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Zachary J. Nolen

DOCTORAL DISSERTATION

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Abstract:

Land use change from agricultural intensification has been highlighted as one of the major drivers of global declines in insects, reducing species diversity and abundance and shifting community composition towards generalist species. However, it remains a challenge to determine how land use change impacts the genetic diversity of species and populations even if this diversity is vital to their long-term persistence. Reductions in genetic diversity are expected to reduce adaptive potential and increase the frequency of deleterious mutations, potentially reinforcing demographic declines. In this thesis, I investigate to what extent grassland habitat loss associated with agricultural intensification in southern Sweden has resulted in genetic diversity declines in grassland butterflies, and what genomic metrics best capture these declines. Utilizing genomic data across multiple species, I infer if differential demographic decline in generalists and specialists is reflected in patterns of genetic erosion. Through a spatial comparison of populations of three grassland butterfly species, I uncover that specialist species show stronger genetic structure, with one specialist species experiencing sufficient isolation to lead to higher rates of recent inbreeding compared to the other species. Associating genetic diversity with land cover variables, I find that genetic diversity is lower in populations with less grassland area in the surrounding landscape due to higher rates of inbreeding. Comparisons between contemporary samples and museum specimens of these same three species reveal that genetic diversity has declined over the past century, and that the genetic structure and inbreeding in the specialist species has arisen recently. Importantly, inbreeding has increased homozygosity of putative weakly deleterious mutations in this species, which may depress fitness of inbred individuals and reinforce decline. To determine if these patterns are general, I estimated the extent of genetic differentiation, reductions in effective population size, and recent inbreeding in Sweden's most common grassland butterflies compared to the European Grassland Butterfly Indicator species. While estimates of heterozygosity varied by species, rates of inbreeding were distinctly higher in specialists, as were levels of genetic differentiation between localities. Trajectories of contemporary effective population size over time reveal that while many widespread species maintain high effective population sizes, specialists have dropped to sizes below 500 and even 50 over the past 30-50 years. Estimations of recent inbreeding and contemporary population size thus provide valuable indicators of genetic decline and should be evaluated in conservation assessments to incorporate information on reduced viability from genetic erosion. To make the methods used to perform such assessments more accessible I develop an automated bioinformatic pipeline for the analyses performed in this thesis, that I describe and provide as a resource. Together, my findings highlight that land use change has reduced functional connectivity and increased inbreeding in specialist grassland butterflies, and conservation efforts targeting habitat restorations that restore gene flow are necessary for the persistence of specialist grassland insect species.

Key words: Conservation, insect decline, landscape genomics, runs of homozygosity, genetic load, functional connectivity, genetic erosion

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"An experimental science is supposed to perform experiments that find generalities. It's not just supposed to tally up a long list of individual cases and their unique life histories. That's butterfly collecting."

Richard Lewontin

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Life stages of *Plebejus argus*, the silver-studded blue or ljungblåvinge. Illustrations by Ye Xiong.

Abstract

Land use change from agricultural intensification has been highlighted as one of the major drivers of global declines in insects, reducing species diversity and abundance and shifting community composition towards generalist species. However, it remains a challenge to determine how land use change impacts the genetic diversity of species and populations even if this diversity is vital to their long-term persistence. Reductions in genetic diversity are expected to reduce adaptive potential and increase the frequency of deleterious mutations, potentially reinforcing demographic declines. In this thesis, I investigate to what extent grassland habitat loss associated with agricultural intensification in southern Sweden has resulted in genetic diversity declines in grassland butterflies, and what genomic metrics best capture these declines. Utilizing genomic data across multiple species, I infer if differential demographic decline in generalists and specialists is reflected in patterns of genetic erosion. Through a spatial comparison of populations of three grassland butterfly species, I uncover that specialist species show stronger genetic structure, with one specialist species experiencing sufficient isolation to lead to higher rates of recent inbreeding compared to the other species. Associating genetic diversity with land cover variables, I find that genetic diversity is lower in populations with less grassland area in the surrounding landscape due to higher rates of inbreeding. Comparisons between contemporary samples and museum specimens of these same three species reveal that genetic diversity has declined over the past century, and that the genetic structure and inbreeding in the specialist species has arisen recently. Importantly, inbreeding has increased homozygosity of putative weakly deleterious mutations in this species, which may depress fitness of inbred individuals and reinforce decline. To determine if these patterns are general, I estimated the extent of genetic differentiation, reductions in effective population size, and recent inbreeding in Sweden’s most common grassland butterflies compared to the European Grassland Butterfly Indicator species. While estimates of heterozygosity varied by species, rates of inbreeding were distinctly higher in specialists, as were levels of genetic differentiation between localities. Trajectories of contemporary effective population size over time reveal that while many widespread species maintain high effective population sizes, specialists have dropped to sizes below 500 and even 50 over the past 30-50 years. Estimations of recent inbreeding and contemporary population size thus provide valuable indicators of genetic decline and should be evaluated in conservation assessments to incorporate information on reduced viability from genetic erosion. To make the methods used to perform such assessments more accessible I develop an automated bioinformatic pipeline for the analyses performed in this thesis, that I describe and provide as a resource. Together, my findings highlight that land use change has reduced functional connectivity and increased inbreeding in specialist grassland butterflies, and conservation efforts targeting habitat restorations that restore gene flow are necessary for the persistence of specialist grassland insect species.

Popular Summary

If you grew up spending summer days outside, you may have noticed something peculiar as you've gotten older – there are fewer insects than when you were younger. It isn't our imagination. Insects are declining by about 1-2% every year and have been declining for close to a century, at least. While it might sound nice to have fewer mosquitos and horse flies, it also means fewer pollinators for flowers and crops, less food for birds in the spring, and fewer decomposers restoring nutrients to the soil. It means less diverse ecosystems with fewer of the parts they rely on to function, making them less resilient.

A major driver of this decline in insects is our use of the land. Over the past century, our footprint on the landscape has grown considerably, largely through expansion of urban areas and intensification of agriculture. The latter has been especially detrimental to insects – landscapes that were once complex mixtures of small farms, grasslands, and forests, supporting a wide diversity of plants and insects, now largely may be covered by only a handful of crops or abandoned and overgrown. With less and less suitable habitat available, insects end up persisting in small populations on isolated patches, which makes them more vulnerable to extinction.

These declines also mean that individual populations of insects are likely becoming less diverse genetically. In wild populations, not all individuals reproduce equally, which changes how common different variants of genes, called alleles, are in each generation. At some point, by chance, only one allele for a given gene gets passed on, reducing genetic diversity. In small populations, the time it takes for this to happen is much shorter than larger ones. Losing genetic diversity can make it harder for a population to persist, as when environments change, variation that might have enabled them to adapt might no longer be there. This makes conserving biodiversity in all its forms – ecosystem diversity, species diversity, and genetic diversity – important for ensuring long-term stability of the natural world.

Reduced genetic diversity can also pose a more immediate threat, as sometimes beneficial alleles may be the ones that are lost, leaving behind only a more harmful alternative in the population. While this is unlikely for very harmful alleles, due to natural selection, it is possible for slightly harmful ones to slip by. These can accumulate from generation to generation, subtly reducing the fitness of all individuals. This process is magnified by inbreeding, as in smaller populations individuals will be more related on average, and more likely to share similar harmful alleles that their offspring will inherit. Together, accumulation of slightly harmful alleles and increased inbreeding can mean that small populations end up in an 'extinction vortex', where they decline to a small size, but then are unable to rebound due to genetic problems reinforcing this decline.

In this thesis, I investigate if this extinction vortex is happening in grassland butterflies in Sweden. Much of what we know about insect decline comes from these species, and we know that in countries with a lot of agriculture, like Sweden, they are declining even faster than the average rate. I use genomic methods, which allow me to analyze all the genetic material that makes up each individual butterfly I study (its ‘genome’). Comparing genomes from multiple individuals can tell us a lot about population history and how well alleles are exchanged between populations as butterflies move through the landscape.

The first question this thesis addresses is if genetic diversity is lower in some landscapes than others. I investigate this in three species, one that is generalist, meaning it doesn’t have strict habitat needs and can persist in places like roadsides and gardens, and two that are specialists, which require healthy grasslands with certain plants. I find that the generalist is quite well connected, so genetic diversity is maintained by individuals dispersing from patch to patch, often bringing back alleles that might be lost in one patch but not another. There is little influence from the landscape on genetic diversity in this species, high dispersal keeps it even across the region. However, for one of the specialists, connectivity is rather low, and populations are isolated and have lost genetic diversity from inbreeding. Our impact on the landscape drives this isolation, as populations with little grassland in the surrounding landscape, especially if there is a lot of arable land, are more inbred.

I then investigate if these specialist butterflies became recently isolated due to agricultural expansion in the past century. I do this by sequencing genomes of pinned butterflies in the Biological Museum at Lund University. Generally, I find that, in the past, all three species were well connected in Sweden, but that the specialist has recently become isolated and lost genetic diversity because of increased inbreeding. I also find that while alleles likely to be very harmful have reduced in number, inbreeding means that slightly harmful ones are getting expressed more, making inbred populations less viable. Together with the first study, this suggests that populations of this specialist species have recently become isolated in agricultural landscapes, increasing inbreeding and making them less fit.

Thirdly, I investigate if these patterns are common in other grassland butterflies, looking at several widespread and specialist species. I generally find that the specialists are more isolated and inbred, whereas the widespread ones tend to have high connectivity and low inbreeding. This suggests that genetic diversity loss may be a pressing issue for many specialist insects. Finally, I have produced a pipeline for these analyses to make such investigations in other species more accessible to researchers and conservationists.

To conclude, this thesis highlights that agricultural intensification has not only reduced grassland butterfly abundance, but also their genetic diversity and connectivity. This is especially a threat to specialist species, that are now inbreeding. If these species, and the grassland communities they play a role in, are to persist, restoration of grassland habitat that can reconnect these populations is a necessary conservation action.

List of Papers

Paper 1

Nolen, Z.J., Rundlöf, M., Runemark, A., 2024. Species-specific erosion of genetic diversity in grassland butterflies depends on landscape land cover. Biological Conservation 296, 110694.

Paper 2

Nolen, Z.J., Jamelska, P., Torres Lara, A.S., Wahlberg, N., & Runemark, A., 2025. Species-specific loss of genetic diversity and exposure of deleterious mutations following agricultural intensification. Manuscript in revision. Preprints of each version are available on bioRxiv, doi:10.1101/2024.10.07.616612.

Paper 3

Nolen, Z.J., van Riemsdijk, I., & Runemark, A., 2025. Specialization predicts population isolation and inbreeding in grassland associated butterflies. Manuscript.

Paper 4

Nolen, Z.J., 2025. PopGLen—a Snakemake pipeline for performing population genomic analyses using genotype likelihood-based methods. Bioinformatics 41, btaf105.

In addition, I have been co-author on the following papers during my doctoral studies which are not included in this thesis:

Nolen, Z.J., Yildirim, B., Irisarri, I., Liu, S., Crego, C.G., Amby, D.B., Mayer, F., Gilbert, M.T.P., Pereira, R.J., 2020. Historical isolation facilitates species radiation by sexual selection: Insights from *Chorthippus* grasshoppers. Molecular Ecology 29, 4985–5002.

Neves, J.M.M., **Nolen, Z.J.**, Fabré, N.N., Mott, T., Pereira, R.J., 2021. Genomic methods reveal independent demographic histories despite strong morphological conservatism in fish species. Heredity 127, 323–333.

Li, Y., Bateman, C., Skelton, J., Wang, B., Black, A., Huang, Y.-T., Gonzalez, A., Jusino, M.A., **Nolen, Z.J.**, Freeman, S., Mendel, Z., Kolařík, M., Knížek, M., Park, J.-H., Sittichaya, W., Pham, T.-H., Ito, S., Torii, M., Gao, L., Johnson, A.J., Lu, M., Sun, J., Zhang, Z., Adams, D.C., Hulcr, J., 2022. Preinvasion Assessment of Exotic Bark Beetle-Vectored Fungi to Detect Tree-Killing Pathogens. Phytopathology 112, 261–270.

Boman, J., **Nolen, Z.J.**, Backström, N., 2025. On the origin of an insular hybrid butterfly lineage. Evolution 79, 510–524.

Author contributions

Paper 1

Zachary J. Nolen: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Maj Rundlöf: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. Anna Runemark: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Paper 2

Zachary J. Nolen: Conceptualization, Methodology, Formal analysis, Investigation (Lead), Data curation, Writing – original draft, Visualization, Funding acquisition. Patrycja Jamelska: Investigation (Supporting). Ana Sofía Torres Lara: Investigation (Supporting), Writing - review & editing. Niklas Wahlberg: Methodology, Writing – review & editing, Supervision. Anna Runemark: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Paper 3

Zachary J. Nolen: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Software, Funding acquisition. Isolde van Riemsdijk: Formal analysis, Investigation, Writing - original draft, Visualization, Software, Resources. Anna Runemark: Conceptualization, Methodology, Writing – original draft, Supervision, Funding acquisition.

Paper 4

Zachary J. Nolen: Conceptualization, Investigation, Software, Validation, Writing – original draft.

Introduction

Global biodiversity is declining at an unprecedented rate, with overwhelming evidence that the Earth is experiencing a sixth mass extinction event (Barnosky et al., 2011; G. Ceballos et al., 2015, 2017; Cowie et al., 2022). Anthropogenic land use change, direct exploitation, pollution, and climate change are all major drivers of this decline (Díaz et al., 2019; IPBES, 2019; Keck et al., 2025), estimated to already have increased extinction rates 100-1000 times higher than that of the background rate (G. Ceballos et al., 2015, 2017; Dirzo et al., 2014; Pimm et al., 2014). With evidence pointing to these rates accelerating, biodiversity loss is one of the greatest crises we face today.

Our understanding of the rate of biodiversity decline differs considerably across taxonomic groups. Insect declines have been relatively poorly understood compared to declines in vertebrates (Wagner, Grames, et al., 2021), but are receiving widespread attention after multiple studies quantified large scale declines in several regions (Edwards et al., 2025; Hallmann et al., 2017; Seibold et al., 2019; van Klink et al., 2020). Such widespread declines in organisms that are usually so abundant has concerning implications, especially as insects are a key component of a variety of food webs and provide diverse ecosystem services (Bowler et al., 2019; Seibold et al., 2019; Weisser & Siemann, 2013). Declines in insects are therefore likely to result in cascades that destabilize ecosystems, fueling further biodiversity decline.

While the prevalence of declines in insect species diversity and abundance are becoming clear, the impacts these declines are having on genetic diversity has seen limited study (Webster et al., 2023). Genetic diversity declines as populations decline, which may reduce their potential to adapt to environmental change (Lande & Shannon, 1996) and increase the frequency and homozygosity of deleterious mutations that reduce their fitness (Hedrick & Garcia-Dorado, 2016). As these genetic threats to persistence can reinforce the demographic declines that brought them about, effective conservation action requires integration of risks from genetic erosion into conservation assessments (Schmidt et al., 2023a). Considerable discussion has been had on what metrics should be utilized for international targets for conserving genetic diversity (Hoban, Bruford, et al., 2023; Hoban et al., 2020; Laikre et al., 2020; O'Brien et al., 2022), and what type of diversity is most informative to conservation (García-Dorado & Caballero, 2021; Kardos et al., 2021; Teixeira & Huber, 2021).

In this thesis, I investigate the genetic patterns associated with insect decline through the lens of grassland butterflies, a group declining strongly in agricultural landscapes (Habel, Ulrich, et al., 2019). By applying a broad set of genomic methods to population-level datasets of multiple species, I assess to what extent genetic erosion has occurred in these taxa while simultaneously assessing which metrics can most efficiently capture its signal in practice. Through datasets that vary in their spatial and temporal resolution, I evaluate how the surrounding landscape influences genetic diversity and functional connectivity, and if patterns of isolation are long-term or developed as a product of landscape change. By using a multi-species approach, I assess whether habitat specialization results in elevated risks of genetic erosion or if it is occurring in a broad range of species. These inferences provide insights into which species should be prioritized when incorporating genetic metrics into conservation assessment.

Grassland insect decline

Several recent studies have attempted to quantify the magnitude of regional and global insect declines (Hallmann et al., 2017, 2020; Lister & Garcia, 2018; Sánchez-Bayo & Wyckhuys, 2021; van Klink et al., 2020), with a recent meta-analysis estimating an 11% decline per decade in terrestrial insects across all datasets analyzed (van Klink et al., 2020). While accurately estimating global insect decline is challenging due to differences in methodology across studies, periodic swings in population sizes, and data that is unevenly distributed across regions and taxa (Wagner, Grames, et al., 2021), the reality of a substantial, globally distributed decline in insects is widely supported (Saunders et al., 2025; Wagner, Grames, et al., 2021). No single driver can be considered responsible, but land use change along with insecticide use are considered by most experts to be the primary culprits (Dicks et al., 2021; Raven & Wagner, 2021; Wagner, Grames, et al., 2021). Agricultural intensification over the past century has been the dominant land use change that impacts insects (Raven & Wagner, 2021; Wagner, Grames, et al., 2021). In Europe, such intensification has largely taken the form of consolidation of small farms into large, industrial monocultures (R. A. Robinson & Sutherland, 2002). This has resulted in the conversion or abandonment of many of the continent’s semi-natural grasslands, habitats that can harbor exceptionally high local levels of temperate biodiversity (Shipley et al., 2024).

Insects are being lost along with these grasslands, and regional rates of insect decline in European grasslands can substantially exceed the global average. For instance, arthropod abundance declined by 78% in grasslands in Germany over a ten year period (Seibold et al., 2019) and grassland butterfly abundance has decreased by 36% across Europe in a similar time frame (van Swaay et al., 2022). These declines are causing shifts in community composition (Keck et al., 2025), with habitat specialist insect species declining more strongly than generalists (Ekroos et al., 2010; Gossner et al., 2016; Habel, Trusch, et al., 2019; Wagner, Fox, et al., 2021; Warren et al., 2021). Such community composition changes can lead to cascades of declines to other species and even taxonomic groups (Kehoe et al., 2021), such has been suggested by concurrent declines in insectivorous birds (Bowler et al., 2019) and flowering plants (Biesmeijer et al., 2006).

Conservation of genetic diversity

Long term stability of grassland ecosystems facing pressure from agricultural intensification requires effective management focusing on biodiversity at multiple levels (Shipley et al., 2024). Along with the ecosystem and species diversity, genetic diversity is a main component of biodiversity, and its maintenance is necessary for resiliency of species and ecosystems (O'Brien et al., 2025). While the impacts of habitat loss and fragmentation on genetic diversity have received some study in vertebrates (Lino et al., 2019), they have received little attention in insects (Webster et al., 2023).

Genetic diversity is lost over time as stochastic fluctuations in allele frequencies from generation to generation bring alleles to fixation (Kimura, 1957; Wright, 1931). While the rate of this process is slow in large populations, it accelerates as population size decreases (Crow & Kimura, 1970). This reduction in standing genetic variation, the substrate upon which natural selection acts, can reduce the adaptive potential of a population, increasing its risk of extinction in the long-term (Lande, 1995; Lande & Shannon, 1996). In the short-term, the increased relative strength of drift compared to selection can allow partially recessive, weakly deleterious mutations to increase in frequency in the population (Bertorelle et al., 2022; Kimura et al., 1963; Lynch et al., 1995). At the same time, greater background relatedness in small populations means that rates inbreeding increase, even when mating is random (L. F. Keller & Waller, 2002). Inbreeding increases homozygosity, increasing the expression of partially recessive deleterious mutations, which is the main cause of inbreeding depression (Charlesworth & Willis, 2009).

Together, inbreeding and drift can allow deleterious mutations to reach elevated frequencies and fixation, reducing the mean fitness of the population. Populations may then enter a type of extinction vortex, where demographic declines due to e.g. habitat degradation increase mutational load and reduce adaptive potential, reinforcing the initial decline (Gilpin & Soule, 1986).

While this might suggest that low genetic diversity is an indicator of high extinction risk, the link is not so straightforward, making genetic assessments for conservation challenging. Genetic diversity within a species can be influenced by a myriad of factors, e.g. propagule size (Ellegren & Galtier, 2016; Romiguier et al., 2014), long-term demographic history (Hewitt, 2000), or body size (Mackintosh et al., 2019; Romiguier et al., 2014). Such influences mean a species' absolute level of genetic diversity may be an unreliable predictor of its extinction risk (Schmidt et al., 2023a). A few recent studies have even documented situations where populations have persisted with low levels of genetic diversity or high rates of inbreeding for long periods (Hofman et al., 2016; Reed, 2010; J. A. Robinson et al., 2016, 2018, 2022). These findings suggest that low genetic diversity and small population sizes may not always condemn a species to extinction from mutational meltdown. Observed dissociations of neutral genetic diversity and fitness have led some to suggest that neutral genetic diversity is overvalued in conservation and more priority must be given to functional diversity for management decisions (Kyriazis et al., 2021; J. A. Robinson et al., 2018; Teixeira & Huber, 2021).

While functional genetic diversity can offer valuable insights to conservation, many researchers have taken issue with the idea of prioritizing it over neutral genetic diversity (DeWoody et al., 2021; García-Dorado & Caballero, 2021; Kardos et al., 2021; Willi et al., 2022). A common counterargument to the critique that genetic diversity is poorly related to conservation status is that such a relationship should only appear if conservation status itself already accurately assesses extinction risk, which it likely does not (Kardos et al., 2021), and if confounding components of genetic diversity are accounted for. When accounting for phylogenetic relatedness (Spielman et al., 2004) and/or assessing within rather than across taxonomic groups (Genereux et al., 2020; Schmidt et al., 2023a), threatened taxa have been found to have lower levels of genetic diversity. Additionally, there is a large body of literature showing the positive association with genetic diversity and fitness (see DeWoody et al., 2021 for a compilation of such studies), begging the question whether resiliency in some small populations is a rare accident being mistaken for a norm (García-Dorado & Caballero, 2021), a survivorship fallacy that conservation biologists have been aware of for decades (Kardos et al., 2021; Ralls et al., 2020; Soulé, 1987). Perhaps most indicative of the value of neutral genetic diversity informed conservation action is that when genetic rescue has focused on maximizing genetic diversity, it has largely been successful (Frankham, 2015; Ralls et al., 2020; Whiteley et al., 2015).

Using genomic methods to infer conservation priorities

The debate surrounding the roles of neutral and functional genetic diversity in conservation assessment highlights that integration of genetic diversity into conservation assessments must be done in a way that considers its confounding variables. Simple thresholds for heterozygosity cannot be applied across species, as what constitutes 'low' genetic diversity in one may not for another, and instead must capture the vulnerability of a species to genetic erosion (Schmidt et al., 2023a). The most direct way of assessing genetic erosion is by estimating genetic diversity at multiple time points, and such genetic monitoring is being implemented in several countries (O'Brien et al., 2022). However, monitoring will not capture declines before the first sampled time point, missing out on considerable genetic erosion that has likely already occurred, given that genetic erosion has likely been occurring for many taxa since at least the industrial revolution (Leigh et al., 2019). To rapidly incorporate genetic information into conservation assessments, we must also utilize metrics that can inform on genetic erosion even when only a single contemporary sample is available.

Genomic methods offer considerable opportunity to help facilitate this aim, and several researchers have weighed in on potential benefits they can offer to conservation (Allendorf et al., 2010; Formenti et al., 2022; Funk et al., 2012; Harrisson et al., 2014; Supple & Shapiro, 2018; Webster et al., 2023). High-quality reference genomes for wild organisms are being generated at an unprecedented rate (Figure 1) due to efforts from several consortia (e.g. Lewin et al., 2018; McCartney et al., 2024; The Darwin Tree of Life Project Consortium, 2022), making genomic conservation assessments possible for more species each day. While the ability to assess variation across the whole genome improves the precision with which we can estimate genome-wide metrics, genomic methods also enable analyses that were previously not available to traditional marker-based conservation genetics. Here, I will briefly cover a few of the methods that have provided insights into genetic erosion in recent genomic studies.

Effective population size (N_e), which classically estimates the size of an ideal population that would experience the same rate of genetic drift as the observed (Wright, 1931), is a fundamental metric utilized in conservation genetics (Franklin, 1980). As a measure of the rate of genetic drift it has direct relevance to estimating the risk of genetic erosion, and for that reason has become one of the headline indicators for genetic diversity decline for the Convention on Biological Diversity (Hoban, da Silva, et al., 2023, 2024). This indicator is primarily concerned with contemporary N_e, estimates of which largely reflect the rate of drift in recent generations, compared to historical N_e, which reflects a harmonic mean of historical effective population sizes (Fedorca et al., 2024). Genomic data offers the opportunity to assess fluctuations in N_e across all these time scales, from a few to millions of generations, using a variety of methods (Nadachowska-Brzyska et al., 2022). Such methods provide the opportunity to estimate contemporary N_e as an indicator of genetic erosion, while also reconstructing how recently bottlenecks occurred, helping to separate recently declined populations from long-term small ones.

Inbreeding depression is one of the key genetic risks of population decline (Hedrick & Kalinowski, 2000), and understanding rates of inbreeding in wild populations has helped inform genetic rescue. Genomic data enables estimation of runs of homozygosity (*RoH*), long identical by descent segments stemming from matings between relatives in an individual's ancestry (Shafer & Kardos, 2025). Inference of the inbreeding coefficient from *RoH* (F_{RoH}) has become a popular way to infer inbreeding in wild populations (McQuillan et al., 2008; Shafer & Kardos, 2025). The length and abundance of *RoH* can carry considerable information about population history, with distinct relationships to population size, bottlenecks, and consanguinity (F. C. Ceballos et al., 2018), enabling identification of populations experiencing recent genetic erosion.

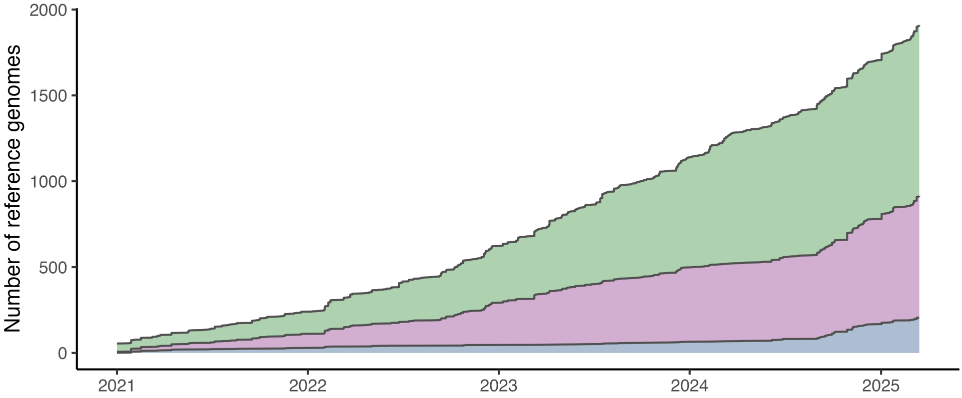

Figure 1. Genomic resources for insects, and especially moths and butterflies, have dramatically increased in the past few years. Reference genomes are a fundamental resource for genomic studies, and have been generated at unprecedented rates in recent years. In this figure, the total number of chromosome-level insect (green), Lepidoptera (purple), and butterfly (blue) reference genomes available on NCBI since 2021 is shown. Butterflies have been particularly well sequenced; though they make up only ~10% of described Lepidoptera species and <2% of described insect species, they currently represent 22% and 11% of all high-quality reference genomes available for these groups, respectively. Data from NCBI Genome Datasets filtered to chromosome-level reference sequences, accessed 2025-03-20.

Reconstruction of the genomes of specimens housed in museums provides an opportunity to directly estimate genetic erosion that has already occurred. Reference based methods enable mapping of highly fragmented, degraded DNA from historical specimens to reconstruct entire genomes from past individuals (Card et al., 2021). While limited by the availability of samples, such methods enable estimation of genetic diversity, population genetic structure, inbreeding, and proxies of mutational load in historic populations that can then provide a baseline to compare modern ones to (Díez-del-Molino et al., 2018; Gauthier et al., 2020).

Genomic data contains information on both neutral and functional variation, the latter of which can be utilized to assess proxies of genetic load (Bertorelle et al., 2022). While these methods do not directly assess fitness, they utilize counts of putative deleterious mutations, inferred from functional annotations (e.g. Cingolani et al., 2012) and/or genomic conservation scores (e.g. Davydov et al., 2010), to approximate genetic load. Counts are often partitioned into heterozygous and homozygous counts, with the former presumed to be masked and the latter realized under an assumption of at least partial recessivity for deleterious mutations (García-Dorado & Caballero, 2000; Huber et al., 2018; Manna et al., 2011). Investigations into genetic load in this way allow for examining the complex dynamics that can occur when populations decline, such as purging of strongly deleterious mutations, but increased realization of weakly deleterious ones (Dussex et al., 2023).

Although they come with their own challenges, genomic methods offer powerful tools for co-analysis of both neutral and functional genetic diversity. Utilizing such methods allow for inferences of genetic erosion from multiple angles, which can help to then identify and refine metrics incorporated into conservation assessment (Fedorca et al., 2024; Hoban, Paz-Vinas, et al., 2024; O'Brien et al., 2022). For instance, a recent study in rattlesnakes added nuance to the functional vs neutral genetic diversity debate, finding the correlation between genome-wide functional and neutral diversity expected by theory, but that this correlation was weaker for projected future levels of neutral genetic diversity (Mathur et al., 2023). Studies such as these that investigate patterns of genetic erosion with genomic methods can not only identify populations in need of conservation action, but simultaneously inform the development of metrics to assess them.

Investigation into genetic erosion in insects remains limited. Inferences made from studies on endangered terrestrial vertebrates are not necessarily directly informative of expected dynamics in insects due to intrinsic differences between the two groups (Webster et al., 2023). Insects, due to their considerably larger population sizes and largely being composed of r-strategist species, may instead be more likely driven to extinction by extrinsic factors before genetic factors contribute (Romiguier et al., 2014; Webster et al., 2023). As such, there is a considerable gap in our understanding and assessment of genetic risks posed to insect populations experiencing demographic declines. This requires empirical studies across multiple insect species to capture the wide spectrum of ecological characters found in insects.

Butterflies have been an important model group for improving our understanding of the dynamics of insect decline. This makes them prime candidate systems for exploring how genomic metrics can inform insect conservation (Sucháčková Bartoňová et al., 2023). As one of the most well studied groups of insects, butterflies have a long history of recorded demographic trends going back centuries in some regions (Habel et al., 2016; van Strien et al., 2019) and are utilized as indicators of trends in other terrestrial insect species (Thomas, 2005; van Swaay et al., 2008). Species can range from generalist to highly specialized and exhibit differential demographic responses to anthropogenic change (Ekroos et al., 2010; Habel et al., 2016), which may be reflected in differential genetic erosion. Furthermore, nearly half of currently available insect chromosome-level reference genomes come from Lepidoptera species (Figure 1). Jointly, this makes butterflies and moths the currently best candidate groups for multi-species investigation of population decline and associated genomic erosion in insects. Finally, as butterflies serve as indicator species for biodiverse grassland habitats, they have the potential to shed light on the effect of grassland loss on insect functional connectivity and genomic erosion.

Thesis Aims

The broad aims of this thesis are to (1) investigate if grassland butterflies exhibit patterns consistent with genetic erosion in human-dominated landscapes and (2) identify which genomic metrics can most effectively capture genetic risks posed to these species to efficiently inform conservation assessments. I address these aims utilizing wild butterflies from southern Sweden, where formerly biodiverse semi-natural grasslands have largely been abandoned or converted to arable and forest land over the past two centuries (Karlsson, 2021). As grassland habitat has declined, so too has the abundance of butterflies associated with these habitats (Pettersson & Arnberg, 2024; van Swaay et al., 2022), potentially resulting in genetic erosion. These declines are not equivalent across taxa, rather, species assemblages have become increasingly dominated by generalist compared to specialist species (Ekroos et al., 2010; Habel et al., 2016). Using population genomic approaches across multiple species, I investigate if, and to what extent, these demographic declines have resulted in genetic diversity declines and if they differ between habitat generalists and specialists.

In **Paper 1**, I investigate to what extent present day land cover contributes to variation in genetic diversity, functional connectivity, and rates of inbreeding in grassland butterfly populations. To do this, I estimate the relationships between the proportion of grassland, arable, and forest land in the surrounding landscape and the levels of genetic diversity and differentiation within and between populations.

In **Paper 2**, I investigate if the variation in genetic diversity, differentiation, and rates of inbreeding among populations estimated in Paper 1 has existed historically, or arisen in the past century as a product of habitat decline and fragmentation. To assess this, I generate whole genome sequencing data from pinned specimens in museum collections that correspond to a subset of the populations sampled in Paper 1, allowing a direct comparison of estimates between the two time periods. In addition, I assess if population decline has led to reductions or increases in the abundance of putatively deleterious mutations, indicative of the potential fitness impact of changes in genetic diversity.

In **Paper 3**, I examine how common the population-level variation in genetic diversity, differentiation, and inbreeding observed in the focal species of Papers 1 and 2 are across a larger set of 11 grassland-associated butterfly species. The aim of this broader survey is to understand how habitat loss in an agriculturally dominated landscape generally affects functional connectivity and inbreeding in grassland-associated butterflies. The species chosen for this paper include the most common species found in Sweden, as well as a subset of the European Grassland Butterfly Indicator species, which contains both widespread and specialist species.

In **Paper 4,** I develop a general, reproducible workflow for the population genomic analyses required to answer the questions of Papers 1, 2, and 3, focusing on tools targeting low coverage data. The aim of this workflow is to provide automated and comparable population genomic analyses of the different study species. An additional aim is to make the workflow generalized, customizable, and documented, allowing it to be accessible both for addressing evolutionary research questions and usable for conservation assessments.

Methods

Study systems and sampling

Across the three empirical papers of this thesis (**Papers 1, 2,** and **3**), I generated whole genome resequencing data for 19 grassland butterfly species, ranging from individual- to population-level samples (Table 1). Individual-level samples involved single individuals representing a species for a given time period. Population-level samples refer to those utilizing multiple individuals sampled at the same locality in the same point in time, with 'population' in this thesis largely referring to individuals of the same species that inhabit the same locality. In **Papers 1** and **2**, analyses focused primarily on population-level analyses of three focal species of blues (Lepdioptera: Polyommatinae): *Polyommatus icarus*, *Plebejus argus*, and *Cyaniris semiargus*. These species were chosen as relatively common species from a single clade that capture different levels of habitat specialization, with *P. icarus* being a habitat generalist, and both *P. argus* and *C. semiargus* being grassland habitat specialists. Additionally, since systematic monitoring of butterflies began in 2010 in Sweden, these species are known to have experienced periods of demographic decline, with consistent declines in *P. icarus* and *C. semiargus* and fluctuating patterns in *P. argus*, which has overall been relatively stable (Pettersson & Arnberg, 2024). These declines reflect a sensitivity to drivers of insect decline and likely began well before systematic monitoring, as butterfly decline in European countries has been estimated to be occurring since at least the end of the 19th century (Warren et al., 2021).

We collected individuals of the three focal species in grassland sites across southern Sweden in the provinces of Skåne, Blekinge, Småland, and Öland (Figure 2). This region of Sweden is characterized by heavily human-dominated land use from urban areas, production forestry, and large-scale crop agriculture (Figure 2), much of which has been converted from grassland over the past two centuries (Karlsson, 2021). Per species, we sampled 4-10 individuals from 22 sampling localities where they occurred, resulting in 11, 7, and 6 sampled populations in **Paper 1** for *P. icarus*, *P. argus*, and *C. semiargus*, respectively (Figure 2). Three, four, and four of these sampled populations (for *P. icarus*, *P. argus*, and *C. semiargus*, respectively) were carried over from **Paper 1** to **Paper 2**, where they served as representatives for modern levels of genetic diversity. I then selected historical population-level samples (4-11 pinned specimens collected in a single locality and year) from the entomological collections of the Biological Museum at Lund University. These specimens were estimated to be collected in localities 3-50km (median 16.44km) from the corresponding modern localities between 1917-1956, being representative of conditions during the early second wave of agricultural modernization in Sweden (Ihse, 1995). The accuracy of distances between historical and modern localities depends upon how close the specimen was sampled to the location given on the label, most often the nearest parish to the point of collection.

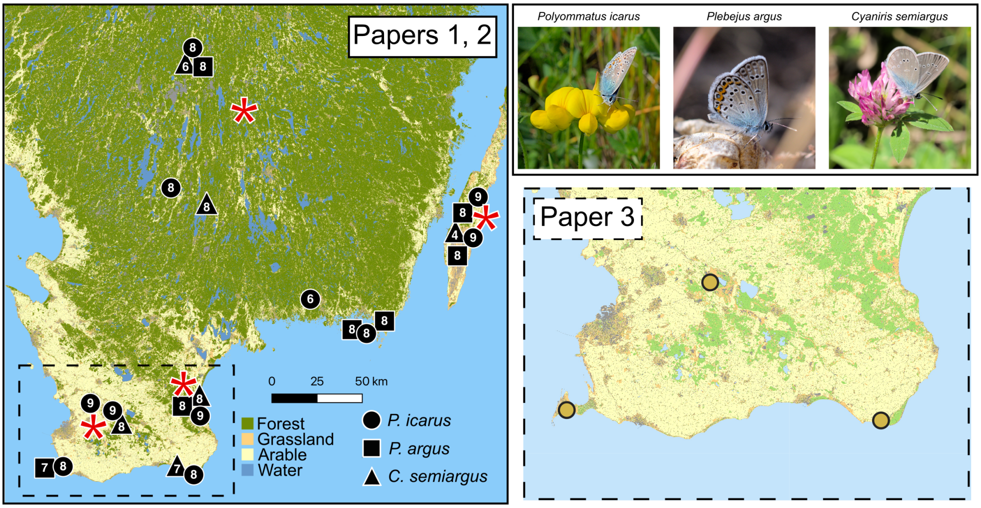

Figure 2. Focal study species and partitioning of the study area across the thesis papers. (Left) Map of the study region for Papers 1 and 2. Black points represent sampling sites from Paper 1 of contemporary butterflies of the three focal butterfly species, where the point shape represents the species and the number inside the point represents the number of individuals collected at a given sample site. Map is colored by the land cover of the study area, which is associated with genetic diversity and genetic differentiation in Paper 1 for four land cover categories: forest, grassland, arable land, and water. Red stars illustrate locations for which pinned historical specimens were sequenced for Paper 2, where they are compared with proximal contemporary specimens to assess changes in genetic diversity, differentiation, inbreeding, and mutational load over time. Dashed box represents study extent of Paper 3, where a narrower spatial focus was given to a wider species set. (Upper Right) Images of the three focal study species in Papers 1 and 2. *P. icarus* and *C. semiargus* were additionally included in Paper 3, as they are Widespread and Specialist European Grassland Butterfly Indicator species, respectively. (Lower Right) Study area extent for Paper 3 in southern Skåne, which examined patterns of genetic diversity, differentiation, and inbreeding in Sweden's most common butterfly species and European Grassland Butterfly Indicator species in a largely arable landscape. Points on the map depict the three general areas that were surveyed in this paper, two coastal and one inland, but are not precise representations for all species. Figure adapted from figures in Papers 1, 2, and 3.

During the field sampling of the focal species for **Papers 1** and **2**, single individuals of other Polyommatinae species were sampled opportunistically in the study area to serve as modern individuals for a set of supplemental Polyommatinae species in **Paper 2** (Table 1) to assess patterns of genetic diversity decline across the clade. Single pinned individuals collected from 1932-1967 at corresponding localities were selected from the entomological collections to act as historical comparisons to these individuals.

Table 1. Study species sampling and grouping across the thesis papers. Each study species is listed along with the papers in which data for the species is included. Study species are drawn from three subsets: species of blue butterflies (Polyommatinae, categorized into focal species and supplemental species for the thesis papers), Sweden's most common species in 2020, and the EU grassland indicator species (which are subcategorized into widespread and specialist species). The sampling level describes whether the analyses were performed using multiple individuals sampled per population (population-level), or if single individual samples were used for analyses (individual-level). Finally, the conservation status of the species from the 2020 Swedish Red List is given.

Species	Papers	Species subsets	Sampling level	Swedish Red List 2020
Polyommatus icarus	1, 2, 3	Polyommatinae (focal) Indicator Widespread	Population	LC
Plebejus argus	1, 2	Polyommatinae (focal)	Population	LC
Cyaniris semiargus	1, 2, 3	Polyommatinae (focal) Indicator Specialist	Population	LC
Agriades optilete*	2	Polyommatinae (suppl.)	Individual	LC
Aricia agetis	2	Polyommatinae (suppl.)	Individual	LC
Celastrina argiolus	2	Polyommatinae (suppl.)	Individual	LC
Polyommatus amandus*	2	Polyommatinae (suppl.)	Individual	LC
Phengaris alcon*	2	Polyommatinae (suppl.)	Individual	EN
Scolitantides orion*	2	Polyommatinae (suppl.)	Individual	EN
Plebejus argyrognomon*	2	Polyommatinae (suppl.)	Individual	CR
Aphantopus hyperanthus	3	Common (Sweden 2020)	Population	LC
Aglais urticae	3	Common (Sweden 2020)	Population	LC
Aglais io	3	Common (Sweden 2020)	Population	LC
Maniola jurtina	3	Common (Sweden 2020) Indicator Widespread	Population	LC
Anthocharis cardimines	3	Indicator Widespread	Population	LC
Coenonympha pamphilus*	3	Indicator Widespread	Population	LC
Lycaena phlaes	3	Indicator Widespread	Population	LC
Ochlodes sylvanus	3	Indicator Widespread	Population	LC
Cupido minimus*	3	Indicator Specialist	Population	NT

*Species did not have a chromosome-level reference genome at the time of analysis and was mapped to the reference of the closest available, which is specified in the relevant paper.

Based on the results of **Papers 1** and **2**, we returned to sample sites in Skåne, Sweden's southernmost province where the landscape is dominated by arable land, to sample a wider set of species in the region for **Paper 3**. We sampled a set of 11 species, selected from two sources: (1) the five most common species recorded in Sweden in the 2020 Swedish Butterfly Monitoring Survey (Pettersson et al., 2022) and (2) the European Grassland Butterfly Indicator species list (van Swaay et al., 2022), limited to species present in the province without a protected status (Table 1). For **Paper 3**, we collected population-level samples in the three regions of Skåne depicted in Figure 2.

Molecular methods

We extracted DNA from the thorax or thorax and head of modern butterfly specimens, homogenizing the tissue by crushing with a pestle. Extractions of modern specimens largely used and followed the manufacturer's protocols for the Qiagen DNeasy Blood and Tissue Mini Kit or the Applied Biosystems MagMAX CORE Nucleic Acid Purification Kit. Extractions using the latter kit were performed utilizing the KingFisher Flex System. Tissue remaining from modern specimens after extraction (wings, abdomen) is retained at -80C or has been registered and archived in envelopes in the entomological collections of the Biological Museum at Lund University.

For extracting DNA and preparing sequencing libraries from pinned specimens, we utilized a more specialized protocol for historical Lepidoptera specimen described by Twort et al. (2021). As DNA in pinned specimens is expected to be degraded and of low quality, all steps in this process required additional care to avoid contamination. We therefore limited lab work to a laboratory dedicated to historical DNA, sterilizing work areas using bleach and UV light exposure, and performed all steps in a UV recirculating cabinet.

To minimize destruction to the pinned specimens, while also maximizing the amount of DNA extracted, we extracted DNA from the abdomen of male specimens. Abdomens of female Lepidoptera are not suitable for such extractions as they may contain spermatophores that would result in sequencing more than just the genome of the pinned individual. To extract DNA from the abdomens, we submerged them in extraction buffer, allowing this to digest the soft tissue, carrying over the digest to subsequent steps and resuspending the abdomen in glycerol to be returned to the pinned specimen (Figure 3). We then prepared Illumina libraries from the individual DNA extracts, customizing the PCR cycle count per sample (12-18x) and performing at least six PCR replicates per sample to increase library complexity.

Libraries from both modern and historical specimens were separately sequenced on Illumina NovaSeq 6000 or X platforms as paired-end 150bp reads by the National Genomics Infrastructure Stockholm within SciLifeLab.

Figure 3. Collecting contemporary butterflies and processing historical specimens. (Left) I caught contemporary butterflies by hand netting, bringing them back to Lund University where I froze them at -80C for subsequent DNA extraction. (Right) I selected historical specimens from the entomological collections of the Biological Museum at Lund University which could be used as population-level samples by selecting multiple individuals sampled in the same year at the same locality for DNA extractions. I extracted DNA from the abdomens of male specimens without homogenizing the tissue to maintain anotomical structures. I then resuspended the abdomens in glycerol and stored them with the original specimen as shown here.

Genomic analyses

Across **Papers 1**, **2**, and **3**, I evaluated similar genomic metrics in each of the species to assess genetic diversity, differentiation, population genetic structure, inbreeding, proxies of mutational load, and contemporary effective population size. To run these analyses in a comparable way on each species, I compiled most of the processing into a generalized, automated population genomic workflow using Snakemake (Mölder et al., 2021) in **Paper 4**. This workflow is made to be flexible to both modern and historical DNA samples with varying levels of sequencing coverage, and so uses primarily genotype likelihood-based methods, which are suited to low coverage data as they incorporate the uncertainty in genotype calling (Nielsen et al., 2011). I have written it in such a way that it can be customized to adapt to a variety of whole genome resequencing datasets with the aim of this pipeline being a resource for researchers and conservation practitioners alike.

Raw sequence data processing

All analyses were performed using paired-end short read (2x150bp) whole genome resequencing data sequenced from DNA extractions from each individual. Reads were mapped to the closest available chromosome-level reference genome for each species (Bisschop et al., 2021; Ebdon et al., 2022; Hayward et al., 2022; Lohse, Hayward, Laetsch, et al., 2023; Lohse, Hayward, Vila, et al., 2023; Lohse, Mackintosh, et al., 2021; Lohse, Weir, et al., 2021; Lohse, Wellcome Sanger Institute Tree of Life programme, et al., 2023; Mead et al., 2021). Analyses were performed across the whole autosomal genome, excluding regions marked as repetitive or with extreme high or low global sequence depth in each dataset.

In **Paper 2**, where I compare genomic data derived from pinned museum specimens with that of freshly caught individuals, separate processing paths were taken for processing historical and modern sequencing data. Historical DNA is expected to be fragmented, with insert sizes shorter than a single paired end read (Gilbert et al., 2007; Stiller et al., 2006). Therefore, rather than mapping read pairs, I collapsed overlapping paired end reads into single sequences. I additionally mapped historical and modern data using separate alignment algorithms, using bwa aln for historical and bwa mem for modern (as in **Papers 1 & 3**), as they perform better for short and long read lengths, respectively (Li & Durbin, 2009). Historical DNA is also expected to have modifications at the end of fragments due to post-mortem damage (Stiller et al., 2006), which can also impact alignment performance, requiring some modifications to the alignment algorithm to permit these mismatches. For analyses that involved comparisons with historical samples, I removed transitions account for the effects of post-mortem DNA damage. However, in addition to post-mortem damage, results must be considered in the context of the differences in sequence quality between the modern and historical samples, as the short fragment length in historical samples may bias them towards the reference compared to the modern ones (Dolenz et al., 2024).

Genetic diversity metrics (Papers 1, 2, and 3)

I estimated genetic diversity using three primary metrics: individual heterozygosity, nucleotide diversity (π), and Watterson's θ. Individual heterozygosity can be estimated on an individual basis and was used for species with both population- and individual-level sampling. Nucleotide diversity and Watterson's θ require multiple individuals to calculate and were estimated per population only for species with population-level sampling. As our data had variable sequencing depth, I estimated these metrics with a genotype likelihood-based approach using ANGSD (Korneliussen et al., 2014).

Genetic differentiation and population structure (Papers 1, 2, and 3)

To approximate functional connectivity between populations, I estimated genetic differentiation as pairwise population F_{ST} for all population-level sample pairs within each species. Additionally, I visualized the clustering of individuals in each species dataset using principal component analysis (PCA), and formally tested for genetic clustering using admixture analyses. As with the genetic diversity metrics, I performed these analyses using a genotype likelihood framework using PCAngsd (Meisner & Albrechtsen, 2018), and NGSadmix (Skotte et al., 2013), respectively.

Inbreeding coefficients (Papers 1, 2, and 3)

To estimate levels of recent inbreeding, I inferred runs of homozygosity for each individual using BCFtools/RoH (Narasimhan et al., 2016). As estimation of runs of homozygosity can be influenced by analysis parameters (Silva et al., 2024), I estimated these from genotype likelihoods and genotype calls. I utilized inferred runs of homozygosity >100kb in length to infer the recent inbreeding coefficient, F_{RoH}, which corresponds to the proportion of the autosomes that is in runs of homozygosity over a specified length (McQuillan et al., 2008). Runs of homozygosity >100kb in length in these species correspond to identical by descent haplotypes that share a common ancestor within the past ~180 generations. To interpret how much of the variation in heterozygosity was explained by variation in the inbreeding coefficient F_{RoH}, I estimated a corrected estimate of heterozygosity outside of runs of homozygosity using the following adjustment from Quinn et al. (2023): $H/(1-F_{RoH})$, where H is the genome-wide estimate of individual heterozygosity.

Burden from putatively deleterious mutations (Paper 2)

Where I produced genomic data from both modern and historical specimens, I estimated the change in putatively deleterious mutation counts over time as a proxy for the change in mutational load. To estimate this, for each single nucleotide polymorphism, I used the Ensembl Variant Effect Predictor (McLaren et al., 2016) to assign an impact based on the available genome annotations. In genic regions, these impacts are ‘HIGH’ e.g. loss of function mutations, treated as strongly deleterious, ‘MODERATE’ e.g. missense mutations, treated as weakly deleterious, and ‘LOW’ e.g. synonymous mutations, treated as neutral. I assessed total mutation burden per individual as the total count of alternate alleles for a given impact category divided by the total count of alternate alleles at all sites, in order to account for differential ability to call alternate alleles in the modern and historical samples (as in Dehasque et al., 2024; Femerling et al., 2023). I also estimated how much of this burden is likely expressed, thus having fitness consequences, by performing the same count for only homozygous alternate alleles, assuming that most deleterious mutations are at least partially recessive (García-Dorado & Caballero, 2000; Huber et al., 2018; Manna et al., 2011).

In addition to a functional annotation-based estimate of mutation burden, I estimated mutation burden at conserved position. I estimated Genomic Evolutionary Rate Profiling (GERP) scores for positions in each reference genome using 107

Lepidoptera chromosome-level outgroup genomes using the method implemented in the GenErode pipeline (Kutschera et al., 2022). I identified positions with the top 1% of GERP scores for a species as the most highly conserved, with derived alleles being presumed deleterious at these positions. As a relative estimate of mutation burden per individual at these sites I quantified the count of derived alleles, multiplied by their GERP score, divided by the total count of derived alleles. As with the functional annotation-based estimate, I separately calculated this estimate with only homozygous derived genotypes included.

Linkage disequilibrium effective population size (Paper 3)

To estimate contemporary effective population size and its trajectory over the past century, I utilized the linkage disequilibrium approach implemented in GONE (Santiago et al., 2020). This approach uses single nucleotide polymorphisms within a population sample to infer effective population size changes in recent generation using patterns of linkage disequilibrium at varying recombination rates. I utilized a constant recombination rate per species for this method, which assumes that the recombination rate of pairs of loci corresponds to their physical distance, assuming 50cM per chromosome, as is typical for butterflies (Mackintosh et al., 2019). To account for regional variation in effective population size trajectories and because this method requires that the sample does not have any genetic substructure to be accurate (Novo et al., 2023), I performed this analysis separately for each sampling locality.

Land cover analyses

In **Paper 1**, I associate variation in heterozygosity within sampled populations and genetic differentiation between sampled populations with land cover surrounding and between sampling localities to assess landscape predictors of genetic diversity and functional connectivity. I utilized the 2018 SMD Swedish Land Cover Database v1.1 (*Nationella Marktäckedata (NMD)*, 2018) and the Landscape Metrics R package (Hesselbarth et al., 2019) to calculate the proportion of arable, forest, water, and grassland land cover in concentric circles ranging from 500m to 20km around each sampling locality (Figure 4A). I additionally calculated the proportion of the same four land cover types in a 10km wide buffer between each sampling locality (Figure 4B).

To determine how land cover relates to genetic diversity, I separately fit linear mixed models for each species with heterozygosity as a response to the proportion of a given land cover category within a given radius, including sample site as a random effect. For each combination of land cover type and radius, I selected the best fitting model as the one with the lowest AIC, ranking models with a difference in AIC of less than two as equivalent fits.

To determine how land cover between sampling localities relates to genetic differentiation between sampled populations, I fit linear mixed models per species with F_{ST} as a response to fixed effects of distance and the proportion of a given land cover type between site pairs. I additionally fit a model per species with distance as the only fixed effect. To account for multiple membership of sampling localities in population pairs, I included a membership matrix as a random effect in each model. I selected the best fitting model with the same criteria as for the genetic diversity models.

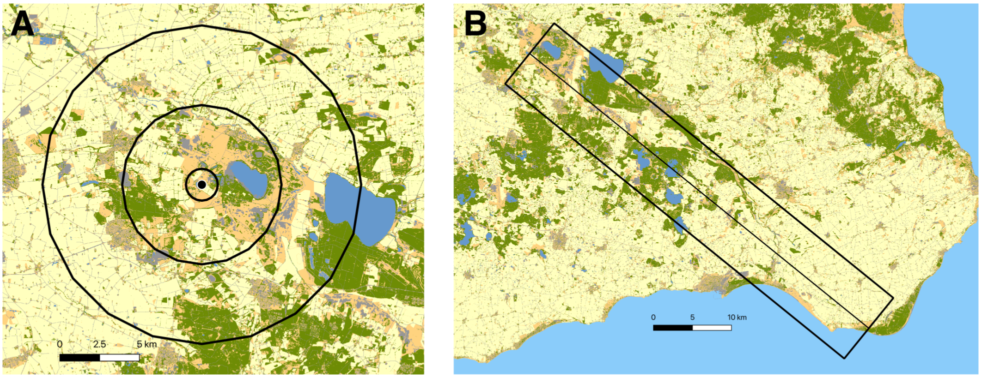

Figure 4. Land cover analysis buffers around (A) and between (B) sample sites. Land cover proportions were calculated within concentric circles ranging from 500m to 20km for each sample site, giving an assessment of local to landscape scale abundance of the four land cover categories: grassland, forest, arable, and water. To estimate the proportions between sites, a 10km wide rectangular buffer, centered on and spanning between the the two sample sites was used. Figure from Figure S2 in Paper 1.

Results and Discussion

In **Paper 1**, I utilize within species variation in genetic diversity to investigate if landscape driven differences in abundance have resulted in differences in genetic diversity. Of the three focal blues in this paper, only the most specialized species, *Cyaniris semiargus*, has consistently strong correlations between individual heterozygosity and the composition of the surrounding landscape. Heterozygosity is greater in *C. semiargus* populations sampled at sites with more grassland area in the broader landscape ($r > 5$km, Figure 5A), and lower in populations embedded in locally arable landscapes. The generalist, *Polyommatus icarus*, and the heathland specialized *Plebejus argus*, showed considerably lower variation in heterozygosity, and while some of this variation could be attributed to landscape variables, including positive relations to grassland area, patterns were largely driven by populations on islands or at the coastline.

The relatively uniform levels of genetic diversity in *P. icarus* and *P. argus*, appear to be related to their persistence as well-connected meta-populations across southern Sweden. Both species show patterns of isolation by distance (Figure 6A), though the pattern is somewhat weaker in *P. icarus* compared to *P. argus*, which may relate to the latter's more specialized ecology. Specialists are less likely to enter the matrix surrounding habitat patches (Brückmann et al., 2010; Habel et al., 2020; Jambhekar & Isvaran, 2022), which may reduce gene flow between *P. argus* subpopulations compared to the generalist *P. icarus*, increasing the isolation by distance. For both species, these levels of gene flow across the region are still sufficient for heterozygosity to be maintained relatively evenly across sampled populations. Even modest levels of gene flow can enable subpopulations in a meta-population to maintain similar heterozygosity levels to panmixia (Allendorf et al., 2022). The variation in heterozygosity in *C. semiargus* suggests that gene flow is lower in this species, consistent with distinct genetic clustering of all sampled populations (Figure 6B). Differentiation is still driven by a pattern of isolation by distance, but even the closest localities (~40km) are more differentiated in *C. semiargus* than distant ones (>200km) in either of the other two species. Together, this indicates that gene flow may recently have decreased or ceased between *C. semiargus* populations, with differentiation slowly eroding the historical patterns of isolation by distance.

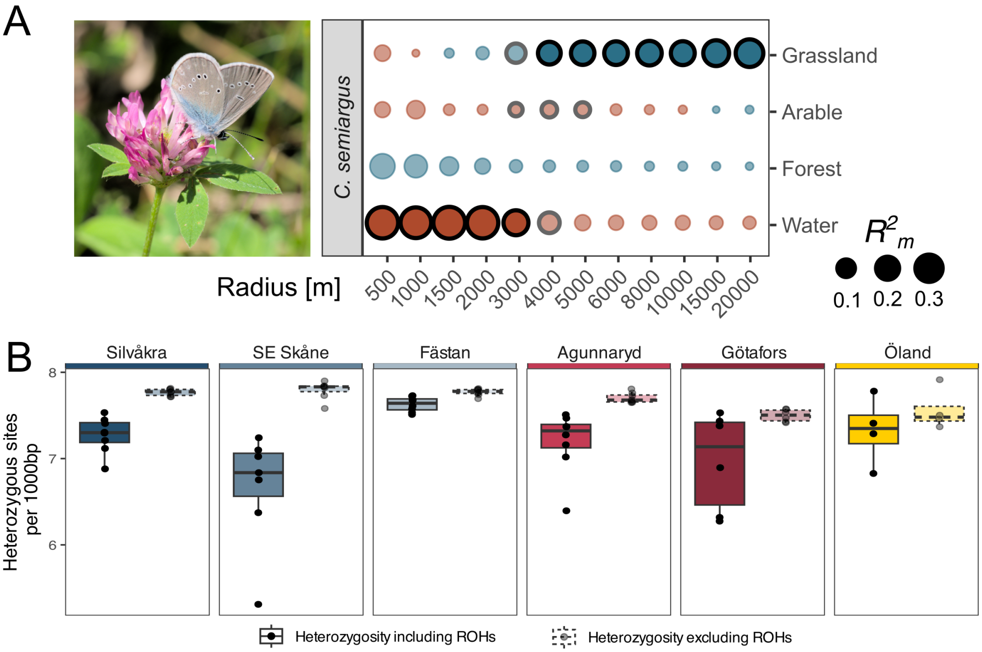

Figure 5. Greater inbreeding rates at localities embedded in landscapes with less grassland in *C. semiargus*. (A) Model fits for linear models of individual heterozygosity as a response to the proportion of four land cover types (y-axis) within a given radius (x-axis) around sample sites. Blue circles represent a positive relationship between the land cover and heterozygosity, with red circles representing a negative one. Circle size represents the marginal coefficient of determination for the model. Models with the highest AIC score for a given radius are circled in black, with models that fit similarly (ΔAIC < 2) for a given radius are circled in gray. See Paper 1 for the same model fits for *P. icarus* and *P. argus*. (B) Individual heterozygosity estimates, grouped by sampling location for *C. semiargus* where runs of homozygosity from recent inbreeding have been included in the estimate (solid boxes) and excluded (dashed). Much of the variation in heterozygosity that is determined by land cover variables is due to variation in recent inbreeding. Figure adapted from Paper 1.

The possibility that *C. semiargus* has experienced recent isolation of subpopulations is supported by estimates of inbreeding from runs of homozygosity. Almost all the variation in heterozygosity between subpopulations is explained by variation in rates of inbreeding related to bottlenecks in the past 80, and especially 40, years (Figure 5B), largely aligning with reductions in grassland area during the second wave of agricultural modernization in the region (Ihse, 1995; Karlsson, 2021). This time frame is estimated using the lengths of the runs of homozygosity in individual genomes, and their distribution can help to shed light on the character of decline (F. C. Ceballos et al., 2018). In this case, runs of homozygosity suggest *C. semiargus* populations are largely experiencing bottlenecks and/or isolation. This pattern is concerning, as inbreeding depression is documented in small, isolated butterfly populations (Nieminen et al., 2001; Saccheri et al., 1998), suggesting that in localities where grassland habitat in the surrounding landscape has become scarce, populations may enter an extinction vortex fueled by inbreeding.

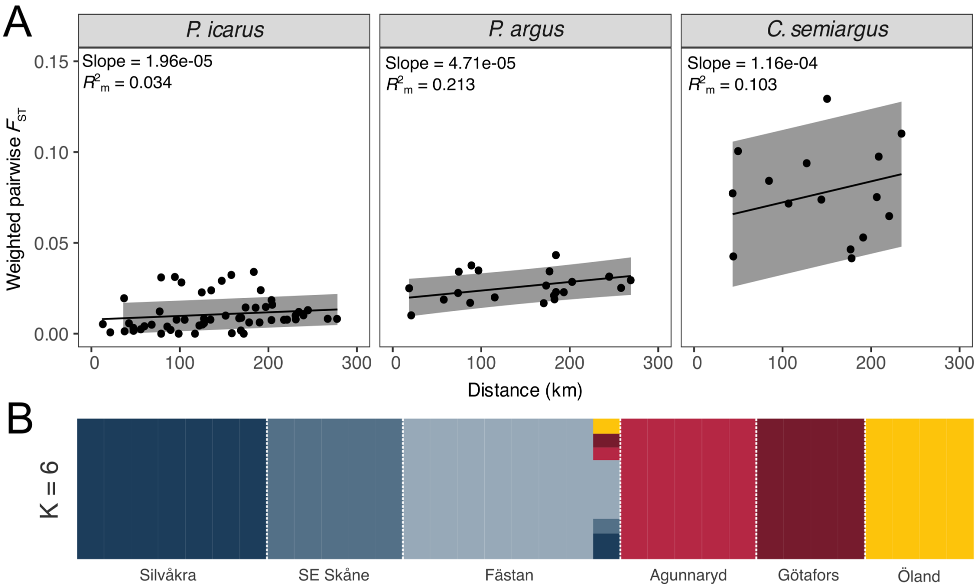

Figure 6. Substantial isolation of *C. semiargus* compared to *P. icarus* and *P. argus*. (A) Isolation by distance in the three focal species of Paper 1, expressed as regressions of pairwise F_{ST}, a measure of genetic differentiation, between sampling localities by their geographic distance. (B) Best fit result for genetic clustering of *C. semiargus* individuals. Each bar represents an individual, ordered by sampling locality, while each color corresponds to the probability of an individual belonging to one of *K* genetic clusters in the model. The best fit for *C. semiargus* separates all localities into distinct genetic clusters, in line with higher F_{ST} estimates in this species compared to *P. icarus* and *P. argus* at all geographic scales. Figure adapted from Paper 1.

While the abundance of these long runs of homozygosity appears to indicate that isolation and inbreeding in *C. semiargus* is recent, genetic data from museum specimens analyzed in **Paper 2** help to confirm this. By sequencing pinned museum specimens collected in similar regions to our modern sampling localities, we identify that levels of heterozygosity in all three species were higher historically, with decline being greatest in *C. semiargus*, corresponding to roughly a 3.3% reduction in heterozygosity per decade (Figure 7). The more extensive decline in *C. semiargus* seems to be a product of increased isolation and inbreeding, as suggested by the results of **Paper 1**. Historical populations of all three species had generally low levels of genetic differentiation and low inbreeding coefficients (Figure 7), and both *P. icarus* and *P. argus* have seen slighter increases in genetic differentiation. However, in these species I find no evidence that the decline in grassland area resulted in increases in inbreeding.

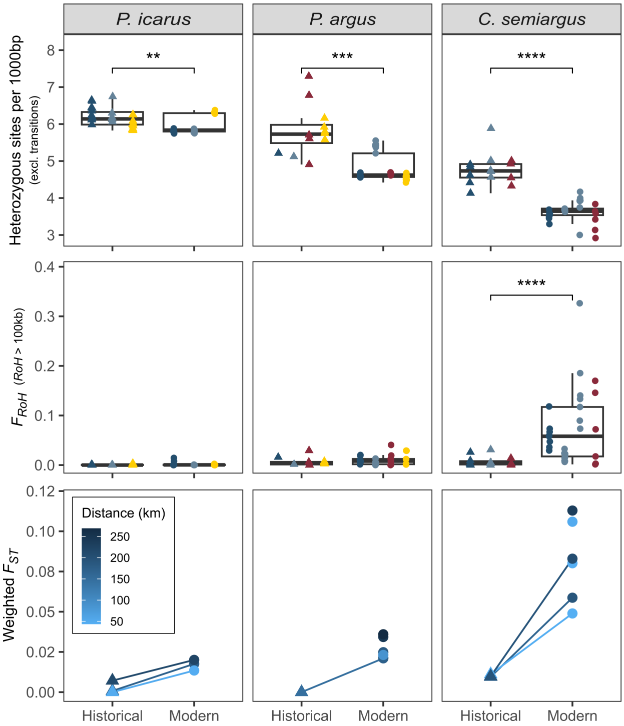

Figure 7. Comparisons between historical and modern specimens infer changes in genetic diversity, inbreeding, and differentiation over time. For the three focal species of Paper 2, changes in individual heterozygosity (upper), the inbreeding coefficient F_{RoH} (middle), and genetic differentiation estimated with F_{ST} were compared across historical (1932-1967) and modern samples (2020-2022). Points in the upper and middle plots are colored by sampling locality, with corresponding localities across time sharing colors. Points and lines in the lower plot are colored by the geographic distance between localities in the pairwise comparison. Differences between time periods for heterozygosity and F_{RoH} were evaluated statistically with an unequal variance t-test, with significant differences shown ($p < 0.05$): * $p <= 0.05$, ** $p <= 0.01$, *** $p <= 0.001$, **** $p <= 0.0001$. Figure adapted from Paper 2.

As all three species have genome annotations available, it is possible to infer proxies for mutational load from functional diversity. Analyses of mutational load can shed light on the potential fitness effects resulting from reductions in genetic diversity. There is no evidence for change in the total count nor homozygosity of putatively deleterious mutations in *P. icarus* (Figure 8A), indicating that its modest reduction in genetic diversity may not yet have had substantial fitness consequences. In contrast, the total count of putative strongly deleterious mutations has reduced over time in *P. argus* and *C. semiargus*, and additionally for putative weakly deleterious mutations in *P. argus* (Figure 8A). Purging of strongly deleterious mutations during a bottleneck is expected from theory (Glémin, 2003; Kirkpatrick & Jarne, 2000; Wang et al., 1999), as they become more frequently exposed to selection when they are brought into homozygous state by inbreeding and drift. Selection may then efficiently remove these mutations from the population, a pattern which has been increasingly observed in genomic studies of wild populations (Dehasque et al., 2024; Dussex et al., 2021; Grossen et al., 2020; Kleinman-Ruiz et al., 2022; Xue et al., 2015). However, the observed reduction in weakly deleterious mutations in *P. argus* is surprising, as weakly deleterious mutations require rather high levels of recessivity to be purged (Glémin, 2003). More deleterious mutations tend to be more recessive, with the estimated mean dominance coefficient for weakly deleterious mutations being around 0.2-0.25 (García-Dorado & Caballero, 2000; Huber et al., 2018; Manna et al., 2011). However, the reduction is rather modest (<5%) and may indicate purging of only the most recessive mutations of this type, as has been described in the Iberian Lynx (Kleinman-Ruiz et al., 2022).

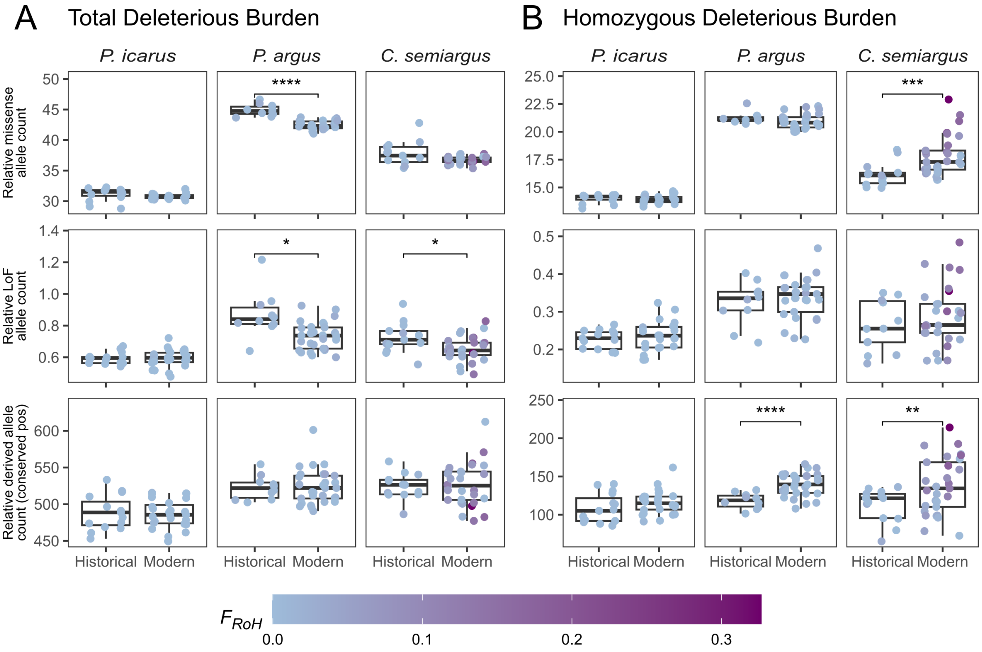

Figure 8. Changes in the burden of deleterious mutations over time. Comparisons of individual deleterious mutation burden, a genomic proxy of mutational load, between historical and modern individuals of the three focal species of Paper 2. (A) Total deleterious burden estimated per individual as the count of putatively deleterious mutations of a given class per 1000 alternate alleles. The three deleterious mutation classes shown are missense mutations (upper; presumed weakly deleterious), loss of function mutations (middle; presumed strongly deleterious), and derived mutations in conserved regions in Lepidoptera (lower; presumed strongly deleterious). Points correspond to individuals and are shaded by the individual inbreeding coefficient F_{RoH}. (B) Homozygous deleterious burden estimates across time as in A, but only counting homozygous genotypes. In both A & B, ifferences between time periods were evaluated statistically with an unequal variance t-test, with significant differences shown ($p < 0.05$): * $p <= 0.05$, ** $p <= 0.01$, *** $p <= 0.001$, **** $p <= 0.0001$. Figure adapted from Paper 2.

However, given that both classes of deleterious mutations tend towards at least partial recessivity, their presence in homozygous state will have more impact on individual fitness. Therefore, the homozygous, rather than total, count of putatively deleterious mutations will better approximate mutational load. While none of the three species have experienced changes in homozygous counts for strongly deleterious mutations, *C. semiargus* has experienced an increase in the number of homozygous weakly deleterious mutations per individual (Figure 8B). Importantly, this is largely an effect of inbreeding, as the individual homozygous mutation count is highly correlated with the individual inbreeding coefficient F_{RoH} (Figure 9). This suggests that, even without estimates of functional genetic diversity, population level variation in heterozygosity and inbreeding coefficients can provide a good proxy for mutational load in grassland butterflies. A similar relationship between F_{RoH} and mutational load has been observed in other organisms where both have been estimated genomically (Kardos et al., 2023; M. C. Keller et al., 2011; Szpiech et al., 2013; von Seth et al., 2021), suggesting that assessing genetic erosion with F_{RoH} may provide a good proxy for mutational load, making it an efficient option for screening wild populations in conservation assessments using neutral diversity alone.

We lack fitness data to confirm if these genomic estimates of burden from deleterious mutations truly translate to reductions in fitness in these populations. However, evidence of a negative relationship between heterozygosity and extinction rates in recently declined, isolated populations of the butterfly *Melitaea cinxia* (DiLeo et al., 2024; Saccheri et al., 1998), lends credence to a connection between genome-wide levels of genetic diversity and fitness in this scenario. Investigation into genomic proxies for mutational load in wild systems such as this, where collection of fitness data is possible, would help to shed light on how well the assumptions of these proxies are met in wild populations.

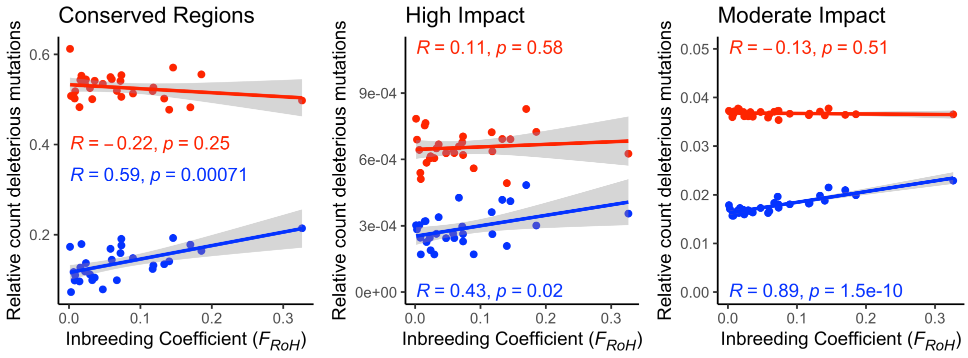

Figure 9. Homozygous, but not total, deleterious burden is associatied with individual inbreeding coefficients in *C. semiargus*. The relationship between individual inbreeding coefficients (F_{RoH}) and the relative count of putatively deleterious mutations (deleterious burden) for modern *C. semiargus* individuals. Deleterious burden is separated into three categories: conserved regions, representing mutations present in regions with high genomic conservation across Lepidoptera presumed to be strongly deleterious; high impact, representing largely loss of function mutations presumed to be strongly deleterious; and moderate impact, representing largely missense mutations presumed to be weakly deleterious. Total counts in red include both heterozygous and homozygous genotypes, whereas counts in blue only include homozygous genotypes, reflecting better the realized fitness effects under presumed partial recessivity for deleterious mutations. While inbreeding does not increase the total deleterious burden, it increases homozygosity of all deleterious mutation categories. However, this effect is strongest for weakly deleterious mutations. Figure from Paper 2, Figure S7.

Reductions in genetic diversity associated with habitat fragmentation have also been detected in three butterfly species in Finland (Fountain et al., 2016; Gauthier et al., 2020), highlighting that these patterns may be general. The parallel findings across studies and species suggest that genetic diversity decline in grassland butterflies may be relatively common in areas where grasslands have become fragmented. I assessed these declines in the three focal species of **Paper 2**, along with seven additional species of Polyommatinae, under a genetic indicator framework developed for Sweden, which categorizes projected retentions of heterozygosity over 100 years into categories of 'Acceptable', 'Warning', and 'Alarm' (Andersson et al., 2022; Johannesson & Laikre, 2020). I found that implementation of such metrics would add considerable new information about genetic erosion as a threat to grassland butterflies, as several 'Least Concern' species were observed with 'Warning' levels of genetic erosion (Figure 10). Rates of erosion in *C. semiargus* would be considered 'Alarm' (Figure 10), which suggests its current 'Least Concern' status should be elevated. While two endangered species experienced the greatest levels of genetic erosion, one actually showed some increase in genetic diversity from the historical to modern samples. Jointly, these findings highlight that genetic metrics can give complementary insights of direct relevance to conservation.

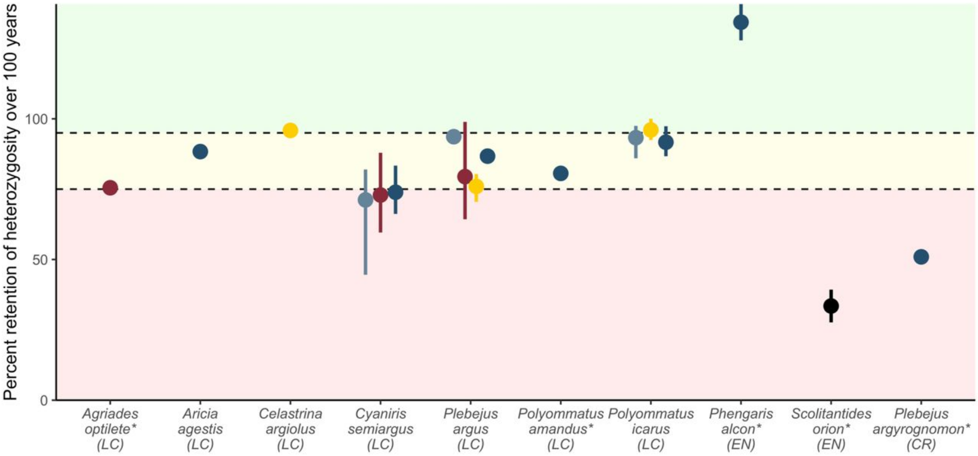

Figure 10. Projected change in heterozygosity over 100 years estimated from contemporary and museum butterflies for 10 species. Species have been grouped by conservation status on the x-axis, given in parentheses below the species name. Points represent the mean predicted retention of heterozygosity over 100 based on an extrapolation of declines in heterozygosity measured for all pairs of historical/modern specimens per species. Bars represent the range of possible estimates across all possible specimen pairs. Point and bar colors represent the region of Sweden the samples came from (see Figure 1 in Paper 2 for map). Background colors represent three genetic decline thresholds utilized in Sweden categorized into 'Acceptable' (green), 'Warning' (yellow), and 'Alarm' (red) declines. Species with a * are mapped to the reference of a closely related species, as described in Table 1. Figure from Paper 2.

While my findings highlight rather frequent declines in genetic diversity in grassland butterflies, comparisons of historical and modern DNA should be considered with caution. Historical DNA is degraded, persisting as small fragments with damaged bases that may complicate their processing. In **Paper 2**, fragments were on average ~59bp in length, compared to the paired 150bp reads that would be mapped for modern samples. While rates of post-mortem damage were rather low, the smaller fragment size of historical samples means that reads map to the reference with less context for the region they occur in, likely inducing a higher error rate compared to modern samples. Usually, this takes the form of a bias towards the reference allele as it becomes harder to map alternative alleles (Dolenz et al., 2024), but in this case, I find historical samples are generally further from the reference compared to modern samples. To address this in the assessment of mutational burden, I scale the counts of deleterious mutations by the total count of alternate alleles for each individual (as in Dehasque et al., 2024; Femerling et al., 2023), helping to account for some of this bias and improve reliability for those results. Such a correction is not as straightforward for other analyses, and they may be prone to artifacts from differences in sequence quality in historical and modern samples. For example, a potential excess of rare, false positive mismatches could cause heterozygosity in historical samples to be overestimated. One promising way for historical DNA studies to improve alignment accuracy is to utilize a variant graph incorporating information about alternate alleles into the reference, improving the mapping rate of short fragments that contain these alternate alleles (Martiniano et al., 2020). This can not only reduce bias towards the reference allele, but also reduce the need for relaxed mapping parameters that might result in false positive mismatches (Figure 11).

While there is some caution to be taken with the results of **Paper 2**, together with **Paper 1**, they suggest a pattern of genetic erosion where habitat loss and fragmentation leads to reduced population connectivity. This, in turn leads to declines in genetic diversity through increased inbreeding, increasing mutational load. This pattern is limited to *C. semiargus*, with less indication of negative fitness consequences in *P. icarus* and *P. argus*. As all three of these species share a 'Least Concern' conservation status, this suggests that inclusion of genetic components to conservation assessments in grassland butterfly species would be valuable, as genetic factors are reducing population fitness in a way that is currently not accounted for. In **Paper 3**, I assess if the patterns seen in *C. semiargus* are present in an additional nine species of grassland butterfly, and what metrics might most directly indicate a risk posed by genetic erosion.

The eleven species included in **Paper 3** vary considerably in genetic diversity, with species means ranging from 5.37 - 23.22 heterozygous sites per 1000bp (Figure 12C). While specialist indicator species have some of the lowest levels of genetic diversity of the sample set, so too do two of the most common species in Sweden. This finding reinforces that without additional context, absolute levels of genetic diversity are a poor indicator of how well a species is doing genetically.

In contrast, a clearer pattern emerges when assessing genetic differentiation and inbreeding. Levels of genetic differentiation are generally low for the most common species in Sweden and widespread indicator species, with five of these nine species showing genetic differentiation (F_{ST}) of 0.000 between all sampled populations (distances ranging from 40-60km), and populations of the remaining four never showing differentiation higher than 0.015 (Figure 12B). In contrast, both specialist indicator species (one of which is *C. semiargus*), show levels of genetic differentiation ranging from 0.05-0.10 at 40-60km distances (Figure 12B). The specialist indicator *Cupido minimus*, where two sampling localities in southwest Skåne were included, even exhibited genetic differentiation of 0.024 between populations separated by 10km, higher than in all the common and widespread species at greater geographic scales (Figure 12B).

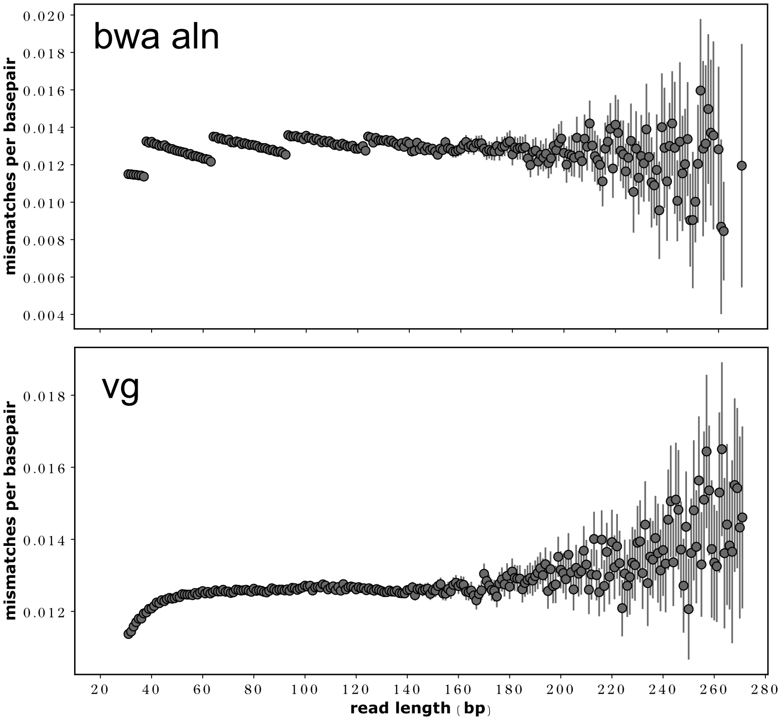

Figure 11. Improved evenness of mismatch rates across fragment sizes when mapping to a reference augmented with known variants. (Upper) Mismatch rates by fragment size when aligning reads from a historical *P. icarus* individual from **Paper 2** to a linear reference genome using bwa aln. Mismatch allowances are set by the aligner by fragment size, which can lead to some fragment sizes being biased towards false positive mismatches, while others are to false negatives. (Lower) Mismatch rates from the same fragments, but aligned to a variant graph constructed using the linear reference genome and variants called in modern individuals. Visualizations produced with AMBER (Dolenz et al., 2024).

Isolation between populations of the specialist indicator species has resulted in greater rates of inbreeding compared to the common and widespread indicator species. Average estimates of F_{RoH} for the two specialist species (*C. semiargus* = 0.112; *C. minimus* = 0.126) exceed the maximum estimates for any of the common or widespread species (maximum individual estimate = 0.1, in *A. hyperanthus*; Figure 12D). Such ubiquity of inbreeding suggests that populations of these species experience reduced fitness from isolation due to habitat fragmentation, based on the relationship between F_{RoH} with land cover and proxies for mutational load in **Papers 1** and **2**, respectively.

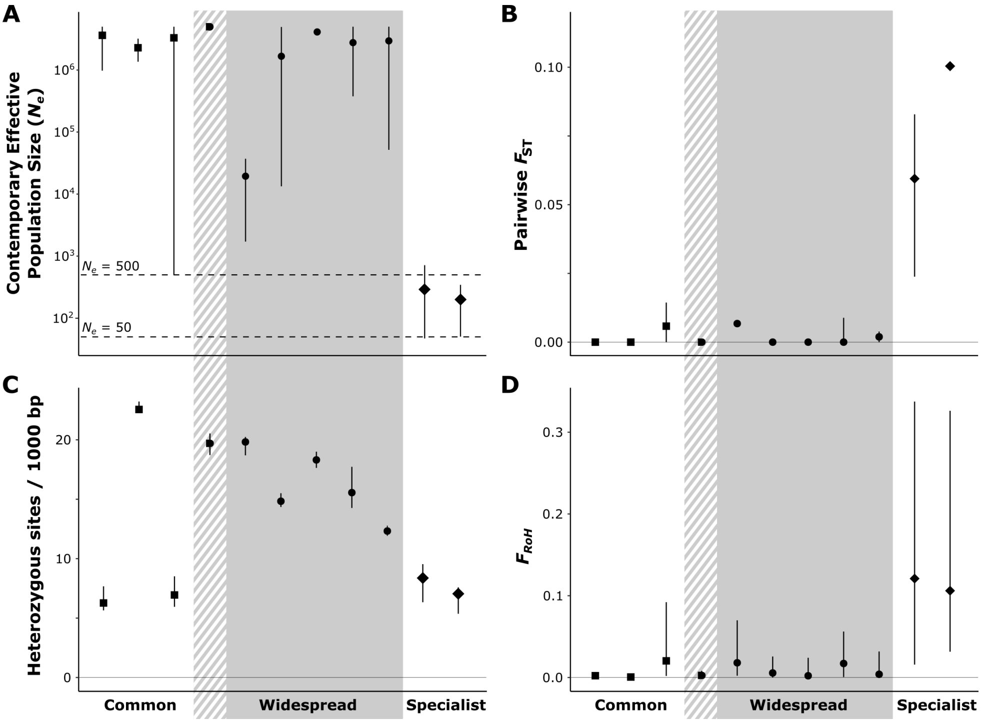

Figure 12. Patterns of contemporary effective population size, genetic diversity, differentiation, and inbreeding in grassland butterflies. Species in each plot are grouped into 'Common', selected from the five most common species in Sweden in 2020; 'Widespread', species from the widespread species category of the European Grassland Butterfly Indicator; 'Specialist', species from the specialist species category of the same Indicator. In each plot, points and ranges depict the mean and total range of estimates within each species. (A) Contemporary effective population size estimates in each sample population for the fifth generation before sampling, depicted on a log scale. Horizontal dashed lines depict sizes of 500 and 50, suggested thresholds for N_e in wild populations (Andersson et al., 2022; Franklin, 1980; Johannesson & Laikre, 2020). (B) Individual heterozygosity per 1000bp for each species. (C) Genetic differentiation estimated as pairwise F_{ST} between sample populations in each species. (D) Individual inbreeding coefficients estimated from runs of homozygosity (F_{RoH}) for each species.

Without historical samples, I cannot directly estimate levels of inbreeding in the past for these species but estimates of demographic changes in contemporary effective population size help to illustrate that the levels of inbreeding in specialist indicator species are related to recent bottlenecks. All species studied in **Paper 3** have experienced contemporary effective population sizes >10,000 within the past century in at least one of the sampled areas. Most common and widespread indicator species retain sizes this large (and considerably greater, with several maintaining sizes estimated above 1,000,000; Figure 12A). In contrast, both specialist indicator species have seen large declines in the past 50, and especially 25, years in all localities, leaving most sampled populations with effective population sizes lower than 500, and one even lower than 50 (Figure 12A), thresholds that would classify populations as having 'Warning' and 'Alarm' levels of genetic erosion, respectively (Andersson et al., 2022; Johannesson & Laikre, 2020). That these declines originated in the past few decades due to isolation is supported by the composition of F_{RoH}, which in both species consists primarily of runs of homozygosity with common ancestry within the past 50 years.

It is notable that even common and widespread species have experienced some instability in the trajectories of contemporary effective population size estimates. In several species, patterns consistent with bottlenecks and rebounds have occurred, primarily in southwest Skåne. These might be indications of recolonization dynamics, which are common among grassland butterflies (Hanski & Singer, 2001). As the magnitude of these fluctuations vary by region, it is possible they may aid in identifying regions where habitat connectivity is weaker, leading to larger fluctuations from more dramatic recolonization events.

Across **Papers 1, 2,** and **3,** I describe land use driven patterns of reduced connectivity, resulting in reductions of genetic diversity and increased inbreeding in grassland specialist butterflies. Such patterns are likely, based on proxies for mutational load and expectations of reduced adaptive potential, to reduce their ability to persist long-term under current landscape configurations. Where I have tested proposed indicators for conservation assessment of genetic erosion (decline in heterozygosity and contemporary effective population size), the affected specialist species would be flagged as at risk by at least one of the indicators. As these species currently are listed as 'Least Concern' and 'Near Threatened' in Sweden, genetic measures identify risks that are currently not captured in conservation assessment. The pipeline developed in **Paper 4** provides an efficient way to process genetic data collected for the purpose of making such assessments, but collection and generation of such data still carries a sizeable cost.

The results from this thesis can help to suggest efficient avenues for efforts to incorporate genetic diversity into conservation assessments for insects that account for their wide variation in neutral genetic diversity. Genomic estimates of recent inbreeding from runs of homozygosity (F_{RoH}) and contemporary effective population size provide the most informative, single time point assessments. When sampling of individuals is limited, F_{RoH} is especially suitable as it can be estimated from only one individual. However, it should be noted that within localities, F_{RoH} could be considerably variable across sampled individuals, so single samples may not be representative.

Estimating these metrics across multiple localities in a species can identify regions where genetic erosion is higher, highlighting areas most in need of habitat protection and restoration. Such efforts should be prioritized in specialist species, as we find they are especially vulnerable to genetic erosion, and thus provide the most efficient indicator of whether the landscape configuration may result in inbreeding. This makes it possible to identify geographic areas where genetic erosion is a concern, even with limited resources for genetic assessment.

Conclusions and future perspectives

Addressing global biodiversity decline is a daunting task, one made only more so by the variety of spatial and biological scales at which it is occurring. Considerable attention has been given in recent years to our gaps in understanding of declines in insects and in genetic diversity. Nevertheless, few studies have investigated to what extent genetic diversity decline impacts wild insect populations. This thesis utilizes genomic data to shed light on the extent of genetic erosion in grassland butterflies as a product of land use change. I find that grassland specialist butterfly species have experienced an increase in isolation, that has resulted in increases in inbreeding that increase mutational load. Local isolation and inbreeding are known to increase extinction risk in butterfly meta-populations (Nieminen et al., 2001), indicating that the genetic patterns we observe likely pose a threat to the persistence of these organisms.

Current conservation assessments do not adequately capture the risks associated with genetic erosion in these species and highlight the urgent need to incorporate genetic metrics into conservation assessment. Conservation geneticists have long advocated for greater inclusion of genetics into conservation assessments (Laikre, 2010; Laikre et al., 2009, 2020; Schmidt et al., 2023b), though integration is improving (Hoban, Bruford, et al., 2023). Adequate measures capturing risk from genetic erosion have been challenging to estimate from traditional markers, but have become more accessible to wild organisms due to advances genomic resources and methods (Formenti et al., 2022). The studies included in this thesis demonstrate that runs of homozygosity and contemporary effective population sizes estimated using genomic toolkits provide actionable information for conservation assessments and management, adding to a growing body of literature finding support for the value of these metrics in conservation (Brüniche-Olsen et al., 2018; Hoban et al., 2021; Shafer & Kardos, 2025).

As the extent of inbreeding in these butterflies largely appears to be related to reductions in grassland area in the surrounding landscape, habitat restoration plays a crucial role in maintenance of genetic diversity in these species. Local assessments of inbreeding and contemporary effective population size in butterflies can help to identify grassland areas where genetic erosion poses a risk to the insect species inhabiting them. Such assessments can play a vital role in meeting the goals of legislation targeted at curbing biodiversity decline, such as the EU Nature Restoration Law, by identifying habitats in need of restoration to curb genetic diversity decline (O’Brien et al., 2025). Inclusion of genetic monitoring into already existing monitoring programs (van Swaay et al., 2022) could provide an opportunity to effectively screen landscapes for regions in need of restoration to conserve grassland insect genetic diversity under an already existing systematic surveying framework. Subsequent usage of landscape genomic approaches at finer spatial scales than those utilized in this thesis could then provide insights into the configuration of landscape features best promoting functional connectivity. Fine scale landscape genomic methods allow for inference of how different land cover features resist gene flow (e.g. Peterman, 2018). Such methods even have power to detect small differences in resistance to gene flow, even when gene flow is generally high in a landscape (Trense et al., 2021).

The studies of grassland butterflies in this thesis illustrate that genetic erosion is a considerable conservation concern, even in relatively common species of insects. Such ubiquity of this loss of biodiversity necessitates efficient action, and these studies, along with a growing body of conservation genomic literature, identify efficient metrics for assessing genetic erosion. However, these methods can require considerable bioinformatic expertise and simply managing the analyses can have considerable overhead. The pipeline I develop in this thesis can provide an efficient method to quickly report on several useful population genetic metrics, including inbreeding estimated from runs of homozygosity and the identity by state matrices needed to evaluate landscape resistance surfaces. I hope that accessible resources such as this can facilitate increased inclusion of genomic metrics into conservation, as I believe they have strong power to identify and provide solutions to conservation concerns that are otherwise invisible to us.

Acknowledgements

It's Friday, and I'm looking forward to seeing many of you in just a few hours to enjoy some drinks at the Friday Pub. As I finish the last run throughs of this thesis, I can't help but see all the ways each of you has contributed to it. The character of everything in here has been molded by the community in our department, and communities that have surrounded it. This book is made up of all the discussions we've had, the feedback you've shared, the frustrations we've tackled, the ways you've inspired me, the work we've pursued together, and the joys that have made it possible to persist through the whole process. More than just your names are on these pages, and I am so thankful for each of you.

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Genomic studies of isolation, diversity loss, and inbreeding in grassland butterflies

PAPER 1

Nolen, Z.J., Rundlöf, M., Runemark, A., 2024. *Species-specific erosion of genetic diversity in grassland butterflies depends on landscape land cover.* Biological Conservation 296, 110694.

PAPER 2

Nolen, Z.J., Jamelska, P., Torres Lara, A.S., Wahlberg, N., & Runemark, A., 2025. *Species-specific loss of genetic diversity and exposure of deleterious mutations following agricultural intensification.* Manuscript in revision.

PAPER 3

Nolen, Z.J., van Riemsdijk, I., & Runemark, A., 2025. *Specialization predicts population isolation and inbreeding in grassland associated butterflies.* Manuscript.

PAPER 4

Nolen, Z.J., 2025. *PopGLen—a Snakemake pipeline for performing population genomic analyses using genotype likelihood-based methods.* Bioinformatics 41, btaf105.

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