Molecular ecology in Vienna: hot topics in a chilly place

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The international VIPCA conference on 'Molecular Ecology' took place in Vienna, Austria, in early February 2012. The meeting showcased the diversity of molecular tools and conceptual approaches at the disposal of practitioners in this flourishing field, which lies at the interface of ecology, evolution and molecular biology.

The Vienna International Plant Conference Association (VIPCA) conference on 'Molecular Ecology' was organized by Alisher Touraev (VIPCA, Austria), Neal Stewart Jr (U Tennessee, Knoxville, USA) and Alex Widmer (ETH Zurich, Switzerland), who together opened the meeting with sequential 'welcome addresses'. The conference featured a mix of invited and contributed talks from participants from around the globe, who braved the unusually frigid Viennese weather that sadly limited voluntary exposure to the city's rich historical atmosphere. Because the meeting featured an overview of the many diverse research traditions that comprise the umbrella discipline of molecular ecology—from landscape and conservation genetics to phylogeography and behavioural ecology—it is nearly impossible to summarize the event in an easily digestible manner. Instead, I have chosen to highlight a few particularly interesting talks that exemplify some of the best science presented in Vienna.

Alpine population connectivity

The ecological and evolutionary consequences of climate change are key questions in many research programmes; a particularly fruitful study was presented by Philippe Matter, a PhD student supervised by Andrea R. Pluess, Chris Kettle and Jaboury Ghazoul (ETH Zurich, Switzerland). His fascinating talk on 'Historical and contemporary gene flow along an altitudinal gradient in *Trifolium montanum*' won the prize for best oral presentation at the conference. Matter studied population structure and pollen flow between five meadows situated along a 600m altitudinal gradient

in the Swiss Alps. The mountain clover species under study is common in Switzerland in calcareous grasslands up to ~2,000m above sea-level, and is primarily pollinated by bees and bumble bees. Matter's research is motivated by the 3°C increase in temperature predicted in the next century and expectations that vegetation zones might 'move up' the mountains by 600m—that is, tracking their niches. Such scenarios underpin the quest for local adaptation and focus attention on evolutionary forces that either mediate or hinder such adaptation.

By combining field work and molecular analyses in the laboratory, Matter assessed the population genetic structure across the altitudinal gradient, independently estimated the extent of pollen flow among these populations and quantified flowering phenology at each elevation. By sampling many adult plants per meadow and genotyping those plants at 11 highly variable microsatellite loci, his analyses revealed a surprising degree of homogeneity in allelic richness and allele frequencies across the five meadows. Even though some genealogical signals of substructure were evident at short distances <50m within meadows, the lack of clear subdivision among meadows implies that recent gene flow must have been extensive. Matter cautioned that the relevant timescale remains uncertain, but that it seemed probable that both seed and pollen dispersal contributed to genetic homogeneity in the face of increasing fragmentation of these steep mountain meadows due to partial abandonment of their traditional management.

By using a complementary approach, Matter characterized gene flow by pollen

more directly. For this purpose, he genotyped ten offspring each from a total of 82 seed families, and by subtracting maternal multi-locus genotypes from progeny genotypes, he obtained a 'pollen pool'—that is, allele identities and their frequencies—for each of the seed families. Contrary to expectation, Matter found no decay of sibship relatedness with physical distance between mothers, indicating extensive pollen flow in a single generation. Indeed, the statistical framework to estimate model parameters could not be used because the tail of the pollen dispersal curve was beyond the spatial scale of sampling. Paternity analyses provided further evidence for pollinator movements among all meadows without directional bias, consistent with the data indicating a lack of genetic structure among meadows.

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Finally, Matter's exhaustive counting of open flower heads at weekly intervals over one summer demonstrated that the peak of flowering shifts by about one week per 150m elevational change, not nearly enough to impose temporal barriers against pollen flow across the 600m elevational gradient. Taken together, his results provide convincing evidence for the strong role of pollen flow in maintaining a genetically cohesive metapopulation despite habitat fragmentation and the considerable altitudinal amplitude.

If local adaptation were to be shown in this system, for example, by reciprocal transplant experiments between the elevational extremes, strong selection against immigrant genes would have to be invoked. Viewed from the perspective of climate change, however, pollen flow might ensure the exchange of adaptive genes from lower to higher elevations.

Evolution of metal tolerance

For me the most exciting talk was given by Marc Hanikenne (U Liège, Belgium) on the evolution of metal hyperaccumulation in *Arabidopsis halleri*. This relative of the plant model system *A. thaliana* has itself become a model for study of the molecular biology, physiology and evolution of metal hyper-tolerance and hyperaccumulation, which are complex traits shared by hundreds of plant species associated with naturally or anthropogenically metal-contaminated soils [1]. Previous work in *A. halleri* has established markedly increased zinc (Zn) and cadmium tolerance compared with *A. thaliana* and particularly high levels of Zn accumulation in shoots of *A. halleri*.

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An obvious and rewarding research question in this field has been to characterize the molecular bases of these physiological differences between closely related species. Along these lines, previous work using cross-species transcript profiling has shown that many genes encoding metal transporters are overexpressed in *A. halleri*. One particularly well-characterized gene is *HEAVY METAL ATPASE 4 (HMA4)*, and elegant experiments involving RNA interference lines have conclusively shown the requirement of the metal pump HMA4 for Zn hyperaccumulation in *A. halleri*, and uncovered its role as a physiological 'master-switch'. The latter role was inferred because the HMA4-dependent high rate of root-to-shoot translocation of Zn seems to trigger high expression of Zn-uptake genes in the roots [2].

Hanikenne and colleagues had previously shown that HMA4, a single-copy gene

in *A. thaliana*, has undergone a complex tandem triplication in the lineage leading to *A. halleri*, with the three functional HMA4 copies spread over ~150kb, interspersed with transposable elements and some functional genes [2]. By using different promoter-GUS reporter constructs that comprised the three paralogue-specific HMA4 promoters, they could show that both *A. halleri* and *A. thaliana* transformants have strong expression in roots and shoots, demonstrating that all three HMA4 promoters from *A. halleri* drive high gene expression, compared with the *A. thaliana* HMA4 promoter [2]. These results strongly argue for the parallel contributions of both copy-number expansion and cis-regulatory changes to the massively higher HMA4 transcript levels in *A. halleri*.

Hanikenne presented new data indicating that natural selection is acting on the genomic region encompassing the three HMA4 loci, but that there are twists of sorts compared with the standard expectations under directional selection. He and his co-workers sequenced PCR-amplified gene fragments from a sample of plants collected in seven central European locations, comprising both metallicolous (M) and non-metallicolous (NM) *A. halleri* populations. These genomic fragments cover parts of the three HMA4 genes and flanking regions adjacent to the HMA4 region. Hanikenne discussed the 'landscape' of nucleotide diversity π —a statistic expressing the average number of pairwise differences per site in a sample of sequences; analogous to heterozygosity—across the sequenced fragments, and how this profile can be interpreted in the context of the functional changes observed in *A. halleri* HMA4 in comparison with its related species.

Intriguingly, Hanikenne and his co-workers found that the three HMA4 paralogues have not evolved independently, and he interpreted this as evidence for selection in a specific, strictly functional context. A more standard footprint of natural selection in these new sequence data is the skew towards low-frequency polymorphisms, as summarized by Tajima's *D* and related statistics that are based on the site frequency spectrum. This pattern characterizes the independently evolving low-polymorphism regions, including the three HMA4 promoter fragments. Considered jointly, these data suggest that evolutionary history has shaped a pattern of nucleotide polymorphism that seems to support the model of functional changes and their putatively

selected underlying mutations; this pattern, however, is far more complex than originally anticipated. This detailed example highlights the types of molecular change underlying the evolution of a complex, species-specific phenotype with obvious links to ecology. Such work has the potential to guide future research with an applied focus on phytoremediation and biofortification.

Adaptation in historical context

A complementary presentation by Pierre Saumitou-Laprade (U Lille, France) focused on the evolutionary history of *A. halleri* across its geographic range in Europe, the goal of the talk being aptly expressed by its subtitle, 'Putting adaptation back in a spatial and historical context'. Whilst the level of comparison in Hanikenne's talk was mainly between species that diverged >5 million years ago, Saumitou-Laprade and colleagues addressed the geographic variation in adaptive traits in *A. halleri*, such as quantitative variation for Zn tolerance, in the context of population history. This requires the tools of population genetics and phylogeography.

...metal hypertolerance and hyperaccumulation [...] are complex traits shared by hundreds of plant species associated with [...] metal-contaminated soils

Saumitou-Laprade's work discussed here included a thorough sampling of both M and NM populations across the natural range of European *A. halleri*. Experiments in which plants were exposed to increasing levels of Zn confirmed that regardless of soil type, *A. halleri* plants are much more Zn-tolerant than individuals of *A. thaliana* and *A. lyrata*. These findings underscore the constitutive nature of *A. halleri* Zn tolerance, implying that the trait might have evolved when the sister lineages *A. halleri*–*A. lyrata* first diverged [3]. Moreover, plants from M populations are on average more Zn-tolerant than those from NM populations, consistent with expectations under local adaptation [4]. However, there is variation in Zn tolerance within soil types, particularly in NM populations. High variance across NM populations and enhanced tolerance in M populations illustrate quantitative variation from the NM to the most tolerant M populations, suggesting that the

evolution of enhanced Zn tolerance largely depended on existing genetic variation [5].

This is where the molecular and conceptual tools of population genetics and phylogeography are important. In principle, the fragmented distribution of M populations, mostly found at the northern edge of the European species range, could be due to multiple origins of the M phenotype from independent NM source populations; alternatively, it could be compatible with a single origin of increased metal tolerance and subsequent spread of the M phenotype through colonization of metal-contaminated sites. To distinguish between these alternative scenarios, Saumitou-Laprade and colleagues conducted broad-scale analyses of population genetic structure and phylogeography, using three sequence fragments encoded by the chloroplast (cpDNA) and six highly polymorphic, nuclear-encoded microsatellite loci (nDNA).

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The slowly evolving cpDNA sequences showed a phylogeographical 'break' separating populations south of the Alps from those north of the Alps. Such a genealogical signal is evidence for long-term geographical isolation, perhaps concurrent with the last glacial maximum during the ice ages. Microsatellite

data confirmed the two differentiated gene pools but allowed further inferences owing to the biparental inheritance of nuclear markers: the researchers identified a set of geographically intermediate populations that seem to be products of admixture between the 'pure' north-western and south-eastern clusters—the latter are concordant with the two phylogeographical units identified with cpDNA. These intermediate populations from Slovenia, Slovakia and Poland are consistent with the idea of a hybrid zone reflecting post-ice age secondary contact [6].

Importantly, joint consideration of cpDNA, nDNA and phenotypic data revealed that selection for increased metal tolerance occurred repeatedly and independently on different genetic backgrounds. For example, there is strong genetic differentiation between the M groups from the Harz Mountains in north-central Germany compared with those from Silesia in southern Poland, but only weak differentiation between either M group and their geographically closest NM neighbours further south. This is clear evidence for recent divergence between regional M and NM populations, consistent with those metal-polluted sites being the products of historical mining and recent industrial activities.

Saumitou-Laprade described continuous efforts to identify the genetic bases for the parallel evolution of higher Zn tolerance that must have occurred in the different NM-to-M transitions suggested by the population-genetic analyses. To this end, whole-genome resequencing of bulk population samples from the three

identified phylogeographical regions—the north-west, south-east and hybrid zone—is under way, contrasting M and NM populations from each region. The obvious goal is to identify genes and individual single-nucleotide polymorphisms underlying the quantitative differences in Zn tolerance between M and NM populations. Whilst this approach seems straightforward, the high variance in Zn tolerance could imply that the resequencing data might turn out to be inconclusive. In any case, such genome scans and the statistical treatment of the resulting high-throughput data are areas of intense interest in molecular ecology. Finally, the work discussed by Saumitou-Laprade has implications for future functional studies in this and other systems, as it uncovers important aspects of evolutionary history and thus informs about the most meaningful choice of samples and biological comparisons.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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