

Yuanzhen Liu¹, Yuanzhen Liu², Anna Olsson¹, Tuuli Larva¹, Aoife Cantwell Jones³, Richard J. Gill³, Björn Cederberg⁴, Sean Birk Bek Craig², Jesper S. Bechsgaard², Jesper S. Bechsgaard⁵, Lynn Dicks⁶, Lynn Dicks⁷, Hans H. Bruun⁸, Toke T. Høye⁹, Trine Bilde², Trine Bilde⁵, Philip F. Thomsen², Philip F. Thomsen⁵

¹Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

²Department of Biology, Aarhus University, Aarhus C, Denmark

³Department of Life Sciences, Georgina Mace Centre for The Living Planet, Imperial College London, Ascot, UK

⁴Swedish University of Agricultural Sciences, Swedish Species Information Centre, Uppsala, Sweden

⁵Center for Ecological Genetics, Aarhus University, Aarhus C, Denmark

⁶Department of Zoology, University of Cambridge, Cambridge, UK

⁷School of Biological Sciences, University of East Anglia, Norwich, UK

⁸Department of Biology, University of Copenhagen, Copenhagen, Denmark

⁹Department of Ecoscience, Aarhus University, Aarhus C, Denmark

Recent declines in insects, particularly for anthophilous insects like bees, raise ecological concerns but remain challenging to evaluate due to scarce long-term monitoring data. Climate change and land use are considered major contributing factors that can be linked to population size changes over time. Arctic species are especially vulnerable as they approach their northern limits, making the assessment of their population viability important. Land use also strongly influences species abundance/occurrence by altering the availability of floral resources. Genome analysis provides a powerful tool to assess population declines and fragmentation. By reconstructing effective population size (N_e) trajectories over millennia, we uncover differences in evolutionary histories between common and rare/endangered species. Here, we analysed genetic variation in seven Swedish montane bumblebee species, finding that long-term N_e correlates with geographic distribution, and population fragmentation is minimal over large distances, suggesting free mating among populations. The arctic specialist species *Bombus hyperboreus* showed population declines since the last ice age and signs of inbreeding, potentially indicating increased genetic load. We also conducted analyses of N_e trajectories of four bee species in Denmark, which revealed contrasting patterns of demography across the last thousands of years, suggesting the influence of historical factors on the current status of species. Genetic monitoring and N_e trajectory reconstruction provide a baseline for understanding current pollinator or generally insect declines and improve conservation strategies.

Genomic Analysis of Wild Bee Populations in Scandinavia Informs Biodiversity Conservation

Yuan-zhen Liu^{1,2} et al.

¹Uppsala University, Sweden

^{2*}Aarhus University, Denmark

Email: yzliu@bio.au.dk



UPPSALA
UNIVERSITET



Acknowledgement



Matthew Webster



Turid Everrit



Anna Olsson



Tuuli Larva



Philip F. Thomsen



Jesper S. Bechsgaard



Sean B. Craig



Marie Vestergård



Lynn Dicks



Toke T. Høye



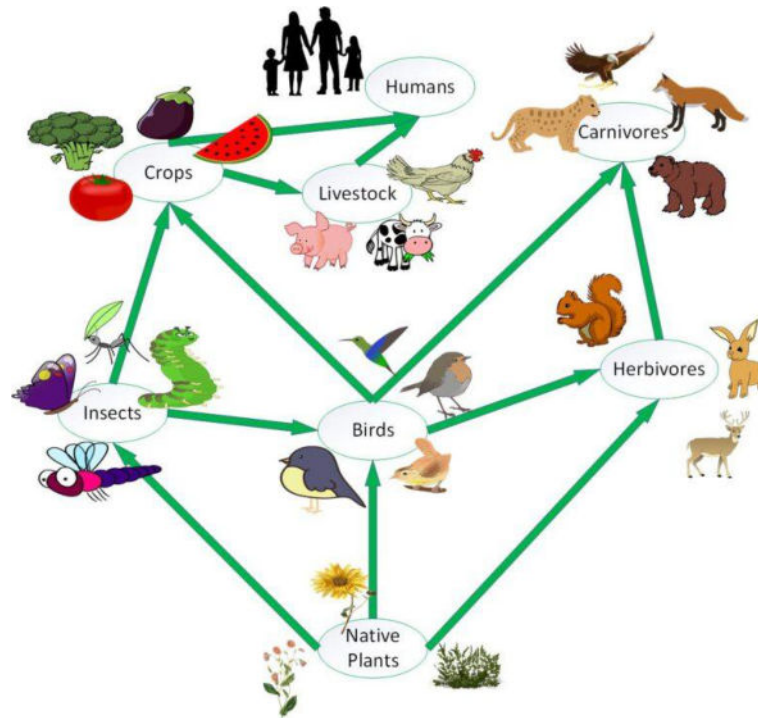
Hand H. Bruun



Trine Bilde



Insects in ecosystem functioning



© sagmoraine.org

87.5% wild plants require animal pollination - most are insects (Ollerton, 2011)

75% crop-plant species - insect pollination (Klein et al., 2007)

85% cultivated crops - wild bee pollination (IPBES, 2019)

62% in yield quantity and quality by honeybee and wild bees (Stein et al., 2017)

Wild pollinators provide a resilient and complementary pollination service that fosters crop yield (Kremen et al., 2002)

Insect diversity

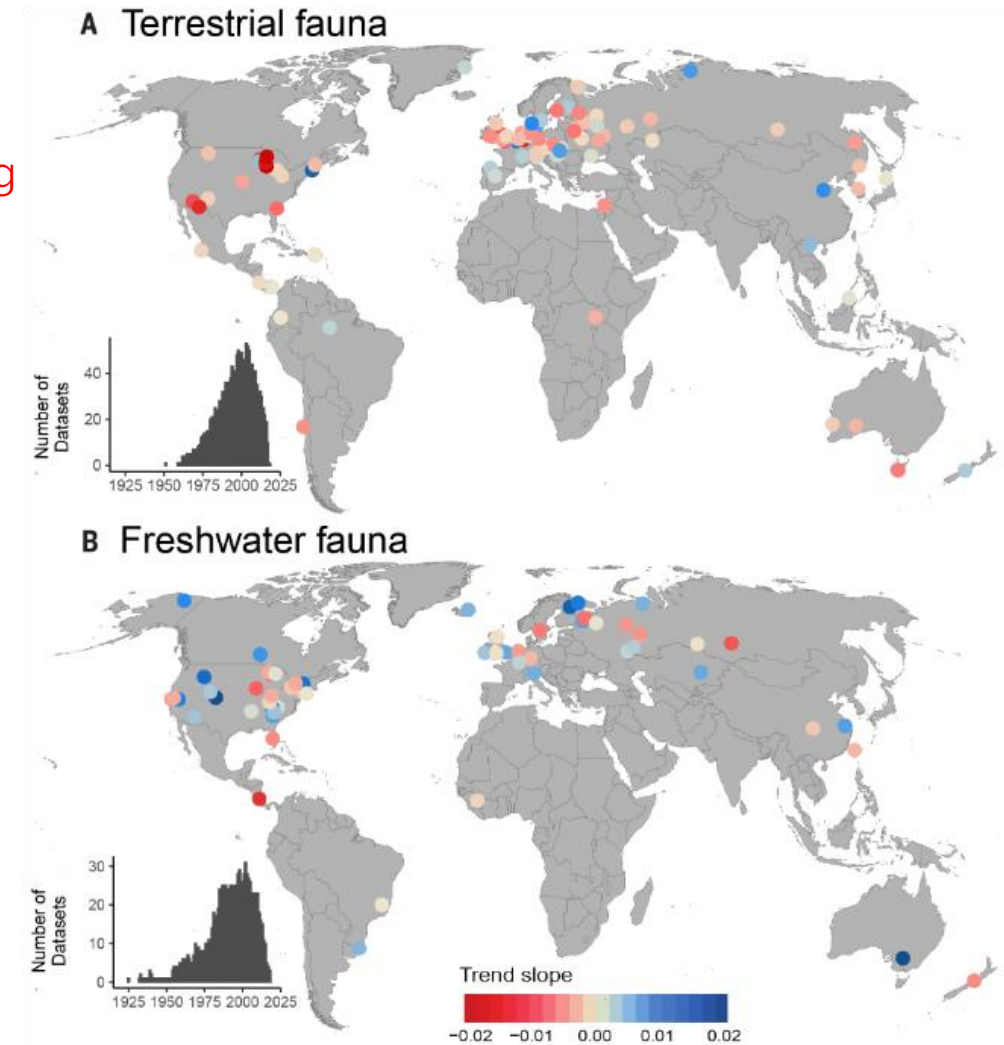
Abundance

**Cause cascading effects
on food webs and
jeopardize ecosystems**

Pollinator declines

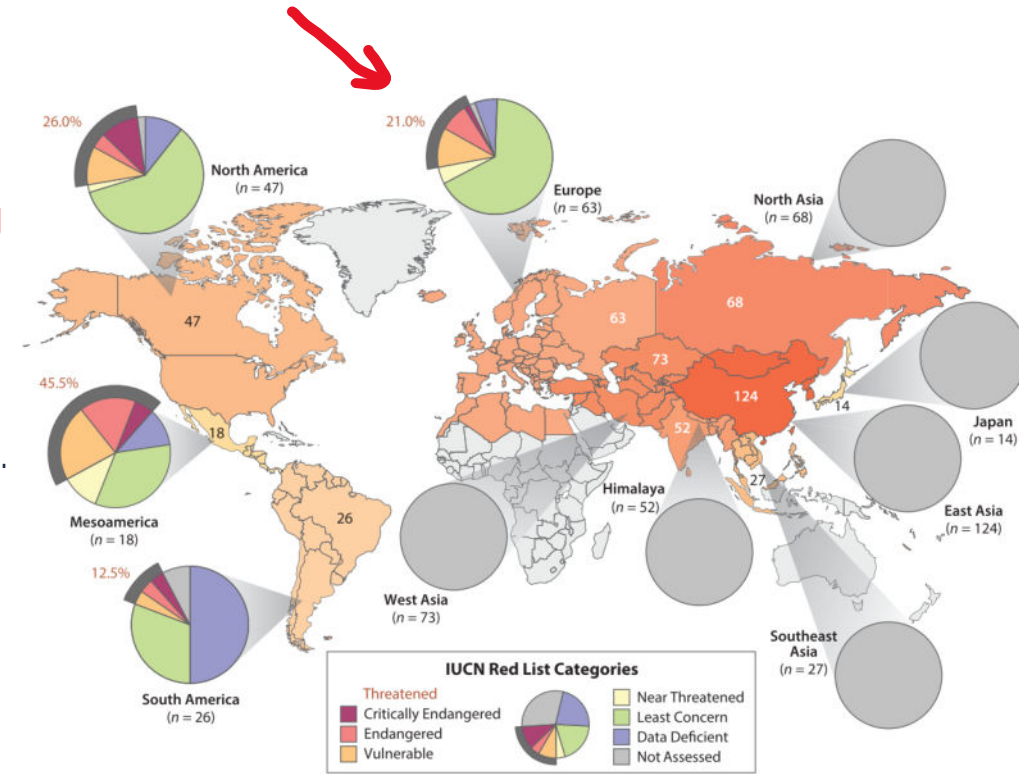
- Insect declines have raised global concerns, including wild bees that receive increased attention.
- Incomplete global patterns due to scarce **long-term monitoring data**.

Dirzo et al., 2014; Wagner et al., 2020; Van Klina et al., 2020; Didham et al., 2020; Webster et al., 2023; Cooke et al., 2025



Pollinator declines

- Insect declines have raised global concerns, including wild bees that receive increased attention.
- Incomplete global patterns due to scarce long-term monitoring data.
- Among wild bees, bumblebees are critical pollinators in mountains and cold regions, highly vulnerable to climate change (Arctic and alpine regions, ~36% species → >80% range).



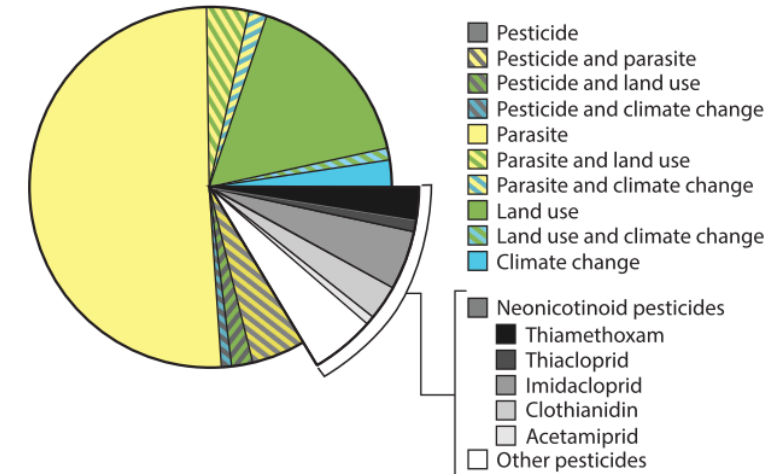
Data from 1985-2018 (Cameron and Sadd, 2020)

Dirzo et al., 2014; Rasmont et al., 2015; Goulson et al., 2015; Wagner et al., 2020; Van Klina et al., 2020; Didham et al., 2020; Webster et al., 2023; Cooke et al., 2025

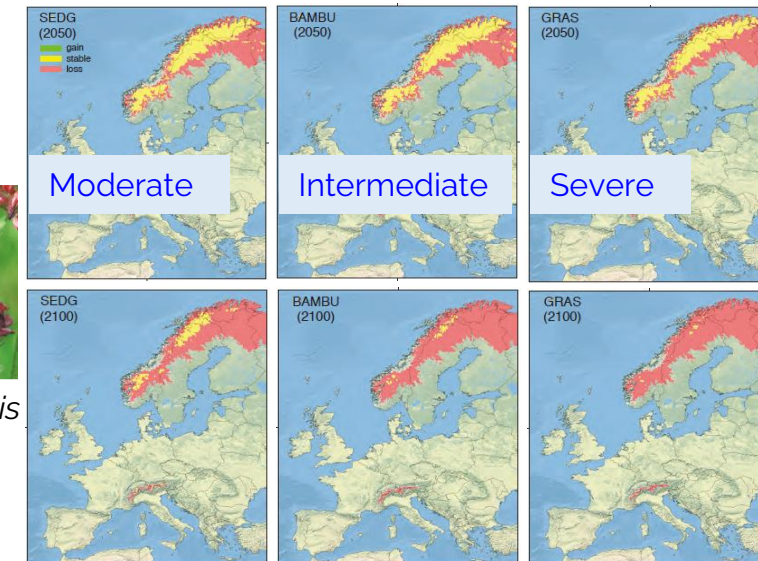
Pollinator declines

- Insect declines have raised global concerns, including wild bees that receive increased attention.
- Incomplete global patterns due to scarce long-term monitoring data.
- Among wild bees, bumblebees are critical pollinators in mountains and cold regions, highly vulnerable to climate change (Arctic and alpine regions, ~36% species → >80% range).

d Proposed causes of bumble bee decline in peer-reviewed literature



Bombus polaris



Dirzo et al., 2014; Rasmont et al., 2015; Goulson et al., 2015; Wagner et al., 2020; Van Klina et al., 2020; Didham et al., 2020; Webster et al., 2023; Cooke et al., 2025

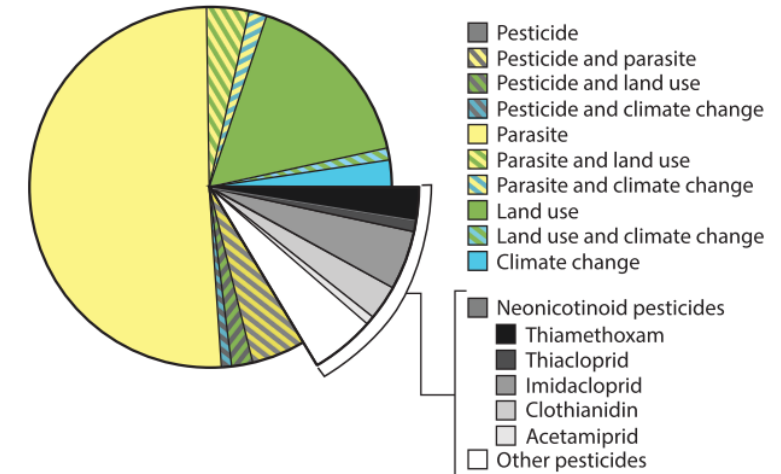
Pollinator declines

- Insect declines have raised global concerns, including wild bees that receive increased attention.
- Incomplete global patterns due to scarce **long-term monitoring data**.
- Among wild bees, bumblebees are critical pollinators in mountains and cold regions, highly vulnerable to **climate change** (Arctic and alpine regions, ~36% species → >80% range).
- Monitoring **population viability** (genetic diversity) of wild pollinators in general is urgently needed to inform future management strategies.

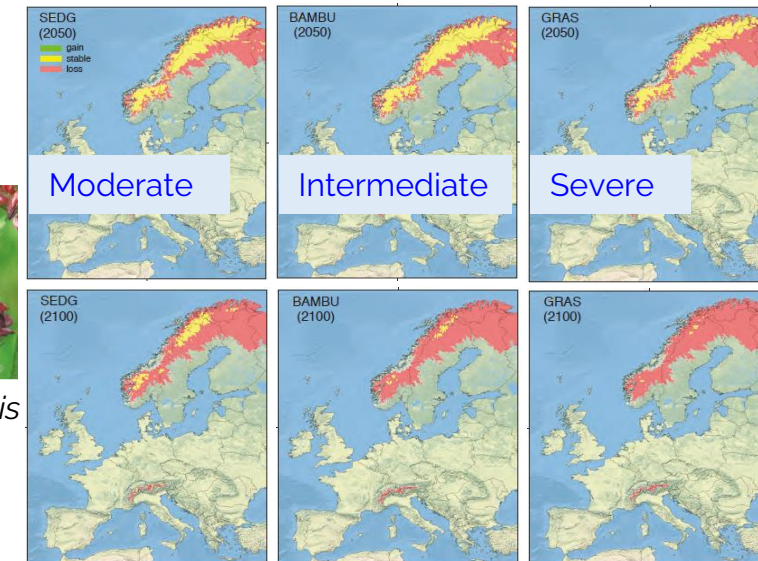
Genomic methods can uncover genetic diversity by investigating **population structure** and **size changes** over evolutionary time, avoiding false baselines from monitoring periods.

Dirzo et al., 2014; Rasmont et al., 2015; Goulson et al., 2015; Wagner et al., 2020; Van Klina et al., 2020; Didham et al., 2020; Webster et al., 2023; Cooke et al., 2025

d Proposed causes of bumble bee decline in peer-reviewed literature



Bombus polaris



Genomic methods for demographic inference

Effective population size (N_e) is defined as the size of an ideal population, undergoing the same genetic drift as the study population, indicating levels of neutral genetic variation.

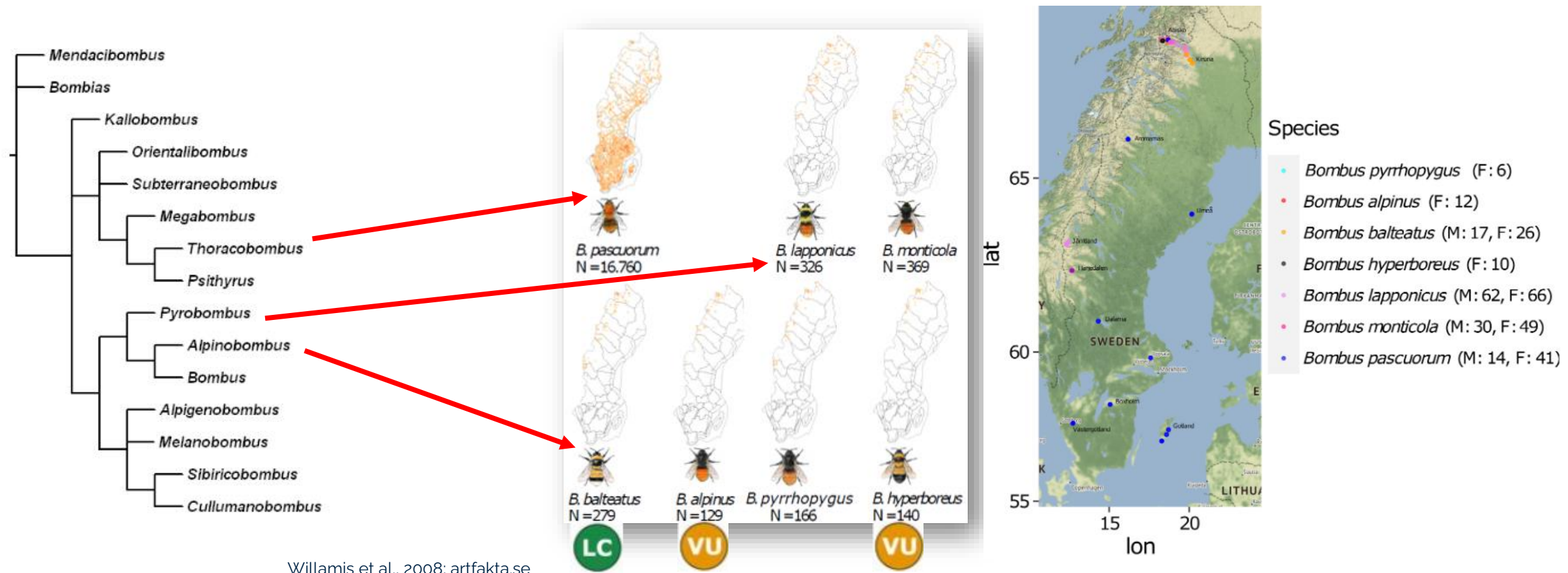
Strengths and weaknesses of different programs

- Coalescent hidden Markov models (HMMs) (*MSMC2*, ~8 ind) → >200 generations (Ind-Seq)
- Both coalescent and SFS (*SMC++*, ~1,000 ind) → >200 generations (Ind-Seq)
- Site frequency spectra (SFS) (*Stairway Plot 2*, ~1,000 ind) → >40 generations (Pool-Seq)
- Bayesian + deep learning (*ABC*, ~50 ind) → >100 generations (Ind-Seq)
- Linkage disequilibrium (LD) (*GONE*, ~100 ind) → <200 generations (Ind-Seq)

Schiffels et al., 2014; Want et al., 2020; Liu et al., 2015 & 2020; Terhorst et al., 2016; Santiago et al., 2020

Genetic diversity and trend for Swedish samples

Monitor genetic diversity and population trends: 7 priority threatened species (Rasmont et al., 2015)



Willamis et al., 2008; artfakta.se

Genetic diversity and trend for Swedish samples

Materials and methods

- ◆ Bumblebee samples (6~128/species)
- ◆ Sequencing: Illumina NovaSeq6000
(Ave. 16 Mreads, 2x150 bp paired-end reads, >15X DP)
- ◆ Data processing pipeline (333 samples):
bwa → samtools → GATK best practice → hard-filtering
(BCFtools/VCFtools → DP & ExcessHet)

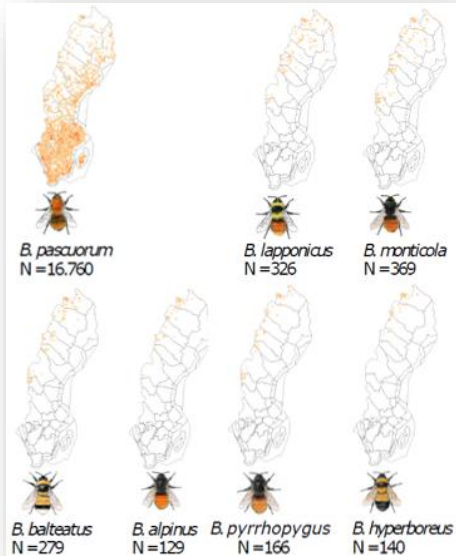
Analyses

- ◆ Pop structure (PCA, Admixture & NJ) → pop fragmentation & cryptic species
- ◆ Long-term N_e : using Watterson's estimator → levels of genetic variation
 - ◆ $\theta = 2pN_e\mu = 3N_e\mu$ ($\mu = 3.6 \times 10^{-9}$, *Bombus terrestris*) (Liu et al., 2017)
 - ◆ $\theta_w = \frac{K}{a_n}$
- ◆ Demographic inference (SMC++) → recent demographic histories
- ◆ Inbreeding levels (runs of homozygosity – PLINK) → genetic load

Genetic diversity and trend for Swedish samples

Levels of genetic variation (Watterson estimator → long-term N_e)

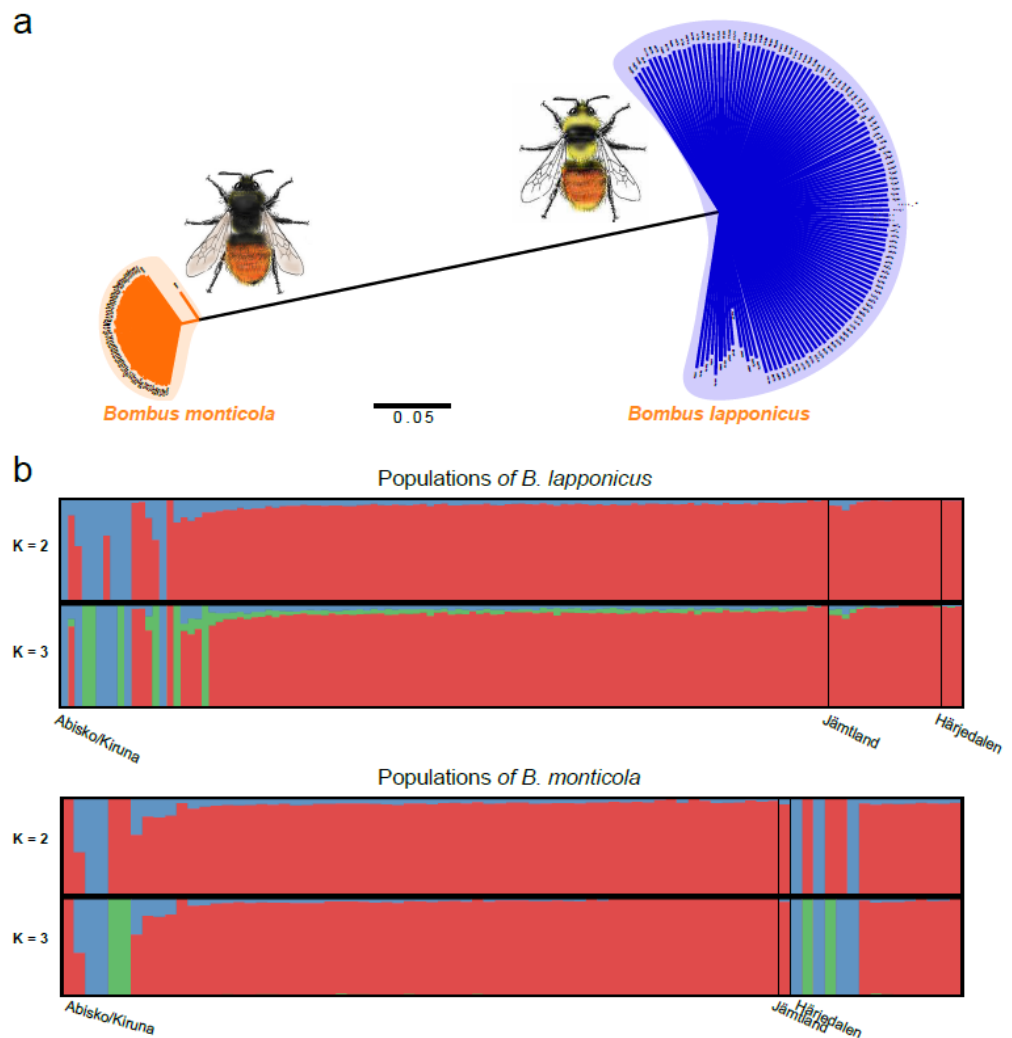
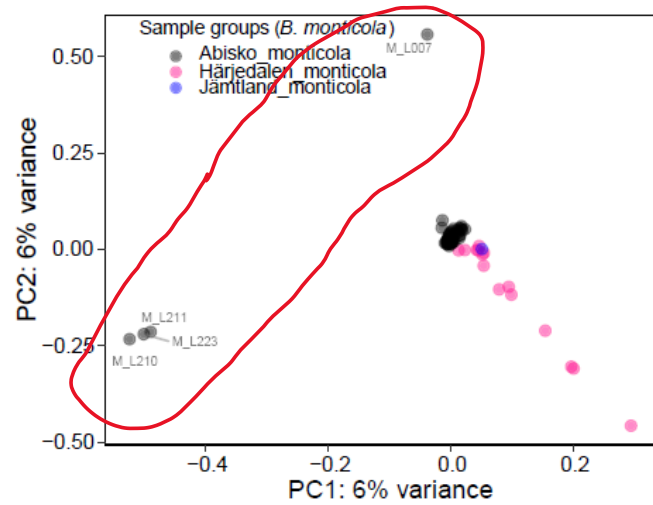
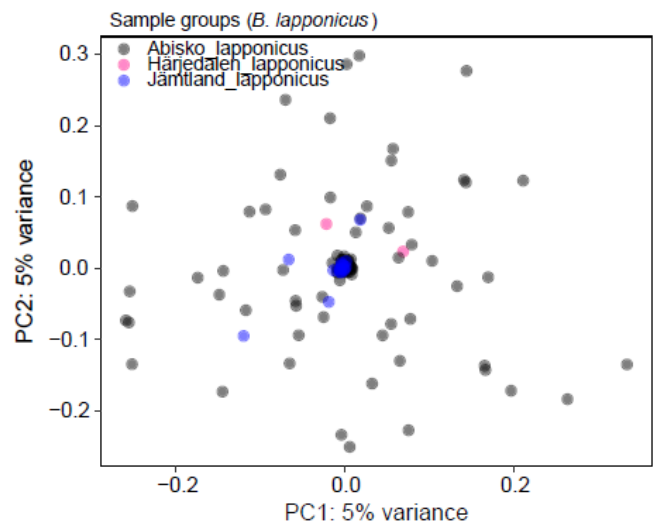
<i>Bombus</i> sp.	Male	Female	Total haploid genomes	No. of SNPs	Watterson's theta per base (W) (%)	Nucleotide diversity (π) (%)	Effective population size (N_e)	Observed heterozygosity (H_{OBS})
<i>B. alpinus</i>		12	24	570,149	0.06	0.08	56,534	0.33
<i>B. pyrrhopygus</i>		6	12	1,390,927	0.18	0.24	170,545	0.37
<i>B. balteatus</i>	17	26	69	2,370,054	0.20	0.24	182,673	0.28
<i>B. hyperboreus</i>		10	20	660,899	0.07	0.10	68,978	0.31
<i>B. lapponicus</i>	62	66	194	3,095,046	0.21	0.23	194,583	0.28
<i>B. monticola</i>	28	48	124	1,085,476	0.08	0.09	73,814	0.30
<i>B. pascuorum</i>	14	41	96	3,800,079	0.24	0.27	222,742	0.22



Generally, $N_e > 50,000$ among species reflects differences in their range and abundance

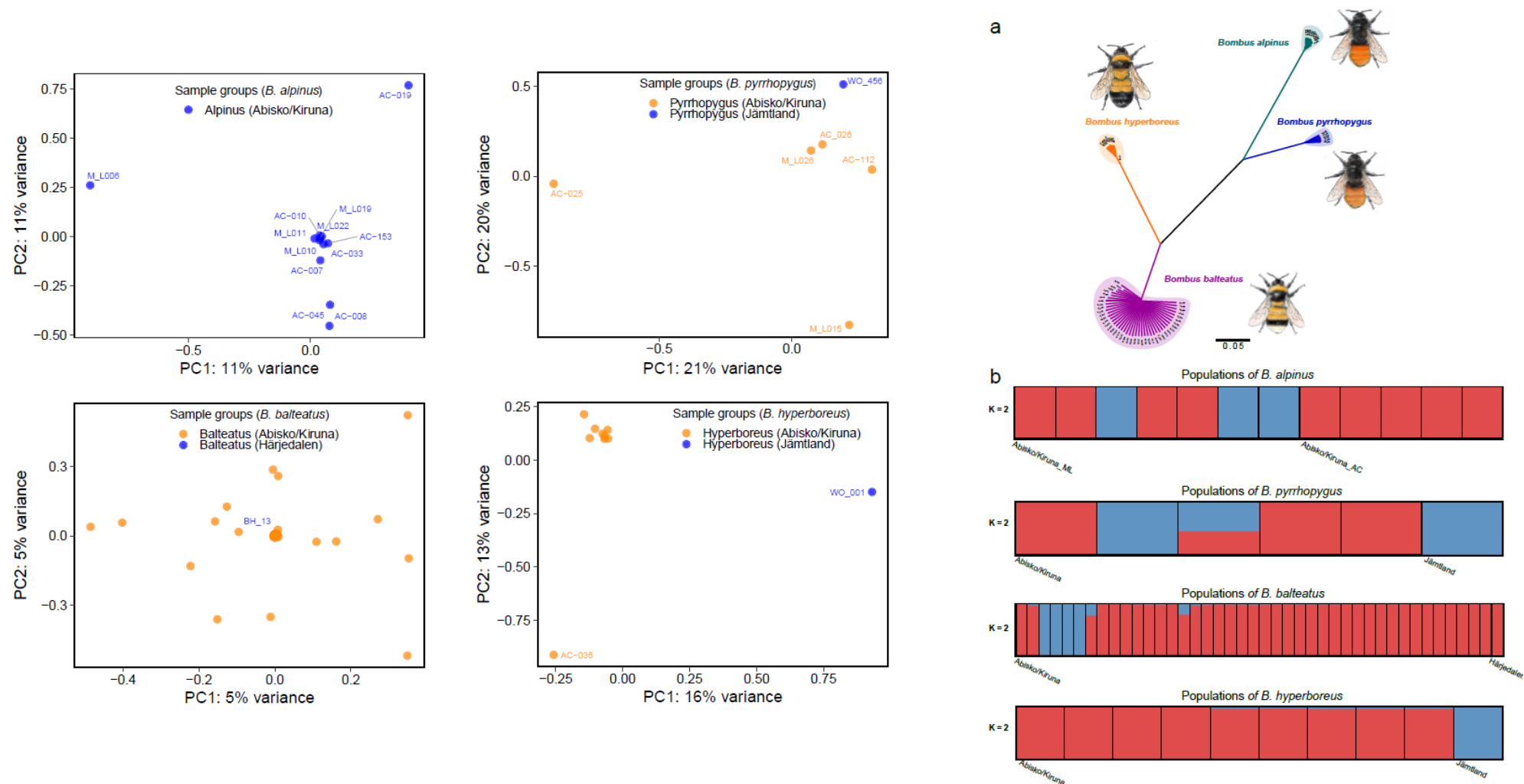
Genetic diversity and trend for Swedish samples

Population structure (Pyrobombus)



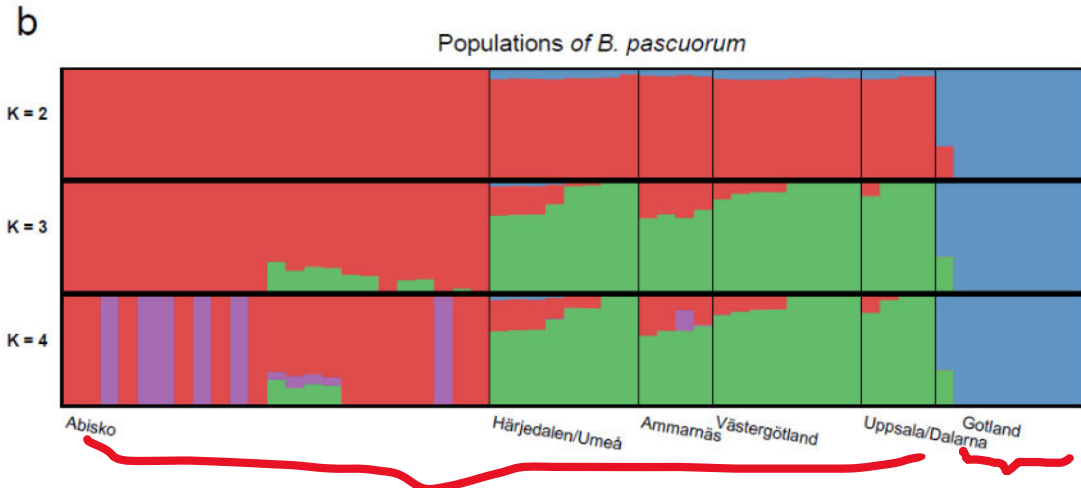
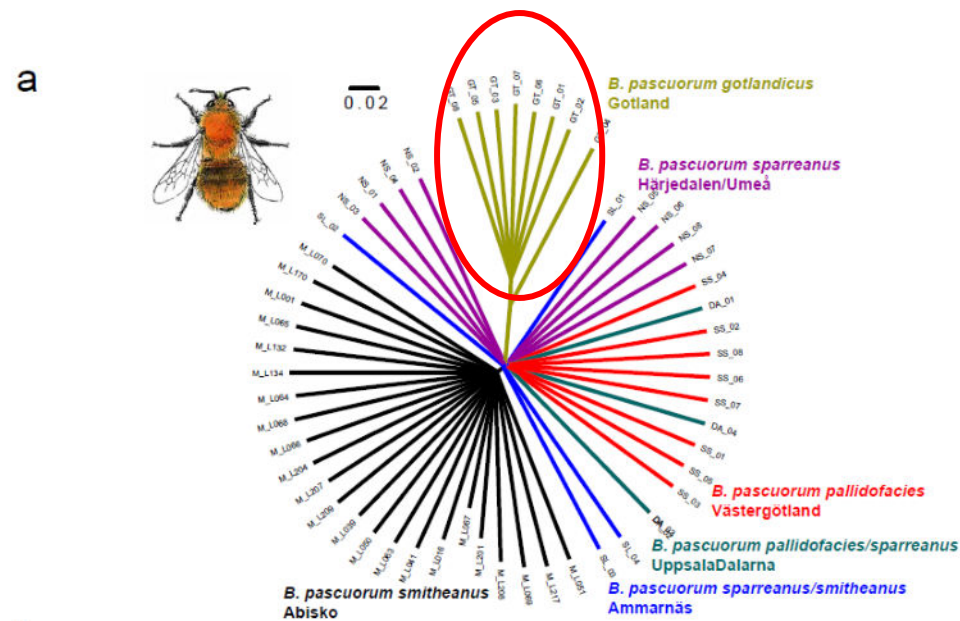
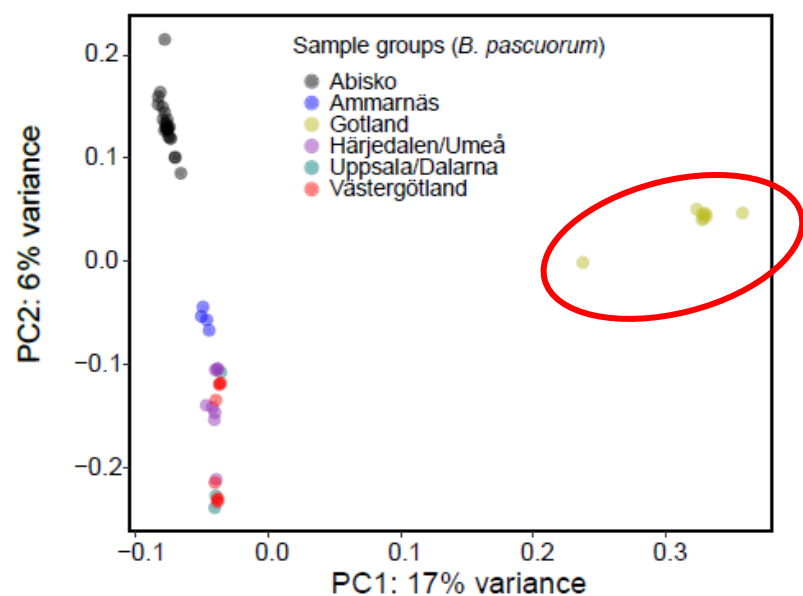
Genetic diversity and trend for Swedish samples

Population structure (Alpinobombus)



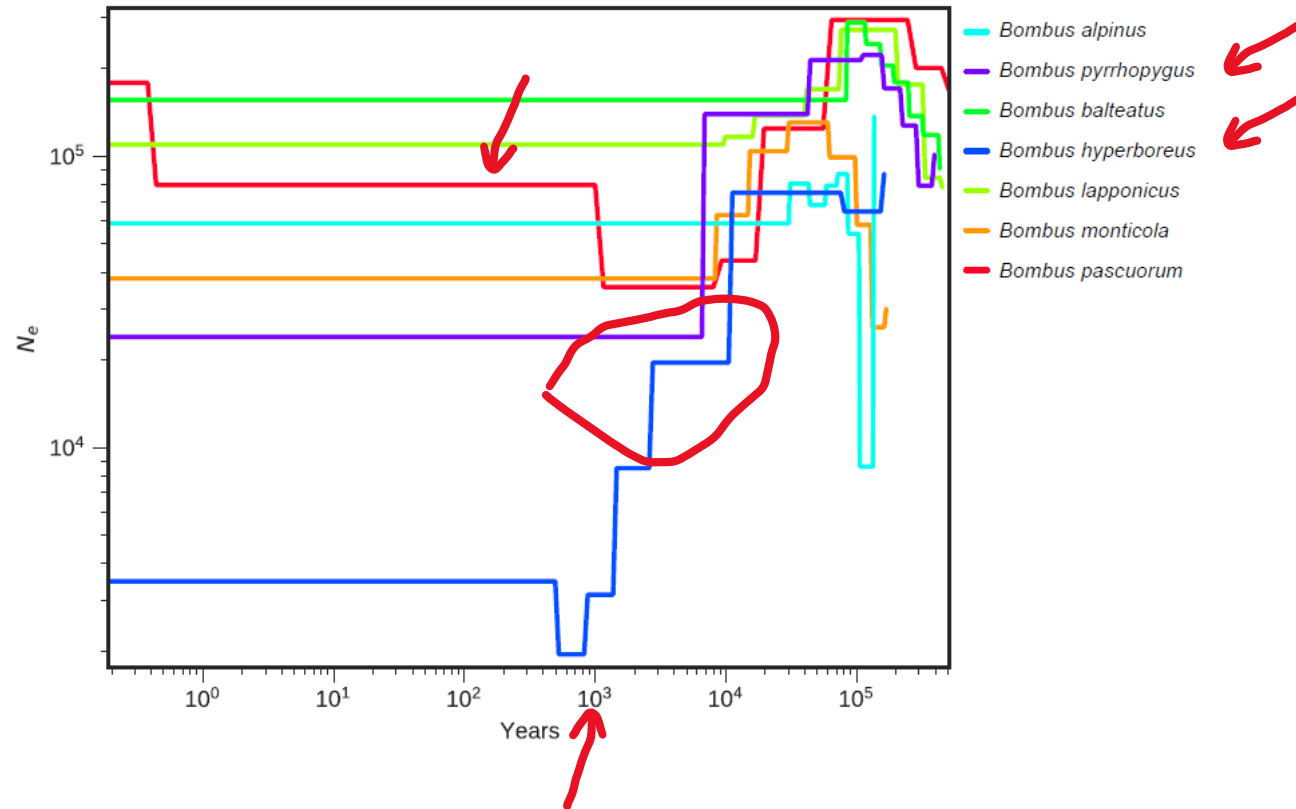
Genetic diversity and trend for Swedish samples

Population structure (Thoracobombus)



Genetic diversity and trend for Swedish samples

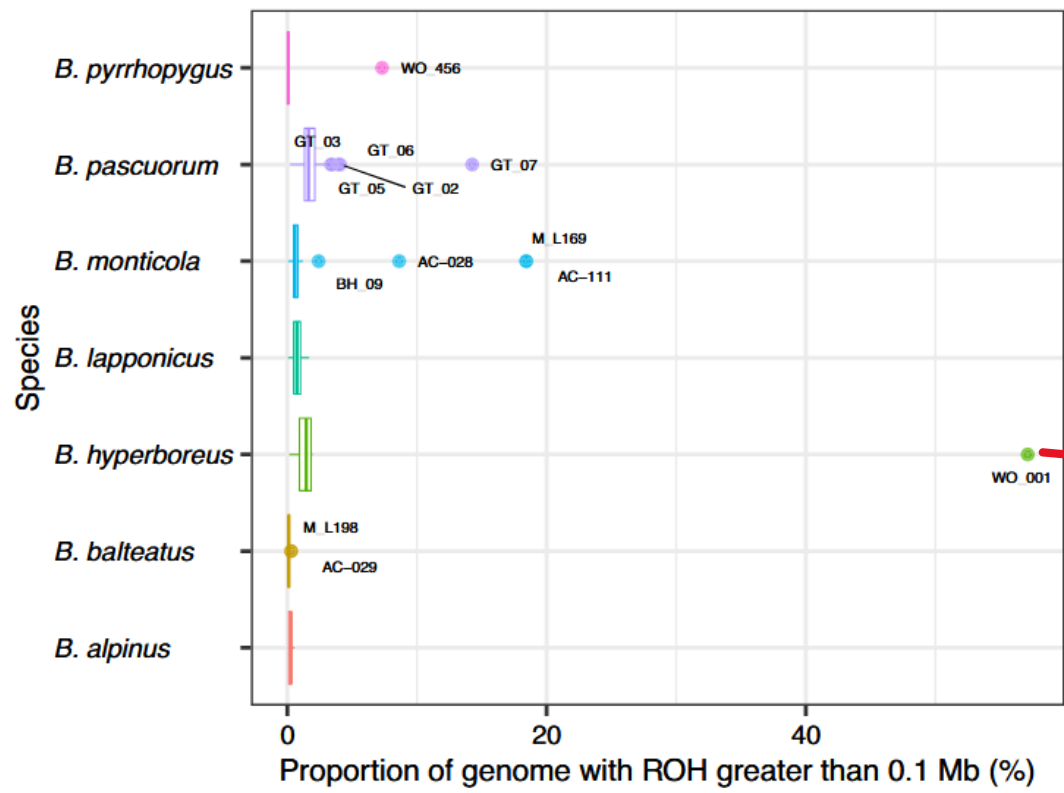
Demographic history



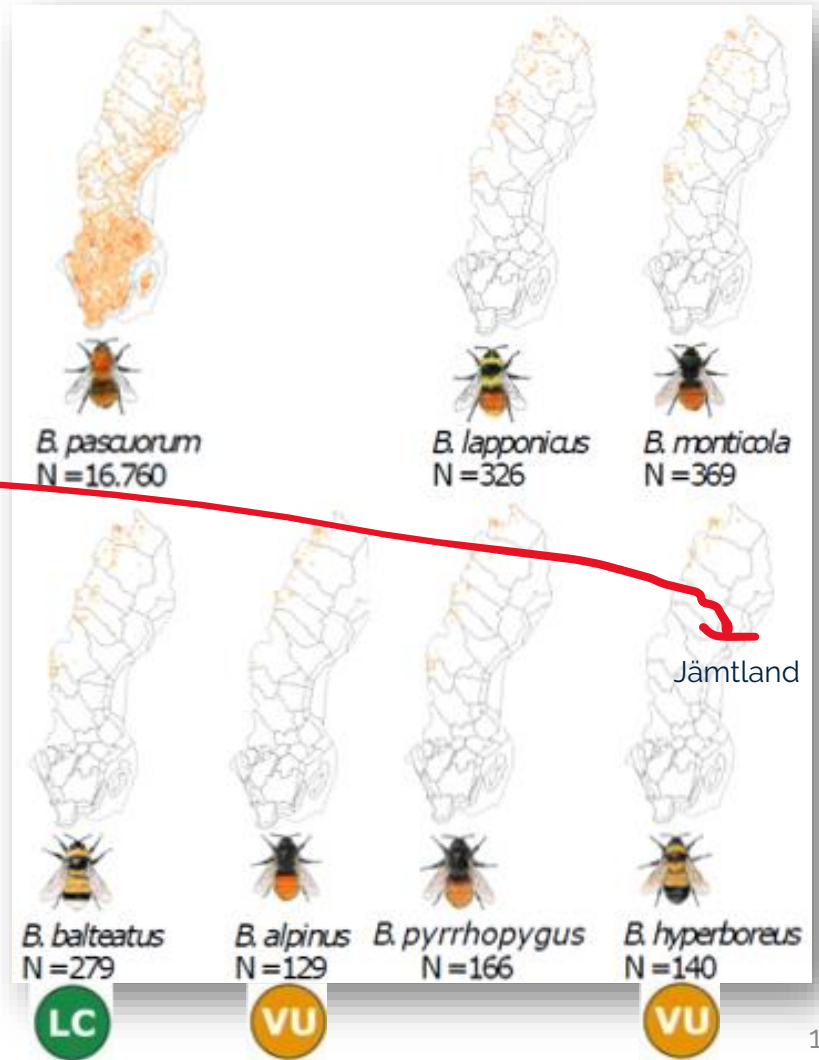
B. hyperboreus has already experienced substantial (*B. pyrrhopygus* to a less extent) population declines over the last millennia. They may have been more widespread earlier in the current post-glacial period.

Genetic diversity and trend for Swedish samples

Inbreeding levels



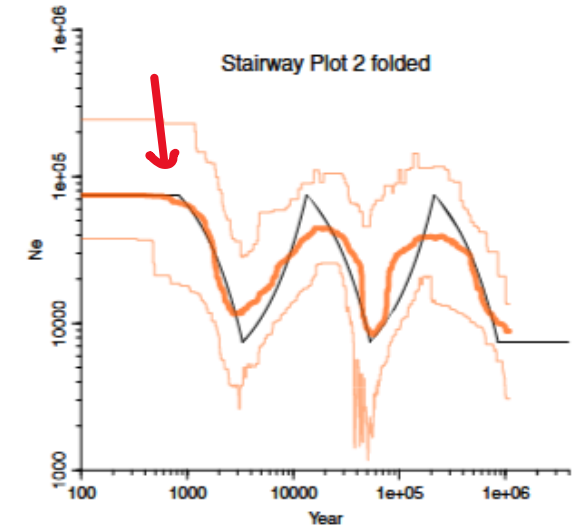
Inbreeding is occurring in *B. hyperboreus*, particularly on the edges of its south range, most likely to be influenced by climate change.



Infer recent N_e trajectories using *Stairway Plot 2*

Advantages

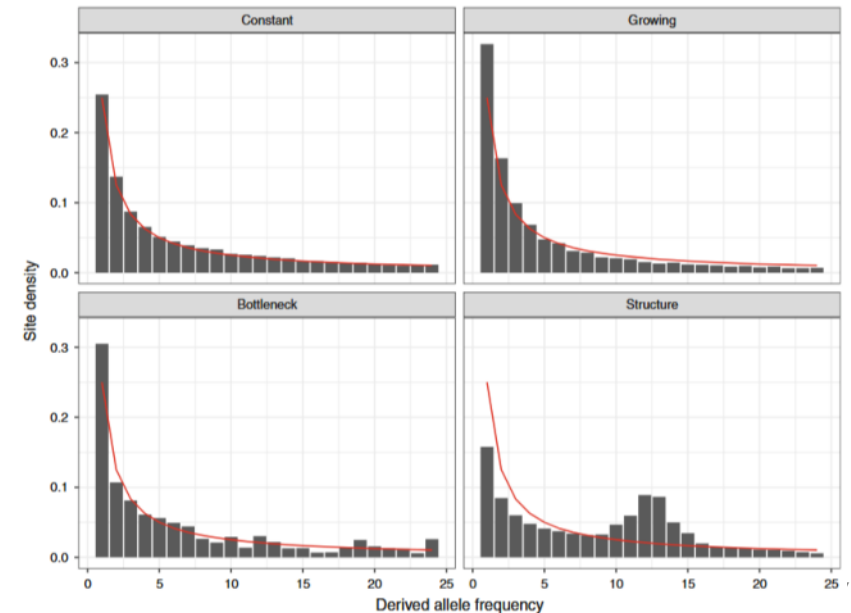
- Using **SFS** data (Pool-Seq, Ind-Seq in low depth)
- Nonparametric method (non-model organism)
- Overfitting control
- Masking out part of the SFS (singletons)
- High resolution >40 generations ago (generation time: **24** years)



Gustavo et al., 2020; Liu et al., 2020

Unexplored aspects for insects

- × Sample size (10, 40, 100 diploids)
- × Recent timing of changes (at 50, 100, 500, 1000 generations)
- × Short generation time (1 year)
- × Sizes of N_e (5000, 50000, 500000)



Infer recent N_e trajectories from Danish samples

Common/Non-endangered



A. haemorrhoa

B. pascuorum

Rare/Endangered



A. marginata

B. veteranus

Workflow

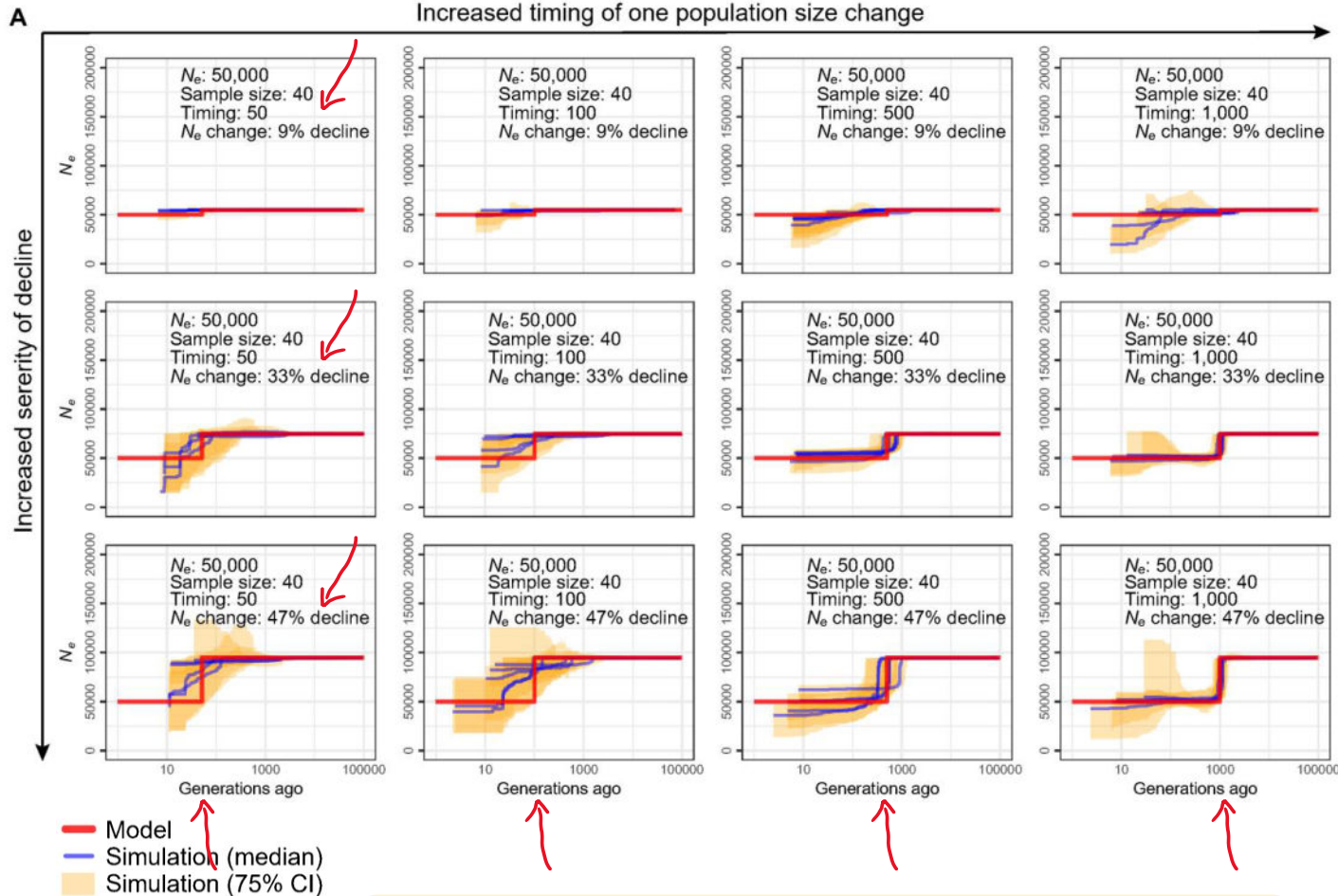
- ◆ Pool-Seq data (2x150 pair-end reads) → mapping to ref. genomes
- ◆ Variant calling (freebayes) + depth filtering (trade-off) → SFS
- ◆ Stairway Plot 2 (depth/chr: 1x, 3x, 5x, 7x, and 10x)
- ◆ Without/with singletons (containing false variants from sequencing errors)

Sample pool	Genome size (Mb)	Mean depth (x)	Mean depth/chr (x)	No. called sites (3x)	SNPs
<i>Andrena haemorrhoa</i> (N = 39)	331	446	6.7x	262,542,042	2,091,104
<i>Andrena marginata</i> (N = 40)	373	443	6.5x	264,299,782	728,481
<i>Bombus pascuorum</i> (N = 34)	308	366	6.8x	240,241,129	1,462,586
<i>Bombus veteranus</i> (N = 29)	355	402	8.8x	247,714,265	1,376,788

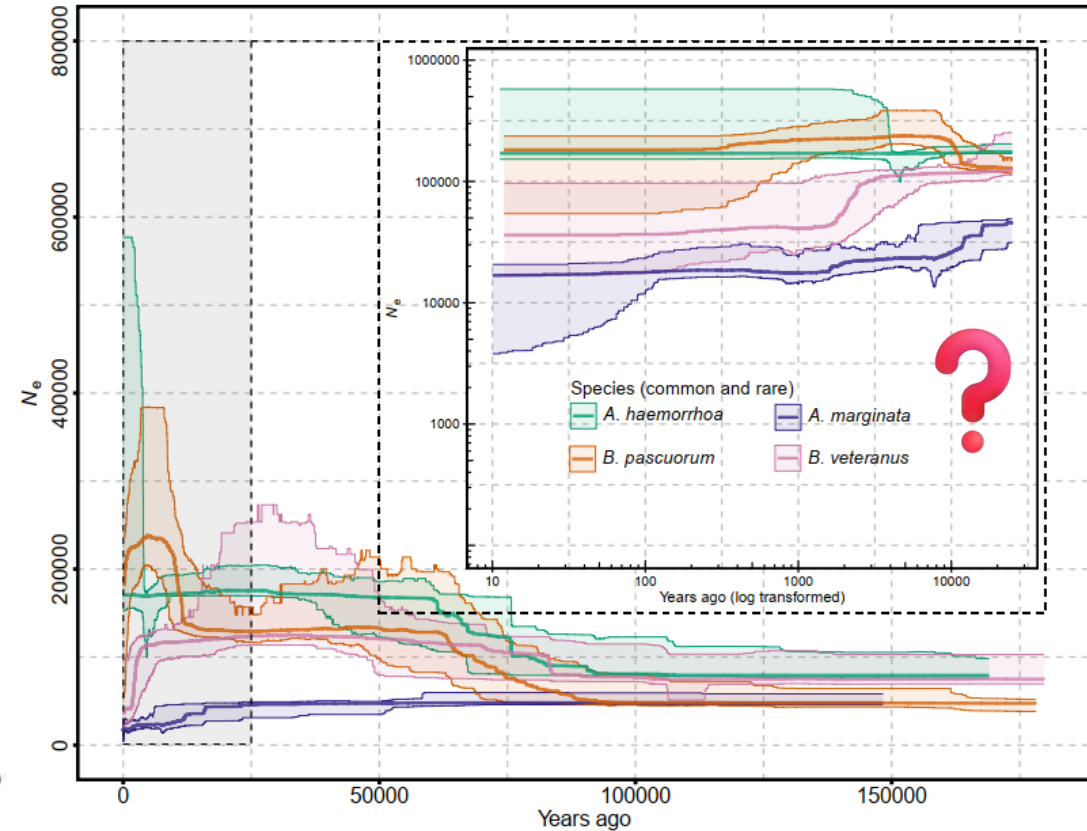
Aim: Establish a genomic framework for reconstructing reliable recent N_e trajectories

- ◆ species rarity
- ◆ current conservation status
- ◆ potential drivers of ongoing insect decline

Infer N_e trajectories from simulations and Danish samples



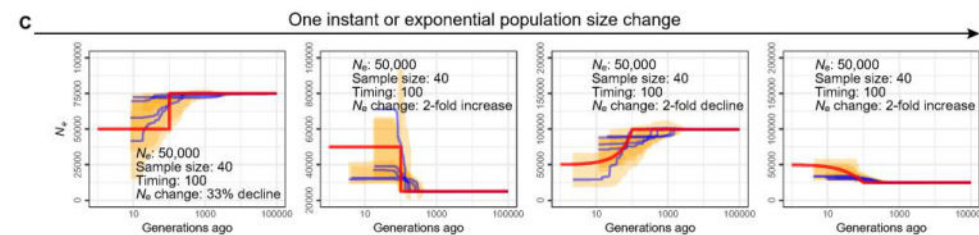
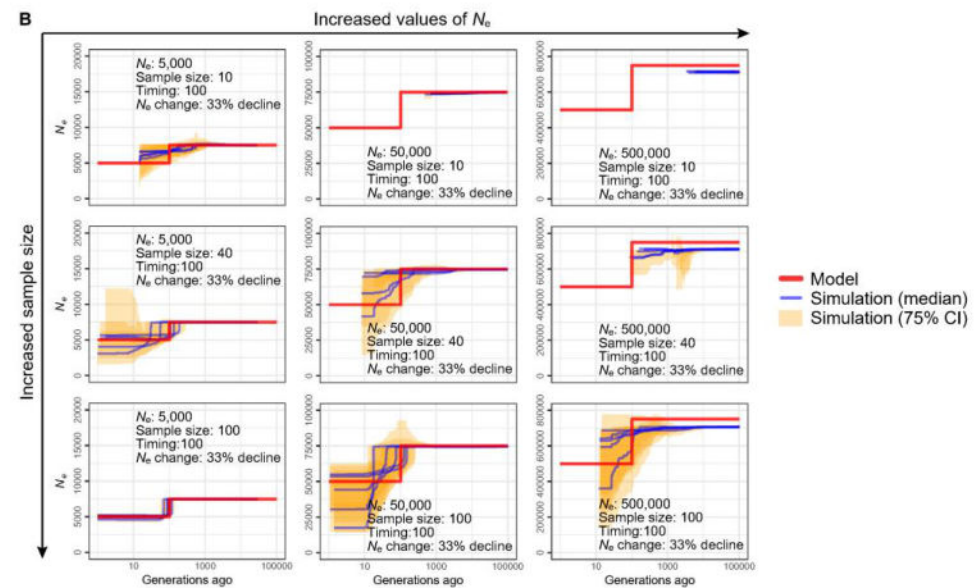
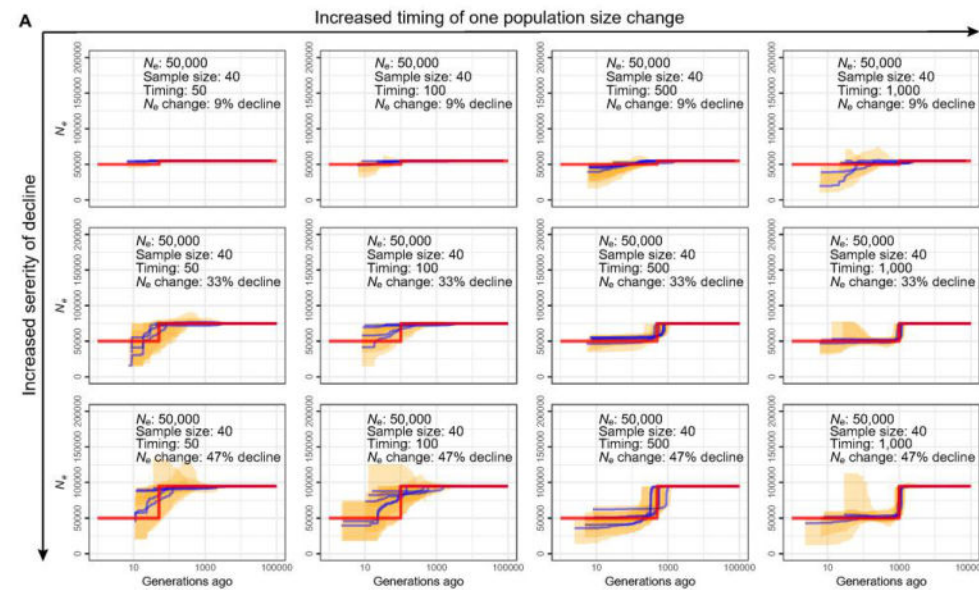
- ✓ Recent timing of changes > 100 generations
- ✓ Sample size ~40 diploids
- ✓ $N_e \leq 50,000$



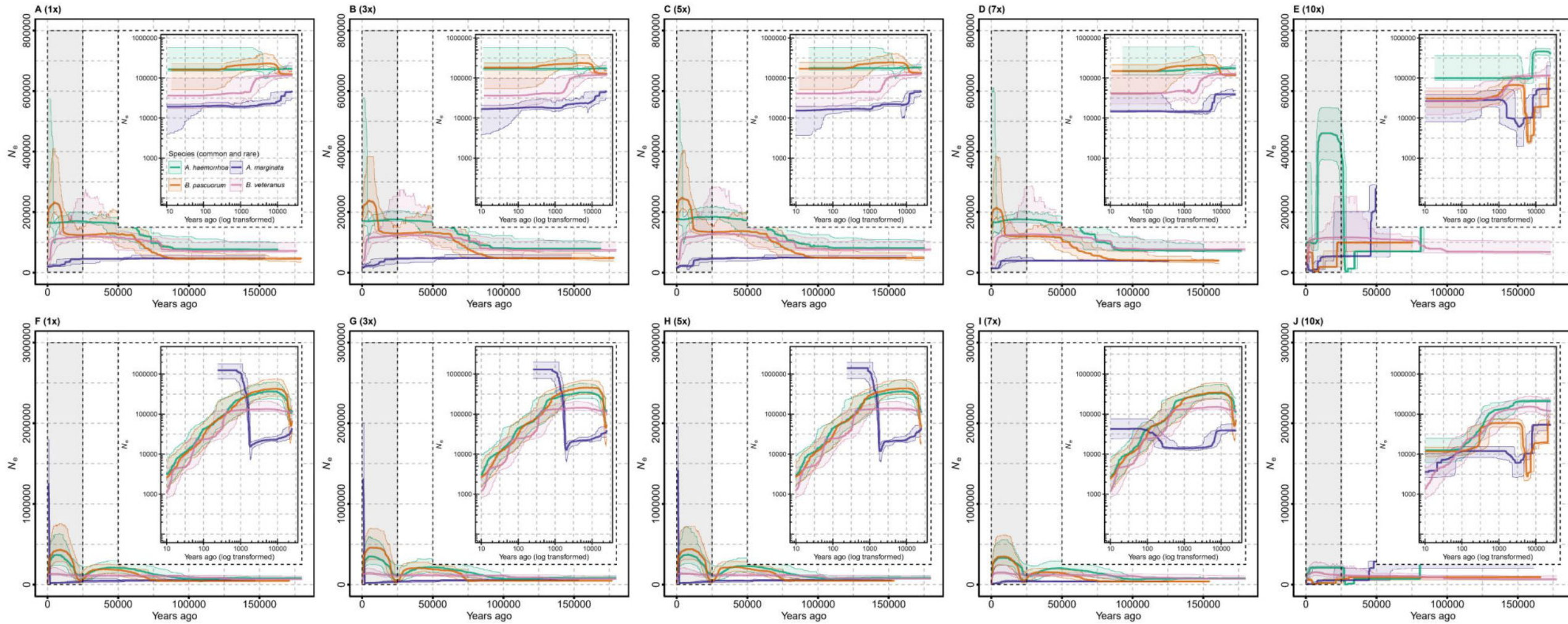
Reconstructing N_e trajectory offers a long-term perspective on species rarity, current conservation status, and potential drivers of insect declines.

Thank you for your attention!





N_e trajectories without/with singletons



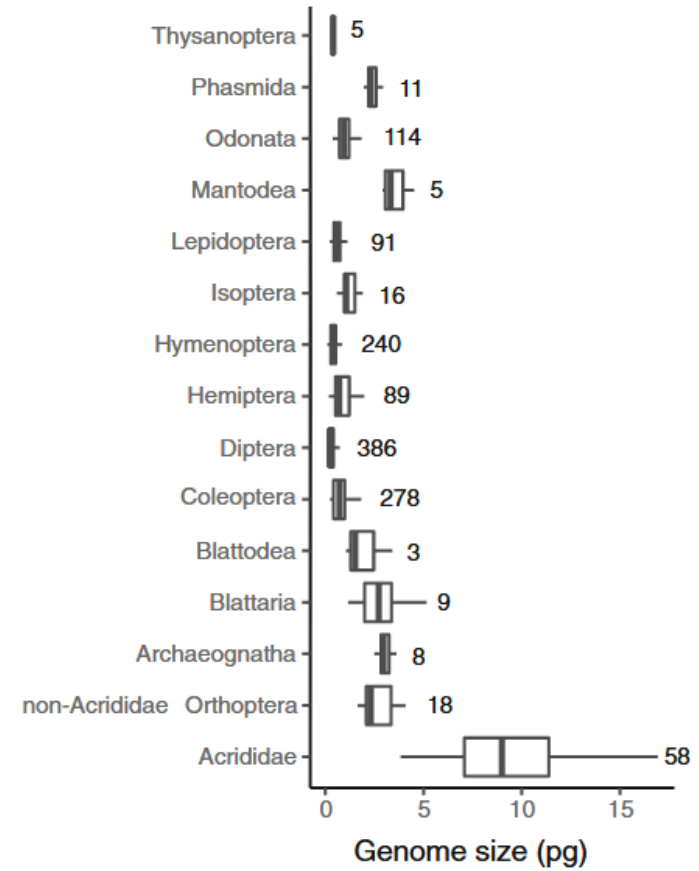
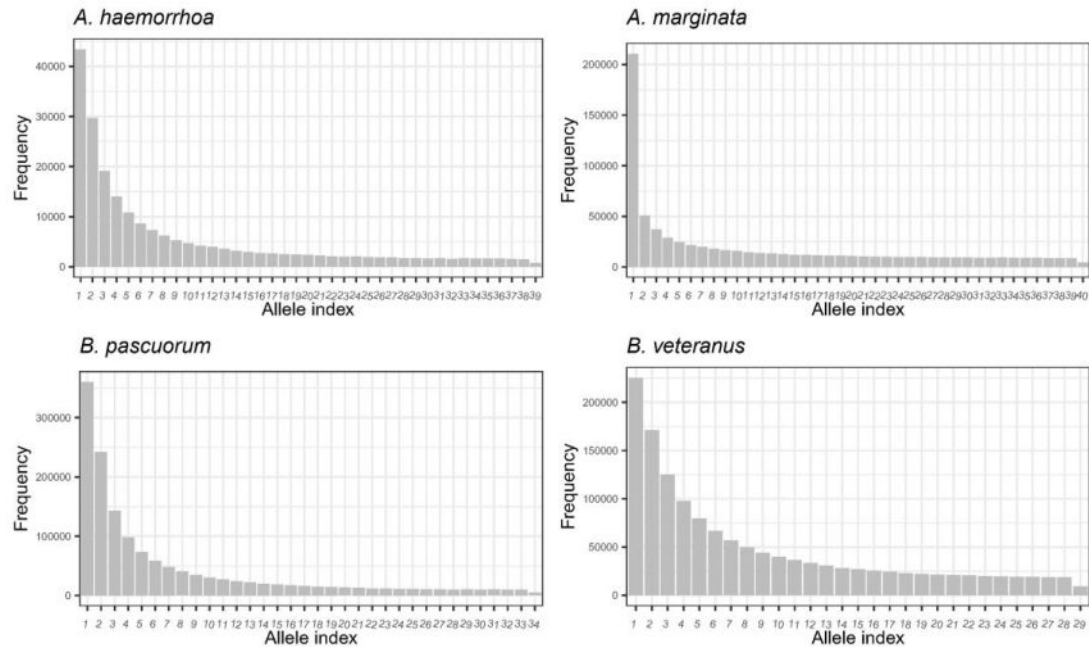
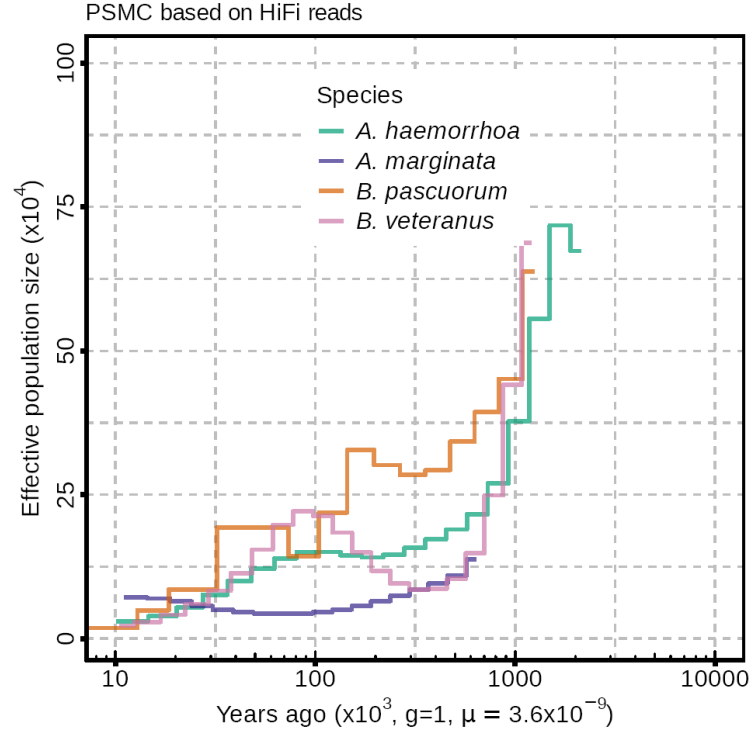


FIGURE 1 GS variation of different insect orders. GS estimates (1331 records) were obtained from the animal genome size database, release 2.0 (Gregory, 2022). Fourteen insect orders have more than three species with GS measurements. Their average GS and ranges are shown as boxplots. The order orthoptera is separated into family Acrididae and non-Acrididae for comparison. Besides each box is the number of species with GS measurements