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**Project Acronym:** LandGen

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Dear Fonds National de la Recherche,

Two unforeseeable circumstances could justify deviations from the original plan for the year 2022 (second year of the project).

First, despite our hard work and overall successful sampling with a near-complete coverage of the study areas, (our total number of specimens, 4870, exceeded our expectations), we only sampled enough individuals for two species (*S. pipiens* and *M. florea*). Indeed, although we caught the other two species (*R. campestris* and *V. pollucens* with 45 and 156 individuals, respectively) and we could theoretically run the planned analyses on them, robust and conclusive research analyses would need more data points.

More focused sampling could be thought of as a solution. However, as our teams explored all habitats at different times of day, across the whole sampling period, we are confident that those two species are simply not abundant in urban areas, rather than this being a sampling bias or a lack of collecting skills. Our specialist on Diptera had been confident that we could catch them in sufficient numbers but, alas, this was not the case. This is an unfortunate result, both for its implication about the conservation of those two species in the face of urbanization, and for our project. The literature is lacking about hoverflies as pollinators, and we plan to communicate this result (low abundance of those two species) in another scientific paper, even though we cannot use the data from those species as originally planned.

A second unforeseeable issue that was completely out of our control is that the two species we caught in sufficient numbers are showing low genetic structure. While this does not mean that we cannot analyze the data, the signal of the effect of different landscape features on pollinator movement and connectivity may be weaker, making it harder to identify accurately the relationship between environment and ecological resistance. Alain Frantz and Julian Wittische (postdoc) have previous experience analyzing datasets with low genetic structure and we will use the latest methods to overcome this issue, but it might be that we cannot reach strong conclusions

Both of those unfortunate circumstances point us towards changing the original plan to better fulfil the general objective. That is, to better understand the landscape connectivity of pollinators, notably in the face of urbanization.

To achieve this we believe propose the following three changes to the original plan for 2022:

1. We should not sample *R. campestris* and *V. pollucens* in rural study areas. Their low abundance makes it risky because despite focusing on urban areas in 2021, we covered many peri-urban rural habitats and we are doubtful that they will be abundant even in remote rural areas.
2. We should not plan to sample *S. pipiens* and *M. florea* in rural study areas. Their low genetic structure in in urbanized study areas, which generally present very fragmented habitats, does not suggest that their structure would be higher in homogenous (relative to anthropogenic disturbances) rural landscapes.
3. We should instead focus on four new species of pollinators, which may give us more chances to detect a stronger genetic structure and help us to better fulfil the original objective. In 2021, only two out of four species ended up being exploitable for our project. Depending on sampling success, we may not be able to use all new species but by considering four new species instead of two, we increase our chances to have more usable datasets. We should sample in two urbanized study areas, around Luxembourg City, and southwest Luxembourg, as last we did last year (but excluding Cologne see point 4 below). Choosing a species of another order/family, with different life history traits could be a solution. We have evaluated the feasibility of using another pollinator species based on the non-target species our teams netted/observed, inventories, citizen science (number of observations on iNaturalist in Luxembourg) and on the literature. Following this, we suggest four new target species:
4. *Andrena cineraria* (Hymenoptera; Andrenidae)because many of our staff have experience catching it thanks to another unrelated project, because we already have access to hundreds of adequately stored specimens, and because beyond phylogeny, its life history traits are very different from those of the two species we successfully sampled.
5. *Osmia cornuta* and *Osmia bicornis* (Hymenoptera; Megachilidae) because they are known to be abundant and easily observable, and frequent insect hotels, which are common in urban landscapes. Additionally, a high quality multiplex microsatellite marker set have been very recently developed (Van Eeckhoven et al., 2022). Finally, although both species separated about 3.9 million years ago, Osmia cornuta, and Osmia bicornis belong to the same clade within the genus Osmia (Branstetter et al., 2021), and comparing the effect of the landscape on related species have led to informative results in the past (Engler et al., 2014). Both species’females are easy identifiable (males are haploid, hence not usable by us).
6. *Bombylius major* (Diptera; Bombyliidae) was found by every fieldworker in 2021 and is very easy to identify in the field. *B. major* is the 4th most observed fly in Luxembourg (iNaturalist). Additionally, they are a known parasitoid of *Andrena* species and this fact makes them an even more interesting choice to us. Indeed, we are also considering a *Andrena* species (see 1 above) and comparing the landscape genetics of trophically related species (here two pollinators at the adult stage, one parasiting the other at the larval stage) can lead to a lot of insight in their ecology (James et al., 2011). Finally, and we expect to find *B. major* hovering close to *Andrena cineraria* nests, so it would indirectly help to catch that species as well.
7. Provided the FNR agrees with the aforementioned plan to switch to other species to seek the fulfilment of our original objective, we would not go back to sample in Cologne because the four chosen species have abundance peaks early in the season compared with our previous hoverfly species and we need to concentrate fieldwork intensely. It would be a better strategy than stretching our fieldwork teams “thin” on multiple study areas over 6 months as we did last year, although it was the right move for the hoverflies, which flied many months. To be clear, we would continue fieldwork to fill the gaps after the abundance peaks but we want to ensure a strong sampling season early on, as it is crucial to the achievement of our objectives.

We understand those suggested changes may appear substantial, but we believe we must include a more diverse array of pollinators to increase the potential of our project to further the understanding of pollinator connectivity and movement.  
  
Sincerely,  
  
Julian Wittische and Alain Frantz

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