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Genetic characterization of western European noble crayfish (*Astacus astacus*) populations for conservation management

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Introduction

One goal in conservation biology is to conserve the genetic diversity of endangered species to protect their adaptive potential. Therefore, it is essential to explore the genetic composition of natural populations to define evolutionary significant units (ESUs). An ESU can be defined as a population or group of populations that is sufficiently differentiated from all the other populations of this species and requires an individual management or conservation strategy (*1*). In cases where no genetic data is available conventional management plans treat different catchments as separate ESUs for restocking (*2*). The aim of this study was to resolve the genetic structure of western European noble crayfish populations for future conservation strategies in western Europe. The genetic data was compared with a European-wide dataset. In particular, we addressed the following questions:

Main Questions

1. **Do the western European noble crayfish differentiate from the central European noble crayfish?**
2. **Do the populations present in the different river catchments (Meuse, Seine, Scheldt, Rhine) form independent ESUs?**

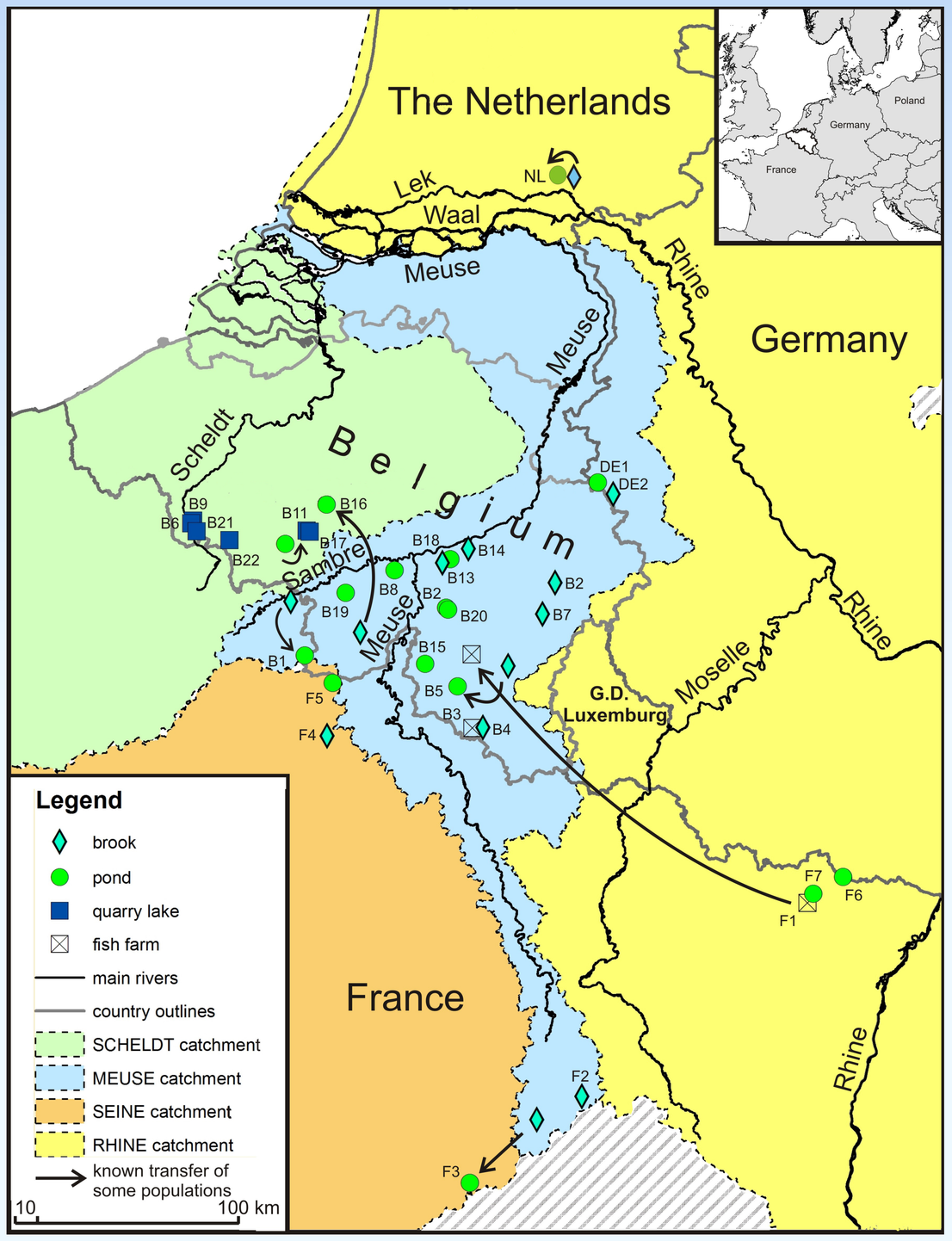

Figure 1. Map showing the sampling sites of noble crayfish populations in France, Belgium, the Netherlands and western Germany. Arrows indicate previous translocations of noble crayfish.

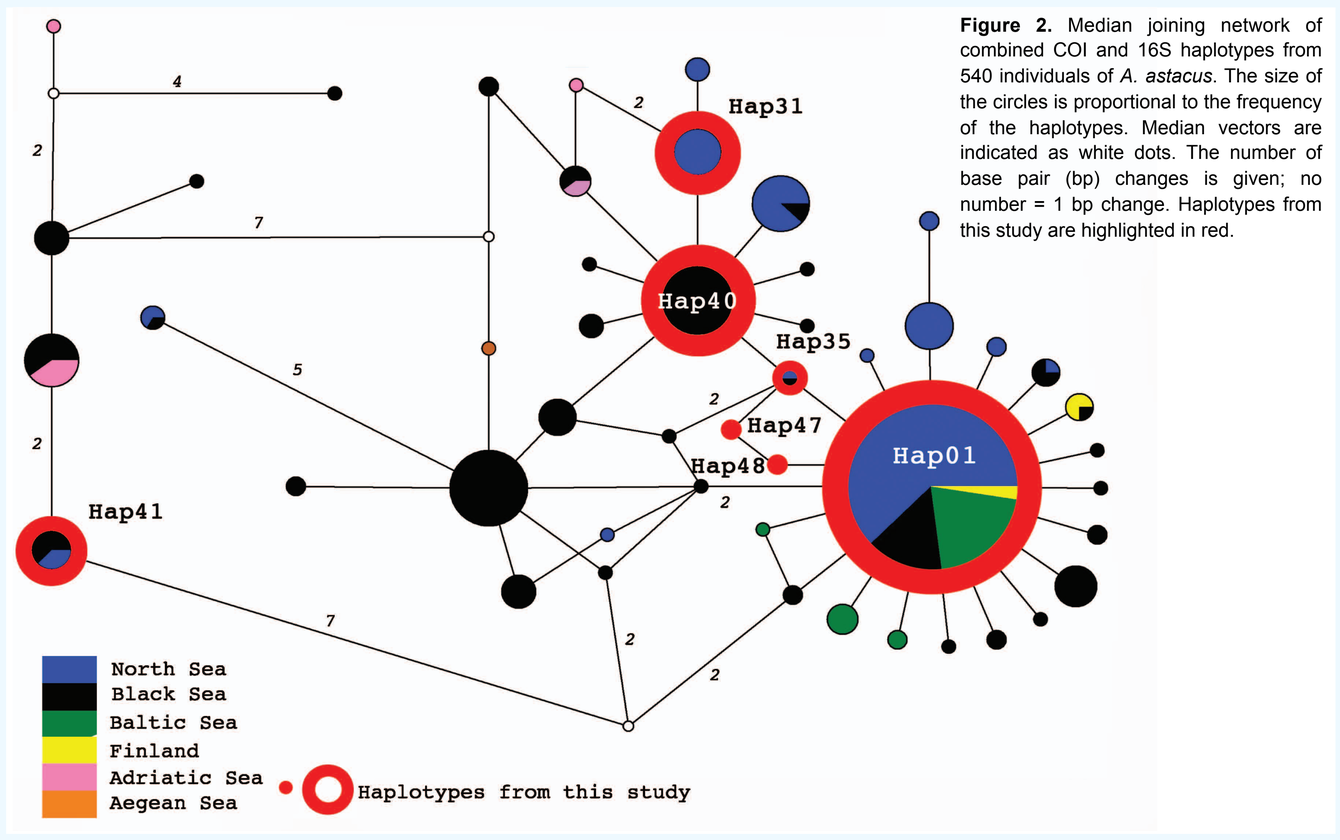

Figure 2. Median joining network of combined COI and 16S haplotypes from 540 individuals of *A. astacus*. The size of the circles is proportional to the frequency of the haplotypes. Median vectors are indicated as white dots. The number of base pair (bp) changes is given; no number = 1 bp change. Haplotypes from this study are highlighted in red.

Results and Discussion

Among 309 sequenced individuals we detected eight distinct haplotypes (Fig. 2). In total, 89.6% of the samples exhibited the most common European haplotype Hap01. However, we found two private haplotypes in population BE11 (Hap47 + 48), which differed from Hap01 by only one or two mutations and could be the result of homoplasy. In the Belgian hatchery in Bitche (F1) and in the French population F7 we detected the haplotype Hap01, but also a distinct Croatian haplotype Hap41.

Based on microsatellites, the FCA showed that the French individuals are distributed most widespread in the graph indicating a comparably high genetic variation (Fig. 3) Populations from the French Rhine (F6, F7, F1=hatchery) and populations from the French and German Meuse (F2, F3, DE1) clustered in groups separated from all other populations, also separated from the remaining European dataset (data not shown). Populations present in the Belgian Meuse and Scheldt catchments, in the French Seine catchment and the Dutch population showed high genetic similarity. They also resemble central European populations (data not shown). The differentiation between the hatchery (F1) and all Belgian natural populations was high. The hatchery and population F7 exhibited the highest heterozygosity and the highest number of private alleles (data not shown).

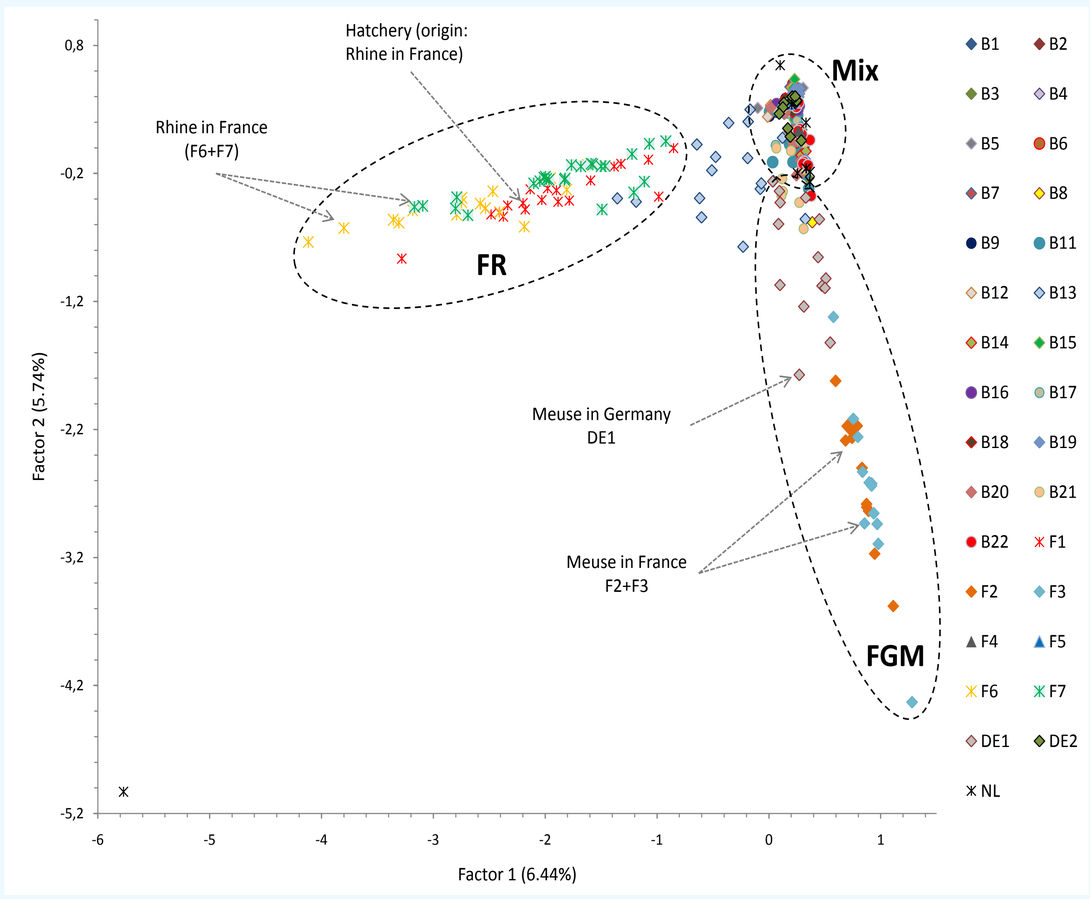

Figure 3. Factorial Correspondence Analysis (FCA) over six micro-satellite loci. Each point represent one individual. The distance between points reflects the degree of genetic differentiation among individuals. We could reveal three groups: "FR", "FGM" and "Mix"

Conclusions

1. **The Belgian and Dutch populations did not show an exceptional genetic structure within the European dataset. However, noble crayfish from the French Meuse and Rhine catchment are differentiated from the European noble crayfish.**
2. **The Belgian Meuse and Scheldt river catchments did not exhibit any significant differentiation and can be treated as one ESU. However, population BE11 (Scheldt) needs special management to protect the endemic Belgian haplotypes. The French Rhine and Meuse catchment should be treated as two distinct ESU. Although the last remaining Dutch population is not distinct from the remaining populations surrounding it, being the last entitles it to special management.**

Methods

Tissue material from 563 specimens from 31 sites distributed in four countries (Fig.1) was collected. Sampling sites were located in the river catchments of the Meuse (N=17), Rhine (N=3), Scheldt (N=8), Seine (N=2) as well as a Belgian hatchery (F1) with crayfish originating from the Rhine basin in France. We sequenced a 350 base pair (bp) fragment of the mitochondrial cytochrome oxidase subunit I (COI) and a 500 bp fragment of 16s rRNA (16S) for ten individuals per population. Haplotypes were identified and compared with a median joining (MJ) network (NETWORK 4.510) consisting of 46 haplotypes from 540 specimens from the dataset of Schrimpf (2013, *3*).

A microsatellite analysis was performed based on six polymorphic loci (*4, 5*) for up to 20 individuals per population. The values of expected heterozygosity (H_E) and a factorial component analysis (FCA) were computed with the software GENETIX v. 4.05. The number of private alleles was estimated with the software GDA v.1.0. Pairwise F_{ST} values between all sites were calculated with ARLEQUIN v.3.5.1.3.

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