Landscape population genetics and the role of organic farming

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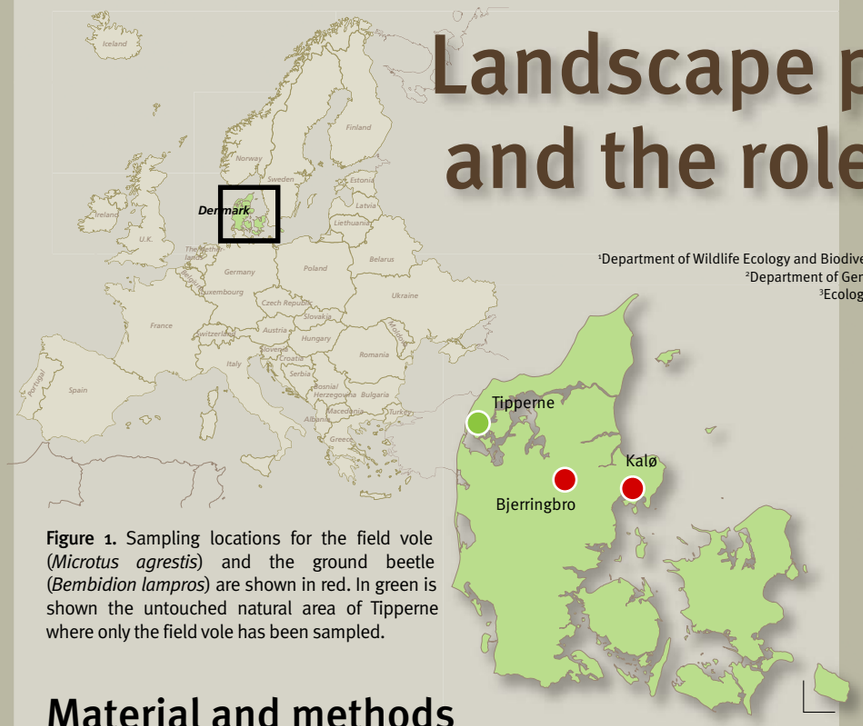

Figure 1. Sampling locations for the field vole (*Microtus agrestis*) and the ground beetle (*Bembidion lampros*) are shown in red. In green is shown the untouched natural area of Tipperne where only the field vole has been sampled.

Introduction

This project aims at understanding the effect of different farming systems on the genetic diversity of common agricultural species.

It is well known that organic farming generally improves the biodiversity and abundance of species in the agricultural landscape (Hole *et al.*, 2005). A reduction in species number and abundance has been shown as a result of the intensification of farming suggesting a relationship between farming intensity and species abundance (e.g. Stoate *et al.*, 2001). Anyway, none of the studies that investigated the effects of pesticides presence and farming intensity has investigated the effect on the genetic diversity and isolation of the populations.

It has been shown that, despite the theoretical expectations, also very abundant species like *Abax parallelepipedus* can be divided in isolated and genetically distinct populations within very few years in response to human activity (e.g. construction of streets: Keller *et al.*, 2004).

Therefore, we chose two common agricultural species (field vole, *Microtus agrestis*, and a non-pest ground beetle, *Bembidion lampros*) belonging to different taxa and with different dispersal abilities, to investigate the effect of pesticide use and intensiveness of farming on their genetic structuring and diversity.

Material and methods

Sampling

Individuals of the two species (field vole, *Microtus agrestis*, and ground beetle, *Bembidion lampros*) have been sampled for two subsequent years in Kalø (Lat: 56 18 00 N – Long: 010 29 00 E, Denmark) and Bjerringbro areas (Lat: 56 23 00 N – Long: 009 40 00 E, Denmark). These two areas have different levels of farming intensity and they present both organic and conventional fields. For the field vole, samples from an untouched natural area were also obtained (Tipperne area, Lat: 55 52 00 N, Long: 08 13 48 E Denmark). Sampling locations are shown in Figure 1.

As the migration rate and distance of the ground beetle are not well known a detailed sampling scheme has been followed in order to investigate population structuring at different levels. We sampled the field hedges in autumn (when the beetles move to the edges to overwinter; Petersen, 1999) at a distance of 50 m for each sampling location within the same field and on both side of each hedge (the sampling scheme is shown in Fig. 2).

Field vole samples have been collected during autumn-winter 2007 and autumn-winter 2008. Beetle samples have been obtained in August 2008 and will be collected again during August 2009.

Genetic analyses

We analyzed 15 microsatellite loci and a hyper-variable part of the cytochrome-b (Jaarola and Searle, 2002) of the field vole. The 15 microsatellite markers had already been developed for the same species (Jaarola *et al.*, 2007) or for a very similar species (*Microtus arvalis*, Gauffre *et al.*, 2007)

For the ground beetle, there was no published primers. In order to develop microsatellite markers we performed a genome scan analysis using 454 technology. For the determination of the microsatellite sequences we used Sputnik software (Abajian C., unpublished) as suggested by Sharma *et al.*, 2007. Primer design was performed using Primer3 (Rozen and Skaletsky, 2000). The loci will now be tested on 10 individuals belonging to different populations in order to asses their level of polymorphism.

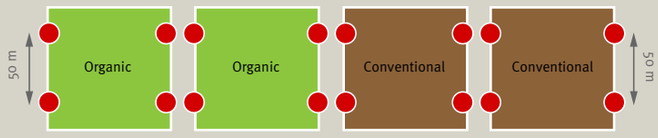

Figure 2. Scheme for the ground beetle (*Bembidion lampros*) sampling in an area comprehending conventional and organic fields. Sampling area is on the field hedges.

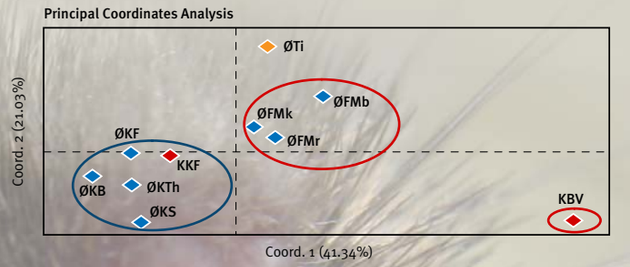

Figure 3. Principal Coordinates Analyses based on first year microsatellite data for the field vole (*Microtus agrestis*). Blue dots indicates organic fields, red dots conventional fields and orange dot is the untouched natural area of Tipperne (Denmark). Blue circle include sample from Kalø (Denmark) and red circle samples from Bjerringbro area (Denmark).

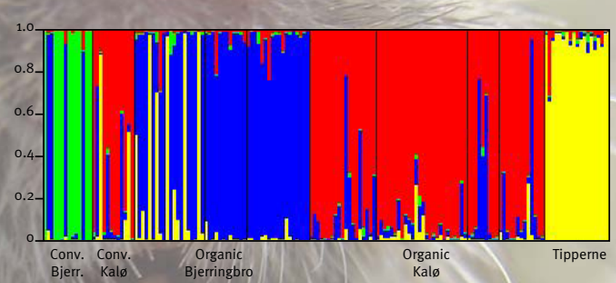

Figure 4. Bayesian clustering analysis performed in Structure (Pritchard *et al.*, 2000) with K=4 and 1.000.000 iterations on first year microsatellite data for the field vole (*Microtus agrestis*).

Preliminary results

Field vole microsatellite analyses

We performed a preliminary analysis on the first year microsatellite data for the field vole. All the loci included in the analyses accorded to the Hardy-Weinberg expectations. The Principal Coordinates Analyses (performed in GenAlEx; Peakall and Smouse, 2005) shown in Figure 3 clearly shows a separation between the different sampling areas and within Kalø area also between conventional and organic fields. The same differentiation between sampling areas has been obtained using Structure (Fig. 4; Pritchard *et al.*, 2000)

Marker selection in Bembidion

The genome scan of the *Bembidion lampros* genome produced about 200.000 contigs within which we were able to determine a huge amount of microsatellite loci using Sputnik (ca. 4000). We were able to select 34 loci that showed a perfect or nearly perfect repetition, a proper sequence on both sides of the marker sequence and a di- or trimeric repetition. These markers will now be tested.

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