively, such small-scale habitat variability can lead to locally adapted subpopulations [4], and these genotypes that are adapted to a more narrow range of conditions may respond poorly to future conditions.



Figure 1. Mosaic of snowbeds and exposed ridges in the spring (May 2011) on Wannengrat, Switzerland.

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Therefore, to understand processes involved in potential responses to changing conditions, it is important to consider not only climate differences between different altitudes but also differences between microhabitats [12–14], because environmental variation at a local-scale can be high [15]. In this chapter, I explore the microhabitat-driven patterns (Section 2) and processes (Section 3) and its impact on genetic diversity. Although there is a focus on alpine ecosystems and sessile organisms throughout this work, the concepts are generalizable. I also summarize the methodologies (Section 4) used to study phenotypic and genetic variation in microhabitats. To understand the interaction among fine-scale environmental variation, genetic diversity and the evolutionary responses of populations and organisms in a changing climate, the following questions can be asked:

- Are patterns of genetic differentiation and gene flow driven by small-scale environmental differences?
- Do morphological and fitness-related traits show heritable variation and is selection currently acting on any of these traits so that they can evolve given changing conditions?
- What is the microhabitat-driven pattern of genomic divergence?
- What is the genomic architecture of ecologically relevant traits at the microhabitat level?

2. Drivers of genetic isolation at a fine scale

The transfer of alleles between populations is known as gene flow. When this allelic transfer is limited or interrupted, there is genetic isolation. Understanding patterns of genetic variation and gene flow across the fine-scale mosaic will help to predict the response of species to climate change. As an example, under climate warming in alpine ecosystems, snowmelt is expected to occur earlier in the season [16]. Restricted gene flow between subpopulations growing in different microhabitats can be linked with local adaptation [17]. In this scenario, genotypes of long-lived dominant species in late snowmelt habitats may have difficulties to persist under warming conditions. On the other hand, genotypes in early snowmelt habitats would need to migrate to new localities, and this might be difficult in long-lived species even if suitable localities are nearby. Alternatively, lack of differentiation between subpopulations in different microhabitats and rampant gene flow between them could lead to genotypes able to grow in both microhabitats and thus persist *in situ* during climate change. Genetic variation contained in subpopulations in early and late snowmelt microhabitats could also differ because of factors such as asymmetric gene flow. This will influence whether genetic variation is lost from one of the microhabitats. In this section, I will cover the main drivers that may limit gene flow across microhabitats. In Section 3, I will consider its major evolutionary and genetic consequences, specifically for adaptation and diversification in populations.

2.1. Mismatch in flowering time and pollen flow

Variation in the timing of flowering between subpopulations in different snow microhabitats can be a major driver of small-scale genetic structuring [9] through the restriction of

pollen-mediated gene flow (as compared to seed-mediated gene flow), regardless of whether flowering time is genetically or environmentally regulated [9, 10, 18]. Small-scale genetic differentiation (measured by the F_{ST}, which is the fixation index, a measure of population differentiation due to genetic structure) has been reported in the majority of studies on snowmelt-driven genetic differentiation [11, 19–21].

2.2. Asymmetry in seed dispersal

Although there may be differentiation in populations' phenology (the timing at which periodic life cycle events happen, like bud breaking, flowering, fruiting and bud setting) between microhabitats due to snowmelt timing (Section 2.1, **Figure 2**), sub-populations growing in different microhabitats do not have to be genetically differentiated [23, 24]. This pattern has been observed in *Empetrum hermaphroditum* (Hagerup) [25] and *Ranunculus adoneus* (Gray) [9] but perhaps most extensively examined in *Salix herbacea* L. a clonal, dioecious, dwarf shrub dominant in the arctic, subarctic and in alpine ecosystems in central Europe [26]. In the Swiss Alps, *S. herbacea* is an ideal species for addressing the impacts of climate change, as it grows along a pronounced elevational gradient (2100–2800 m asl) and occupies a wide range of microhabitats such as rocky, early-exposure ridges, and late-season snowbeds.

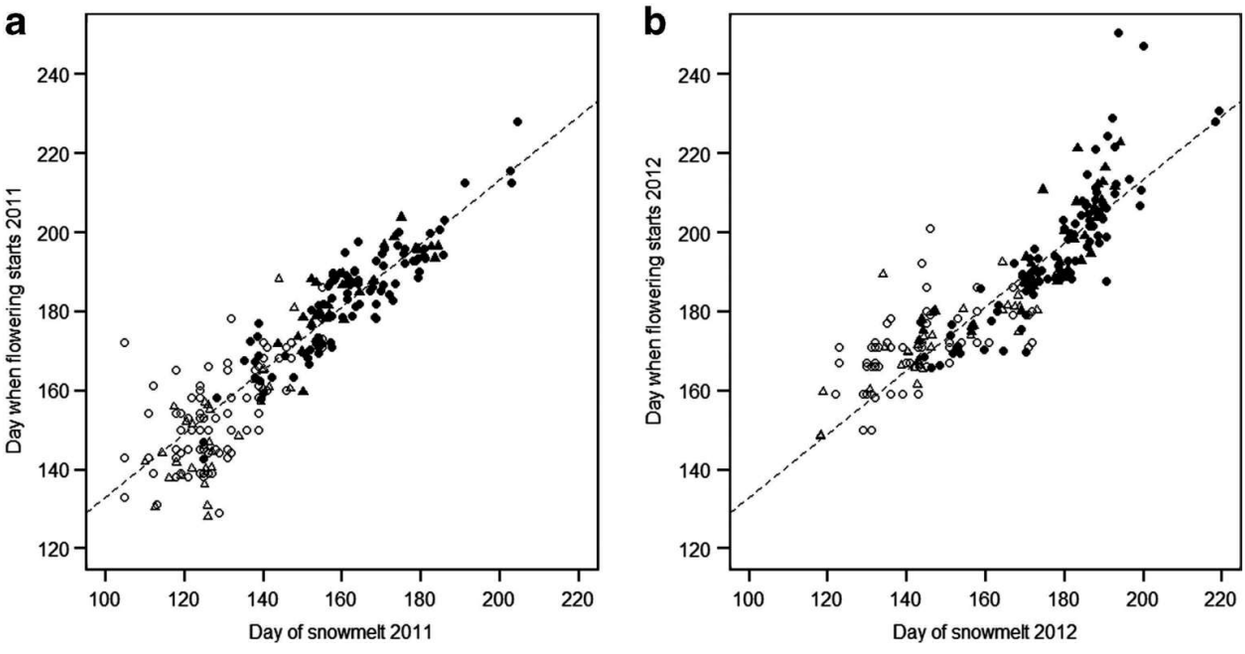


Figure 2. Day of snowmelt predicts when flowering starts for 274 female *Salix herbacea* patches growing on ridges (○) and snowbeds (●) and 85 male *S. herbacea* patches growing on ridges (Δ) and snowbeds (▲) surveyed in (a) 2011 and (b) 2012. Dashed lines are regression lines ($R^2 = 0.827$, P value < 0.001). Modified from Cortés et al. [22].

Although *S. herbacea* populations growing in different microhabitats could be differentiated phenologically, they could not be differentiated genetically [22] using 7 highly polymorphic molecular markers, F_{ST} and N_em estimation and STRUCTURE analysis (see Section 4.4.1). F_{ST} was 0.028 ± 0.003 and 0.035 ± 0.004 for within-microhabitat and between-microhabitat comparisons, P-value = 0.691. Lack of population structure was supported by a STRUCTURE

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analysis. This absence of population differentiation, even in microhabitats with highly different snowmelt dates, may be mediated by high and asymmetric seed dispersal [22].

Seed dispersal can counteract isolation driven by barriers to pollen flow, like snow, because seed dispersal occurs later in the season when all winter snow has melted [27]. Gene flow via seed dispersal may result in asymmetric source/sink-like patterns driven by wind, topology and the success of seed establishment [28].

In the *S. herbacea* example, late-snowmelt microhabitats (snowbeds) were genetically more diverse than early-snowmelt sites (i.e. allelic richness: 8.93 ± 0.27 and 6.81 ± 0.29 for snowbeds and ridges, respectively), and gene flow, measured as the number of migrants per generation, was asymmetric toward the snowbeds (**Figure 3**). Overall, these results are consistent with snowbeds acting as sinks of genetic diversity and seed dispersal preventing snowmelt-driven genetic isolation [22].

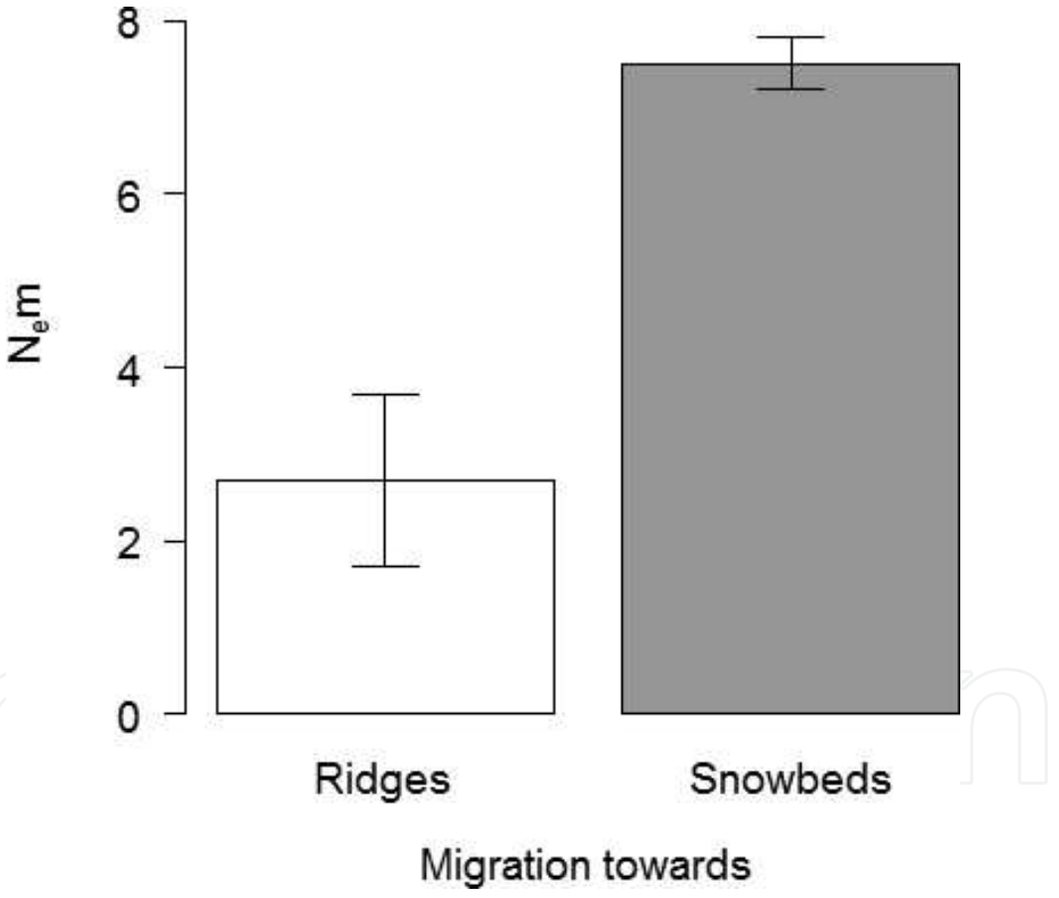


Figure 3. Estimates of the number of migrants per generation (N_em) between microhabitats differing in snowmelt timing (ridges and snowbeds) in *Salix herbacea* from three transects in the Swiss Alps. Modified from Cortés et al. [22].

3. Genetic adaptation and diversification at a fine scale

Most research on the responses of species to changing snowmelt and temperature conditions has focused on species migration toward higher altitudes, where researchers can track

the species' climate requirements [29–32]. However, if migration potential is limited, the only way organisms can persist is by adjusting to the new environmental conditions [18, 33]. Adjustment though phenotypic plasticity might be particularly important in long-lived species, as it can occur within the lifetime of an individual [2]. However, plasticity may be constrained or even maladaptive, if populations are exposed to novel conditions outside the range of conditions they encountered in their evolutionary history [33]. Alternatively, adaptation from standing genetic variation may happen by increasing the frequency of existing variants that can cope with the new conditions [34]. While adaptation is dependent on the genotypes, plasticity itself depends on the environment.

Genomic divergence, which is the genetic differentiation throughout the genome, has been studied mostly among species and well-differentiated populations [35, 36] but few of them have been studied at a very local scale from a genome-wide point of view. Genomes are regarded as porous since different regions present multiple signatures and levels of gene flow, drift, selection and ancestral variation [36]. Genome-wide divergence is heterogeneous, with peak-like or plateau-like sections of high divergence surrounded by genomic regions with lower divergence, a landscape described metaphorically like 'islands' and 'continents' of divergence [35]. High divergence in specific sections may be due to disrupting selection from novel or standing genetic variation [37] or random drift [38]. On the other hand, regions with low divergence may be maintained by balancing or uniform selection, continuous gene flow or ancestral-shared polymorphism [39, 40].

An approach that combines between-microhabitat genomic divergence with selection gradients and association mapping of ecologically relevant traits is useful to understand which regions in the genome are likely to differ between populations in different microhabitats, and therefore harbour genetic variation unique to each, and how these genomic regions may relate to phenological, growth and fitness traits (**Figure 4**) [41–44]. Ultimately, this combined approach will allow differentiating plastic and adaptive variation.

3.1. Selection and evolutionary responses

Three essential components are necessary for evolution to occur: there must be trait variation, which must be heritable and selection should be acting on it [45]. A multivariate form of the breeder's equation [46] illustrates this paradigm well and allows for the prediction of the evolutionary response of a trait to selection over one generation ($\boldsymbol{R}$), as expressed in Eq. (1):

$$\boldsymbol{R} = \boldsymbol{G}\boldsymbol{\beta} \tag{1}$$

where $\boldsymbol{G}$ is the variance-covariance matrix of additive genetic parameter estimates ($\boldsymbol{G}$ matrix, or a proxy for heritabilities and traits' trade-offs), and $\boldsymbol{\beta}$ is the vector of standardized selection gradients for the focal traits [47]. The evolutionary response can be calculated using selection-gradient estimates derived from fitness proxies (i.e. fitness regressed as a function of standardized trait values) and marker-based heritabilities [48], using highly polymorphic molecular markers (see Section 4.4.1) as is explained in Section 4.5.3.

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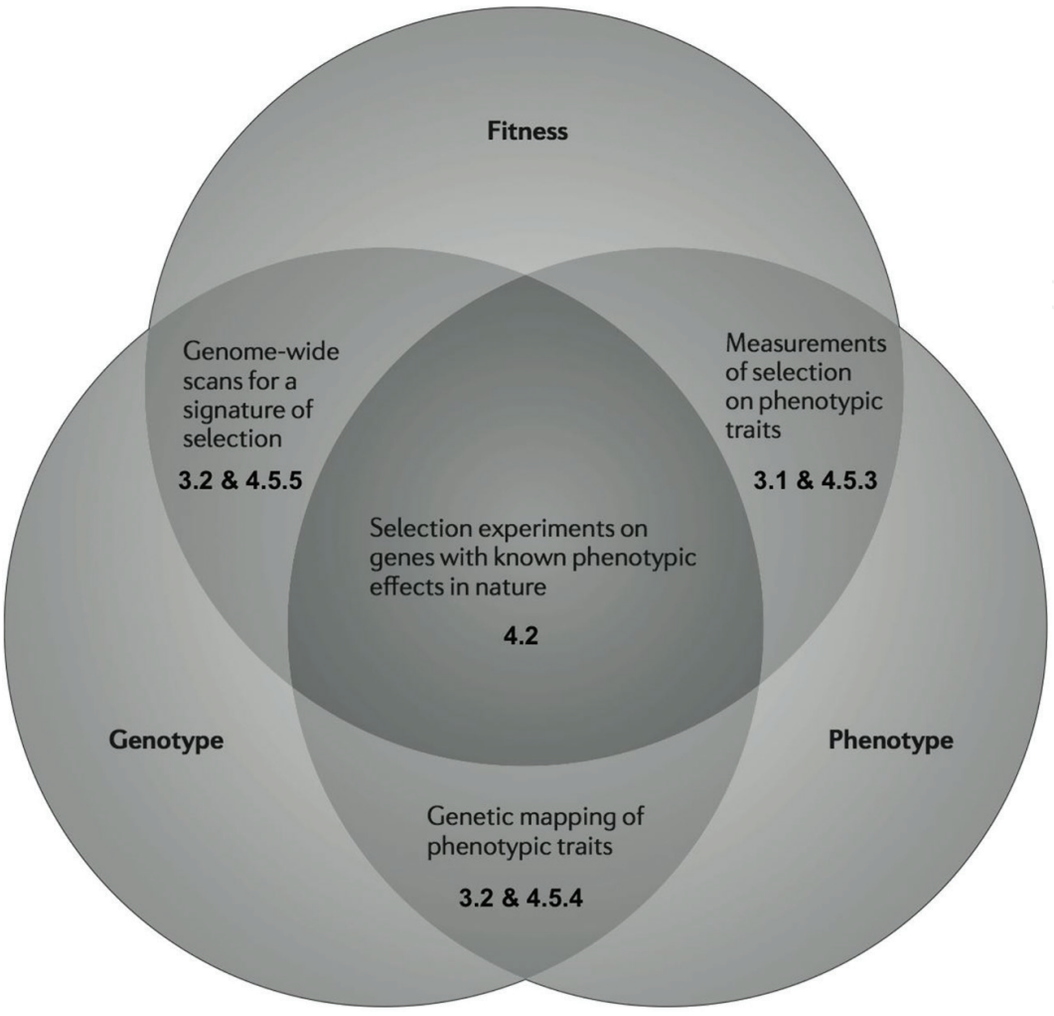


Figure 4. Connections between various approaches for studying the genetics of ecologically relevant variation. Numbers indicate sections in this chapter dealing with the specific concepts. Modified from Barrett & Hoekstra [41].

After the *S. herbacea* case study introduced in Section 2.2, marker-based relatedness estimates in natural populations were used to calculate heritabilities for phenological and morphological traits. For instance, there was selection toward smaller leaves and shorter thermal duration until leaf expansion when using clonal reproduction (change in stem number) as a fitness proxy, in both ridge and snowbed microhabitats (**Table 1**). Conversely, there was selection toward longer thermal durations until flowering in both ridge and snowbed microhabitats when using sexual reproduction (proportion of flowering stems) as a fitness proxy. Selection on thermal durations until flowering diverged in the two microhabitat types when using clonal reproduction as a fitness proxy. This suggests that selection pressures on phenology may vary with ongoing climate change.

Additionally, when using the multivariate form of the breeder's equation (Eq. (1)) to estimate potential evolutionary responses of traits in the *S. herbacea* case study, while accounting for genetic correlations among traits and selection on these traits [12], the strongest predicted response was found for leaf size and the interval between snowmelt and leaf expansion (R = −5.238 days per

generation) when using clonal reproduction as a fitness proxy. Given these traits, the adaptive potential could allow *S. herbacea* to adapt to climate change. Since earlier snowmelt is the most likely oncoming scenario [49], evolutionary response might shift toward the reaction that is currently observed on ridge microhabitats. Therefore, longer thermal duration until flowering is hypothesized, as a natural way to avoid early season frost damage [12, 14].

Fitness	Trait (standardized)	β	df	F	p
Proportion of flowering stems	Leaf size	−0.023	67	0.032	0.86
	Interval snowmelt to leaf expansion	0.029	67	0.342	0.561
	Thermal duration until leaf expansion	−0.041	67	0.426	0.516
	Thermal duration until flowering	0.211	67	4.153	**0.046**
	Leaf size × MH	0.054	67	0.076	0.784
	Interval snowmelt to leaf expansion × MH	0.179	67	1.110	0.296
	Thermal duration until leaf expansion × MH	−0.095	67	0.376	0.542
	Thermal duration until flowering × MH	−0.075	67	0.163	0.688
Change in stem number	Leaf size	−5.232	116	−3.279	**0.011**
	Interval snowmelt to leaf expansion	−3.655	116	−2.217	0.051
	Thermal duration until leaf expansion	−3.646	116	−2.218	**0.020**
	Thermal duration until flowering	2.467	116	1.418	0.85
	Leaf size × MH	3.758	116	1.614	0.073
	Interval snowmelt to leaf expansion × MH	4.058	116	1.308	0.184
	Thermal duration until leaf expansion × MH	1.644	116	0.696	0.805
	Thermal duration until flowering × MH	−4.821	116	−2.047	**0.043**

Linear mixed models were run separately for the two relative fitness proxies: proportion of flowering stems (h^2 = 0.049) and change in stem number (h^2 = **0.071**) and included the traits leaf size (h^2 = **0.386**), interval between snowmelt and leaf expansion (h^2 = **0.178**), thermal duration until leaf expansion (h^2 = **0.469**) and flowering (h^2 = **0.399**), and their interactions with microhabitat type (microhabitat—MH: positive interaction with ridges if $\beta > 0$ and positive interaction with snowbeds if $\beta < 0$), with the plot nested within the transect as a random effect. Estimates of narrow-sense heritability (h^2) are based on the multivariate animal model with a marker based relatedness matrix. Significant values (P-value < 0.05) are in bold based on the F statistic (F), the degrees of freedom (df) and its P-value (p). Significant h^2 values are also in bold. Modified from Sedlacek et al. [12].

Table 1. Standardized selection gradients (β) across microhabitats differing in snowmelt timing (ridges and snowbeds) in *Salix herbacea* in the Swiss Alps.

3.2. Adaptation and plasticity

Persistence of populations and species given climate change may be mediated by phenotypic plasticity [2] or by adaptation from standing variation by increasing the frequency of existing

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variants that can cope with the new conditions [3]. Epigenetic mechanisms, which are those modulated by environmental factors that switch genes on and off and affect how cells read those genes, may also affect how plants respond to climatic variability [50]. In Section 4, I review ways to access the roles of plasticity and adaptation in explaining trait variation across the fine-scale mosaic. In this section, I discuss the utility of genome-wide analysis to infer microhabitat-driven divergent selection and the genetic basis of trait variation. Although the following results concern *S. herbacea* growing under different snowmelt regimens, this type of analysis also extends to other scenarios such as local scale variation in the occurrence of drought [51–53].

In the *S. herbacea* case study, eight strong, between-microhabitat divergence peaks and two weaker peaks were detected on seven different chromosomes (**Figure 5**). These regions coincided with regions of low SNP (see Section 4.4.2 for a complete definition of this type of molecular marker) density, extensive linkage disequilibrium (a measure of dependency among loci) and negative Tajima's D values (a statistic that describes whether molecular evolution is random). This suggests that novel genetic variation may arise and be fixed in snowbeds and ridges separately in contrast to standing variation that is differentially recruited between microhabitats. The same highly divergent sections persisted when the between-microhabitat F_{ST} was calculated per transect, and they coincided with "valleys" in the F_{ST} when it was computed within microhabitats and across transects. This pattern is an indication that the observed divergent peaks are not due to genetic drift [54], which is the random change in the frequency of alleles. This indicates that genomic divergence can occur in the presence of gene flow and strong environmental differentiation at a very fine geographic scale. The ten between-microhabitat divergence regions spanned a total of 219 genes, which may help to infer functional traits that diverge between microhabitats. This approach is known as forward genetics or bottom-up inference because it makes conclusions regarding unseen traits by first looking at the underlying genetic variation.

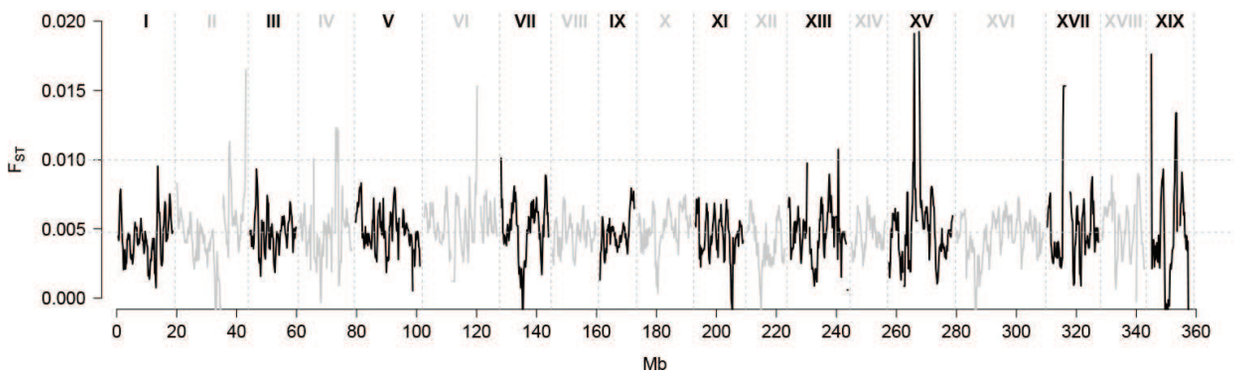


Figure 5. Between-microhabitat genomic divergence in *Salix herbacea*. Sliding window analysis for the average between-microhabitat fixation index (F_{ST}). The window size is 1×10^6 basepairs (bps) and the step size is 200 kilobases (kb). Results of all windowed analyses are plotted against window midpoints in millions of bps. Black and grey colours highlight different chromosomes identified by roman numerals. The lower and upper grey–dashed horizontal lines indicate the genome-wide average and the threshold for the identification of outliers, respectively. Modified from Cortés et al. [54].

In addition to the population genomics approach used to study microhabitat-driven divergence and identify traits that may have diverged between environments, association

mapping is usually performed to explore genetically based variation in ecologically relevant traits across microhabitats [54, 55], part of what is known as reverse genetics or top-down inference, as it looks at the genetic basis of specific traits. In the *S. herbacea* case, 57 genomic sections spanning 66 SNP markers were significantly associated with the surveyed traits for which high heritabilities were reported (see Section 3.1). These associated markers explained for each trait, on average, 19% of the observed variation. Ten regions included candidate genes for seven of the nine analysed traits.

4. Heterogeneous microhabitats as a field laboratory to study genetic reactions to climate change

Regions in the world that are experiencing extremely high diversification rates, such as the alpine tundra ecosystems known as páramos [54, 56], and convergent adaptation, like in mountainous microhabitats [57, 58], are good candidates to serve as evolutionary experiments for today's scientists. In this section, I explore the ecological and genetic sampling strategies and the analytical methods commonly used to cover biological variation across altitudes and microhabitats when addressing the main questions suggested at the beginning of this chapter.

4.1. Natural surveys across microhabitats

Plot-based and transect surveys are the two main sampling strategies that allow the study of microhabitat-driven variation. Three-to-five different transects spanning a range of north-east (sun/shade) exposure and covering the main elevational range are the best compromise between sampling effort and feasibility. These surveys at a microhabitat level follow an experimental approach known as space-for-time (SFT) substitution [15], in which current spatial heterogeneity is used as a proxy for predicting ecological time series (i.e. reactions to future conditions).

4.1.1. Plot surveys

In this type of sampling, few representative categorical altitudes (e.g. high and low) are chosen to cover the desired altitudinal distribution. Depressions and ridge-like microhabitats may be chosen at each altitude based on indicators such as topology and vegetation. In each altitude/microhabitat combination, one big plot (~10 × 10 m) is designated. Within each plot, several patches (~100) should be sampled randomly. This sampling is best for assessing isolation between microhabitats (e.g. ecological, trait-based or genetic isolation). Heritabilities and evolutionary responses for different traits are best computed in natural populations with this sampling strategy [12] because it allows including many individuals in close proximity with a high potential of being genetically related in different degrees.

4.1.2. Transect surveys

In this kind of sampling, the main elevational range is covered continuously. Around five to ten elevational bands along transects, with one or more small study plots (~3 × 3 m), must be set up in different microhabitat sites (e.g. early-season exposure from snow and late-season

exposure) to grasp a complete overview of the environmental variation. In each plot, patches (at least two) must be selected randomly. When compared to the plot survey, the transect survey has many smaller plots with fewer total patches but covers the desired altitudinal and microhabitat variation at a better resolution by having more total plots. This is ideal to assess ecological responses [15] or genetic isolation-by-distance and to explore trait and microhabitat-driven genomic architecture [15, 22].

4.2. Transplant experiments

To rigorously test how organisms respond to microhabitat-driven changes through phenotypic plasticity, as well as whether populations experience local adaptation (home-site advantage), reciprocal transplant experiments are needed (**Figure 6**) [59]. Transplant experiments are typically carried out across altitudinal gradients. However, reciprocal transplant studies explicitly examining the effects of local-scale variation (e.g. altered snowmelt timing) are scarce. Almost all reciprocal transplants have examined short-lived perennial herbs, and experiments with long-lived woody species are rare due to the difficulty in establishing clones of perennial, slow-growing species. Yet, it is important to understand how long-lived species will respond to changes in snowmelt timing, as they are a dominant functional vegetation type in alpine areas. Transplant experiments can be carried out in long-lived species using long-term monitoring [60] or clonal propagation [59].

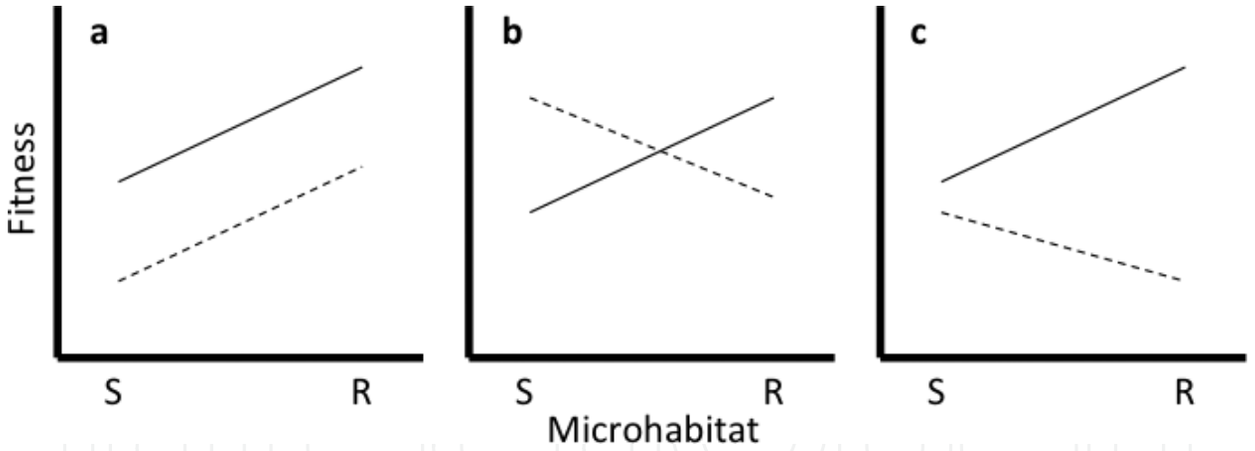


Figure 6. Scenarios where (a) plasticity, (b) local adaptation and (c) plasticity with a genetic basis explain trait variation across different microhabitats: snowbed (S) and ridge (R). Distinct types of lines (S: dashed, R: continuous) are different genotypes reciprocally transplanted to each microhabitat. Illustration based on Sedlacek et al. [59].

4.3. Phenotyping

Phenotyping is the systematic assessment of trait variation. It is an essential component that must be accounted for in ecological and genetic studies. Soil temperature data loggers, nutrient probes and field observations can be used to estimate drought severity [61], frost events [14], snowmelt timing [8], nutrient availability [13] and other soil properties [33]. Monitoring of individuals carried out weekly during the growing season and across microhabitats during

several growing seasons is the most exhaustive and informative survey method, although fewer snapshots can also be used.

4.4. Genotyping

Genotyping is the systematic assessment of genetic variation. Adaption and diversification are recognized as important processes that generate diversity [62, 63]. However, their effects on genetic divergence and on the generation of morphological and ecological variation are poorly understood. Low- and medium-throughput techniques (as in Section 4.4.1) together with newly developed high-throughput next-generation sequencing methods (as in Section 4.4.2) offer the promise of major advances in the study of these interactions.

4.4.1. Microsatellite genotyping

Microsatellites loci (also called single sequence repeats or SSRs, which are regions of repetitive DNA that vary in the number of repeated DNA motifs) are commonly used to assess population structure [64], as shown in Section 2.2, and may help to estimate relatedness [65], as shown in Section 3.1, due to their high polymorphism [63, 66]. The PCR (polymerase-chain-reaction, a procedure used in molecular biology to replicate DNA exponentially) reactions are usually multiplexed into several PCR runs. Two or more multiplexed PCR runs can be pooled afterward and separated by capillary electrophoresis. Allele sizes are estimated using software such as GeneMapper v.3.7 (Applied Biosystems).

4.4.2. Genotyping-by-sequencing

Recent developments allow scientists to survey entire genomes to answer questions beyond the population genetics paradigm, in what is starting to be recognized as population genomics (as in Section 3.2). Genotyping-by-sequencing (GBS) is one of the cheapest and most used methods to generate massive amount of SNPs (single-nucleotide polymorphism, a type of molecular marker representing a change in a single base pair). GBS libraries are prepared according to Elshire et al. [67] using different enzymes for digestions. Raw Illumina DNA sequence data from the libraries can be aligned to reference genomes using BWA aligner [68] and processed through the GBS analysis pipeline as in TASSEL-GBS [69].

4.5. Common statistical approaches to compare microhabitats

4.5.1. Linear models

Linear models (e.g. ANOVA, ANCOVA, linear regression) are the first option to assess the effects of microhabitats and altitude on flowering time, and how temperature, humidity and snowmelt vary between microhabitats. In order to assess whether microhabitat and phenological differences trigger genetic isolation, pairwise F_{ST} (fixation index) values can be computed among plots or populations using, for instance, GENEPOP [70]. The number of alleles or heterozygosity, which are standard measures of genetic diversity, may be compared between microhabitats as well (as in Section 2.2), using linear mixed models [71] with the microhabitat

as the fixed effect and the transect as the random effect (covariate). These can be done in R (R Core Team), with the packages *lme4* or *lmerTest* [72].

4.5.2. Population structure

Population structure is usually examined using the software STRUCTURE [73]. It is suggested that several independent runs are performed with different K (number of assumed populations) values using an admixture model with a minimum of 100,000 iterations for the burn-in and 100,000 subsequent iterations for the MCMC analysis. The optimal K is posteriorly determined using the rate at which the likelihood changes across different K values [74]. Pairwise migration rates (N_em) and effective population size (N_e) are also meaningful statistics calculated across microhabitats, as in Section 2.2. They can be estimated following coalescent theory and a maximum-likelihood–based approach using software such as, for instance, MIGRATE [75].

4.5.3. Trait heritability and selection

When it comes to trait variation, narrow sense heritability (h^2) is estimated in natural populations using a multivariate animal model [76] with a marker-based relatedness matrix [48]. To test for selection on different traits, proxies of relative (i.e. relative to the mean across all sites or populations) clonal and fixed sexual reproductive fitness are compared against the standardized phenotypic traits using multiple regressions with linear mixed models to yield selection gradients [77]. This analytical approach is illustrated in Section 3.1.

4.5.4. Genetic mapping

To understand trait architecture in natural populations, trait-marker association studies are used [78]. Standard trait-marker association analysis can be easily implemented in FaST-LMM [79] or BiForce [80], the latter detects epistatic interactions (i.e. second-order trait-marker associations) and dominance effects.

4.5.5. Scans for genome-wide selection

As a descriptive approach, genome-wide sliding window analysis can be used to determine F_{ST}, as it is in Section 3.2, and the proportion of variable SNPs that are fixed between microhabitats using, for instance, ARLEQUIN [81, 82]. Linkage-disequilibrium (LD) and Tajima's D [83] are also usually computed in the same windows with the R package *PopGenome* [84].

5. Conclusion

Fast-evolving microhabitat-driven genomic divergence and, at the same time, genetically based trait variation at a larger scale may play a role in the ability of species and populations to persist in diverse and variable conditions in heterogeneous ecosystems. Populations from

different microhabitats may be isolated, and they may act as sinks or sources of genetic diversity. From a genomic point of view, multiple genetic regions that diverge between microhabitats may arise at very local geographic scales even in the presence of gene flow, due to strong environmental differentiation. Additionally, regions of high genomic divergence are possibly related to traits under selection that may matter for the prediction of evolutionary responses. In this chapter, I have shown how small-scale environmental variability helps understanding the way organisms may react to changing conditions by looking at processes such as genetic adaptation and diversification at a very fine environmental scale. In the oncoming years, I expect to see an increase in the number of genetic studies aiming to resolve the genomic architecture of environmentally relevant trait variation at a fine scale, improving in that way our understanding on how species and populations may cope with rapidly changing climatic conditions.

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