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Is the mountain ant *Myrmica kotokui* a single species?: an approach from molecular phylogeny and chemical taxonomy

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Modern molecular phylogenetic techniques have revealed that some morphological species are divided into several genetically distinct cryptic species. To evaluate the biodiversity of a taxonomic group, it is essential to identify cryptic species. In addition, biogeographical studies that determine the distributions of cryptic species are of crucial importance in conservation biology. However, recently, it has been pointed out that the molecular phylogenetic analysis alone cannot resolve the identification of cryptic species. Each gene is expected to have an independent genealogical history, and their topologies would differ from each other and from the species tree which reflects the history of speciation. Therefore, to identify the cryptic species, we should integrate many types of information, such as molecular phylogeny, chemical taxonomy and morphology. For instance, Schlick-Steiner et al. (2006) succeeded in identifying seven cryptic species in a *Tetramorium* ant species complex which are difficult to distinguish morphologically, by using mtDNA phylogeny, morphology, and cuticular hydrocarbons.

We reconstructed a molecular phylogeny of *Myrmica kotokui* specimens collected from six mountain ranges in the Japanese Alps. The phylogeny showed four highly differentiated clades and they tend to be elevationally segregated between lower and upper mountain zone (Ueda et al. 2012). However, it is not clear whether each of the DNA clades based on the molecular phylogeny represents cryptic species. Thus, we analyzed the cuticular hydrocarbons (CHCs) of the ants that were used for the molecular phylogenetic analysis, and tested whether the CHCs differ between the DNA clades. CHCs are the wax covering the surface of insects, and protect them from desiccation. In ants that have advanced chemical communication, CHCs are also used to discriminate nest-mates from non-nest-mates and conspecifics from hetero specifics. Because CHC profiles are expected to be species specific, it is useful to evaluate cryptic species in ants.

The CHC profiles of the *M. kotokui* specimens were analyzed by using gas chromatography-mass spectrometry (GC-MS). Three different types of CHC profiles (A, B and C) were observed. Comparison between the CHC types and the DNA clades showed that a CHC type (C) corresponded to the two clades living in mid and higher elevation, whilst the other two CHC types (A and B) corresponded to two clades living in lower elevation and the two types were intermixed in each clade. The results suggest that the highland type (C) is a reproductively isolated cryptic species, and that introgressive hybridization occurred between the lowland types (A and B).

Keywords: cryptic species, cuticular hydrocarbon, altitudinal gradient, introgressive hybridization, Japanese Alps