P110

Genetic differentiation and structure within the Reticulitermes species complex in Southwestern Europe: a multi-approach study to complete a speciation story

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Reticulitermes subterranean termites spread across South-western Europe for ages, but their distribution and their genetic relationships have evolved though several paleoclimatic and geological variations. Our study proposes to trace genetically and geographically the evolution of the Iberian species complex which has led to the separation of two lineages: Reticulitermes grassei and R. banyulensis. A large sampling area of 145 sites in Spain, Portugal, France and Morocco was investigated combining the analysis of diverse markers (cuticular hydrocarbon GC profiles, mitochondrial COI and COII sequences, ITS2 nuclear sequences and a panel of 10 microsatellite loci). Considered separately, each marker provided a fragmented picture of the evolutionary history of the two taxa at different time scales. Both mathematical analyses (ACP) of cuticular hydrocarbon profiles and phylogenetic analysis (BY, MP, ML, NJ) of mitochondrial and nuclear genes showed a clear separation between the R. grassei and R. banyulensis. In comparison to other European subterranean termites, these results would date their divergence back to possible vicariant events in the Late Miocene. However, the detection of intermediate chemical profiles and asymmetric mtDNA introgressions in some Spanish colonies suggests porosity in their genetic relationships. In fact, the current genetic structure of Iberian populations would be better explains by the isolation/dispersion alternations in Quaternary glacial periods. The genetic structure analysis of 15 localities accurately sampled along transects enabled the identification of the post-glacial colonization routes from southern Spain where heterozigosity is high to south-western France resulting in strong genetic bottlenecks after the passage across the Pyrenees. Finally, this study provides a nuanced answer to the question 'have R. grassei and R. banyulensis reached species status?'. If the presence of a mosaic of mixed colonies in Spain suggests that speciation was not completed on the Iberian Peninsula, French populations were completely separated according to a parapatric model.