Research News

Glacial refugia: sanctuaries for allelic richness, but not for gene diversity

Alex Widmer and Christian Lexer

Glacial refugia are generally expected to harbor higher levels of genetic diversity than are areas that have been colonized after the retreat of the glaciers because colonization often involves only a few individuals. A new paper by Comps *et al.* challenges this expectation by demonstrating a more complex situation in the European beech *Fagus sylvatica*, for which some measures of genetic diversity are higher in newly colonized areas than in refugia. The key to understanding this counter-intuitive result rests both in the estimators used to measure genetic diversity and in the processes affecting these estimators during postglacial recolonization.

Studies of glacial refugia and postglacial recolonization have profoundly changed our views of how species evolve and diverge. Species can no longer be thought of as static entities that are maintained via persistent gene flow. Instead, ranges of species are dynamic, and repeated extinctions followed by recolonization and secondary contact are now recognized as important determinants of the structure and divergence of populations. Phylogeographical studies of taxa with a history of range changes that are a result of the Quaternary ice ages represent fascinating examples of the dynamic nature of the evolution of species.

Studies on the phylogeography of postglacial colonization are usually based on the analysis of genetic variation within and among extant populations inhabiting both potential refugia and formerly glaciated habitats. A general assumption inherent to most of these studies is that glacial refugia harbor higher levels of genetic diversity than do areas that have been colonized after the retreat of glaciers[1]. A new paper by Bernard Comps and co-workers[2] suggests that this assumption might be too simplistic. The authors show that genetic diversity can be either higher or lower in the recolonized areas, depending on the estimator used to assess genetic diversity. This study is important because it cautions against the simple expectation of decreased genetic diversity in colonizing populations. It also illustrates how different estimators of genetic diversity can react to the underlying population genetic processes. Moreover, the phylogeographic conclusions[2] are firmly based on an extensive sample size, and are supported by nongenetic, independent data. We therefore expect that insights derived from this paper could inform the analysis and interpretation of genetic data in other animal and plant taxa.

European beech phylogeography

Comps *et al.*[2] analysed genetic variation at 15 allozyme loci from 18 440 European beech *Fagus sylvatica* trees distributed over 389 populations, spanning the whole geographical range of the species. This data set has been accumulated over the past decade because *F. sylvatica* is an economically and ecologically important forest tree. The work of Comps *et al.* demonstrates the enormous benefits of merging experimental results from different laboratories.

In contrast to oak (*Quercus* spp.), another important tree genus for which large data sets are available, patterns of beech genetic diversity in central and western Europe are not complicated by the presence of interfertile species that might affect genetic diversity via introgression. Chloroplast (cp) DNA polymorphisms in European oaks (*Quercus robur* and *Quercus petraea*) are characterized by a similar geographic pattern for both species, but very weak inter-specific differentiation, suggesting past introgression among the two genomes[3,4]. Clearly, *F. sylvatica* represents a much simpler model, its only close relative being *Fagus orientalis*, an allopatric species native to Asia Minor[2].

Because *F. sylvatica* is wind pollinated (anemophilous), a detailed pollen record is available[5], which has allowed the independent identification of potential refugia and most probable routes of postglacial recolonization. This aspect is of particular importance, because it implies that inferences drawn from genetic data can be tested on a second, independent data set – the pollen record. By contrast, neither herbaceous nor animal-pollinated (zoophilous) plants typically leave significant traces of their former distribution in the subfossil record, and the same is true for small, soft-bodied animals. The availability of independent data on which patterns of molecular variation can be calibrated makes *F. sylvatica* an exciting model to use for studying patterns of postglacial recolonization.

Expected patterns of genetic diversity

What pattern of genetic diversity can be expected when populations recolonize an area from a refugium? Dispersal into a new habitat can lead to the foundation of new populations. Such populations are typically assumed to consist of a single or few individuals and, therefore, represent a small subsample of the genetic diversity of the source population. Although predictions about the level and distribution of genetic variation within

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Box 1. Estimators of genetic diversity

Surveys of genetic variation and phylogeographic analyses typically use a limited set of parameters to estimate genetic variation. These parameters can react differently to bottlenecks and are, therefore, not equally well suited to trace historical processes.

Nei's[a] gene diversity, H: the probability that two alleles sampled at random from a population are different; also known as expected heterozygosity. Bottlenecks lead to a limited decrease of *H* at neutral loci, especially when compared with allelic richness[b].

Proportion of polymorphic loci (P): insensitive to bottlenecks because common alleles can persist even in severe bottlenecks.

Inbreeding coefficient F_{IS}: also known as heterozygote deficit; a measurement of the reduction in heterozygosity of an individual as a result of nonrandom mating within its subpopulation. F_{IS} is less suited to reflect historical processes because it has a different, more rapid dynamic than does gene diversity[c].

Allelic richness: a measurement of the number of alleles per locus. It is highly dependent on effective population size and is, therefore, more useful for identifying historical processes such as bottlenecks[d] and population admixture[e]. However, allelic richness must be standardized because it is very sensitive to uneven sample sizes. This can be achieved using the rarefaction method[f,g].

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and among populations differ between models[6,7], there is general agreement that transitory reductions in effective population size, also called bottlenecks or founder events, lead to a reduction of genetic diversity – the pattern typically expected in most phylogeographic analyses.

However, the intensity of reduction in genetic diversity differs between estimators[2] (Box 1). At neutral loci, a much stronger decrease is expected for allelic richness than for gene diversity, which is the expected heterozygosity, *H*. As expected, *F. sylvatica* shows a reduced level of allelic richness in populations in Northern Europe, compatible with a loss of rare alleles during recolonization. The expected reduction of gene diversity, however, was not found. Comps *et al.*[2] found a negative correlation between allelic richness and gene diversity, with recolonized areas displaying reduced allelic richness, but increased gene diversity. The salt in this soup is that most phylogeographic studies calculate only gene diversity, rather than allelic richness, and might, therefore, be seriously misled in their attempt to identify refugial areas on the basis of that estimator.

How reliable then are the conclusions drawn for *F. sylvatica*[2] and are they likely to also hold for other organisms, or is there just something peculiar about *F. sylvatica*? As highlighted, *F. sylvatica* is suitable as a study system because a very detailed pollen map exists for it[5]. The picture of glacial refugia and postglacial recolonization drawn for *F. sylvatica* is highly congruent for both data sets, pollen and allozymes, respectively. However, there is indeed something peculiar about *F. sylvatica*. *Fagus sylvatica* is a wind-pollinated forest tree. Such trees typically have a delayed reproduction, and chances could be high that numerous juvenile migrants arrive at a newly colonized site before reproduction begins and therefore dampen the intensity of the founder event. Moreover, adult trees might serve as pollen traps, and low density of adult trees in recently founded populations could allow long-distance gene flow via pollen. These characteristics could account for differences between forest trees, such as beech, and annual plants[8]. Two other processes that have been invoked in *F. sylvatica* (selection and secondary contact between populations originating from different refugia) might have a similar effect in other organisms. It is suggested that, for *F. sylvatica*[2], each of these factors might have added to the observed increase of gene diversity in the recolonized areas, but one cannot be singled out as being most important.

Prospects

We hope that the study by Comps *et al.* encourages students of postglacial recolonization to make innovative use of the different estimators of genetic diversity, and to be open towards unexpected results – the typical expectation of reduced genetic diversity in recolonized areas might be too simple to be true. Also, it will be important to see whether the patterns observed in *F. sylvatica* are also found in organisms with different life histories, mating systems and distributions. Generalizations can only be made once genetic data from a variety of plant and animal taxa have been interpreted in the greater context of shared history.

Now that the history of beech colonization of Europe has been elucidated, what work remains? Clearly, the importance of admixture effects (hybridization between populations originating from different glacial refugia) and selection in producing patterns of genetic diversity, such as those observed by Comps *et al.*, remain to be investigated. We suggest that genetic map-based approaches involving genome-wide sampling of genetic markers, similar to the experimental design of introgression studies in natural hybrid zones[9], might be informative. Both population admixture and selection might be detectable through patterns of linkage disequilibrium between pairs of genetic marker loci for which estimates of recombination frequencies are available.

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Alex Widmer*
Christian Lexer
Indiana University, Biology Dept, Bloomington, IN 47405, USA.
*e-mail: awidmer@bio.indiana.edu

Intramitochondrial recombination – is it why some mitochondrial genes sleep around?

Mark Dowton and Nick J.H. Campbell

A new paper by Kajander *et al.* undermines the general view that mitochondria do not recombine. The authors discovered the existence of 'sublimons', rearranged mitochondrial genomes present at very low levels in healthy human patients. Crucially, the different rearranged mitochondrial genomes can theoretically be interconverted through intramitochondrial recombination. The putative operation of intramitochondrial recombination should impact on our ideas of how mitochondrial genes evolve, particularly with respect to how mitochondrial genomes rearrange.

Some biological phenomena are crucial to get right, because they become central assumptions that are used to interpret many subsequent observations. One such assumption is that animal mitochondria do not recombine. For 20 years, this has shaped our perceptions of how mitochondrial genes and genomes evolve. Although many observations are consistent with that view, some are not. Recent findings by Kajander *et al.*[1] challenge the traditional view that mitochondria do not recombine[2] and, in our opinion, they offer an intriguing explanation for observations that are at odds with this popular belief.

In experiments carried out during the 1980s, somatic cell hybrids – containing a mixture of mitochondria from two closely related mammalian species – did not produce hybrid mitochondrial genomes. However, these experiments were designed to detect just one type of recombination, that between mitochondrial genomes from different species ('intermitochondrial' recombination). For reasons that are unclear to us, these observations led to the general view that all types of recombination were rare or absent in animal mitochondria. Although there has been recent debate over the existence of recombination in animal mitochondria[3–6], this is again concerned with intermitochondrial recombination. The operation of intramitochondrial recombination has not yet been critically assessed. In our view, the article by Kajander *et al.* provides evidence that intramitochondrial recombination does occur.

There have been tantalizing hints that intramitochondrial recombination does occur in animal mtDNA. Rand and Harrison[7] were among the first to suggest that intramitochondrial recombination might explain the presence of precisely tandemly repeated mitochondrial sequences. However, slipped-strand mispairing was an equally plausible explanation[2], one that conformed to the conventional wisdom that recombination does not occur in animal mitochondria. However, more difficult to dismiss were the instances of concerted evolution of repeats in remote regions of the mitochondrial genome from three separate phyla[8–10]. Slipped-strand mispairing could not explain this phenomenon, because the repeated regions were conserved among divergent taxa, and recombination seemed the only plausible explanation. More concrete evidence came from Lunt and Hyman[11] who found small mini-circle mtDNA molecules in nematode mitochondria, showing that mitochondria contained the necessary components to cut double-stranded mtDNA, and rejoin the ends. We also knew that mammalian mitochondria contained the necessary enzymatic machinery to accomplish homologous recombination[12], but the paradigm that mitochondrial genomes do not recombine remained resilient.

The recent work of Kajander *et al.*[1] should overturn this paradigm. These authors describe the presence of 'sublimons', rearranged mtDNA molecules present at very low levels, in healthy human patients. Although the presence of these sublimons in patients with mitochondrial pathologies has been known for some time[13], it is the discovery that they represent a proportion of the mtDNA population in healthy individuals that is likely to impact on our views of mitochondrial genomics.

Evidence that these sublimons are created by intramitochondrial recombination comes from the identification of mitochondrial break points present simultaneously within an individual. Rearranged genomes with a given pair of primary break points are present within an individual as partial duplications, deletion monomers, dimers and multimers. Intramitochondrial recombination could theoretically produce

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