**UPDATE – PROJECT PROGRESS**  
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Project Acronym: LandGen

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Dear Fonds National de la Recherche,

As requested, please find a report providing a brief update on the progress of the project. This builds upon our last document which suggested changes to the original project, which were accepted by the FNR. To recapitulate briefly, we planned to sample 4 new species (*Osmia bicornis*, *Osmia cornuta*, *Andrena cineraria*, *Bombylius major*) in the same Luxembourgish study areas we sampled the year before, using the same sampling design (1-km2 cells).  
  
Despite concentrating our efforts in time and a near-complete coverage of the study areas, we sampled *O. bicornis* in only 12 cells and *O. cornuta* in only 26 cells. We had much more success with *B. major* despite its short flying season, and managed to catch 601 specimens in 327 cells (over 457 total cells, all of which were visited at least once). We have extracted and amplified *B. major* DNA, and we are currently genotyping our individuals which should be completed soon. Regarding, *A. cineraria*, we were initially struggling to find individuals in enough cells and, although we reached 37 cells, it was not enough to be able to conduct analyses for this species. Still wanting to study this species and not let the specimen we collected be wasted for our initial purpose, we strived to leverage our network and managed to secure usable specimen from several other projects including the wild bee atlas (Dr. Alexander Weigand) and MONIPOL (LIST). We are currently collecting those specimen from different labs to prepare for their DNA extraction. Together with ours, we could reach about 150 specimen in our study area, which should be conductive to decent population genetic analyses.

Similar to the 2021 fieldwork, we will unfortunately have to drop two species (both *Osmia*) from our project because of the low number of sampling cells we found them in. This does not mean that those species are particularly threatened. Indeed, we found very large numbers of *Osmia* individuals in some sites. Our lack of coverage for those two species, notably in natural and semi-natural areas, may be an artefact of their anthropophily and gregariousness, which is notably exacerbated because of the very trendy insect hotels found across urban areas.

Regarding the two original target species we had caught in sufficient numbers in 2021 (*S. pipiens* and *M. florea*) and for which we had found low genetic structure, we have continued to refine our analyses, using many different statistical approaches. We also reached out to other specialists to discuss our results. The general conclusion has not changed, and fragmentation associated with urbanization does not strongly affect their ability to disperse and recolonize habitat patches. Because genetic structure and isolation-by-distance are so low, it is mathematically impossible to relate patterns of genetic differentiation to environmental features and investigate isolation-by-resistance throughthe optimization of landscape genetics models. However, while we cannot conclude that fragmentation limits connectivity in those two species, this does not mean that landscape perturbation does not affect their population dynamics in other ways such as lowering their overall numbers, or that fragmentation does not hinder gene flow for *B. major* or *A. cineraria*, which we will investigate this winter.

The manuscript about the two hoverflies will be submitted to the journal Evolutionary Applications in in October.

Sincerely,

Julian Wittische and Alain Frantz