

## Commentary

# A challenge for hyperaccumulating plant models: 'cycling' as fast as *Arabidopsis thaliana*

## Studying the genetics of metal tolerance and hyperaccumulation

Two species of the Brassicaceae family have emerged as hyperaccumulating plant models: *Thlaspi caerulescens* (renamed *Noccaea caerulescens*) and *Arabidopsis halleri* (reviewed in Verbruggen *et al.*, 2009). In addition to being phylogenetically close to the major model plant, *Arabidopsis thaliana*, the ability to hyperaccumulate zinc (Zn) and cadmium (Cd) is a trait almost exclusively confined to these two species; consequently, molecular genetic tools developed for *A. thaliana* can be applied to the study of the genetic mechanisms of metal tolerance or hyperaccumulation in *N. caerulescens* or *A. halleri*. Even though *N. caerulescens* and *A. halleri* are the most relevant model species to investigate the genetics of metal adaptation, several issues remain unsolved, partly because of experimental constraints such as their long generation time: accessions of *N. caerulescens* are rarely annual, and require up to 32 wk (including vernalization time) to flower (Peer *et al.*, 2003). Little is known about the life cycle of *A. halleri* as a result of the scarcity of studies on reproductive traits and its clonality. Nevertheless, some accessions have appeared to be clearly biennial in controlled conditions (H. Frérot, pers. obs.). Conversely, *A. thaliana* behaves largely as an annual species in controlled conditions, and flower primordial development is visible as early as 2 wk after germination (Koorneef & Scheres, 2001). In this issue of *New Phytologist*, Ó Lochlainn *et al.* (pp. 409–414) show

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that faster cycling and nonvernal lines of *N. caerulescens* have been obtained through fast neutron mutagenesis (< 15 wk to complete flower development); this development opens new perspectives for understanding Zn and Cd hyperaccumulation genetic mechanisms in this model species.

## A more comprehensive detection of target genomic regions

Quantitative trait loci (QTL) mapping is a powerful forward genetic approach used to unravel genetic determinism of metal hyperaccumulation. In the literature, several studies report the investigation of genetic determinism of Zn/Cd hyperaccumulation or tolerance by QTL mapping using standard mapping populations of *N. caerulescens* (e.g. Deniau *et al.*, 2006) or *A. halleri* (e.g. Frérot *et al.*, 2010). In *A. thaliana*, QTL analyses for mineral nutrition or metal tolerance were mainly based on recombinant inbred line (RIL) families issued from crosses between the most common lines used in laboratories, namely 'Columbia', 'Landsberg erecta' and 'Cape Verde Islands' (reviewed in Buescher *et al.*, 2010). The production of a RIL is a property of self-compatible organisms, which is the case for *A. thaliana* and *N. caerulescens*, but not for *A. halleri*. Recombinant inbred lines are of great interest because they can be indefinitely maintained through seeds; they need to be genotyped only once but can be repeatedly phenotyped, which facilitates genotype × environment interaction studies. Usually, it is easier to produce RILs in a short generation-time species, such as *A. thaliana*, because the repeated selfings could otherwise take many years. By reducing the life cycle duration and removing the vernalization requirement of *N. caerulescens*, the work of Ó Lochlainn *et al.* opens the way for obtaining RILs from this species within a reasonable period of time.

Quantitative trait loci regions are generally large (up to 10 cM) and can include *c.* 1000 genes; for example, one of the major QTL for Zn tolerance in *A. halleri* (Zntol-1) corresponded to a 24 cM confidence interval and was estimated to contain 739 genes (Roosens *et al.*, 2008b). Therefore, it is necessary to improve the QTL resolution by fine mapping before candidate genes can be identified and selected. In *A. halleri*, confidence intervals of QTLs for Zn tolerance were reduced, especially for Zntol-1 (from 24 to 5 cM), by adding 16 markers to the initial *A. halleri* × *A. lyrata* map from Willems *et al.* (2007) and genotyping 137 additional BC<sub>1</sub> individuals (Roosens *et al.*, 2008a). A very efficient method of fine-mapping, which has been applied to many crop plants, and also to *A. thaliana*, is the analysis of near-isogenic lines (NILs). Near-isogenic lines have been used in various studies on *A. thaliana* to refine QTLs previously identified with RILs (reviewed in Keurentjes *et al.*, 2007). The work of Ó Lochlainn *et al.* should enable the production of NILs of *N. caerulescens* to confirm the location of previously identified QTL.

Once a QTL region has been confirmed and refined, a strategy to identify the actual gene(s) affecting the trait has to be developed. Transcriptomic results are quite a reliable indication that a particular gene deserves attention because it has shown altered expression in a metallophyte species in comparison with a nonmetallophyte species. Even though transcriptomic studies on *N. caerulea* (against *Thlaspi arvense*) or *A. halleri* (against *A. thaliana*) involved a large screening of several thousands of genes, special focus has been devoted to genes encoding metal transporters or chelators (e.g. Hammond *et al.*, 2006). If NILs of *N. caerulea* could help to drastically narrow the support interval of QTLs, and then the number of potential candidate genes, perhaps fewer *a priori* assumptions about gene function could be put forward.

### Motivating perspectives for evolutionary biologists

From an evolutionary point of view, it is interesting to investigate the variation of tolerance or hyperaccumulation phenotype in genetic  $\times$  environment interactions. Fast cycling lines of *N. caerulea* could be used to produce RILs or NILs that can then be cultivated under different metal pollution levels, to uncover QTL  $\times$  environment interactions. This would also provide an indication about the expression profile of the gene present in the QTL region according to the metal concentration, and thus help focus on a few candidate genes; expression profiles of several Zn-regulated candidate genes are quite well known (Talke *et al.*, 2006).

Recombinant inbred lines or NILs also offer the opportunity to make multiple, even destructive, measurements. Metal tolerance and hyperaccumulation are complex traits, which can be dissected into several morphological or physiological end points. Tolerance in *A. halleri* or *N. caerulea* has been measured through root growth, chlorotic symptoms, shoot biomass or photosystem yield (reviewed in Meyer *et al.*, 2010). Other analyses requiring leaf sampling (e.g. for chlorophyll or other leaf pigment contents) could be performed only if replicates of mapping lines were available. Similarly, hyperaccumulation consists of the active root-to-shoot translocation of elements, and is classically measured by global shoot metal concentration. However, hyperaccumulation involves many physiological pathways, including xylem loading in the root, xylem unloading in the shoot, or vacuolar sequestration (reviewed in Verbruggen *et al.*, 2009) – as an example, there is evidence that Zn complexation to organic acids and vein : tissue partitioning of this metal could be segregating traits in an *A. halleri*  $\times$  *A. lyrata petraea* segregating progeny (Sarret *et al.*, 2009). These traits were estimated through destructive microscopic techniques and chemical analysis, so replicates of mapping populations would be necessary to analyze both shoot metal concentration and localization.

Only a few different accessions have been involved in crossing programmes for QTL analysis: four accessions of *N. caerulea* were mentioned in the literature, namely Lellingen (Luxembourg), La Calamine (Belgium), Prayon (Belgium) and Ganges (France) (Zha *et al.*, 2004; Assunção *et al.*, 2006; Deniau *et al.*, 2006). In *A. halleri*, only two accessions, either from France (Auby) or from Germany (Langelsheim), were referred to in the literature (Filatov *et al.*, 2007; Willems *et al.*, 2007). Nonetheless, it was demonstrated that *A. halleri* displays a phylogeographical structure, with genetically differentiated population groups separated by the Alps (Pauwels *et al.*, 2005). This suggests that distinct genetic mechanisms of metal tolerance and hyperaccumulation could potentially exist depending on the geographical origin of accessions. Moreover, it is still unclear whether nonmetallicolous populations share a common genetic basis of metal tolerance and hyperaccumulation with nearby metallicolous populations. Provided that a consistent phylogeographical analysis is also conducted in *N. caerulea*, exactly the same questions may arise. The production of multiple crosses (between metallicolous accessions from distinct geographical regions, or between metallicolous and nonmetallicolous accessions in the same geographical region) followed by QTL analyses should shed light on this topic – this will take quite some time unless fast cycling lines can be used.

Fast cycling lines from different accessions of *N. caerulea* or *A. halleri* may be the choice material in studying the evolutionary dynamics of metal tolerance or hyperaccumulation through experimental evolution designs. The evolution of these traits from nonmetallicolous populations, or their possible loss from metallicolous populations under specific selection pressures, has never been investigated, partly because of the long generation time of these species. As an illustration, a recent publication on experimental evolution of flowering time in wheat annual populations was the result of 12 yr of research (Rhoné *et al.*, 2010). By the production of up to four generations of seed in a single year, fast cycling lines will allow the production of up to 12 generations in only 3 yr. Moreover, the use of fast cycling lines should help in assessing the micro-evolutionary dynamics of adaptive alleles identified by QTL analysis.

### Conclusion

Ó Lochlainn *et al.* show that faster cycling and nonvernal lines of *N. caerulea* have been obtained through fast neutron mutagenesis. The main advantage of this method is the possibility to obtain RILs or NILs mapping populations within a reasonable time, which should allow the fine-mapping of QTL regions, the investigation of QTL by environmental interactions, the possibility to make several, even destructive, measurements relative to tolerance or

hyperaccumulation and the production of multiple crosses involving different metallicolous or nonmetallicolous accessions. By using fast cycling lines from different accessions, evolutionary dynamics of metal tolerance and hyperaccumulation can be investigated through experimental evolution. As *A. halleri* is the other model species for metal-adaptation studies, it may be of great interest to also obtain fast cycling lines of this species. Here, the advantage would only be for population studies because this species is clonal but not self-compatible (RILs or NILs to the same recurrent parent cannot be produced). On the other hand, mutagenizing *A. halleri* could be a strategy to break down its self-incompatibility.

However, by creating random deletions over the whole genome, fast-neutron mutagenesis can cause the breakdown of genetic interactions (also known as epistasis). Genetic interactions can occur between several structural genes involved in a metabolic network. In the specific framework of plant mineral nutrition, it is known that the same genes can affect several physiological processes relative to different mineral elements: these genes affect the so-called 'ionome' (Salt *et al.*, 2008). In this case, the use of fast neutron mutagenesis will probably hide the actual complexity of genetic mechanisms controlling metal tolerance and accumulation by preventing the study of epistasis. This may be the main drawback of the method.

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