



ESF - Exchange Grant - Final Report

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EXCHANGE GRANT

Reference Number : **651**
Submission date : **15/03/2005 11:57:23**

ESF ACTIVITY

Unit(s) : **LESC**
Activity Title : **Integrating population genetics and conservation biology: Merging theoretical, experimental and applied approaches**
Activity Acronym : **CONGEN**

PROJECT

Title of the proposed research project : **The impact of dormant propagule banks on the genetic structure of recently founded populations**
Date of visit (starting date) : **09/05/2005**
Duration : **17 week(s)**
Travel Granted : **200 €**
Applicant's Name : **Raquel Ortells, Valencia, Spain**

MAXIMUM AMOUNT GRANTED

TOTAL ESF Grant FUNDING : **7000 €**

HOST INSTITUTE(s)

Professor Luc De Meester, Leuven, Belgium

ACTUAL EXPENDITURE

Travel Cost : **0 €**

Purpose of the visit

To genetically study artificially created ponds as they offer a unique opportunity to track changes in the genetic structure from the very first moment of population founding and thus, to study the impact of founder effects and the interactions among genotypes.

In the fall of 2001, 25 new farmland ponds were created in Flanders as part of a restoration project. The colonization of these ponds by cladocerans was monitored monthly during the first year and bimonthly in the following years.

In cyclical parthenogens dispersal is associated with the sexually-produced resting egg. After hatching, parthenogenetic reproduction allows for a rapid population growth rate. One single egg is thus capable of colonize and quickly spread in the pond monopolizing the resources. Due to high population growth rates, the formation of an extensive egg bank, and the potential for local adaptation, those genotypes first colonizing the pond have the potential to abort the establishment of new immigrants. This phenomena has been theoretically suggested as the Monopolization Hypothesis, which tries to explain that, despite effective dispersal, populations that are very close can show high genetic differentiation due to long and lasting founder effects. Under this hypothesis the allele frequencies of the first population establishment are very resilient to change, because any immigrant coming later will have to cope with a high number of individuals already adapted to the local conditions. However, if the population is colonized by one or a few clones, the effects of inbreeding depression after the first growing season can reduce the local fitness and thus, facilitate the successful establishment of new immigrants. Once a certain number of genotypes have been established, local adaptation takes a leading role in determining the population genetic structure and the Monopolization Hypothesis becomes fully operational. According to this, the main objectives of this project were:

- To document the initial stages of colonization at a population genetic level
- To asses the process and progress of genetic diversity of cladocerans in natural conditions
- To infer the successful establishment of genotypes in order to determine the strength and persistency of founder effects
- To test local adaptation vs. inbreeding depression during the initial stages of population build-up

In this short stay I have concentrated on the first two objectives, as I have analyzed only one growing season of the cyclically parthenogen *Daphnia magna*.

Description of the work carried out during the visit

I chose one artificially-created pond in the west coast of Flanders, Damme, which is temporary, small and shallow. Louette and De Meester (2004) found that after four months the pond was colonized by *Daphnia atkinsoni*, and that after 8 months, *D. magna* and *Symocephalus vetulus* appeared, the latter only sporadically. For this study, I chose *Daphnia magna*.

Colonization is strongly influenced by the regional characteristics and the presence of source habitats. I therefore also studied 12 ponds of similar characteristics found in the region, in an area of 3 km. I took sediment samples and found *D. magna* ephippia in 5 of these ponds.

In a previous study, Louette and De Meester (2005) found, with allozyme markers, 2 genotypes colonizing the pond and no new alleles through the year. The temporal samples were kept in ethanol and the sediment samples collected every year

were kept in dark and cold conditions. Unfortunately, no ephippia of *D. magna* was found in the sediments of 2002, and that of 2004 accidentally disappeared. So, I studied 12 temporal samples corresponding to the first growing season of *D. magna*, the resting egg bank after that growing season and one produced two years later and resting egg banks from 5 regional ponds. I analysed these samples genetically by screening them for 8 polymorphic microsatellite markers. With the data I run assignment tests (to ensure the origin of the genotypes) and classic *f*statistics (to look at the genetic structure of the system).

Main results obtained

Despite microsatellite are highly variable, I only found one more clone than Louette & De Meester (2005) did with allozymes. Three clones reached the pond during the first three months and established through the year. Also consistent with their results, no new allele was found over time. Due to the unequal representation of genotypes, clonal diversity was variable along time. One clone clearly dominated, while the others were found at lower frequencies, and one clone was not detected during the second year. Regarding allele frequencies, no new alleles were found in the resting egg bank of 2003. After confirmation through assignment tests, this means that the three genotypes build up the resting egg bank, providing genetic diversity for future growing seasons.

Due to the uneven frequencies of genotypes, selfing, compared to out crossing was very high. 75% of the genotypes from the resting egg bank were formed by crossings between individuals from the same clone.

All the alleles found in 2002 and in the resting egg bank were found in the regional samples at similar frequencies, so it is very likely that the source habitat is within this region. Confirming this, the index of genetic differentiation between the regional ponds and the resting egg bank in 2003 was low ($F_{st}=0.035$).

In the resting egg bank two years later we found two new alleles, in very low frequencies. However, taking into account the sample size of the resting egg bank in 2005 ($N=75$), these frequencies are very important and mean that there were at least three new immigrants in the pond. They very probably came from the regional pool, as these alleles were also found there. In fact, the genetic differentiation this year is now lower ($F_{st}=0.029$), which suggests ongoing gene flow.

To test the probability of finding new alleles in the pond I did a simple test assuming that the pond is colonized by three genotypes each year: I pooled all the genotypes in the regional ponds as in a metapopulation and randomized them. Then I choose ten individuals and count the frequency of new alleles and repeat this for several times. The frequencies I obtained from this analysis and the real ones from the pond are not different. So what I really have found in Damme pond is a reflex of the allele pool in the metapopulation.

According to the assignment test, the most likely source in Damme is the biggest one of the regional ponds, which suggests a possible mainland-island scenario.

One possible reason why I didn't get the expected Monopolization output may be that the genetic differentiation in the region is very low, and the ponds were very similar, so there is not enough scope for local adaptation to start with. The most probable dispersal vectors are geese, commonly found in the area.

In summary, 3 clones colonized Damme pond from the first three months. No new alleles were found during the temporal period or in the resting egg bank at the end of the growing season. The resting egg bank was build up with these clones, increasing the genetic diversity for future growing seasons, but with a high frequency of selfed

offspring compared to outcrosses. New alleles were found in the resting egg bank of 2005, which are probably the result of migration from the regional ponds, given the similarity in allele frequencies

Future collaborations with the host institution

Thanks to the so interesting results obtained, future collaborations in the host institute have been scheduled. I have been awarded with a two year grant from the Spanish Ministry of Science and Education. Future plans include:

1. Increasing the number of markers in order to increase the power in detecting new immigrants.
2. Extracting vertical cores and try to find a structure from which to infer the genetic changes observed.
3. Increasing the scope by analyzing more ponds and more species within a pond, searching for a pattern. A very interesting result will be when comparing the former results with an area with absence of geese.
4. If time allows, comparing the results obtained from cladocera with rotifers. Several reasons indicate a very different output according to the main differences between these two taxa. First, rotifer generation times are shorter, so selection and local adaptation is expected to be stronger and faster. Second, rotifer males are haploid, so they represent a filter for recessive deleterious mutations making the effects of inbreeding depression less determining.

Projected publications resulting from the grant

The results mentioned above have been exposed in the VII Symposia on Cladocerans, held in Herzberg, Switzerland, from the 3rd to the 9th of September. ESF funding was clearly acknowledged in the talk I presented. These results, together with additional ones produce in the shortcoming future, will be submitted to a high ranking journal with expertise in genetics and evolutionary ecology. The ESF funding will be stated in the acknowledgments.

References

- Louette G, De Meester L (2004) Rapid colonization of a newly created habitat by cladocerans and the initial build-up of a *Daphnia*-dominated community. *Hydrobiologia* 513:245-249.
- Louette G, De Meester L (2005) High dispersal capacity of cladoceran zooplankton in newly founded communities. *Ecology* 86:353-359.