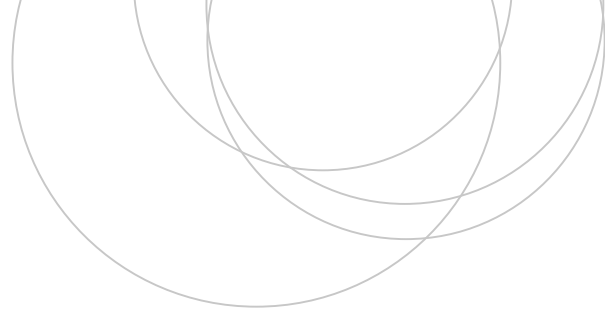




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Local adaptation to serpentine soils: a review of adaptive traits and underlying genetic bases

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ABSTRACT

Local adaptation studies offer a powerful conceptual approach for evolutionary ecology studies. Particularly, they provide evidence of the evolutionary processes that influence lineage diversification that can potentially lead to speciation. This paper reviews literature on local adaptation and focuses on the adaptation of plants to serpentine soils as they present an important example of research on this area. First, I present fundamental aspects of local adaptation and consider ecological and breeding studies. To follow up, I discuss the multiple approaches in genomic studies that focus on the identification of candidate loci involved in adaptation. Example studies are described as well to provide useful background for the subsequent discussion of adaptation to serpentine soils. Finally, I consider different ecological studies involved in the identification of adaptive traits to serpentine soils and genomic studies that seek to analyze the genetic basis of these traits. I highlight specific mechanisms, such as nickel hyperaccumulation, and physiological models that have been proposed. The genetic basis of these mechanisms is reviewed, yet no general tendency is concluded as simple and complex bases have been detected. Lastly, I propose that future studies should focus on combining plant breeding with genomic approaches.

RESUMEN

Los estudios de adaptación local ofrecen un poderoso enfoque conceptual para los estudios de ecología evolutiva. En particular, proporcionan evidencias de los procesos evolutivos que influyen en la diversificación de linajes que potencialmente pueden conducir a la especiación. En este artículo se revisa la literatura existente sobre la adaptación local y se enfoca en la adaptación de plantas a suelos serpentínicos, ya que presentan un importante ejemplo de investigación en esta área. Primero, presenta los aspectos fundamentales de la adaptación local y considera los estudios ecológicos y de reproducción. Para continuar, discute los múltiples enfoques en los estudios genómicos que se centran en la identificación de genes candidatos involucrados en la adaptación. También se analizan estudios para proporcionar antecedentes útiles para la posterior discusión de la adaptación a suelos serpentínicos. Finalmente, se consideran los diferentes estudios ecológicos involucrados en la identificación de rasgos adaptativos a suelos serpentínicos y los estudios genómicos que buscan analizar la base genética de estos. Se destacan los mecanismos específicos, como la hiperacumulación de níquel, y los modelos fisiológicos que han sido propuestos. Se revisa la base genética de estos mecanismos, sin embargo, no se concluye una tendencia general ya que han sido detectadas bases simples y complejas. Por último, se propone la combinación de estudios de fitomejoramiento con estudios genéticos como enfoque para los futuros estudios.

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1. Introduction and objectives

Natural landscapes are highly heterogeneous and spatial environmental variation is ubiquitous, which results in selection pressures that differ among habitats. Divergent selection can lead to population differentiation, maintenance of genetic variation and, potentially, speciation. Species often adapt to local biotic and abiotic conditions and express higher fitness in the local habitat in contrast to the fitness they display in other habitats, a phenomenon known as local adaptation (Savolainen *et al.* 2013; Selby *et al.* 2018). Studies of local adaptation provide insight and understanding of the underlying processes by which populations respond to local selection regimes. These studies are crucial for the understanding of the process of natural selection relative to other evolutionary processes and more broadly for the development of evolutionary ecology (Kawecki & Ebert 2004).

Local adaptation offers a powerful conceptual approach for studies in evolutionary ecology. An important example of research on local adaptation is the frequent adaptation of plants to specific soil types. This type of soil adaptation provides evidence of strong natural selection and has been a productive model for research (Brady *et al.* 2005). Among the different edaphic factors and their associated plant communities, serpentine soils are considered an ideal research model as they present a stressful environment with strong limitations on plant growth (Kazakou *et al.* 2008). Understanding how plants survive such hostile environments is possible by, first, testing for local adaptation with manipulative ecological and growth experiments, and subsequently studying the underlying genetic basis of specific population characteristics and features. Advances in genomic studies have enabled identification of candidate genes that facilitate adaptation to serpentine (Arnold *et al.* 2016). Overall, numerous ecological and genetic approaches have been developed to investigate local adaptation.

The aim of this paper is to review literature on local adaptation and to focus on the adaptation of plants to serpentine soils. To accomplish this, I develop and execute the following tasks which serve as the objectives in this review. In the first section, I present several fundamental aspects of local adaptation by reviewing selected studies. Next, I consider ecological and breeding studies, and more recent genomic studies that focus on identifying candidate loci involved in adaptation. I review examples of studies that shed light on local adaptation, in order to provide a useful background for the subsequent discussion of adaptation to serpentine conditions. The final section of the paper focuses on the identification of the genetic basis of local adaptation to serpentine soils and explains how future studies could extend our knowledge in this field.

2. Methodology

Due to the Covid-19 pandemic, this project has suffered big changes. However, the focus of the study has remained the same: genetic identification of adaptation to serpentine soils. Sierra Bermeja's natural area has a high degree of this type of soils, and species that grow on them could have evolved to some sort of adaptation to be able to survive. Taking them as study populations, our goal was to test the phylogenetic relationship between populations of facultative species on and off serpentine soils. After sampling in Sierra Bermeja, a large sampling set was collected to make the corresponding analysis to reach our goals. Nonetheless, when classes changed to remote delivery, laboratory work had

not started. Hence, the project was changed into a bibliographic review to follow the government's guidance on social distancing.

To obtain relevant literature on the topic, the platform “Web of Science” has been mainly used, which provides access to numerous databases. Combined with this platform, multiple keywords relevant to the topic have been employed to help to identify and obtain relevant literature on the topic of focus: local adaptation to serpentine soils. Specifically, the following keywords have been used: adaptive evolution, adaptive traits, association mapping, heterogeneous environments, local adaptation, population differentiation, population genetics, serpentine soils, transplant experiment, and QTL mapping. By the end of this meticulous search, multiple primary source articles and secondary source reviews have been obtained, which provided comprehensive citation data on the topic. After analyzing these sources and their applicability to our review, additional literature has been included by the examination of the cited literature in each source.

3. Fundamental aspects of local adaptation

As environmental variation is ubiquitous, populations of many species become adapted to their local abiotic and biotic conditions (Savolainen *et al.* 2013). Under local conditions, populations generally perform better than they do when planted elsewhere. Population performance here is in the sense of average individual Darwinian fitness, or just ‘fitness’, the ability of organisms to survive and reproduce (Orr 2009). When individuals have their highest fitness at their native site, compared to individuals from other populations introduced to that site, they are locally adapted (Savolainen *et al.* 2007; Savolainen *et al.* 2013). Local adaptation is the result of populations of individuals undergoing genetically-based phenotypic differentiation due to contrasting selection for different phenotypes in distinct habitats. This process of differentiation of lineages of populations due to local adaptation can potentially contribute to speciation (Sobel *et al.* 2010; Wright *et al.* 2013).

Studies of local adaptation are essential for gaining insights into the evolutionary processes that influence lineage diversification and potentially lead to speciation, including natural selection, gene flow, and genetic drift. These fundamental evolutionary processes are able to change allele frequencies in populations. First, evolution by natural selection occurs when differences in the survival, mating success, or fecundity among individuals occur because of phenotypic differences with an underlying heritable genetic basis. This process predominates in large populations (McPeck 2014). Second, genetic drift predominates in small populations as it consists of random fluctuation in gene frequency, which reduces population genetic variability (Kliman *et al.* 2008). Third, gene flow is the transfer of alleles from one population to another (Maley 2014). By studying local adaptation, one can identify these processes acting in a population in a specific habitat. The interaction between these processes will affect the degree of local adaptation (Kawecki & Ebert 2004). As Williams (1996) explains, in the absence of homogenizing evolutionary processes, each native population evolves traits advantageous for the local environmental conditions, which increase fitness in each specific habitat, even if these traits have negative consequences for fitness if present in other habitats. This results in individuals in native populations having higher average fitness in their local habitat than would individuals from other

populations that originate elsewhere. However, this process of local adaptation can be hindered by gene flow as it promotes genetic homogenization (Linhart & Grant 1996). Therefore, even if divergent natural selection is the driving process of local adaptation, other processes, in particular gene flow, play an essential role in determining whether patterns of local adaptation develop (Kawecki & Ebert 2004). All of this emphasizes the idea of the tension between natural selection, which has a differentiating effect, and gene flow, which has a homogenizing effect. Overall, local adaptation depends on the balance between these different evolutionary processes.

Population differentiation resulting from local adaptation depends on the balance between different evolutionary processes. Even if the two principle processes determining local adaptation are natural selection and gene flow, specifically, strong selection and low gene flow, other evolutionary processes can influence the development of local adaptation (Kisdi 2002; Kawecki & Ebert 2004). For instance, another process that influences the development of local adaptation is genetic drift. This plays a larger role in small populations, where random effects (i.e. stochasticity) can influence gene frequencies. Similar to selection, genetic drift reduces additive genetic variance, which refers to the total heritable genetic effects on a polygenic trait, i.e. one influenced by multiple loci (Singh & Singh 2017). Therefore, a reduction in genetic variance for a trait that arises from drift means that an increased proportion of the relevant loci are *fixed* for a particular allele at random, and not necessarily one that contributes to increased fitness. As a result, local adaptation is reduced in small populations through strong effects of drift (Yeaman & Otto 2011; Blanquart *et al.* 2012). All of these factors, selection, gene flow, and drift, play a role in determining the extent of local adaptation (Kawecki & Ebert 2004).

Besides evolutionary processes, other factors can promote local adaptation, such as the costs of, or constraints on, adaptive phenotypic plasticity (Kawecki & Ebert 2004). Adaptive phenotypic plasticity refers to the changes directly induced by different environments in an individual's behavior, morphology, or physiology (Price *et al.* 2003). Plasticity is advantageous in unpredictable heterogeneous environments. For local adaptation to occur, adaptive phenotypic plasticity must have a cost. Without this constraint, a genotype that produces the optimal phenotype in each local habitat would become fixed in all habitats. Consequently, adaptive phenotypic differentiation among habitats would be achieved without any underlying genetic differentiation. Therefore, cost or constraints on adaptive phenotypic plasticity are crucial for local adaptation to develop (Kawecki & Ebert 2004).

Since natural selection varies in space, variation in fitness occurs through genotype-environment interactions (GxE interactions) (Kawecki & Ebert 2004). In the case of local adaptation, GxE interactions impact fitness vis-à-vis variation in phenotypes that mediate a response to the environment (Ottman 2008). Genotype-environment interactions (GxE) for fitness can take several forms, nonetheless, the most relevant one for local adaptation is antagonistic pleiotropy. This type of GxE interaction is characterized by alternate alleles that are favored in different habitats and genetic polymorphisms maintained by selection (Anderson *et al.* 2012). Antagonistic pleiotropy entails that no single genotype is superior in all habitats, which leads to fitness trade-offs in adaptations to different habitats (Kawecki & Ebert 2004). These trade-offs for particular phenotypes across different habitats lead to local adaptation (Hall *et al.* 2010).

Local adaptation can have a cost in species fitness in contrasting habitats (Kawecki & Ebert 2004). In other words, adaptation to one environment results in reduced fitness to another one, also known as genetic trade-off (Hereford 2009). However, this is not the only possible scenario. Occasionally, local adaptation leads to strong adaptive fitness effects in the native habitat but selectively neutral effects in other locations, a phenomenon called conditional neutrality (Anderson *et al.* 2012). Hall *et al.* (2010) point out, “local adaptation can arise either from trade-offs of alternative alleles at individual loci or by complementary sets of loci with different fitness effects of alleles in one habitat but selective neutrality in the alternative habitat”. These different scenarios are shown in **Figure 1**. Nevertheless, these

two possible situations or hypotheses are not mutually exclusive since both genetic trade-offs and conditional neutrality can occur in one species (Wadgymar *et al.* 2017).

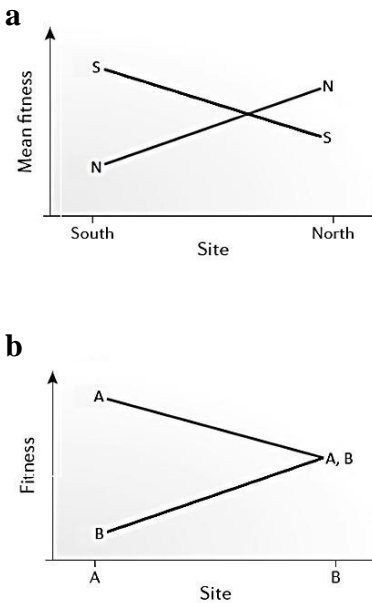


Figure 1 Comparisons of fitness between populations in different sites. **a)** Antagonistic pleiotropy. Locally adapted populations show higher fitness in their native site compared to others **b)** Conditional neutrality. The local allele A shows fitness advantage at site A compared to the non-native allele B, however, the alleles do not differ in their fitness elsewhere (site B) (Savolainen *et al.* 2013).

4. The long history of studying local adaptation

Different experimental approaches have been developed to estimate the amount of local adaptation in natural populations (Blanquart *et al.* 2013). Nonetheless, even if studies of local adaptation have a long history of empirical investigations, the best methodology for measuring and identifying local adaptation is still to be determined. In this section, I focus on how manipulative ecological and growth studies were developed and review studies that aim to identify local adaptation in different environments.

To assess local adaptation an experiment that demonstrates the GxE interaction for fitness is required, where genotypes are sampled from the local populations of habitat and population from different habitats, and then directly compared under the same environmental conditions. Most empirical studies assess local adaptation by using reciprocal transplants or common garden experiments. These types of studies can be done either directly in the field or in the laboratory, greenhouse, experimental plots along with others, by re-creating the essential properties of different habitats (Kawecki & Ebert 2004). These experiments usually quantify the specialization of a population to their native

environment by measuring the difference between the performance of the population in the native environment and elsewhere (Blanquart *et al.* 2013).

4.1. Reciprocal transplant experiments

Reciprocal transplant experiments are a useful approach to study local adaptation as individuals from at least two populations are reared in their respective native and non-native environments (Kawecki & Ebert 2004; Savolainen *et al.* 2013). These types of experiments allow differences in fitness, morphology, phenology along with others to be assessed (Bender *et al.* 2002). However, their main goal is to test local adaptation by exhibiting how the average fitness of local individuals is higher than the average fitness of individuals from elsewhere (de Villemereuil *et al.* 2016). Reciprocal transplants are designed to identify local adaptation overall. They are very useful for genomic studies as well (Bischoff *et al.* 2006; Leimu & Fischer 2008; Garrido *et al.* 2012; Gould *et al.* 2018). By identifying variation in responses that can be measured to assess local adaptation, such as survival and growth, it is possible to map the underlying genetic of these parameters' differences later. For instance, Selby & Willis (2018) report three reciprocal transplant studies at different sites and different years to identify a gene of major effect that controls adaptation to serpentine soils in *Mimulus guttatus*. In each reciprocal transplant experiment, transplant survival as well as juvenile and adult size traits are recorded. Transplant survival is scored three weeks after transplantation and afterward weekly. Subsequently, survival time is calculated taking into account the mean planting date at each site. Survival curves are constructed using a package in R. Concerning morphology traits, height, length, rosette diameter, and flowering date are measured weeks after transplantation. All in all, this research is an excellent example of how reciprocal transplants provide crucial information for local adaptation studies.

4.2. Common garden experiments

Common garden experiments are conducted by collecting individuals from different populations and growing them in a common environment. These types of experiments are mainly used to study the genetic bases of complex traits, such as morphological or physiological traits (Bender *et al.* 2002). In addition, common garden experiments are used to study GxE interactions by applying the same design of the experiment in different environments. Lastly, they are also used to study the consequences of local adaptation in the ecosystem. Nonetheless, the main difference between the use of reciprocal transplant and common garden experiments is that the first one is designed to detect local adaptation while common garden experiments are designed to study genetic bases of traits, regardless of them being adaptive or not (de Villemereuil *et al.* 2016). Common garden experiments are not only used as field-experiments, they can be applied in laboratory experiments. They are often used to assess local adaptation in parasites, where the environmental factor for parasite adaptation is potentially the genetic composition of the local host population. For instance, Thrall *et al.* (2002) conduct these experiments to assess this type of local adaptation, precisely on the *Linum marginale-Melampsora lini* plant-pathogen system.

4.3. Local vs. foreign and Home vs. Away criteria

In both, reciprocal transplant and common garden studies, GxE interactions for fitness in contrasting environments are indicative of local adaptation. Two different criteria have been proposed to diagnose local adaptation depending on what is being analyzed. The local vs. foreign criterion compares fitness differences in individuals from different populations within habitats. Individuals originating from the local population are expected to have higher fitness values than populations from other habitats. On the other hand, the home vs. away criterion compares the fitness values of the individuals from a population across habitats. In this case, local adaptation would occur in the case in which each group of individuals of a population had its higher fitness value in their native habitat (Kawecki & Ebert 2004). Most of the time, both criteria can be valid. However, Kawecki & Ebert (2004) review that both are not equally adequate for testing the pattern of local adaptation, as the ‘home vs. away’ model can mistake the effect of divergent natural selection with the differences of habitat quality. For instance, the survival of individuals adapted to a poor-quality habitat may increase when transplanted to a resource-rich one. These authors conclude that ‘local vs. foreign’ criterion should be the one used to identify the patterns of local adaptation; as it directly reflects the driving process of local adaptation, divergent natural selection, relative to other evolutionary processes.

5. Quantitative genetics and how it can be used to characterize local adaptation

Field and laboratory manipulative ecological and growth experiments have provided initial insights into the genetic architecture of adaptive traits. However, the genetic basis of local adaptation remains poorly understood. Most traits related to local adaptation are polygenic, quantitative traits, whose identification is still a challenging task (Savolainen *et al.* 2013). After all, polygenic traits are influenced by variation at many loci and quantitative traits do not follow simple Mendelian inheritance (Barton & Keightley 2002; Savolainen *et al.* 2013). Part of this challenging aspect of the identification of traits related to local adaptation is that full genome data studies have been mostly only possible in model-species. These model-species are species that had previously been well-characterized and studied and present a reference genome for further studies in other organisms. Recent approaches have allowed genome-wide studies of non-model species, which has opened a wide range of studies of local adaptation (Savolainen *et al.* 2013). As most traits related to fitness are quantitatively inherited, the current interest in using different approaches to understand quantitative traits in local adaptation studies is huge (Stich & Melchinger 2010). In this section, I discuss different approaches and molecular tools used for ecological genomics related to local adaptation.

5.1. QTL-mapping approach

While quantitative traits do not follow simple Mendelian inheritance, they are a common feature of natural variation in most populations. Because of this, quantitative traits have been a focus of studies that attempt to understand the genetic basis of local adaptation (Kearsey & Farquhar 1998). Through statistical analysis of quantitative traits their genetic loci can be identified, also known as quantitative trait locus (QTL) (Barton & Keightley 2002). To map QTLs genetic markers are used as references, which are known variabilities at the DNA level (i.e. genetic polymorphism). These markers have known locations in the genome that can be used to identify characteristics of genetic polymorphisms present in their DNA (Savolainen *et al.* 2013). Therefore, when mapping QTLs, the association

between a quantitative trait and segregating marker alleles is measured (Kearsey & Farquhar 1998). This association between the two is referred to as genetic linkage, which results when two genes are located near each other on the same chromosome and end up tending to be inherited together (Lobo & Shaw 2008). The closer a marker is from the QTL the probability of recombination occurring between them will decrease and they will be more likely to be inherited together (Boopathi 2013). Different types of genetic markers are known, such as SNPs (single nucleotide polymorphism). Nevertheless, a large number of molecular markers are not required for QTL mapping approaches (Kearsey & Farquhar 1998; Savolainen *et al.* 2013). QTL analysis or mapping is based on searching associations between quantitative traits and marker alleles segregating in the study population. These analyses have two main stages; the first one being mapping the markers, and the second one, associating the qualitative trait with the marker.

Methodologies used previously to the discovery of QTLs to study polygenes were rather laborious and had heavy statistical associations with polygenes. By using these polymorphic markers, mapping studies evolved and QTLs reflecting polygenes could be located. This provided a wide mapping approach as nearly all populations have polymorphisms; hence, QTL mapping can be conducted (Kearsey & Farquhar 1998). Nevertheless, QTL analyses have several limitations the first one being that most QTL mapping is done with populations deriving from pure lines and approaches developed with these populations. Additionally, studies will be limited as both environment and alleles have an effect on genotypes. In this sense, two individuals that have the same genotype could have different phenotypes, or conversely, two individuals that have the same phenotype could have different genotypes. To solve this issue, individual associations between marker and genotypes are looked at. Nevertheless, approaches of this kind have their own limitations as well. For instance, all genes on a chromosome show some sort of linkage among them; hence, a QTL is always related to more than a marker (Kearsey & Farquhar 1998).

To overcome the limitations that QTL mapping has in plants, alternative approaches have been introduced. One of them is interval mapping, which consists of scanning intervals between adjacent pairs of markers and determining the probability of QTLs being at any given point of each interval, in other words, the log of the ratio of likelihoods (LOD). A QTL is presumably where data exceeds a specified significance level. This approach has been extensively used with populations derived from inbred parents. Another alternative approach has been developed by using multiple regression. This approach is quite similar to interval mapping; however, the programming is much simpler and quicker. In addition, it can be used with complex pedigrees and includes numerous fixed effects such as environments. The third alternative approach is marker regression, which integrates all marker information on a given chromosome in a single test. Generally, when working with QTLs, the markers that flank a specific QTL are unknown. This technique provides the huge advantage of integrating all marker information and provides an overall test of the model (Kearsey & Farquhar 1998).

Regarding the reliability of QTL mapping, it depends on the heritability of each QTL. In literature, the term heritability tends to be used to refer to the fraction of the phenotypic variance due to additive genetic variance (Barton & Keightley 2002). The simplest way to increase the reliability of QTL mapping is to increase the number of genotypes which also increases precision. A simple way of achieving this is backcrossing populations, i.e. crossing a hybrid with a parental

genotype. On the other hand, the heritability of individual QTL can be enhanced by having several replicates of each individual, which minimizes environmental variation. Along with this, residual variation, i.e. variations among the regression line being studied, due to QTLs can be identified and removed from the error (Kearsey & Farquhar 1998).

Several authors have used QTL mapping to identify candidate genes and traits of local adaptation in different environments. Agren *et al.* (2013) have mapped QTLs for total fitness in 398 recombinant inbred lines (RILs) from a cross of between locally adapted population from Sweden and Italy and grown 3 consecutive years. The results of this study suggest that by changing a few genomic regions of small effects *A. thaliana* could adapt to different environments. Additionally, they have identified 15 different QTLs that show evidence of trade-offs. This data has been used by Oakley *et al.* (2014) as a reference and they have determined two freezing tolerance QTLs that provide genetic trade-offs across environments for both survival and overall fitness.

5.2. Association-mapping approach

Similar to QTL mapping, association-mapping is based on correlating phenotypes with genotypes. However, this approach considers populations instead of pedigrees (Savolainen *et al.* 2013). In other words, association mapping ‘studies the linked inheritance of functional polymorphisms and adjacent molecular markers with unknown ancestry’ as defined by Stich & Melchinger (2010). As this mapping approach uses an unknown ancestry, it is possible to extend it across thousands of generations compared to QTL mapping (Savolainen *et al.* 2013). Populations studied this way have undergone more generations and linked inheritance will only endure very closely located polymorphisms. Association studies provide a way of testing the genetic basis of local adaptation in a diverse selection of species with a broader range of life histories (Wadgymar *et al.* 2017). The number of markers required for the association-mapping approach depends on the linkage disequilibrium (LD), i.e. the non-random association of alleles at different loci, in study populations. Association mapping is based on LD between molecular markers and functional loci (Stich & Melchinger 2010; Savolainen *et al.* 2013). This last term is defined as ‘the whole set of genomic regions that are alternatively used to carry out the same function’ by Ruiz-Narváez (2011). The LD value can be influenced by several forces such as recombination, mating type, genetic drift, selection, mutation, population substructure, and relatedness. Therefore, these factors must be estimated to evaluate the applicability and resolution of association mapping (Stich & Melchinger 2010).

Although association mapping studies are undoubtedly useful, two major limitations exist in them. The first major limitation is when the trait of interest is strongly associated with population structure. Although several statistical methods correct population structure in association mapping, by correcting it differences between subpopulations are ignored. Therefore, if this association is that strong the power of mapping will decrease as phenotypic differences between subpopulations remain undetected. The second major limitation is related to the allele frequency distribution at the functional polymorphism. This will limit mapping as most alleles being studied are rare, which makes it difficult to account for most phenotypic variation (Stich & Melchinger 2010). Nevertheless, promising results have been obtained with the use of these studies and local adaptation studies have benefited directly. For instance, Porter *et al.* (2017) have used association mapping to identify gene clusters in a population of symbiotic *Mesorhizobium* that reveal

novel serpentine adaptation. Their work proposes several candidate genes for nickel adaptation. All in all, association mapping can be combined with QTL mapping studies which allow the increase of mapping accuracy and resolve some of the issues explained above (Savolainen *et al.* 2013).

5.3. Population genetics

Population genetics unlike the previous approaches start with variation at the DNA level instead of at phenotypic level. Currently, these analyses are executed using a combination of microsatellites, SNPs, and DNA sequencing. For population genetics analyses the best data input comes from high-quality resequencing data. Resequencing population data is obtained by sequencing multiple individuals of population that had a previous reference genome sequence. To achieve this, next-generation sequencing is very appropriate as it allows a fast and efficient way of sequencing and it can be used with non-model organism (Ekblom & Galindo 2011) . The main issue that this approach presents, is that most tests that fall in this category were developed to detect natural selection. Thus, most of them are not developed to study local adaptation. Nonetheless, even if directional natural selection usually acts as selective sweeps which rapidly increases the frequency of the beneficial allele, methods can still be used to search evidence of selection for local adaptation. (Savolainen *et al.* 2013).

Two different groups of approaches can be found on the population genetics approach. The first one focuses on detecting population differentiation through scanning F_{st} . This index references the proportion of the total genetic variability that occurs among populations and is used as a measure of population differentiation. Many of these methods are developed to detect F_{st} outliers as shown in **Figure 2**. However, local adaptation is not the only process that produces F_{st} outliers; other processes such as species-wide selective sweeps, which increase the frequency of particular alleles, can cause them as well. Hence, considering all processes that can cause outliers is crucial for an accurate interpretation. Turner *et al.* (2010) have used this approach in their population resequencing study that reveals local adaptation to serpentine soils in *Arabidopsis lyrata*. The second group of methods analyzes associations between SNP frequency and environmental variables (Savolainen *et al.* 2013). Coop *et al.* (2010) present a method that uses a null model that is based on an estimation of allele frequencies and is tested against an alternative model that incorporates environmental variables to its estimation.

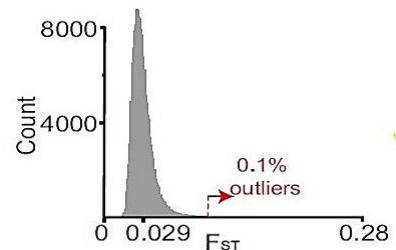


Figure 2 F_{st} index of population differentiation with outliers marked (Arnold *et al.* 2016).

6. Serpentine soil general features & characteristics of local adaptation to the environment

A powerful approach for local adaptation studies is offered by the frequent plant adaptation to specific soil types, in specific serpentine soils (Kazakou *et al.* 2008; Arnold *et al.* 2016). Serpentine soils are formed by the weathering of ultramafic rocks, i.e. rocks containing more than 70% ferromagnesian or mafic minerals (Kruckeberg 2002). These types of soils are distributed around the world patchily. However, they could be classified as ubiquitous. These soils present both physical and chemical challenges for plants growing in them. (Brady *et al.* 2005; Kazakou *et al.* 2008).

Regarding physical conditions, serpentine soils are usually rocks with a granular texture that can be easily weathered which makes them vulnerable to erosion. All of this results in shallow soils (Brady *et al.* 2005; Kazakou *et al.* 2008). Additionally, their capacity to hold moisture is generally low and they lack organic material (Whittaker 1954; Malpas 1992). Therefore, plants living in these environments often tolerate drought (Brady *et al.* 2005). Nevertheless, even if these physical conditions are undoubtedly limiting factors for plant growth, peculiarities of serpentine soils are mostly explained by their chemical composition (Brady *et al.* 2005; Kazakou *et al.* 2008). An example of a slope with these serpentine characteristics is shown in **Figure 3**.



Figure 3 Example of a serpentine soil in Wenatchee Mountains, Washington, USA (photo credit: Brady *et al.* 2005).

6.1. Calcium-to-magnesium availability

Serpentine soils have low Ca content and excess Mg availability.

This feature is inherited from their parent materials being rich in Mg mineralogy (Burt *et al.* 2003). Although calcium deficiency on its own controls serpentine flora, plant growth is mostly affected by the low calcium-to-magnesium ratio (Kazakou *et al.* 2008). This low Ca/Mg molal quotient is the principal reason for these soils being infertile, as it must be at least a unity for optimal plant growth. Moreover, these high levels of Mg are potentially toxic for plants, as they are thought to act antagonistically to plant uptake of Ca (Brady *et al.* 2005).

Several studies have sought to assess how this ratio affects plants. A research conducted by O'Dell & Claassen (2006) points out that this excess of substratum Mg could potentially induce a deficiency in growing root tips, which leads to localized root tip necrosis in non-serpentine accession seedlings grown on a serpentine environment. However, root necrosis has not been observed in the serpentine accession which could be due to a physiological mechanism for coping with high Mg and low Ca availability. These results suggest that serpentine tolerant species are able to survive on these soils since they can absorb sufficient Ca quantities without absorbing excessive Mg.

Different mechanisms have been proposed that allow serpentine species to tolerate this low soil Ca/Mg quotient that serpentine soils have. The first hypothesis supports the idea that serpentine species absorb more Ca and lower Mg than non-serpentine ones. O'Dell *et al.* (2006) have supported this hypothesis by suggesting in their study of *Achillea millefolium* a selective Ca transport that would act at the root-to-shoot transportation level. Alternatively, Mg translocation from roots is predicted to be inhibited. The second hypothesis suggests that serpentine species have a larger tolerance to high Mg concentrations and, as it logically follows, have a higher Mg requirement. Nevertheless, these two hypotheses are not mutually exclusive as greater absorption of Ca compared to Mg could be due to a greater tolerance of Mg (Kazakou *et al.* 2008).

Bradshaw (2005) proposes a physiological model for *Arabidopsis lyrata* where the loss of function of *cax1*, an H⁺/Ca²⁺ antiporter, could produce phenotypes that plants adapted to serpentine soils feature. These phenotypes include survival in solutions of low Ca/Mg ratio, the requirement of high Mg concentrations for maximal growth, or leaf tissue concentration of Mg being reduced. These findings would explain why plants adapted to serpentine soil are able to survive in those challenging conditions and other plants unable to survive. In addition, *Mimulus guttatus* reciprocal transplants experiments have revealed that the species' serpentine tolerance could be related to a mechanism to low Ca/Mg as well, instead of Mg exclusion (Palm *et al.* 2012). All in all, future studies should focus on identifying these types of mechanisms that enable serpentine adaptation as well as the genetic basis of these features and their heritability as they remain mostly unknown.

6.2. Essential macronutrients

Serpentine soils are deficient in essential macronutrients for plants, such as nitrogen, potassium, and phosphorus (Brady *et al.* 2005). In addition, these types of soils have been associated with low organic pools. Limiting nutrients vary depending on the location (Kazakou *et al.* 2008); for instance, Californian serpentine soils have been reported to be limited in nitrogen (O'Dell & Claassen, 2006; O'Dell *et al.* 2006) and conversely, European serpentine communities are strongly phosphorous limited soils (Nagy & Proctor 1997). Nevertheless, the cause for poor plant productivity on serpentine soils has been hypothesized to be a multiple soil macronutrient deficiency instead of a single nutrient deficiency (Chiarucci *et al.* 1999).

To analyze the response of serpentine species to the removal of nutrient limitation several studies have been performed. Aerts & Chapin's (1999) results suggest that species adapted to these stressful environments tend to have a slower growth rate and lower plasticity when nutrients are added to their environment. Alternatively, Nagy & Proctor (1997) stress in their study that some native species of serpentine soils like *Cochlearia pyrenaica* tolerate stress but respond in an opportunistic way to the removal of the nutrient limitation. On the other hand, O'Dell *et al.* (2006) study's differences in tolerance to low N and P are not significant between the serpentine and non-serpentine *Achillea millefolium*, suggesting that tolerance could be a common characteristic. The results of these studies do not explain a general tendency or hypothesis of plants' response to nutrient limitation removal and further research should be undertaken to explain these results.

6.3. Heavy metal levels

High concentrations of heavy metals, such as nickel, chromium, zinc, cadmium, copper, cobalt, and manganese, further make these soils stressful for plants (Brady *et al.* 2005; Kazakou *et al.* 2008). Nevertheless, out of all these potentially phytotoxic heavy metals, nickel has been the main focus of studies. Proctor & Baker (1994) review studies that reveal the phytotoxic effect of Ni on plant growth. One-third of the plants under study suffer toxicity symptoms from nickel and have high foliar nickel concentrations as well. The reason for this last finding is that species tolerant to serpentine soils are able to restrict their foliar nickel concentrations. Despite this, nickel toxicity is not described as a universal characteristic of serpentine soils as its concentration is dependent on other factors, such as Mg concentration.

These elevated concentrations of heavy metals can affect plants in three different ways described by Antonovics *et al.* (1971): through direct toxicity that leads to chlorosis and stunting, antagonism with other nutrients, or inhibition of root penetration and growth. To survive in these high levels of heavy metal and avoid the high toxicity of these environments, several strategies have been described for plants. Nonetheless, most authors define hyperaccumulation as the main strategy to cope with high levels of heavy metals (Reeves & Baker 2000). Hyperaccumulation is defined as the ability of plants to contain 100 times more metal in their shoots than normal species (Baker & Brooks 1989). It is thought to have selective advantages for plants according to several authors like Proctor & Woodell (1975), Pollard (2000), or Boyd (2007). Four different functions have been proposed: drought resistance, interference with other competitor species, inadvertent uptake, and defense. The list of nickel hyperaccumulators currently includes species widely distributed through the plant kingdom, which directly suggests an independent convergent evolution (Kazakou *et al.* 2008). Sobczyk *et al.* (2016) have studied serpentine adaptation in the species *Alyssum serpyllifolium* which grows in serpentine and non-serpentine soils. Their results indicate that nickel hyperaccumulation in this species represents adaptation for growth on serpentine soils. All this considered, further studies should be focused on this hyperaccumulation and the genetic basis that underlies this mechanism.

7. Genetic identification of local adaptation to serpentine soils

Taking into account all of the features that make serpentine soils a stressful environment for plants to grow, certain plants have been able to evolve different adaptations to survive on these harsh environments as previously reviewed on this paper. The study of how plants survive such hostile environments as serpentine soils offer a powerful model of adaptation and advances in genomics have provided new insights to population-wide views and demographic histories (Arnold *et al.* 2016).

Despite this environment being this complex chemically, genetic studies often reveal a simple genetic basis of tolerance to serpentine soils (Pollard *et al.* 2002; Bratteler *et al.* 2006; Burrel *et al.* 2012; Selby & Willis 2018). For instance, a recent study conducted by Selby & Willis (2018) identifies a major locus that controls adaptation to serpentine soils in *Mimulus guttatus*. To identify this locus, reciprocal transplant studies and common garden studies are done to detect differences in their survival on serpentine soils. These differences in field survival then are mapped by performing a bulk segregant analysis in F2 survivors, which involves the display of two opposite phenotype groups for a trait of interest and analyzing their DNA samples. They are able to identify a single QTL whose homozygous individuals for the non-serpentine alleles are not able to survive in the serpentine environment. In a second geographically distant population, the results are the same, which makes their results consistent with the idea of this QTL to underlay adaptation to serpentine soils. Besides, their results indicate that the serpentine allele is largely dominant with heterozygotes having only a slight difference in survival rates compared to the serpentine homozygous allele.

This study of Selby & Willis (2018) therefore supports other previous QTL studies that have shown major gene effect for serpentine tolerance (Pollard *et al.* 2002; Bratteler *et al.* 2006; Burrel *et al.* 2012). Burrel *et al.* (2012) base their study on a genetic analysis of nickel tolerance in *Caulanthus amplexicaulis* variety *barbarae*, which is a North American serpentine endemic plant. This study uses exclusively hydroponics to map QTLs, quantify biomass

accumulation, and determine the heritability of nickel tolerance in this species. The serpentine endemic has a significant growth advantage on high concentrations of nickel ($>30\mu\text{mol/L}$). They identify two major loci that confer nickel tolerance in serpentine soils. As these two loci have large phenotypic effects, the idea of a simple genetic model for nickel tolerance in *C. amplexicaulis* var. *barbarae*, is supported. Bratteler *et al.* (2006) on the other hand, detects 23 QTLs of which 15 are classified as major QTLs by executing a QTL mapping analysis. This author uses an F2 mapping population derived from an intraspecific cross between a serpentine and non-serpentine ecotype of *Silene vulgaris*. When exploring the genetic architecture of 7 morphological, physiological, and life-history traits these results are obtained. Overall, these different studies support the idea that a simple genetic basis can underlie local adaptation for serpentine soils.

In contrast to a major gene effect on serpentine tolerance, other studies such as Turner *et al.* (2008) and Turner *et al.* (2010) are not able to distinguish which of the genes recognized to be involved in serpentine adaptation in *Arabidopsis lyrata* are most critical. Hence, the work of recognizing the genetic basis of local adaptation for serpentine environments is often more complex than what it seemed in the previously mentioned studies (Bratteler *et al.* 2006; Pollard *et al.* 2006; Burrell *et al.* 2012). These studies are based on population resequencing to recognize candidate loci. Turner *et al.* (2008) use *A. lyrata* to measure genetic differentiation between populations growing on serpentine soils. They try locating genomic polymorphisms to identify candidate loci for serpentine adaptation. Their results indicate an overrepresentation of genes involved in ion transport. They set the starting point for Turner *et al.* (2010) to investigate the genetic basis of this local adaptation. In this next research, they investigate local adaptation of *A. lyrata* to serpentine soils and try mapping the polymorphisms responsible for this adaptation. They analyze DNA from individuals from serpentine and non-serpentine soils and sequence it. The polymorphisms they characterize that are strongly associated with the soil type have numerous loci, which are related to calcium/magnesium transport or heavy-metal detoxification. However, they are not able to measure selection on these candidate loci. All in all, these studies reveal how adaptation to serpentine soils often underlies a more complex genetic basis.

Other issues that may arise while studying the genetic basis of local adaptation to serpentine soils is the use of laboratory hydroculture. In this type of approach, the complex interaction between ions or physical properties that may occur on serpentine soils cannot be mimicked. Thus, for accurate characterization of the genetic basis of adaptation to serpentine soils, experiments should be conducted on native soils. Nonetheless, hydrocultures are a great approach for determining what traits fitness QTLs control, but their accuracy should be tested in the field (Selby 2014). Future studies should focus on developing this methodology for a better understanding of the genetic basis underlying local adaptation for serpentine soils.

8. Conclusion

This review synthesizes fundamental aspects of local adaptation and the current methods to study this process. Particularly, populations associated with serpentine soils are the main focus of this paper due to their adequacy as models for local adaptation studies. Identifying traits that enable this local adaptation to serpentine soils is still a daunting task. Multiple hypotheses have been proposed for mechanisms related to traits that permit their survival in

these harsh environments. These traits have multiple aspects, such as physiological and ecological ones, that overall reflect the complexity of local adaptation to serpentine soils. Considerably more work will need to be done to determine these traits. Similarly, identification of the genetic basis of adaptive traits is still a challenge. Further researches should focus on investigating the heritability of these traits, as two main tendencies have been found: simple and complex genetic basis. Additionally, laboratory approaches when studying this genetic basis should be complemented with fieldwork to verify the obtained results. The combination of plant breeding with genomic approaches could provide more in depth analysis and further study should be undertaken this way. Overall, I believe that furthering our understanding of serpentine soils will extend our general knowledge of local adaptation.

9. References

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