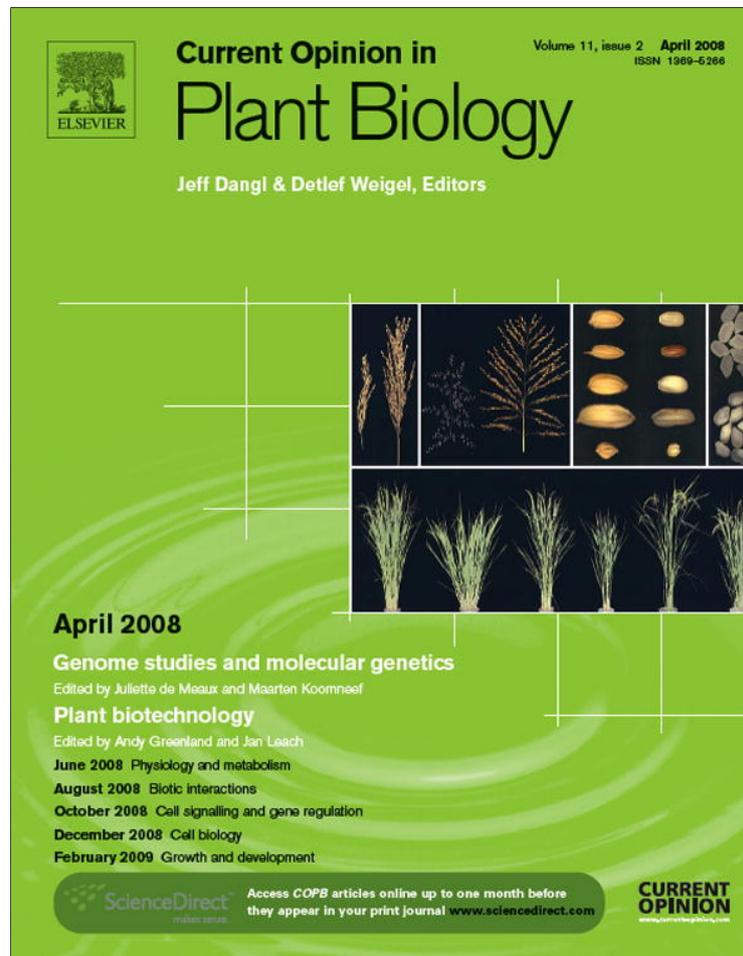


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When population genetics serves genomics: putting adaptation back in a spatial and historical context

Maxime Pauwels, Nancy Roosens, H el ene Fr erot and Pierre Saumitou-Laprade

Recent advances in molecular biology have opened new perspectives for the study of plant adaptation, especially at the intraspecific level. Nowadays, scientists employing -omic results in multiple scientific fields can be optimistic of their chances of revealing mechanisms involved in adaptive population divergence. However, the investment required by integrative studies greatly reduces the number of experiments that can be performed. In this context, a comprehensive choice of accessions under study is crucial. We maintain this choice could be appreciably enlightened by population genetics because it helps putting adaptive population divergence in a spatial and historical context. As an example, we highlight the usefulness of knowledge about population genetic structure in the integrative study of metal tolerance in *Arabidopsis halleri*.

Addresses

Laboratoire de G en tique et Evolution des Populations V eg etales, UMR CNRS 8016, Universit e des Sciences et Technologies de Lille - Lille1, F-59655 Villeneuve d'Ascq Cedex, France

Corresponding author: Saumitou-Laprade, Pierre (Pierre.saumitou@univ-lille1.fr)

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Studying adaptive traits in the molecular era

Studies of local adaptation to environmental conditions can be broadly classified into two major categories. ‘Mechanistic’ studies use formal genetics, organismal or physiological approaches to reveal the genetic, anatomical and biochemical basis of adaptive traits; ‘dynamic’ studies employ population biology and genetics approaches to identify evolutionary factors influencing adaptive divergence among conspecific populations on relatively short time-scales [1].

In the past, both approaches have been performed in parallel, rather than benefiting from each other. Recent

progress in molecular biology and bioinformatics has greatly changed this traditional situation. The coincident availability of efficient technologies for genetic engineering, -omics data acquisition or large-sample genotyping, and computational tools to handle large biological datasets have widely encouraged integrative approaches (i.e. exploiting interdisciplinary results) for ascertaining adaptive genes. Among the most promising proposals, a combination of results from population genomics and quantitative genetics (Box 1) appears as a fundamental stage. Molecular markers that could be independently detected as outliers in population genomic studies [2,3] and associated to adaptive phenotypes in QTL analyses are very likely to be in the vicinity of gene(s) of biological interest for adaptation [2,4].

However, integrative studies typically require huge experimental efforts and commonly drastically reduce the number of biological accessions that can be analysed, especially in non-model species (ultimately, one genotype per phenotypic category in comparative genomics and QTL mapping, one pair of populations with contrasted ecology in association mapping). This reduction raises the question of the representativeness of genotypes that are investigated. Although strong demonstrations are scarce, it is commonly accepted that, within species, the same adaptive phenotype may have independently evolved at multiple times [5–7]. At the molecular level, this would mean that a similar trait could be achieved by different genetic mechanisms in different populations, especially when population genetic structure is elevated and suggests divergent genetic backgrounds in separated populations [8]. For example, Le Corre *et al.* revealed that early flowering phenotypes in *Arabidopsis thaliana* resulted from the recent positive selection of distinct loss-of-function mutations that, although concerning the same gene, might have arisen independently in isolated populations [9]. Accordingly, a single analysis performed several times with phenotypically similar accessions but with distinct origins would potentially give dissimilar (although exact!) results.

Additionally, using association mapping to confirm candidate genes involved in flowering time and pathogen resistance in *A. thaliana*, Aranzana *et al.* and Zhao *et al.* revealed that population genetic structure could result in false associations between trait and marked loci; they highlighted the necessity to control this confounding effect in association mapping [10,11**]. Alternatively,

Box 1 Definitions

- **Population genomics** scans genomic diversity in natural populations with molecular markers to investigate evolutionary processes shaping population genetic structure. **Outlier** markers exhibiting highest levels of population divergence are supposed to reveal selection on linked loci involved in adaptive divergence among populations.
- **Quantitative genetics** uses individuals from either natural populations (**association mapping**) or experimental segregating populations with known pedigree (**QTL mapping**) to detect association(s) among molecular marker(s) and phenotype(s).
- **Metal tolerance** is the ability to tolerate toxic metal exposure. It is observed in species presenting **metallicolous** populations on **metalliferous** soils, that is, soils containing toxic level(s) of metal(s). Tolerance is **constitutive** in a species when all genotypes show higher tolerance than non-tolerant species.

sampling could be designed to reduce neutral population divergence and the rate of false association.

It appears from these considerations that determining rigorous sampling strategies for genomic studies of adaptation suggest being able to pinpoint either genotypes with identical phenotypes but independent evolutionary history or pairs of populations with contrasted ecology but low population structure. Clearly, this requires prior knowledge of spatial, environmental and historical factors influencing patterns of polymorphisms within species. In this review, we present recent population genetic approaches dedicated to the analysis of the relative impact of these factors. From the example of zinc tolerance evolution in *Arabidopsis halleri*, we illustrate how population genetics, putting local adaptation back in the species history, helps to determine accessions of interest for genomic studies.

Recent advances in population genetics

Recently, population genetics has experienced both practical and conceptual improvements. Whereas molecular biology generated genotyping data at multiple levels of biological organization, population genetics slipped into a systematic comparison of theoretical to experimental results. Simultaneously, the development of landscape genetics and phylogeography provided a better understanding of the spatial and historical factors shaping population genetic structure.

Landscape genetics was initially defined as a combination of population genetics and landscape ecology [12]. Landscape geneticists proposed including data on environmental variables in the analysis of the spatial patterns of intraspecific genetic polymorphisms [13^{••}]. This allows addressing new research questions about estimated population genetic structure to test the influence of landscape features beyond the traditionally investigated isolation-by-distance patterns [13^{••},14]. In a review of publications involving landscape genetics, Storfer *et al.* [13^{••}] classified

research topics in five major categories: (1) assessing the influence of actual and historical landscape configuration on gene flow, (2) identifying environmental barriers to gene flow, (3) understanding source–sink dynamics in relation to habitat quality and identifying corridors, (4) understanding at which spatial and temporal scales landscape variables influence gene flow and (5) testing more accurately hypotheses about ecological differentiation/speciation.

Phylogeography (Box 2) was developed to incorporate considerations from phylogeny and systematics (so far concerned by macroevolutionary processes at and above the species level) into microevolutionary studies (namely, population genetics) carried out at the intraspecific level [15]. Practically, Avise *et al.* [15] suggested integrating the analysis of the genealogical relationships between genetic variants (i.e. alleles or haplotypes) into the classical analysis of the microevolutionary forces (especially gene flow) shaping the evolution of their frequencies among populations [16]. This advance is a way to distinguish contemporary and historical gene flow among populations. This historical perspective for population connectivity or divergence allows considering past demographic events whose influence can remain determinant in current population genetic structure. In plant species, the chloroplast genome is specifically used for phylogeographical analyses [17,18] and there are universal PCR primers covering the genome that allow investigation of a very large number of species [19,20]. In Europe, phylogeographical analysis revealed that many tree species, despite a current continuous distribution, are divided in divergent genetic assemblages. This is assumed to result from the containment of survival populations in distinct and isolated refugia during the past glacial age [21]. More recently, phylogeographical surveys also helped to determine the geographical origin of crops [22,23,24[•],25] and hybrids [26–28].

The metal tolerance study case

Metal tolerance* in plants (Box 1) is traditionally considered as a typical example of local adaptation [29,30]. Among current models for the integrative study of zinc tolerance, *A. halleri* is one of the most promising. It has already been involved in numerous studies using genomics [31], transcriptomics [32–34] and QTL mapping [35]. If the genetic mechanism of zinc tolerance remains unresolved, available data suggest a complex trait encoded by several genomic regions [35,36] and related with overexpression of a large set of genes, through either regulation [32–34] or duplication [37]. Among these genes, the more interesting belong to the metal homeostasis network including metal transport, chelation, trafficking and sequestration [31,38].

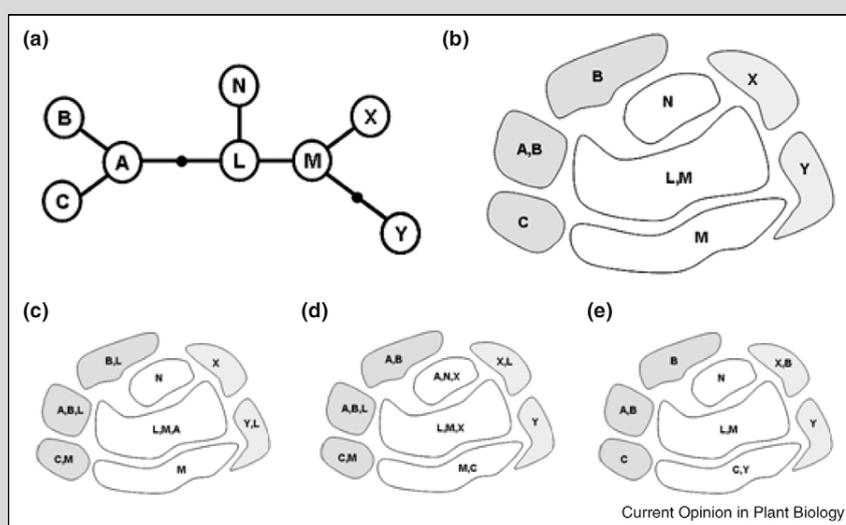
Zinc tolerance in *A. halleri* is usually considered constitutive*. Constitutive tolerance is generally supposed to

Box 2 The basis of phylogeography [16,17]

(a) Genealogical relationships between hypothetical haplotypes A to Y. Extant and missing (i.e. extinct or unsampled) haplotypes are represented by open and dark circles, respectively; each branch in the tree represents a single mutational step. Internal haplotypes (L, M, A) with rather large distribution are considered to be ancestral. External and more localized ones are supposed to be derived.

(b), (c), (d), (e) Geographic distributions of haplotypes according to different histories. Colours and drawings indicate geographic regions where populations occur. (b) Congruence between genealogy and geographic distribution: geographic distribution of haplotypes is restricted and related haplotypes occur close to each other. Population divergence is high. Such a pattern is supposed to indicate a

longstanding restriction of gene flow among populations and a substitution of ancient haplotypes by derived ones in their region of origin. (c) Incongruence: *ancestral haplotypes* occur in most populations. As ancestral haplotypes pre-date the population divergence, their distribution is supposed to ensue more from their persistence than from recent gene flow. (d) Incongruence: large distribution of *derived haplotypes*, more probably owing to recent gene flow. (e) Incongruence: although overall population divergence attests a longstanding restricted gene flow, the simultaneous occurrence of divergent haplotypes in some geographic regions suggests secondary contact zones between previously isolated populations (Figure 1).

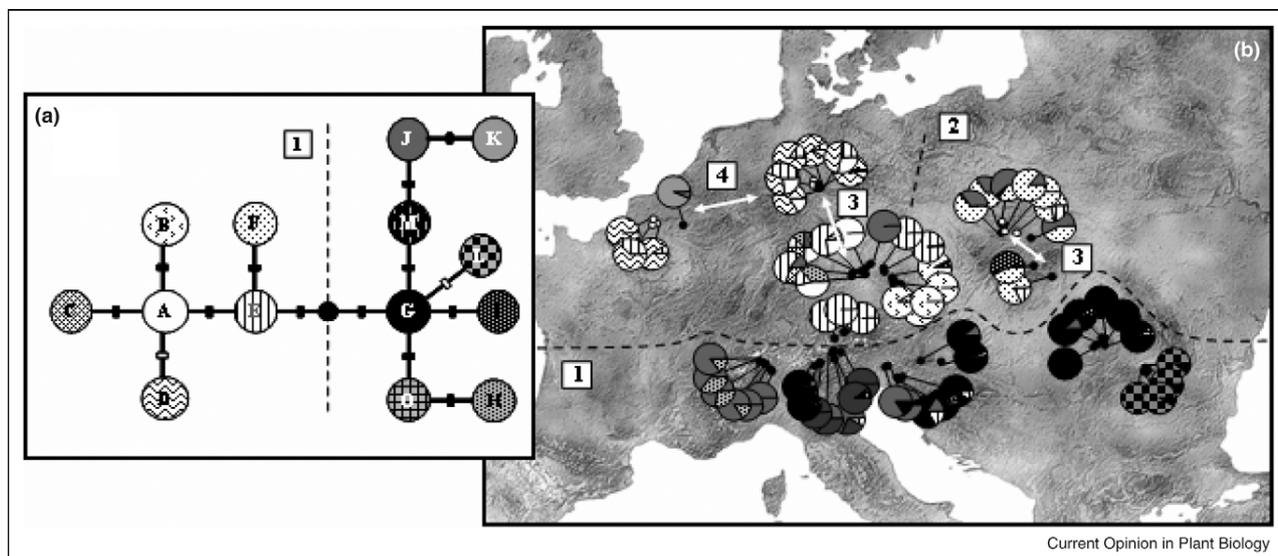
Figure 1

have evolved only once in the early stages of speciation, although the nature of past selective pressures is debated [39]. However, we recently revealed not only variation for the degree of tolerance among individuals, but also population differentiation with higher tolerance in metalicolous populations than in non-metalicolous ones [39]. Differentiation was interpreted as local adaptation to higher zinc contents in soils, through a recent selection of genes conferring enhanced tolerance. Considering the discontinuous distribution of *A. halleri* metalicolous populations, we assessed genetic relationships among population using population genetics. Chloroplast DNA polymorphisms were used to discuss the effects of both spatial and historical factors on population structure (Box 2). Please note that cpDNA results were confirmed by an analysis of nuclear microsatellite variation (Pauwels *et al.*, unpublished.). Results are summarized in Figure 1 (for details, see references [40,41]). They reveal a longstanding restriction of gene flow among populations situated north and south of the Alps Mountains, suggesting divergent genetic backgrounds between both population groups (1). Interestingly, metalicolous populations from

the Harz region in Germany or Silesia in Poland share no haplotype but are composed of haplotypes that are genetically distant (2). This suggests multiple origins of metalicolous populations, with distinct foundation events in distinct geographical regions [40]. It is thus very likely that enhanced tolerance independently evolved several times in *A. halleri*. Conversely, geographically close populations are genetically similar, irrespective of zinc content in soils. (3). This latter observation, in accordance with results from allozyme and microsatellite variation analyses in the related pseudometallophyte *Thlaspi caerulescens* [42,43], suggests that local adaptation to metalliferous sites occurred without restriction of gene flow. Thus, the level of population genetic structure among neighbouring populations is low, except for genes involved in local adaptation to polluted sites, and could correspond to an ideal situation in which genome scan analysis could detect 'outliers' closely linked to adaptive gene(s).

Population analysis results raise some novel considerations for future genomic studies. First, bringing out

Figure 1



Genealogical relationships (a) and geographic distribution (b) of haplotypes in *A. halleri*. For each population in (b), pie chart indicates the proportion of haplotypes with corresponding artwork in the haplotype tree (a). 1: haplotypes north and south of the Alps belong to diverged haplotypes lineages, revealing vicariance of populations caused by a longstanding restriction of gene flow. 2: metalicolous populations (white circles) from the Harz region in Germany and from Silesia in Poland share no haplotype, suggesting a longstanding absence of gene flow and independent foundation events. This supports the hypothesis of independent evolution towards enhanced tolerance. 3: in Germany and Poland, metalicolous populations share haplotypes with geographically close non-metallicolous ones (dark circles), revealing a maintenance of gene flow despite ecological and phenotypic differences. 4: French metalicolous populations share haplotypes with German ones. This exception is known to be artificial, provoked by humans.

population differentiation for zinc tolerance, they suggest that tolerance mechanisms may differ among populations, depending on their edaphic origin (non-metallicolous *vs.* metalicolous). Second, supporting independent evolution towards enhanced tolerance in distant metalicolous populations, they suggest that tolerance mechanisms may differ among metalicolous populations, depending on their geographic origin. Thus, results of molecular studies could be tightly related to the accession(s) studied. So far, genetic and molecular studies revealed genomic regions or genes from comparisons between *A. halleri* metalicolous genotypes from Harz, Germany (or northern France, but see point (4) in Figure 1) and close non-tolerant relatives. Our results address two related questions: (1) will these genes be detected in any population, whatever its edaphic or geographic origin? If yes, they will be associated with constitutive tolerance; if no, they will be associated with local adaptation to metalliferous conditions (enhanced tolerance). In this case, (2) will the genes be detected in any metalicolous populations or can we detect different genes corresponding to different local adaptation events in different regions (e.g. Harz and Silesia)? Another important result of population analysis is that population genetic structure is low at the local spatial scale, suggesting extensive gene flow among neighbouring non-metalliferous and metalliferous populations. In this context, population genomic studies

should favour the comparison of geographically close populations for the identification of genes involved in local enhanced tolerance.

We suggest -omic studies could focalize first on non-metallicolous accessions, in which tolerance history and genetic architecture should be less complex than in metalicolous accessions [39]. Subsequent comparisons should be made between non-metallicolous and metalicolous accessions, in order to distinguish genes involved in enhanced tolerance. Then, studies should consider possible parallel evolution in a distinct geographical area to take into account putative differences in adaptive genes and free themselves from specific effects in each situation.

Conclusion

Naturally occurring variation is increasingly considered as a valuable resource, complementing the results from laboratory mutants, for the dissection of complex adaptive trait and the identification of candidate genes [44, 45, 46]. However, any natural accession results from a unique and complex history of interactions among evolutionary forces. Therefore, analysing genetics of adaptation from natural accessions requires situating them in the overall background of the population history. Nowadays, this could be achieved using population genetics, landscape genetics and phylogeography, in particular, that investi-

gate the genetic relationships among conspecific populations [16,47]. In conservation biology, landscape genetics and phylogeography have been successfully used to define conservation units [16,48].

Contrastingly, population genetics have been largely unexploited when investigating adaptive traits, in particular when molecular biology is involved. From the active field of plant metal tolerance study, we demonstrated that identifying historical and spatial factors shaping population genetic structure greatly helps understanding the evolution of adaptive traits. In particular, it enables interpreting molecular results in the general evolutionary background of the species history. Therefore, we strongly encourage including a species-wide population genetic analysis in future integrative studies of adaptation.

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