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► To cite this version:

Mauro Simonato, Laure Sauné, Carole Kerdelhué, Emmanuelle Magnoux, Jérôme Rousselet. Tracking the colonization patterns of an expanding forest pest and its natural enemies using molecular markers. [Contract] 1, Le Studium - Loire Valley Institute for Advanced Studies. 2017, pp.37-42. hal-03217254

HAL Id: hal-03217254

<https://hal.science/hal-03217254>

Submitted on 5 May 2021

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FELLOWSHIP FINAL REPORT

Tracking the colonization patterns of an expanding forest pest and its natural enemies using molecular markers

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REPORT INFO

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ABSTRACT

Climate change is influencing population dynamics of several pest insect species leading to the expansion of their range. Range expansion can be driven also by human-mediated dispersal, with the establishment of new insect populations in suitable areas far from their native range. In this process, interactions between insects and their natural enemies can change due to new environmental conditions or to different rate of dispersion. In recent years, pine processionary moth (PPM), one of the main forest pests in the Mediterranean region, is expanding its range favored by both higher winter mean temperatures and accidental human-mediated transportation. Here we outlined the genetic structure of PPM along its range in France using 23 microsatellites loci, characterizing the main patterns of expansion of this species and identifying the source populations of new colonies in the expansion areas. These data can be employed for developing assignment tools to genetically characterize PPM for a quick identification of their origin area. Finally, we developed a new set of microsatellite primers for the PPM specialist egg-parasitoid in order to track its dispersion following its host in the expanding areas. The low genetic variability found, not directly useful for tracking parasitoid expansion, shed light on the role of bacterial endosymbionts in the population genetic structure of this species.

1- Introduction

Climate change is influencing the distribution of several insect species, leading to local extinction, range shifts and range expansions (Parmesan & Yohe, 2003; Root et al., 2003). Range expansion can be driven also by human-mediated dispersal, with the establishment of new insect populations in suitable areas far from the native range. This process is enhanced by the increase of international commerce and in particular by the ornamental plant trade.

Besides affecting herbivore life histories and subsequent population dynamics, climate change can also alter the relationships between herbivore insects and their higher trophic levels both in their native range and during range expansion. (Stireman et al 2005; Tylianakis et al. 2008). Lower interactions of predators and prey or differences in the speed of dispersion can indeed lead to an increased number of outbreaks and a boost of the expansion rate of the herbivores (Menendez et al. 2008). It is

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<https://doi.org/10.34846/Le-Studium.148.01.FR.10-2017>

therefore crucial to study both herbivore expanding populations and population dynamics of their natural enemies, to determine the main factors involved in this process in order to improve prediction models of pest distribution, useful for pest management programs. In both cases studying the genetic variability of expanding populations is essential to reconstruct the history of the expanding process, obtaining information about the path of expansion and the possible populations of origin involved (Estoup and Guillemaud, 2010).

Among insect species showing a range expansion in recent years, pine processionary moth (PPM), *Thaumetopoea pityocampa* Denis and Schifferrmüller (Lepidoptera: Notodontidae) is one of the best studied examples in Europe. The PPM is one of the main pine forest pests in the Mediterranean countries, with larvae feeding on pine needles during the winter, and adults spreading and reproducing in the late summer, after a pupation period in the soil lasting from few months to one or more years of prolonged diapause. Larvae are gregarious and develop in a typical silk tent. They bear urticating setae harmful to humans and domestic animals (Battisti et al. 2011). Males seem to disperse more than females and thus the spread of PPM is mainly limited by female dispersal ability (Salvato et al. 2002). The range expansion of PPM towards higher latitudes and altitudes is favored by a better winter survival and an enhanced feeding activity caused by higher mean environmental temperatures (Battisti et al. 2005; Buffo et al. 2007). In France, the range of the PPM has expanded considerably over the last three decades with a northward expansion that reached the southern part of the Paris basin (Battisti et al., 2005). The front has shifted by 2.7 km/year between 1972 and 2004, and has accelerated during the last 10 years (5.7 km/year) (Robinet et al., 2007). The analysis of both mitochondrial and nuclear markers showed that the expansion front populations originated from two distinct lineages coming from western and eastern France (Rousselet et al 2010). The Massif Central has been bypassed by these two lineages and the Region Centre Val de Loire has

been almost completely colonized during the two last decades (except a small area in the North of Dreux). Pioneer colonies were also discovered close to Paris and in eastern France far beyond the limits of the main range of the species (Robinet et al. 2010). The low flight ability of females and the presence of pupal parasitoids only suggested that these colonies were accidentally introduced in these areas by human-mediated transportation (Robinet et al. 2012). Expansion capacity of a species increasing its natural range could be enhanced by low or null pressure of natural enemies in the new environment (Menendez et al 2008). Regarding PPM, its rapid expansion probably is not followed by a parallel expansion of its natural enemies, due to their different dispersion capacity or limited ability to detect the host at low density (Auger-Rozenbeg et al. 2015). There is a rich community of insects attacking PPM at different developmental stages. Among these the most studied are the egg parasitoids as they may be useful as biocontrol agents (Mirchev et al. 2004, Arnaldo and Torres 2006). In a preliminary study about parasitism rate along three expansion gradients of PPM, a significantly lower parasitism at the front edge than in the core areas was observed. In another study on expanding PPM populations in the Italian Alps, the parasitism rate increased with a delay of years after the PPM expansion to higher elevations and it was mainly due to the specialist egg-parasitoid *Baryscapus servadeii* (Zovi et al., 2006). The observed decrease of parasitism rate near the expanding front due to a delayed parasitoid colonization could be important as it may favor PPM expansion as a result of the higher colony sizes in the front edge (Auger-Rozenberg et al. 2015)

2- Experimental details

In order to track the expansion of PPM, 25 sampling site were sampled throughout France, covering the native range as well as the recently afforested areas. In addition, 3 sites from Italy and 2 from Spain were included, chosen in areas with a high density of nurseries trading ornamental trees. All the new pioneer colonies in the Paris Basin and North-East France were considered, sampling 10 localities, among

which 4 were sampled twice in different years. An old pioneer colony in Alençon (currently embedded within the PPM range and having genetically contributed to the PPM populations in the Region Centre VdL) was also included in the dataset. The final dataset, composed by a total of 1042 individuals, was analyzed using 23 microsatellite loci. Genetic diversity inside each population was assessed by the software Genepop. The R package PopGenReport' was used to estimate the allelic richness. In order to determine the number of homogeneous genetic clusters inside the native and expanding PPM range, we used the software Structure (Pritchard et al, 2000). The same software was used for a first assignment test for individuals sampled in the pioneer colonies. For each genotype we estimated the percentage of membership to each of the main clusters found in the PPM main range.

Regarding the study of the population genetic patterns in the main PPM egg parasitoid, *B. servadeii*, 213 individual were considered from a total of 10 populations sampled throughout the distribution range of the parasitoid. One population of the sister species *Baryscapus transversalis* Graham, 1991, the hyperparasitoid of *B. servadeii*, was also included in the study.

After a first screening of a set of 43 microsatellite loci by PCR and run on agarose gel, in order to evaluate their genetic variability, only eight loci were used for the subsequent analyses. Genetic diversity inside each population was estimated with the software Genepop. The R packages PopGenReport' and PoppR, were employed to evaluate the allelic richness and number of genotypes in the samples, whereas the genetic distances among populations were summarized in a Neighbor-joining tree with the package 'treemap'. The presence of the endosymbiont *Rickettsia* was determined as well, using specific primers for the 16S region, in order to understand the influence of this bacterium on the genetic variability of the parasitoid.

3- Results and discussion

The analysis of the genetic variability in the PPM populations throughout its native and expanding range showed a northward decrease of the average number of alleles, suggesting a range expansion mainly driven by a diffusive dispersal. The clustering obtained by Structure shows that the main differentiation observed among PPM populations in France is between Mediterranean and Atlantic populations, as already outlined in Rousselet et al. (2015), where the two main corridors of PPM expansions along the north west and north east axes were identified. This west-east differentiation mirrors also the main clustering found for the mitochondrial haplotypes in the same area (Rousselet et al. 2010). Further sub-clustering in the Structure analysis shows that populations are genetically differentiated at a regional scale, with six main clusters distributed in North West, South West, Mediterranean coast, Alps, North east and Paris basin areas respectively. Interestingly, the genetic sub-clusters found seem to be linked to the biogeographical forest regions that are involved in the PPM population dynamics (Toigo et al. 2017). In any case, the population clustering found at the regional scale represents the level of resolution for identifying source populations of pioneer colonies. The analysis of the genetic variability in the pioneer colonies showed a lower number of alleles in these populations than in the populations of the main range, indicating that these colonies were founded by few individuals (genetic bottleneck). By comparing the genetic distances among populations, it was possible to identify the origin of the pioneer colonies. Seven out of ten colonies originated from regions far from the expanding front of PPM, highlighting a pattern of long distance dispersal. The analysis at the individual level showed also that in some colonies there is a genetic mixture from different and distant source populations, underlined also by the diachronic analysis of genetic composition in the pioneer colonies. This outcome confirms the results obtained in a preliminary study on fewer PPM pioneer colonies and with fewer microsatellite loci. (Robinet et al. 2012) and suggests that human mediated dispersal could be a crucial factor in

the dispersion of this moth. The modeling of PPM expansion was previously based only a Gaussian dispersal kernel (reaction-diffusion model) and consequently a stochastic component has been added to the model (parameterized using the human population density). Moreover, the genetic mixture observed in the pioneer colonies can enhance the expansion process, as it has been shown that heterosis could both reduce extinction risks and increase the population growth of recently settled colonies (Wagner et al. 2017). Life cycle and phenology (and then further expansion) might especially be affected by the mixture between various genetically and ecologically differentiated populations. As phenological changes have impacts on tree, animal and human health and on pest management, this question is of interest both to scientists (climate change study) and stakeholders (impacts on health). From 2018 to 2021, URZF will receive funds from public health authorities (Agence Régionale de la Santé Ile de France, ARS IdF; 26 kEUR per year) to survey the front and pioneer populations and to address this question. Interrelationship between phenology and distribution will be developed as a new research topic in the Forest Zoology Research Unit and the Euro-Mediterranean network P-CLIM (see for example “Lettre Biotechnocentre in October 2018). A proposal will also be submitted in 2018 to the French National Research Agency (Agence Nationale de la Recherche, ANR). This proposal will also associate Steve D. Franck (North Carolina State University) who attended to Le Studium conference in last October.

About the genetic structure of the PPM egg-parasitoid populations, the genetic variability retrieved by microsatellites is undoubtedly not enough to record the colonization patterns of *B. servadeii* in PPM expanding areas at small geographical scale. The analysis of the genetic variability in the populations of *B.servadeii* and *B.transversalis* showed a strong difference in terms of genetic variability between the two species. In particular, in *B. servadeii* populations there is a very low number of microsatellite alleles in all the ten loci

considered. Moreover the alleles are associated in few multi-locus genotypes, an expected pattern when dealing with parthenogenetic individuals. *B. servadeii* is known to reproduce through thelytokous parthenogenesis, a type of reproduction in which females are generated by unfertilized eggs. Interestingly, a screening for the presence of *Rickettsia*, and endosymbiont known to induce parthenogenesis in other hymenopterans (Giorgini et al. 2010), showed that 86% of *B. servadeii* and no one of the *B. transversalis* individuals were infected by this bacterium, underlining the probable role of *Rickettsia* in the genetic pattern observed in the two parasitoid species. Nevertheless, the genetic variability found in this study with microsatellite loci underlines the differentiation occurring between *B. servadeii* and *B. transversalis*, confirming them as distinct species. Moreover, it outlines the differentiation occurring inside *B. servadeii* at the macro-geographical scale, already seen with the mitochondrial markers, with the split between North African populations and the rest of the range.

4- Conclusion

The analysis of the patterns of genetic differentiation in the PPM populations both in the native range and in the expanding areas of this species underlined the importance of using a high number of markers in order to better identify at a finer geographical scale the source populations of pioneer colonies. The subsequent essential step will be to define the optimal number of markers to be employed for a more precise identification, assessing the assignation power of all the microsatellite loci used in this study. Another important step will be to understand the paths followed by PPM in the long distance dispersal, comparing the different hypothetical paths of expansions supported by genetic data, and trying to understand the role of ornamental tree nurseries in the dispersion of this moth.

About the PPM egg-parasitoid, our analysis suggests that microsatellite markers are not the best choice in studying the expansion of *B. servadeii* and that more powerful molecular

markers such as high throughput sequencing should be preferred for the identification of source populations and patterns of expansion of this parasitoid.

5- Perspectives of future collaborations with the host laboratory

In the next future, the collaboration will be carried on the discussion and the development of further analyses related to this project. In particular we have to consider the possibility to develop an IT-tool for the molecular identification of newly settled PPM colonies, assessing the possible population of origin and thus the pattern of introduction of this pest in new areas.

6- Articles published in the framework of the fellowship

The results obtained in the project during the fellowship have been included in two manuscripts:

- Simonato M., Sauné L., Kerdelhué C., Rousselet J., Patterns of expansion of pine processionary moth at the northern edge of its distributional range (manuscript in preparation)
- Simonato M., Auger-Rozenberg M.A., Magnoux E., Pilati M., Courtin C., Kerdelhué C., Rousselet J., Isolation and characterization of microsatellite loci of *Baryscapus servadeii*, the egg parasitoid of pine processionary moth (manuscript in preparation)

Both the manuscripts will be shortly submitted to international journals. Analyses are over and draft is almost ready to be submitted for the second one. Finally, results of DIY ABC analyses still in progress will not be separately published but added to those already done in order to increase the impact factor of the targeted journal.

7- Acknowledgements

This work was supported by the Le Studium, Loire Valley Institute for Advanced Studies, Orléans & Tours, France under Marie

Sklodowska-Curie grand agreement no. 665790, European Commission.

I would like to thank Alexis Bernard for the effort for samples collections and Emmanuelle Magnoux, Beatrice Courtial, Claudine Courtin, and all the URZF-INRA staff of Orléans as well as Laure Saune from CBGP from Montpellier, for all their help and support during my fellowship.

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