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Perspectives

One, two or more species? Mitonuclear discordance and species delimitation

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Delimiting species boundaries is central to understand ecological and evolutionary processes, and to monitor biodiversity patterns over time and space. Yet, most of our current knowledge on animal diversity and phylogeny relies on morphological and mitochondrial (mt) DNA variation, a popular molecular marker also used as a barcode to assign samples to species. For morphologically undistinguishable sympatric species (cryptic species), the congruence of several independent markers is necessary to define separate species. Nuclear markers are becoming more accessible, and have confirmed that cryptic species are widespread in all animal phyla (Fišer, Robinson, & Malard, 2018). However, striking differences between the mitochondrial and nuclear variation patterns are also commonly found within single species. Mitonuclear discordance can result from incomplete lineage sorting, sex-biased dispersal, asymmetrical introgression, natural selection or *Wolbachia*-mediated genetic sweeps. But more generally, the distinct mode of transmission of these two types of markers (maternal vs. biparental) is sufficient to explain their distinct sensitivity to purely demographic events such as spatial range and population size fluctuations over time. In a From the Cover manuscript in this issue of Molecular Ecology, Hijonosa et al. (2019) show that highly divergent mtDNA lineages coexist in a widespread European butterfly (Figure 1). None of the hundreds of nuclear markers analyzed was associated with mt lineages, nor was *Wolbachia* variation. These findings rule out the presence of cryptic species but shed light on complex demographic history of lineage divergence/fusion during the Pleistocene climatic fluctuations, and pave the way to a better integration of both mt and nuclear information in demographic models.

KEYWORDS

climate change, contemporary evolution, hybridization, population genetics – theoretical, speciation

Species represent the main currency in biology, and all descriptors of biodiversity require separating species. Yet, species are not discrete, fixed entities, but ever-evolving groups of populations more or less connected by gene flow. In geographical isolation natural selection and drift act on heritable variations over time eventually leading to genetically distinct, reproductively isolated entities. When their geographical distributions change to partly overlap, the diverging lineages can remain distinct entities if they evolved

reproductive isolation (e.g., genetic incompatibilities and/or ecological or behavioural differences) strong enough to prevent gene flow. Interestingly, even if hybrid fitness is only slightly less than parental fitness in the contact zone, reproductive isolation can be selected for (reinforcement), ultimately leading to two biological species (Butlin & Smadja, 2018). Otherwise, they interbreed and genome-wide recombination will lead to lineage fusion into a single entity. The outcome of secondary contact between divergent lineages therefore

depends on the relative strength of divergent selection (natural and sexual) versus homogenizing gene flow. The development of DNA sequencing techniques has dramatically accelerated the rate of discovery of new species and the documentation of species distributions. For decades mitochondrial DNA was used as the choice molecular marker to identify evolutionary significant units and to infer their phylogenetic relationships. Especially for taxonomically diverse and complex groups such as insects or marine organisms, mtDNA is a marker of choice to identify deeply diverged lineages, including cryptic species, that is, reproductively isolated entities that do not differ morphologically. Furthermore, mtDNA is increasingly used in community ecology as a barcode to quantify animal diversity distributions and to assess biodiversity patterns from environmental samples (eDNA), based on MOTU (molecular operational taxonomic units) defined according to their mitochondrial nucleotide divergence, skipping the tedious step of individual morphological identification of each component of the community (see Adams et al., 2019 for a recent review). But does mtDNA divergence indeed reflect the number of local species?

Mitochondrial DNA is a haploid cytoplasmic nonrecombinant genome maternally transmitted, and as a result it has a four-fold lower effective population size than the nuclear genome. The intensity of genetic drift, that is, the random loss of genetic diversity over time, is directly linked to effective population size (Charlesworth, 2009). As a consequence, the fragmentation of an initially large population into small isolates will lead to the differential fixation of

mt haplotypes in the geographical isolates while nuclear diversity will be much less affected. Furthermore, cytoplasmic bacteria interfering with the reproduction of their host are common especially in insects (e.g., *Wolbachia*), and were shown to evolve through periodical selective sweeps that lead to the local fixation of the associated mitotype through hitch-hiking, enhancing nucleotide divergence among isolates in mt versus nuclear DNA (Hurst & Jiggins, 2005). As a consequence, mt and nuclear DNA respond differently to demographic fluctuations. When a large population is fragmented into small isolates, such as during glacial periods, mtDNA will diverge while the nuclear genome will retain variability. During geographical and demographic expansion (e.g., interglacial periods) the nuclear genomes will come into contact and fully recombine (in absence of reproductive isolation) while divergent nonrecombinant mitotypes will be retained as drift is no longer at play in large expanding populations. This explains why several highly divergent mtDNA lineages can coexist over large geographical range despite the absence of reproductive isolation (Figure 1). The discovery of such highly divergent mtDNA lineages in the absence of any morphological or ecological evidence for differentiation requires the additional analysis of nuclear markers before claiming that a new cryptic species has been discovered.

With the development of NGS technologies and bioinformatics pipelines, genotyping many individuals for thousands of SNPs is becoming feasible, even in non-model organisms. These genome scans were first used by the evolutionary ecologist community to

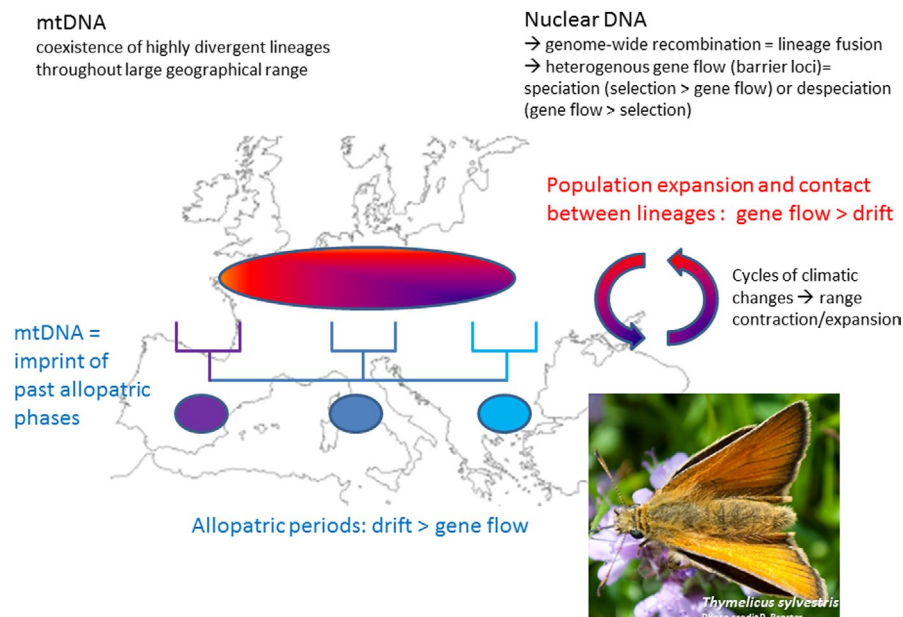


FIGURE 1 Climatic fluctuations have strong impact on species geographical distributions. The small skipper *Thymelicus sylvestris*, a widespread European butterfly, has experienced cycles of range contraction/expansion (Hijonosa et al., 2019). During Pleistocene glacial periods, the species was fragmented into small geographically isolated populations, and drift was the predominant evolutionary force, acting especially on mtDNA (small effective population size). During warming periods, populations expanded, and genome-wide recombination took place between previously diverging lineages, while nonrecombinant highly divergent mt lineages neutrally diffused over large geographical areas, gene flow overcoming drift. Another possible outcome of secondary contact (not observed in *T. sylvestris*) could be heterogeneous gene flow due to selection against hybrids if some isolation mechanisms (barrier loci) evolved during the allopatriotic phase, ultimately leading to speciation or despeciation, depending on the relative force of selection over gene flow. Photo credit: Peter Broster

detect loci showing higher than expected neutral divergence between lineages, and putatively under divergent selection (Foll & Gaggiotti, 2008). The assumption underlying this popular quest for outliers is that demographic fluctuations impact the same way all neutral loci. However, this is not true for mtDNA, and loci mapping to mitochondrial genome should be discarded from the SNP data set prior searching for adaptive loci. Interestingly, Hijonosa et al. (2019) have used a popular method for outlier detection, Bayescan, to search for potentially adaptive loci associated to the mt lineages, but identified as outliers only the mt loci that were present in their ddRAD data set. This suggests that no divergent selection or genetic incompatibilities evolved during the allopatric phase, and that the nuclear genomes freely admixed during secondary contact (lineage fusion). However, it cannot be excluded that some genomic regions are under divergent selection and less permeable to introgression than mtDNA, as recently shown in red snappers where two biological species appear to be maintained despite sharing of deeply divergent mt lineages across nuclear genomes (Pedraza-Marrón et al., 2019). Recent statistical developments including Bayesian inference and approximate Bayesian computation (ABC) applied to NGS data sets, now allow testing for sophisticated demographic scenarios, such as population expansion/contraction over time, divergence between lineages, but also admixture (hybridization) and introgression. It is possible to infer the intensity and direction of gene flow, whether it has been continuous throughout divergence or not, and whether some genomic regions are more or less permeable to introgression (Capblancq, Mavárez, Rioux, & Després, 2019). These 'barriers' to gene flow are all loci involved in reproductive isolation between the divergent lineages (e.g., loci involved in genetic incompatibilities, mate or habitat choice, hybrid fitness: Butlin & Smadja, 2018). Whether diverging lineages will ultimately become two reproductively isolated species depends on the evolution of barrier loci (Figure 1). Hijonosa et al. (2019) found no evidence for such divergent adaptive loci; this suggests that during the allopatric phase, no barrier loci evolved, and secondary contact resulted in lineage fusion. The term 'despeciation' goes a step further simple lineage fusion as it implies that some reproductive isolation mechanisms have evolved during the allopatric phase, so that the speciation process was already at an advanced stage before lineage contact; in my opinion the term 'despeciation' should be used only if some evidence for this can be provided, i.e., if at least some nuclear loci could be identified as 'outliers' or 'barrier loci'.

Episodic gene flow is ubiquitous in natural populations, making the raw material (i.e., a large variety of recombinant genotypes) for natural selection to take place. The eco-evolutionary dynamics of range expansion/contraction and its outcomes (one, two or more species) is hardly predictable, but it represents one of the currently

most exciting and challenging topic in evolutionary biology. Hijonosa et al. (2019) study nicely underpins that mtDNA variation, by itself not ideally suited for species delimitation, provides precious information about past allopatric phases in widespread species. Complex processes of population fragmentation, expansion and admixture need to be addressed by combining several and complementary types of molecular markers and analyzed into an integrated modelling framework taking into account the peculiar demogenetics parameters of each marker that is still in its infancy.

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