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DIANA POSLEDOVICH, ROBERT EKBLOM AND LINDA LAIKRE

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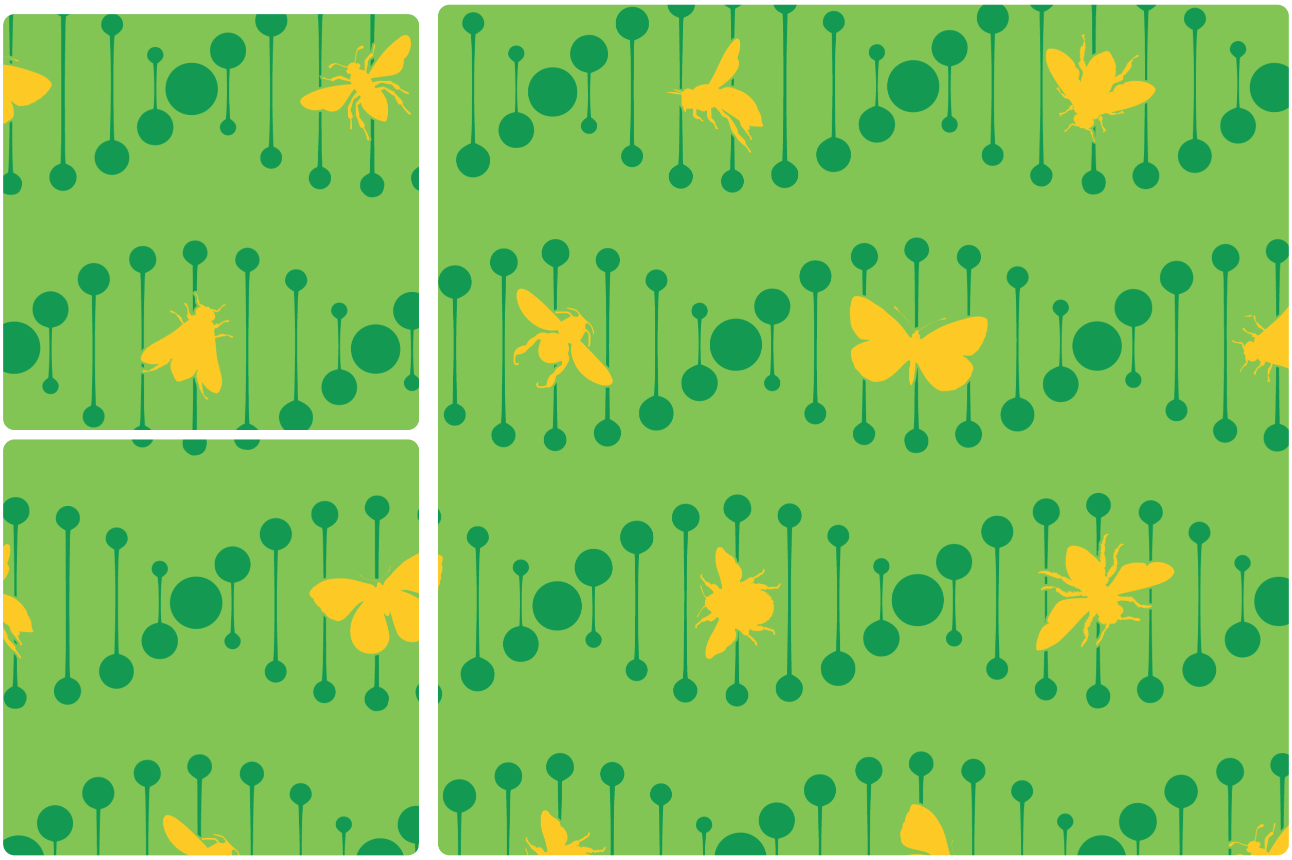

Mapping and monitoring genetic diversity in Sweden

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Förord

Genetisk mångfald är en av tre komponenter av biologisk mångfald och en grundförutsättning för populationers och arters långsiktiga överlevnad och förmåga att anpassa sig till förändringar i miljön, till exempel genom klimatförändringar.

Minskningen av populationer av pollinatörer har blivit ett uppmärksammat problem de senaste årtiondena. Miljöövervakning för genetisk mångfald hos vilda pollinerande arter har därför identifierats som ett prioriterat utvecklingsområde och kopplar till regeringens treåriga satsning att bygga upp övervakning av vilda pollinatörer som genomförs 2020 - 2022.

Övervakningen svarar även på de krav som finns formulerade i etappmålet *Kunskap om genetisk mångfald* samt behov av data och kunskap för uppföljning av preciseringar om genetisk inomartsvariation inom flertalet miljökvalitetsmål. Det bidrar även till underlag inom åtgärdsarbete samt internationell rapportering av status för arter och biologisk mångfald.

Som en del i arbetet med att utveckla övervakning av genetisk mångfald beställde Naturvårdsverket ett uppdrag att ta fram förslag till övervakningsprogram för genetisk mångfald hos vilda arter av pollinatörer. Förslagen, vilka presenteras i denna rapport, utgör viktigt underlag inför myndighetens pågående och fortsatta arbete med att kartlägga och utveckla miljöövervakning för genetisk mångfald. Arbetet är en del i ett uppdrag att ta fram förslag till ett övergripande övervakningsprogram för vilda växt- och djurarter, vilket presenteras i rapporten ”Mapping and monitoring of genetic diversity in Sweden – a proposal for species, methods and costs” (ISBN 978-91-620-6959-9).

Rapporten har tagits fram genom ett samarbete mellan de populationsgenetiska forskarna Dr. Diana Posledovich och Prof. Linda Laikre vid Stockholms universitet samt Dr. Robert Ekblom vid EBC, Uppsala universitet.

Författarna är ansvariga för rapportens innehåll.

Stockholm den 25 maj 2021

Susann Östergård
Enhetschef, Naturanalysenheten
Naturvårdsverket

Preface

Genetic diversity is one of three components of biological diversity and is central for the long-term survival of populations and species, as well as their ability to adapt to environmental changes such as climate change.

The decline in pollinating species has become a recognized problem over the past decades. Development of environmental monitoring of genetic diversity in pollinating species has therefore been identified as an area of priority linked to the Swedish government’s three-year effort 2020-2022 to develop monitoring of wild pollinating species.

Further, monitoring of genetic diversity in pollinating species meet demands that are set by the milestone target *Knowledge about genetic diversity* within the Swedish environmental objectives system. It also contributes with knowledge and data for the assessment of specifications about genetic diversity within several environmental quality objectives, as well as for management and international reporting on species conservation status.

As part of the work on developing monitoring of genetic diversity the Swedish Environmental Protection Agency commissioned a report containing suggestions for a monitoring program of genetic diversity in wild pollinators. The suggestions, which are presented in this report, are an important part of the ongoing and continued work on mapping and developing environmental monitoring of genetic diversity at the Swedish Environmental Protection Agency. The commission also included suggestions for a wider range of plant and animal taxa, which are presented in a separate report: ”Mapping and monitoring of genetic diversity in Sweden – a proposal for species, methods and costs” (ISBN 978-91-620-6959-9).

The report has been developed through a collaboration between the population genetic researchers Dr. Diana Posledovich and Prof. Linda Laikre at Stockholm University and Dr. Robert Ekblom at EBC, Uppsala University.

The authors are responsible for the content of this report.

Stockholm May 25, 2021

Susann Östergård
Head of Nature Analysis Unit
The Swedish Environmental Protection Agency

Contents

1. Sammanfattning

Genetisk inomartsvariation är en viktig förutsättning för populationers långsiktiga överlevnad och för ekosystemens resiliens, t.ex. genom en ökad förmåga för populationer att kunna anpassa sig till ett förändrat klimat. Trots detta finns det idag stora brister i vår kunskap om arters och populationers genetiska variation och hur den förändrats över tid och i relation till olika påverkansfaktorer. Övervakning av arters genetiska variation är således ett viktigt verktyg för förvaltning och bevarandearbete då det ger kunskap om förändringar som vi annars inte kan upptäcka vilket kan fungera som tidiga varningssignaler om hot kopplat till mänsklig aktivitet. Minskningen av populationer av pollinatörer har blivit ett uppmärksammat problem de senaste årtiondena och pollinerande arter har därför identifierats som en viktig grupp för övervakning av genetisk mångfald. I denna rapport ges förslag på övervakningsprogram för pollinatörer. Utifrån redan tillgängliga resurser i form av bland annat genetisk metodik, forskning och historiska samlingar presenteras förslag på arter, metoder för genetiska analyser, stickprovsstorlekar, insamlingslokaler mm., samt en uppskattning av kostnader enligt tre olika ambitionsnivåer. Totalt föreslås 15 arter att ingå i övervakningen – 13 arter av humlor och bin samt 2 arter av dagfjärilar.

2. Summary

Within-species genetic variation is an important factor for long-term maintenance of viable populations and ecosystem resilience, e.g. by increasing the ability for species to adapt to climate change. However, there are still gaps in our knowledge on genetic variation within species and populations and how it changes over time and in relations to different influencing factors. Monitoring genetic diversity is an important tool of management and conservation providing knowledge on invisible changes of a population state and early detection of threats of anthropogenic changes. Declining populations of pollinator species have become a recognized problem over the past decades, raising concerns about stability of pollinator services in the future. Pollinating insects are identified among key species of importance for genetic diversity monitoring. This report provides a suggestion for a program to initiate monitoring of genetic diversity of such species. The work includes an overview of available resources in the form of genetic tools, research and historical collections, and a suggestion of species to be included in monitoring, methods for genetic analyses, sample sizes, collection localities, etc., as well as estimated costs according to three different ambition levels. A total of 15 species are suggested for all three ambition levels – 13 bumblebee and bee species and 2 butterfly species.

3. Introduction

Declining populations of pollinator species have become a recognized problem over the past decades, raising concerns about stability of pollinator services in the future (Biesmeijer et al., 2006; Potts et al., 2016). Monitoring genetic diversity is an important tool of management and conservation providing knowledge on invisible changes of a population state and early detection of threats of anthropogenic changes (see section 1 of the main report).

The aim of this work is to present a suggestion including cost calculations for implementing a monitoring program targeting genetic diversity of some of the pollinator species in Sweden from 2020.

During work to propose a broad program for monitoring of genetic diversity of wild species in general (Posledovich et al. 2021) we identified 34 pollinating insects as of potential interest for monitoring genetic diversity. Of these 34 species 16 were suggested as of high relevance and 18 of medium relevance for the genetic monitoring program. During the revision of the first version of that report (Posledovich et al 2021), SEPA selected 15 of the 16 high ranked species as of particular relevance to investigate further for a more detailed suggestion for monitoring.

Here we elaborate on suggestions for these 15 insect species. SEPA made a ranking among those 15 species from 1-10 (with 1 the highest priority and 10 the lowest) and we provide this ranking in Table 1.

Launching a genetic monitoring program would be beneficial for understanding what processes may affect pollinator intraspecific genetic diversity in time. Both common and declining species are of interest since they will provide insight into the fate of populations with different starting genetic preconditions. The data generated will be of importance for the conservation and long-term maintenance of populations of these species as well as of the ecosystem services that they provide.

The evaluation of these species will be regarded in the light of the following factors:

- **How common/rare/threatened the species are**. Patterns of temporal change in genetic diversity are likely to differ for species with widespread and restricted distribution ranges. It is not necessary that species with small distribution reveal small genetic diversity: on the contrary, more fragmented populations may appear to have a more differentiated genetic structure. On the other hand, threatened species as a rule have already undergone a significant decline in population size and are likely to represent a special case in terms of genetic diversity loss which is important to follow to evaluate how successful conservation management programs are. It is therefore important that species from different categories become subject to genetic monitoring (Appendix sI & sII).

- **How vulnerable the species is expected to be to climate change**: here fall alpine and northern boreal species like mountainous bumblebees (e.g. *Bombus polaris*, see Appendix sI & Table 1) which will not have suitable habitats to retreat under the warming climate. Not only that but also low-elevation and southern populations might be even more vulnerable if they are the first to experience temperatures which a given species cannot tolerate. *Parnassius apollo*, a butterfly with a southern distribution restricted to coastal and island (Gotland) areas and rapidly decreased population size over the course of 20th century, is an example of such a species.

- **How important the species is to provide pollination services**: wild bumblebee species are well-known providers of pollinator services (Willmer et al., 1994) and different species contribute to pollination in different plant communities (Lázaro et al., 2008). Albeit less recognized for their pollination input, nonetheless butterflies do provide their pollination share (Winfree et al., 2011), and moreover, there is some indication that they may spread pollen over larger distances (Herrera, 1987).

- **Whether there is an ongoing genetic research on the species**: launching a monitoring program without having any infrastructure in place would be problematic, therefore research groups with already ongoing genetic studies and established facilities for some species can serve as a basis for adding those species for which no genetic information is available. Such research groups exist both for butterfly (*Pi. napi* and *Pa. apollo*) and bumblebee species (*Bombus pascuorum, B. lapidaries, B. hortorum*).

- **What genetic/genomic resources are available for immediate launch of the genetic monitoring and cost reduction**: it would be appropriate to include species for which reference genomes are already available in order to save resources for creation of new reference data which often takes a long time to develop. However, considering steadily decreasing costs for generating genetic data, the long-term aspect of the genetic monitoring program and conservation importance of the threatened and rare species, it is reasonable to also include species for which no genetic resources are yet available and which will benefit from being included into the program.

- **How much material – both historic and modern – is available in the various entomological collections** which could serve as a starting point for assessment of a species genetic diversity in historical populations and how they change with time. The species in focus have a robust historic-specimens support in various entomological collections.

Among the 15 proposed species, there are two day-butterfly species – one common and one endangered, and 13 bee and bumblebee species, both common well-known pollinators and more rare species endemic to alpine areas and which are likely to be affected by the changing climate. We prioritized among the species based on the above criteria (Appendix sI) and the resulting priority list is found in Table 1.

Table 1 Pollinator species proposed for genetic monitoring program in the order of their priority (ranking from SEPA). Information about species distribution is taken from Swedish Species Information Centre (ArtDatabanken, https://artfakta.se/artbestamning). See Appendix sII for the species distribution maps and Appendix sI for a general overview

SEPA ranking	Species	Common name	Population distribution	Landscape type
1	*Pieris napi*	Green-veined white	All over Sweden	Forest, Agricultural landscape, Urban environment, Mountain, Sea beach
1	*Parnassius apollo*	Apollo	Småland Södermanland Uppland Västergötland Östergötland Blekinge Bohuslän Skåne Gotland	Forest, Agricultural landscape, Urban environment, Sea beach
1	*Bombus hortorum*	Garden bumblebee	All over Sweden except for Lule lappmark	Forest, Agricultural landscape, Urban environment
2	*Bombus soroeensis*	Broken-belted bumblebee	All over Sweden except for Lycksele, Pite and Torne (extinct) lappmark	Forest, Agricultural landscape, Urban environment
3	*Bombus pascuorum*	Common carder bee	All over Sweden	Forest, Agricultural landscape, Urban environment
4	*Bombus lapidarius*		All over Sweden except for Pite lappmark	Agricultural landscape, Urban environment, Sea beach
5+	*Bombus hyperboreus*		Torne, Lule, Pite and Lycksele lappmark	Mountain
5-	*Bombus polaris*	Arctic bumblebee	Torne, Lule, Lycksele, Åsele, Pite lappmark, Härjedalen	Mountain
6	*Bombus balteatus*	Golden-belted bumblebee	Torne, Lycksele, Härjedalen, Lule, Jämtland and Pite lappmark. Extinct from Dalarna	Forest, Urban environment, Mountain
6	*Bombus lapponicus*		Torne, Lycksele, Lule, Härjedalen, Pite and Jämtland lappmark	Forest, Mountain
6	*Bombus monticola*	Mountain bumblebee	Torne, Lule, Härjedalen, Jämtland, Lycksele, Pite lappmark, Västerbotten, Dalarna	Forest, Mountain
7	*Bombus subterraneus*	Short-haired bumblebee	Dalarna, Gästrikland and all provinces to the South of them	Agricultural landscape, Urban environment
8	*Andrena vaga*	Grey-backed mining bee	Dalarna, Hälsingland and all provinces to the South of them	Forest, Agricultural landscape, Urban environment, Wetland
9	*Andrena denticulata*	Grey-banded mining bee	Dalarna, Gästrikland and all provinces to the South of them + East coast provinces	Forest, Agricultural landscape, Urban environment
10	*Halictus rubicundus*	Orange-legged furrow-bee	All over Sweden except for Lule and Pite lappmark	Forest, Agricultural landscape, Urban environment

4. Collections

Having a comparison with historic samples can be an invaluable contribution to understanding the direction of the present-day changes in genetic diversity of pollinator species. A number of both museum and private collections contain a great deal of historic and modern entomological samples of the 15 species which could be used for creating a baseline of a past genetic diversity and genetic structure. Many of the contacted persons were very enthusiastic about providing their material for genetic monitoring purposes. However, practically all of them yet lack digitalized databases and a great deal of on-the-spot investigation of the collections, information gathering and digitalizing is necessary to be performed before such information can be practically used for the monitoring purposes.

As for now, the following museums have the selected 15 species in their entomological collections (Appendix sI provides a more detailed collection overview):

- Natural History Museum in Stockholm (NRM, Department of Zoology, entomological collection) – a large number of specimens with a good coverage of all the species both spatially and temporally;
- Lund University, Biological Museum (SLU) – a large number of specimens with a good coverage of all the species spatially, the sampling time periods need a closer look;
- Natural History Museum in Göteborg (GNM, entomological collection) – some specimens, for exact numbers and localities/time periods a closer look is needed
- Swedish Malaise Trap Project (SMTP, Karlsson et al., 2020), Station Linné – a large number of Hymenoptera specimens from early 2000s, species identification is ongoing; additionally, 24 *Pa. apollo* specimens from Sweden

Specimens available from ongoing research collections and private collectors (see Appendix sI for the full list of collectors):

- Christopher Wheat, Assoc. Prof., Stockholm University (*Pi. napi*)
- Håkan Elmqvist, lepidopterologist, private collector (all Bombus spp., *Pi. napi*, *Pa. apollo*) – good spatial coverage for many species from different time periods
- Magnus Stenmark, head of Ecocom Nord sector and environmental consultant in Calluna AB – a large number of specimens, for spatial and temporal coverage a closer look is needed
- At least 27 other private collectors – for exact numbers, localities and time periods a closer look is needed

Suggestion: preliminary information from the various collections suggests a good spatial and temporal coverage of the species in focus, meaning that historic samples can be included in the genetic monitoring program either for setting a baseline for genetic diversity in the populations or/and revealing demographic history and species response to e.g. climate change/pesticide use over the last 100 years.

5. Type of genetic markers

Modern DNA sequencing techniques become constantly cheaper and more and more available for both research and monitoring purposes. The high-resolution genomic data approach provides an opportunity for extremely detailed investigations of historical population size changes, as well as ongoing dispersal among regional populations. Further, these data are being used to investigate how local populations have been responding to land use (e.g. pesticide application) and climate changes.

We therefore suggest to use whole-genome pool-sequencing for the widely-spread species to reduce the costs for DNA sequencing. Individual genome sequencing can be applied to the red-listed and rare species of which only a few specimens will be sampled and individual approach would provide a better resolution. Further, pool-sequencing cannot be used to monitor important parameters such as the genetically effective population size, and genotype frequencies. Therefore, individual whole genome sequencing should be prioritized when costs for such sequencing are reduced.

6. Cost estimations

Approximate costs were calculated for three ambition levels of genetic monitoring – minimum (satisfactory) **ambition level 1**, medium **ambition level 2** and best quality **ambition level 3**. For exact cost estimations per species and level see Appendix sIII.

1) **Sampling costs**
    a) **Number of sampling locations, samples per populations, sampling time interval**
        - *Pieris napi*: ambition level 1 would include six populations (Abisko, Luleå, Umeå, Sundsvall, Stockholm, Skåne) for which extensive field collection have already been done within the ongoing research and genetic data for which have already been generated by Christopher Wheat’s lab (SU). Ambition levels 2 and 3 would include respectively 3 and 6 additional populations for a better spatial coverage of the species distribution. Yearly sampling of 20 individuals per location for each ambition level.

- *Parnassius apollo*: ambition level 1 would include two populations corresponding to the two hot spots in the distribution of the species (the island of Gotland and the mainland in the South-East Sweden). Ambition levels 2 and 3 would include respectively 1 and 2 additional populations for a better spatial coverage of the species distribution. Yearly sampling using non-destructive method (wing tissue sampling) of 2/5/10 individuals per location is proposed corresponding to our suggested ambition levels 1-3.

- *Bombus hortorum, soroeensis, pascuorum (SEPA ranking 1-3; Table 1):* all three species are distributed across Sweden with the highest occurrence density in the South, where sampling of both modern and historic populations of *B. hortorum* and B. *pascuorum* has already been scheduled within the framework of research project by David Díez-del-Molino (NRM & SU). These populations and the genetic data that are going to be generated within the project can therefore be used as the starting points for the genetic monitoring for these species, while *B. soroeensis* can also follow their pattern to reduce the costs of field collections. Just like for *Pi. napi*, ambition level 1 for these species would include six populations with 20 individuals from each: four southern populations from areas with the most intensive farming for which the sequencing is already being done, plus two relatively more northern populations (from e.g. Västerbotten and Norrbotten) from areas with no intensive farming. Ambition levels 2 and 3 would include respectively 3 and 6 additional populations for a better spatial coverage of the species distribution.

- *Bombus lapidarius, hyperboreus, polaris, balteatus, lapponicus, monticola (SEPA ranking 4-6):* all these species except for *B. lapidarius* have a limited distribution in mountainous areas in northern and/or central Sweden. Their collection would therefore be challenging and, if possible, should involve non-destructive methods since the species are endangered in Sweden. As a result, sampling of only two populations within each monitoring level should be sufficient. If it is going to be possible to sample that many individuals, the suggested sample size per location can be 2, 4 and 6 corresponding to ambition levels 1-3; individual whole genome sequencing can be used in this case.

  Bombus lapidarius has distribution similar to the three above-mentioned high-priority Bombus species and collection and sequencing of its both modern and historic samples has already been scheduled within the research project by David Díez-del-Molino (NRM & SU). Sampling of this species can therefore follow the same pattern as *B. hortorum*, *soroeensis* and *pascuorum* but with decreased number of samples and omitting some of the sites.

- *Bombus subterraneus, Andrena vaga, A. denticulata, Halictus rubicundus (SEPA ranking 7-10)*: most of species from this category have southern and eastern coast distribution. Two to three populations, corresponding to ambition levels 1-3, can be sampled: from the populations within and outside intensive-farming areas, plus an additional population on the East coast. If sampling numbers would allow, ten individuals per population can be yearly analysed using whole-genome pool-sequencing.

b) **DNA extraction, DNA sequencing and preparation of libraries (these costs are going to be more or less similar for all the species)**:

Costs for DNA extraction are estimated to be 40 SEK per sample, for DNA sequencing and library preparation by the SciLifeLab – approx. 2,500 SEK per sample. However, hiring highly-qualified personnel would allow to make library preparations within the work group and lower the costs for sequencing to approx. 1,000 SEK per sample.

2) **Number of employees:**

- *Pi. napi & Pa. apollo*: the work load for the principal investigator (PI) leading the project would vary between 10-15% of time depending on the ambition level of the monitoring. Another additional full-time person would be required for performing the field work, DNA extractions, preparation of libraries, genetic analysis and integration with SLU ArtDatabanken. The same group of two can work with both *Pi. napi* and *Pa. apollo* at the same time, resulting in the same expenses for yearly salaries.

- *Hymenoptera species:* the number of people involved in genetic monitoring of the chosen Bombus, Andrena and Halictus species would require one PI and two highly-qualified persons for ambition level 1, three to four persons for ambition level 2, and four persons for ambition level 3.

In case the same group is assigned to run monitoring of both the Lepidoptera and Hymenoptera species, one PI and 2 qualified persons could be suggested for ambition level 1, 3-4 persons for ambition level 2, and 4 persons – for ambition level 3.

3) **Work with museum collections:** since many museum specimens have been treated with aggressive antifungal chemicals like dichlorvos, their DNA is broken down into small fragments of about 40-50 base pairs, which makes methods other than whole genome sequencing, challenging. Therefore, costs for whole genome sequencing have been calculated for museum specimens of all the species.

- *Pi. napi & Pa. apollo*: for setting a base line for the species' historic genetic diversity and demographic changes within the last 100 years, we suggest to use museum specimens from the very populations from which modern sampling will be done. Two time points (1900s and 1950s) would be useful for assessing demographic history of *Pi. napi* and three time points (1900s, 1950s and 1980s) for *Pa. apollo* (to follow genetic changes both during the population increase between 1900 and 1950 and population decline after the 1950s). Historical samples from now extinct populations of *Pa. apollo* can be included into ambition levels 2-3 of monitoring.

- Bombus hortorum, soroeensis, pascuorum (priority level 1-3): a great deal of work with museum specimens of *B. hortorum* and *B. pascuourum* has already been started within the research project by David Díez-del-Molino (SU & NRM), therefore the data generated by him for three time periods (before 1920, to have a baseline of diversity; 1930-1960 - the bulk of museum collections are from this time; and 1970-1990) can be used directly for the genetic monitoring purposes with no additional costs. Generating such data will come at a cost only for *B. soroeensis*.

- Bombus lapidarius, hyperboreus, polaris, balteatus, lapponicus, monticola (priority level 4-6): historic populations should correspond to successfully sampled modern populations, with two museum individuals per population. Two time points for ambition level 1 (before 1900s as a baseline of diversity and in the middle of the 20th century with the bulk of museum specimens coming from this period) and three for ambition levels 2-3 (+ 1970-90s) are available in the museum collections.

- Bombus subterraneus, Andrena vaga, A. denticulata, Halictus rubicundus (priority level 7-10): two historic populations should correspond to successfully sampled modern populations, with two museum individuals per population. Two time points for ambition level 1-3 (before 1900s as a baseline of diversity and in the middle of the 20th century with the bulk of museum specimens coming from this period) are available in the museum collections.

4) Genetic resources:

- *Pi. napi & Pa. apollo*: a reference genome has already been generated by research group of Associate Prof. Christopher Wheat (SU) and is available for *Pi. napi*. Additional costs however, will be required for generating moderate-quality reference genome for *Pa. apollo*.

- *Hymenoptera species*: out of the 13 hymenopteran species, work on generating reference genomes is ongoing only for three of them (*B. hortorum, B. pascuorum, B. lapidarius* within David Díez-del-Molino's project). These data will be possible to use for the genetic monitoring with no additional costs.

Depending on the budget and the questions being asked within the monitoring program, possible solutions for the remaining 10 species would include:

a) Skip the genomic reference: much can still be done without a high-quality reference using other tools (e.g. microsatellites or SNPs). If the project gets a steady financing, a reference genome can be generated at a later point and used then. Similarly, the work with historic specimens can be postponed.
b) DNA sequences of Bombus species in focus can be mapped to a reference genome of another sister species (e.g. *B. terrestris*). Whether this method is appropriate, should be decided in the course of the work. This will leave out

the three non-Bombus species (*Adrena* and *Halictus*), for which no related-species reference genomes exist.

c) Use 10X Genomics Chromium Technology: this method can offer a cheaper alternative for generating a reference genome; however, it has its limitations (like high heterozygous species) and expediency of its use should be decided in the course of the work.
d) reference genomes can be generated and used for all the hymenopterans in the beginning or at a later point of the genetic monitoring project, making a good investment in possibilities to answer more various questions in the course of the project, given it is meant to be run for a longer period of time.

Since the solutions (a-c) either do not require additional costs or the costs are difficult to estimate before the start of the project, cost estimations provided in Appendix sIII therefore include possibility (d) – generating genomes with traditional methods. The costs for them are shown separately.

5) **Additional costs:** the project will require both sample and data storage, therefore purchase of hard drives for data storage and freezers for sample storage will be necessary.

Based on the above-described suggestions, annual costs for monitoring of genetic diversity for the 15 pollinator species are estimated to be:

A. **<u>If monitoring setup includes preparation of libraries by the implementor group</u>**

One-time costs will include analyses of museum specimens and purchase of equipment for the data and sample storage; for 2 Lepidoptera species it also includes generating of a reference genome for *Pa. apollo* (since it is the only option for this species). Generating reference genomes for the 13 Hymenoptera species is optional, therefore these costs are shown separately if their sequencing will be decided upon.

Ambition level 1: yearly costs for all species of approx. 2,432,000 SEK plus one-time costs of 1,183,000 SEK (of which 500,000 SEK is for generating reference genomes for Hymenoptera species) (thus, **total cost during the first year 3,615,000 SEK**). These costs are distributed between the species groups in the following way:

- 2 Lepidoptera species (*Pi. napi* and *Pa. apollo*): annual costs of approx. 825,000 SEK plus one-time costs of approx. 304,000 SEK
- 13 Hymenoptera species (3 solitary bees and 10 bumblebees): annual costs of approx. 1,607,000 SEK plus one-time costs of 879,000 SEK (of which 500,000 SEK for generation reference genomes)

Ambition level 2: yearly costs for all species of approx. 3,224,000 SEK plus one-time costs of 1,391,000 SEK (of which 500,000 SEK is for generating reference genomes for Hymenoptera species) (thus, **total cost during the first year 4,614,000 SEK**). These costs are distributed between the species groups in the following way:

- 2 Lepidoptera species (*Pi. napi* and *Pa. apollo*): annual costs of approx. 883,000 SEK plus one-time costs of approx. 395,000 SEK
- 13 Hymenoptera species (3 solitary bees and 10 bumblebees): annual costs of approx. 2,341,000 SEK plus one-time costs of 997,000 SEK (of which 500,000 SEK for generation reference genomes)

Ambition level 3: yearly costs for all species of approx. 4,147,000 SEK plus one-time costs of 1,892,000 SEK (of which 500,000 SEK is for generating reference genomes for Hymenoptera species) (thus, **total cost during the first year 6,038,000 SEK**). These costs are distributed between the species groups in the following way:

- 2 Lepidoptera species (*Pi. napi* and *Pa. apollo*): annual costs of approx. 1,052,000 SEK plus one-time costs of approx. 798,000 SEK
- 13 Hymenoptera species (3 solitary bees and 10 bumblebees): annual costs of approx. 3,096,000 SEK plus one-time costs of 1,094,000 SEK (of which 500,000 SEK for generation reference genomes)

B. <u>If monitoring setup includes preparation of libraries by the SciLifeLab</u>

Ambition level 1: yearly costs for all species of approx. 2,432,000 SEK plus one-time costs of 1,759,000 SEK (of which 500,000 SEK is for generating reference genomes for Hymenoptera species) (thus, **total cost during the first year 4,191,000 SEK**). These costs are distributed between the species groups in the following way:

- 2 Lepidoptera species (*Pi. napi* and *Pa. apollo*): annual costs of approx. 825,000 SEK plus one-time costs of approx. 502,000 SEK
- 13 Hymenoptera species (3 solitary bees and 10 bumblebees): annual costs of approx. 1,607,000 SEK plus one-time costs of 1,257,000 SEK (of which 500,000 SEK for generation reference genomes)

Ambition level 2: yearly costs for all species of approx. 3,224,000 SEK plus one-time costs of 2,258,000 SEK (of which 500,000 SEK is for generating reference genomes for Hymenoptera species) (thus, **total cost during the first year 5,481,000 SEK**). These costs are distributed between the species groups in the following way:

- 2 Lepidoptera species (*Pi. napi* and *Pa. apollo*): annual costs of approx. 883,000 SEK plus one-time costs of approx. 719,000 SEK
- 13 Hymenoptera species (3 solitary bees and 10 bumblebees): annual costs of approx. 2,341,000 SEK plus one-time costs of 1,540,000 SEK (of which 500,000 SEK for generation reference genomes)

Ambition level 3: yearly costs for all species of approx. 4,147,000 SEK plus one-time costs of 3,470,000 SEK (of which 500,000 SEK is for generating reference genomes for Hymenoptera species) (thus, **total cost during the first year 7,616,000 SEK**). These costs are distributed between the species groups in the following way:

- 2 Lepidoptera species (*Pi. napi* and *Pa. apollo*): annual costs of approx. 1,052,000 SEK plus one-time costs of approx. 1,698,000 SEK
- 13 Hymenoptera species (3 solitary bees and 10 bumblebees): annual costs of approx. 3,096,000 SEK plus one-time costs of 1,772,000 SEK (of which 500,000 SEK for generation reference genomes)

These estimations can be found in Appendix sIII (sheet “Total cost” and “Itemized costs”) as well as more detailed estimations for *Pi. napi*, *Pa. apollo*, Hymenoptera species with priority 1-3, Hymenoptera species with priority 4-6 and Hymenoptera species with priority 7-10 by ambition level.

7. Potentially interested participants

The laboratory of Associate Professor Christopher Wheat (Department of Zoology, Stockholm University) works extensively with about a dozen lepidopterans in general, and *Pieris napi* in particular, studying the genetics of its populations, their demographic history and how they adapt to local conditions. Specifically, Wheat studies the genetic diversity and population history across the entire range of *Pieris napi* in Sweden, as well as across Europe. His lab has generated an extremely high-quality genome for their work and has already obtained genomic data both from individuals and populations from across all of Sweden and Europe.

In addition, through collaboration with Prof. Niklas Wahlberg, he also has individual genomes of many individuals from across Sweden, that are from the 1940-1950s. This dataset provides the opportunity for extremely detailed investigations of historical population size changes, level of inbreeding, as well as ongoing dispersal among regional populations. Further, this data is being used to investigate how local populations have been responding to land use changes (e.g. response to habitat changes and pesticide use) and climate changes.

The already available genomic data – both from historic and modern populations of *Pi. napi* - generated by the lab can be used as the basis for setting up the genetic monitoring for this species. Wheat has possibilities and is interested in running the monitoring program also for *Parnassius apollo* and the Hymenoptera species.

Researcher David Díez-del-Molino (Centre for Palaeogenetics, Swedish Museum of Natural History and Stockholm University) is currently running a project "Consequences of 100 years of agricultural intensification on the morphology and genomics of bumblebees" which aims to study the demographic changes in Swedish populations of *Bombus pascuroum, B. hortorum, B. lapidarius and B. terrestris* and their adaptive response to land use changes in the last 150 years.

The project is heavily focused on historical specimens from museum collections for which obtaining DNA is not trivial since it requires measures to prevent modern DNA contamination, special DNA extraction methods for highly fragmented DNA, dedicated ancient DNA facilities, etc. So far, work has already been started on both historical and modern bumblebee specimens from central and southern Sweden (Småland, Dalarna, Västermanland, Södermansland, Uppland, Västergötland, Värmland).

The genomic data generated within the project, as well as the bioinformatic methods that will be used to analyse these data, have the potential to be applied for other purposes, such as monitoring the genetic health of bumblebee populations through time. Therefore, it can be a starting point for the genetic monitoring of the Hymenoptera species of interest. Díez-del-Molino is interested in being involved in the genetic monitoring program, specially any insect species for which work with historical samples is planned.

The laboratory of Associate Professor Niclas Backström (Department of Ecology and Genetics, Uppsala University) works on questions related to reproductive isolation, local adaptation and genome evolution in a set of systems, including butterflies, using both classical genetic methods and new genomic tools. Studies on population genetics of the wood white butterflies (*Leptidea* sp.) and the painted lady (*Vanessa cardui*) use a combination of genetic mapping, population genetics and comparative genomics approaches. Backström has therefore possibilities and is interested in being involved in the monitoring program on pollinators.

The laboratory of Professor Matthew Webster (Department of Medical Biochemistry and Microbiology, Uppsala University) works on the evolution of genes and genomes and uses e.g. honey bee (*Apis mellifera*) and bumble bee (*Bombus terrestris*) as models.

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9. Appendices

Appendix sI - A general overview of the species and collections
Appendix sII - Species distribution maps
Appendix sIII – Setups and cost calculations for genetic monitoring

Appendices are available at https://www.naturvardsverket.se/978-91-620-6958-2

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Mapping and monitoring genetic diversity in Sweden

a proposal for species, methods and costs

REPORT 6958

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DIANA POSLEDOVICH, ROBERT EKBLOM AND LINDA LAIKRE

Genetisk inomartsvariation är en viktig förutsättning för populationers långsiktiga överlevnad och för ekosystemens resiliens, t.ex. genom en ökad förmåga för populationer att kunna anpassa sig till ett förändrat klimat. Trots detta finns det idag stora brister i vår kunskap om arters och populationers genetiska variation och hur den förändrats över tid och i relation till olika påverkansfaktorer. Övervakning av arters genetiska variation är således ett viktigt verktyg för förvaltning och bevarandearbete då det ger kunskap om förändringar som vi annars inte kan upptäcka vilket kan fungera som tidiga varningssignaler om hot kopplat till mänsklig aktivitet.

I denna rapport ges förslag på ett genetiskt övervakningsprogram för pollinerande insekter. Utifrån redan tillgängliga resurser i form av bland annat genetisk metodik, forskning och historiska samlingar presenteras förslag på arter, metoder för genetiska analyser, stickprovsstorlekar, insamlingslokaler, samt en uppskattning av kostnader enligt tre olika ambitionsnivåer.

Within-species genetic variation is an important factor for long-term maintenance of viable populations and ecosystem resilience, e.g. by increasing the ability for species to adapt to climate change. However, there are still gaps in our knowledge on genetic variation within species and populations and how it changes over time and in relation to different influencing factors. Monitoring of genetic diversity is an important tool for management and conservation, providing knowledge on changes of a population state and early detection of threats of anthropogenic changes.

The work includes an overview of available resources in the form of genetic tools, research and historical collections, and a suggestion of species to be included in monitoring, methods for genetic analyses, sample sizes, collection localities, as well as estimated costs according to three different ambition levels.

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