

# Genetic markers for species identification: a new cryptic species of wood ant in the Swiss Alps?

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## INTRODUCTION/BACKGROUND

Wood ants (*Formica rufa* group) are one of the most studied groups of ants in Europe. Moreover, due to their huge impact in forest ecosystems, these ants are protected in many European countries. In Switzerland, law protects wood ants since 1966 and, since 1983, they have been classified as globally threatened species by the International Union of Conservation of Nature (IUCN).

Because of their morphological similarity and ability to hybridize, species identification can be very difficult and the taxonomy of the group has always been controversial. At present time, the *Formica rufa* group includes six species (Seifert 1996a, Goropashnaya 2004). During this project we have focused on three of them (*F. lugubris*, *F. paralugubris* and *F. aquilonia*), which have a boreo-alpine distribution and often coexist in the same locality.

Behavioural, physiological and genetic data indicate that the different species are separate taxonomic units (Pamilo *et al* 1992, Cherix *et al* 2004, Maeder 2006), but morphological criteria allowing clear discrimination are rare.

For example, a high level of experience is needed to distinguish *F. lugubris* and *F. paralugubris* and the method is time consuming even for specialists.

*F. paralugubris* has been recently described as a new sibling species of *F. lugubris* (Seifert 1996b). This description was based on comparative investigations on the external morphology of queens and workers. The best results were obtained with multiple discriminant functions based on nest samples means using characters such as body part measures, numbers of setae and hair length. Unfortunately, two main reasons make this procedure quite long and not easy to use. First, precise measurements using micrometers are required and, second, those measurements have to be taken from 5-10 ants of the same nest.

Different taxonomical approaches have also been proposed during the last years (Cherix *et al* 2004, Maeder 2006), but wood ants species identification is still controversial and more reliable methods are strongly recommended. In this work we therefore investigated the suitability of genetic markers as new discriminative tool to distinguish between the different species of the *Formica rufa* group.

## MATERIAL & METHODS

Sampling has been conducted between 2005 and 2007 within the Swiss National Park (Engadin, Graubünden). Created in 1914, this strict natural reserve located in the Eastern Swiss Alps offers the unique opportunity to study the evolution of wood ants populations in unmanaged forests.

Ant workers have been captured at the nest surface from 72 nests located in different valleys of the Swiss National Park. Individuals have been preserved in ethanol until genetic analysis.

After sampling, species identification has been assessed on the basis of morphological criteria according to Seifert (1996a,b) by measuring morphological traits in workers (Seifert 1996b, B.

Seifert pers. com.) and also by comparing, if available, morphological traits in queens with reference material deposited at the Museum of Zoology of Lausanne (Switzerland). After morphological investigation, the 72 sampled nests belong to *F. aquilonia* (24 nests), to *F. lugubris* (34) and to *F. paralugubris* (14).

DNA amplifications have been carried out at the University of Oulu (Finland). Eight to ten workers from each nest were genotyped at 9 microsatellite markers (highly polymorphic) and a total of 650 ants have been genotyped.

To check the concordance between morphology and genetic structure, all the genotypes were screened using a Bayesian admixture procedure implemented in *Structure 2.1* software (Pritchard *et al* 2000). To investigate on the genetic structure of the different nest and species, Wright's fixation indices have been calculated with *FSTAT v. 2.9.2* (Goudet 1995).

## RESULTS

Results indicate that each species is genetically separated from the others, suggesting that genetic nuclear markers (such as microsatellites) are a powerful tool for species identification at a local scale. Nevertheless, according to our data we also observed that our samples, which belong to three morphological species, formed four distinct genetic groups.

*F. paralugubris* and *F. aquilonia* form 2 distinct genetic groups within the park, while samples of *F. lugubris* do not create a homogeneous group but split in two distinct genetic groupings. This interesting result could be explained by hybridization of *F. lugubris* with *F. paralugubris* and/or *F. aquilonia*, or could more likely indicate the existence of an undescribed cryptic species within the Swiss National Park (Graubünden).

## CONCLUSIONS & OUTLOOK

The existence of an undescribed cryptic species would be of great interest in terms of biodiversity and of conservation of these protected species. Chemical and behavioral analysis are currently in progress and should help us to clarify whether the fourth genetic group really represents a separated species or not.

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