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LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

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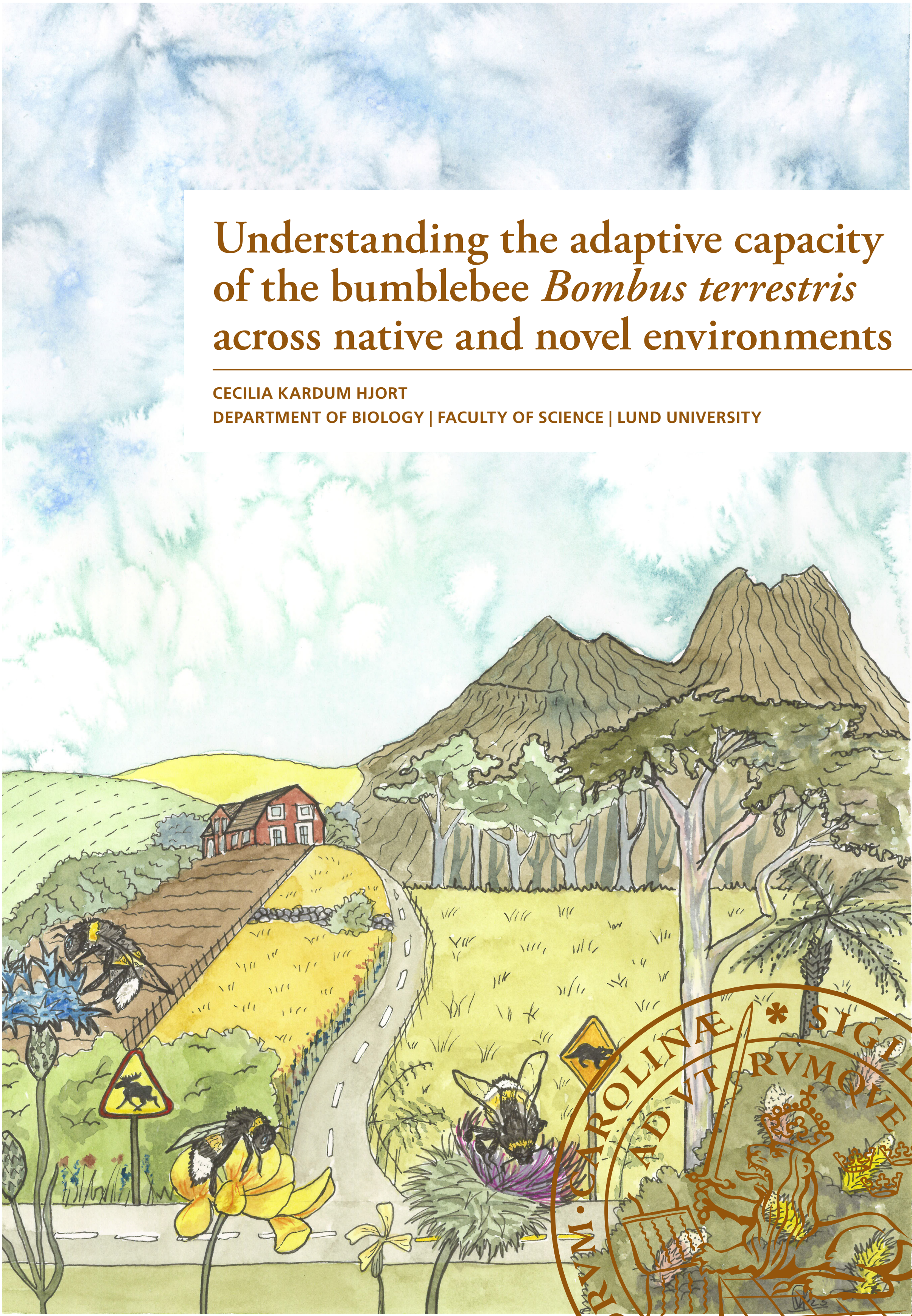
CECILIA KARDUM HJORT

DEPARTMENT OF BIOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY



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Cecilia Kardum Hjort

DOCTORAL DISSERTATION

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Abstract:

Anthropogenic stressors such as agricultural intensification, climate change, and increased densities of non-native managed bees used for crop pollination are contributing to bee declines. Understanding how and why bees have responded to past environmental changes is crucial for predicting future ones and enabling mitigation to maintain the functioning of both natural and agricultural ecosystems. The anthropogenic stressors are expected to put demand on the ability of bees to respond to future change. Thus, the ability of bee populations to persist in a particular area may require adaptive responses via either phenotypic plasticity or evolutionary adaptations in functional traits (e.g. physiological, morphological, and life-history traits). However, for bumblebees, it is not fully understood how gene flow and environmental differences affect such adaptations. In this thesis, I studied the bumblebee *Bombus terrestris* in a native (Sweden) and novel (Tasmania, Australia) setting, aiming to investigate: (1) if the introduction of commercial *B. terrestris* of a different sub-species affects wild Swedish *B. terrestris* via introgressive hybridization using whole-genome sequencing (WGS), and what the evolutionary consequences are; (2) if wild native *B. terrestris* in Sweden is genetically structured and shows selection signatures in relation to land use and climate variables, using double digest restriction-site associated DNA (ddRAD) sequencing; (3) if the recently introduced *B. terrestris* in Tasmania shows morphological variation in relation to the island's heterogeneous environment and climate, facilitating its invasion success; and finally, (4) if there is evidence for selection on functional genes and morphology in *B. terrestris* given the novel environmental and climatic conditions across Tasmania, using RADseq. From these four chapters, I outline several interesting key findings. In **Chapter I**, I found genomic differences between wild Swedish and commercial *B. terrestris* but no evidence for recent genomic introgression between the two even though feral commercial bumblebees were present in the wild. Although, non-native *B. terrestris* are likely ill-adapted to the Swedish environment but may under future climate change be able to persist and mate. **Chapter II** showed that wild *B. terrestris* in a native range consists mainly of a homogeneous population, but with evidence for weak genetic structuring, separating southern Sweden from the central and more northern regions. Our results showed no evidence for signatures of adaptation to agricultural landscape simplification, instead, weak, local genetic adaptation was documented in genes related to insecticide resistance and immune response and associated with higher proportions of agricultural cover and latitude of sites. We suggest that *B. terrestris* generalist and mobile characteristics allow the species to exploit various environments with limited genetic local adaptation and suggest this generalist species may not be as severely affected by land-use change as more specialist bumblebees. **Chapters III** and **IV** showed how *B. terrestris* in its novel range has locally adapted to the selection pressures of a novel heterogeneous environment in Tasmania, Australia, specifically to precipitation and wind. Identified genes were related to cuticle-regulated water loss prevention, immune response, olfactory system, and functions relating to flight muscles. Our results shed further light on how *B. terrestris* successfully colonized and invaded Tasmania despite the genetic consequences of a founding bottleneck. Taken together, the four chapters in this thesis demonstrate how the generalist bumblebee *B. terrestris* in both its native and novel ranges is able to thrive in the face of anthropogenic change and in new heterogeneous environments.

Keywords: Bumblebees, *Bombus terrestris*, pollination, introgression, single nucleotide polymorphisms, whole genome sequencing, landscape simplification, gene flow, morphology, environmental variation, invasion, local adaptation, island

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MADE IN SWEDEN

To my family- Basti, Pappa, Emme and Börje

Table of Contents

Abstract

Anthropogenic stressors such as agricultural intensification, climate change, and increased densities of non-native managed bees used for crop pollination are contributing to bee declines. Understanding how and why bees have responded to past environmental changes is crucial for predicting future ones and enabling mitigation to maintain the functioning of both natural and agricultural ecosystems. The anthropogenic stressors are expected to put demand on the ability of bees to respond to future change. Thus, the ability of bee populations to persist in a particular area may require adaptive responses via either phenotypic plasticity or evolutionary adaptations in functional traits (e.g. physiological, morphological, and life-history traits). However, for bumblebees, it is not fully understood how gene flow and environmental differences affect such adaptations. In this thesis, I studied the bumblebee *Bombus terrestris* in a native (Sweden) and novel (Tasmania, Australia) setting, aiming to investigate: (1) if the introduction of commercial *B. terrestris* of a different sub-species affects wild Swedish *B. terrestris* via introgressive hybridization using whole-genome sequencing (WGS), and what the evolutionary consequences are; (2) if wild native *B. terrestris* in Sweden is genetically structured and shows selection signatures in relation to land use and climate variables, using double digest restriction-site associated DNA (ddRAD) sequencing; (3) if the recently introduced *B. terrestris* in Tasmania shows morphological variation in relation to the island's heterogeneous environment and climate, facilitating its invasion success; and finally, (4) if there is evidence for selection on functional genes and morphology in *B. terrestris* given the novel environmental and climatic conditions across Tasmania, using RADseq. From these four chapters, I outline several interesting key findings. In **Chapter I**, I found genomic differences between wild Swedish and commercial *B. terrestris* but no evidence for recent genomic introgression between the two even though feral commercial bumblebees were present in the wild. Although, non-native *B. terrestris* are likely ill-adapted to the Swedish environment but may under future climate change be able to persist and mate. **Chapter II** showed that wild *B. terrestris* in a native range consists mainly of a homogeneous population, but with evidence for weak genetic structuring, separating southern Sweden from the central and more northern regions. Our results showed no evidence for signatures of adaptation to agricultural landscape simplification (i.e. the proportion of semi-natural habitat and the length of uncultivated agricultural field borders). Instead, weak, local genetic adaptation was documented in genes related to insecticide resistance and immune

response and associated with higher proportions of agricultural cover and latitude of sites. Range expansion of *B. terrestris* was evident via negative Tajima's D, suggestively in a northward direction and more inland into densely forested areas as evidenced by more recent observational data. We suggest that the species' generalist and mobile characteristics allow *B. terrestris* to exploit various environments with limited genetic local adaptation and suggest this generalist species may not be as severely affected by land-use change as more specialist bumblebees. In contrast, **Chapters III** and **IV** show how *B. terrestris* in its novel range has locally adapted to the selection pressures of a novel heterogeneous environment in Tasmania, Australia, specifically to precipitation and wind. Candidate loci found were annotated to genes involved in cuticle differentiation and cuticle-regulated water loss prevention, immune response, olfactory system, functions relating to flight muscles, and fatty acid metabolism. Our results shed further light on how *B. terrestris* successfully colonized and invaded Tasmania despite the genetic consequences of a founding bottleneck. Taken together, the four chapters in this thesis demonstrate how the generalist bumblebee *B. terrestris* in both its native and invasive ranges is able to thrive in the face of anthropogenic change and novel heterogeneous environments. This thesis is a testament to the capacity of *B. terrestris* to be a successful pollinator in native ranges while also being one of the world's most successful invasive bee species.

Populärvetenskaplig sammanfattning

Gruppen bin, som innefattar sociala humlor och honungsbin, och solitära bin, är oerhört viktiga pollinerare av både vilda blommor och odlade grödor. Men de pågående mänskligt orsakade förändringarna bidrar dock till minskningen av bin jorden runt. Just därför är det viktigt att förstå hur bin reagerar på dessa förändringar och om de kan anpassa sig för att fortleva och kunna fortsatt bidra med viktiga ekosystemtjänster inom både naturliga och jordbruksekosystem. Orsakerna bakom minskningen av bin är många, men intensifieringen av jordbruket har i hög grad bidragit. Odlingslandskapet som förut bestod av en spatial blandning av betesmarker, blomrika fältkanter och odlingsfält har på många platser i landskapet ersatts av fler och större odlingsfält. Fälten odlas intensivare och innehåller färre blomresurser i form av ogräs. Dessutom har klimatförändringarna i form av ökade temperaturer och oförutsägbara väderomställningar, ökad urbanisering, samt oavsiktliga konsekvenser ifrån användandet av kommersiella humlor till pollinering av grödor bidragit till minskningen av bin jorden runt.

Alla dessa förändringar förväntas ställa krav på binas förmåga att kunna anpassa sig, men alla bin förväntas inte reagera på samma sätt. Det kan bero på olikheter inom så kallade funktionella egenskaper så som fysiologiska och morfologiska egenskaper, samt livsegenskaper som påverkar hur pass känsliga bin är för exempelvis förändrad markanvändning och klimatpåverkan. Vissa bi-arter kan förflytta sig till bättre livsmiljöer medan andra anpassar sig till sin lokala miljö, antingen genom fenotypisk plasticitet eller evolutionär anpassning om kombinationen av rätt omständigheter så som selektionstryck, genetisk variation och genflöde finns.

Det har nyligen skett en konceptuell förändring av vårt synsätt på evolutionen, att anpassningar till mänskligt orsakade förändringar kan ske snabbare än vi tidigare trott. Det finns många exempel inom gruppen insekter på snabb evolutionär anpassning men för humlor är bevisen inte kompletta. Dessutom för många arter, inklusive humlor, är det osäkert hur genflöde och landskapsskillnader påverkar deras förmåga att anpassa sig. Genflöde kan både vara negativt för en arts anpassningsförmåga, genom ett inflöde av neutrala eller missanpassade gener, men även positivt, då ett inflöde av fördelaktiga gener upprätthåller genetisk variation som kan ligga grund för framtida anpassningar. Därför förutsätts genflöde i många fall bidra till anpassning av förändrade livsmiljöer, om de nya generna bidrar till ökad fitness. Dessutom anses fenotypisk plasticitet som svar på förändrade livsmiljöer vara ett viktigt och snabbt sätt för arter att hantera mänskligt orsakade förändringar och ge arter tid att kunna genetiskt anpassa sig. Följaktligen är sambandet mellan genflöde, fenotypisk plasticitet och genetiska anpassningar till mänskligt orsakade förändringar viktigt att förstå om bin ska fortleva i framtiden.

Vissa humlearter verkar klara miljöförändringar bra. Den mörka jordhumlan (*Bombus terrestris*), som är en viktig pollinatör i både naturliga och jordbruks ekosystem, har inom vissa områden ökat i antal, sannolikt på bekostnad av andra humlearter. Den har dessutom utökat sitt utbredningsområde inom Skandinavien som svar på ökade temperaturer. Den mörka jordhumlan kan bosätta sig och födosöka i flera olika landskapstyper. Den är en utav de mest varmanpassade humlearterna och är förutspådd att göra bra ifrån sig under framtida klimatförändringar. Dessa egenskaper bidrar till att den mörka jordhumlan förväntas vara mindre känslig för just mänskligt orsakade landskaps-och temperaturförändringar, och har förmågan att anpassa sig till dessa förändringar.

Den mörka jordhumlan används även i stor utsträckning för kommersiell pollinering av grödor, till exempel tomater, på grund av dess förmåga att effektivt kunna frigöra pollen med hjälp av vibrationer från vingarna (Buzz pollinering). Men i samband med kommersialiseringen har humlan även spritt sig som en invasiv art i områden där risken för konkurrens med vilda bin och hybridisering med vilda humlor finns. På Tasmanien, Australien, har den mörka jordhumlan lyckats bra och blivit invasiv, där selektionstrycket från den nya miljön kan ha format olikheter inom humlans morfologi som har hjälpt göra den mörka jordhumlan så framgångsrik. Sammanfattningsvis påverkas den mörka jordhumlan av mänskligt orsakade förändringar och har möjligtvis anpassat sig, men samtidigt påverkar den andra pollinatörer negativt inom både vilda och nya områden.

I min avhandling studerar jag den mörka jordhumlan i dess naturliga miljö i Sverige och som en invasiv art på Tasmanien, Australien. Inom dess naturliga miljö undersöker jag om kommersiella mörka jordhumlor hybridiserar sig med svenska vilda mörka jordhumlor och vilka eventuella genetiska konsekvenser sådan introgressiv hybridisering kan ha. Dessutom undersöker jag i Sverige om den vilda mörka jordhumlan uppvisar genetiska skillnader till förändrade landskaps- och klimatförhållanden. Inom Tasmanien undersöker jag om den invasiva mörka jordhumlan uppvisar morfologiska skillnader och skillnader i antal i förhållande till olika miljöer. Dessutom under söker jag om det finns bevis för selektion till de nya miljöerna trots begränsad genetisk variation resulterat av en genetisk flaskhals (’bottleneck’) vid introduktionen till Tasmanien.

Tillsammans visar mina studier hur både den vilda svenska och invasiva mörka jordhumlan har anpassat sig morfologiskt och genetiskt till mänskligt orsakade landskaps- och klimatförändringar, och nya miljöer men även hur kommersiell mörk jordhumla påverkar vild mörk jordhumla. Mer specifikt visar min avhandling att det finns genetiska skillnader mellan kommersiella mörk jordhumla och svensk vild mörk jordhumla. Dessa skillnader mellan dem skulle kunna ha en negativ framtida påverkan om hybridisering sker. Dock hittade vi inga bevis för att hybridisering sker. Den mörka jordhumlan i Sverige verkar vara ganska homogen, men uppvisar trots detta skillnader i genetisk struktur mellan dess södra och centrala utbredningsområde. Våra resultat visar även att den mörka jordhumlan lokalt har anpassat sig till högre andel jordbruksmark och latitud. Den mörka jordhumlan har även utökat sitt utbredningsområde, förslagsvis mer norrut och inåt landet till skogsmiljöer. Inom dess nya utbredningsområde uppvisar den mörka jordhumlan både morfologiska och genetiska skillnader som svar på den nya varierande miljön på Tasmanien, trots att den tasmanska populationen förlorat genetisk variation vid introduktionen. Sammantaget, bidrar min avhandling med en utökad förståelse för hur en generalistisk humleart har anpassat sig till mänskligt orsakade förändringar och till nya miljöer.

List of papers

Chapter I

Kardum Hjort, C., Paris, J. R., Olsson, P., Herbertsson, L., de Miranda, J. R., Dudaniec, R. Y., & Smith, H. G. (2022). Genomic divergence and a lack of recent introgression between commercial and wild bumblebees (*Bombus terrestris*). *Evolutionary Applications,* 15(3), 365–382.

Chapter II

Kardum Hjort, C., Dudaniec, R. Y., & Smith, H. G. Is local adaptation to landscape structure and climate constrained by gene flow in the native bumblebee, *Bombus terrestris*? Manuscript.

Chapter III

Kardum Hjort, C., Smith, H. G., Allen, A. P., Dudaniec, R. Y. (2023). Morphological variation in bumblebees (*Bombus terrestris*) (Hymenoptera: *Apidae*) after three decades of an island invasion. *Journal of Insect Science*, *23*(1), 1–10.

Chapter IV

Kardum Hjort, C., Paris, J. R., Smith, H. G., Dudaniec, R. Y. (2023). Selection despite low genetic diversity and high gene flow in a rapid island invasion of the bumblebee *Bombus terrestris*. *Molecular Ecology,* 00, 1–18.

Author’s contributions

Chapter I

CKH, RYD, LH, and HGS conceptualised the study. **CKH** collected samples in the field. **CKH** performed the statistical analyses with the help of JRP. **CKH** drafted the manuscript. All authors contributed to the manuscript with comments and suggestions.

Chapter II

CKH and HGS conceptualised the study. **CKH**, HGS, PO, and JE set up the design. **CKH** collected samples in the field, performed lab work and statistical analyses with help from RYD and PO. **CKH** drafted the manuscript. All authors contributed to the manuscript with comments and suggestions.

Chapter III

CKH, RYD, and HGS conceptualised the study. **CKH** collected samples in the field. **CKH** performed the statistical analyses with the help of AA. **CKH** drafted the manuscript. All authors contributed to the manuscript with comments and suggestions.

Chapter IV

CKH, RYD, and HGS conceptualised the study. **CKH** collected samples in the field and performed initial lab work. RYD finalised the lab work*. **CKH** performed the statistical analyses with the help of JRP. **CKH** drafted the manuscript. All authors contributed to the manuscript with comments and suggestions.

* CKH returned to Sweden due to the Covid-19 pandemic in 2020.

All authors in the list of papers have given their consent for the use of their work in the thesis.

Authors

Cecilia Kardum Hjort (CKH), Rachael Y. Dudaniec (RYD), Henrik G. Smith (HGS), Josephine R. Paris (JRP), Johan Ekroos (JE), Peter Olsson (PO), Andrew Allen (AA), Lina Herbertsson (LH).

Abbreviations

SNH	Semi-natural habitat
FB	Uncultivated agricultural field borders
CB	Commercial *Bombus terrestris*
WE	Wild experimental (*B. terrestris*)
WC	Wild control (*B. terrestris*)
ddRAD	Double digest Restriction-site Associated DNA sequencing
sdRAD	Single digest Restriction-site Associated DNA sequencing
WGS	Whole-Genome Sequencing
SNP	Single Nucleotide Polymorphism
EAA	Environmental Association Analysis
COI	Cytochrome Oxidase subunit 1
GO	Gene Ontology enrichment analysis
VEP	Ensembl Variant Effect Predictor tool
XP- EHH	Cross-Population Extended Haplotype Homozygosity
MaxTempWarmestMonth	Maximum Temperature of the Warmest Month
ITD	Inter-Tegular Distance

Introduction

Anthropogenic environmental and climate change

Insect pollination is essential for ecosystems and the maintenance of global biodiversity (IPBES, 2016). The most important group of insect pollinators are bees, where wild and domesticated bees are the main pollinators of the majority of wild flowering plants and crops worldwide (Klein et al., 2007; Ollerton et al., 2011; Potts et al., 2016). Among bees, wild bumblebees (*Bombus sp.*) are well recognized for their pollination services of flowers in natural ecosystems (Belsky & Joshi, 2019) and crop pollination (Klein et al. 2007), with bumblebees such as *Bombus terrestris* being important pollinators of oilseed rape (Goulson, 2003). Managed bumblebees are also efficient crop pollinators (Nayak et al., 2020; Rollin et al., 2016), and have in some systems been shown to be more efficient pollinators than honeybees (Nayak et al., 2020; Zhang et al., 2022).

However, there are concerns about declines in both the abundance and diversity of bees on local to global scales (Chagnon, 2008; Goulson et al., 2008; IPBES, 2016; Kosior et al., 2007) as well as resulting changes in bee community composition (Bommarco et al., 2012; Fourcade et al., 2019). While many bee species have declined, some species have expanded or are predicted to expand their distributions, are stable or have even increased in relative abundance (i.e. relative to other bee species) (Dew et al., 2019; Herbertsson et al., 2021; Huml et al., 2021; Kammerer et al., 2021; Kerr et al., 2015; Martinet et al., 2015).

Losses have been attributed to anthropogenic impacts (IPBES 2016) (Figure 1). Understanding how this will ultimately affect global food security and ecosystem resilience is urgent to resolve (Marshman et al., 2019). Although many of the drivers of the observed declines are known, knowledge about what is causing the declines is still incomplete. Agricultural intensification has in many cases acted as a driver of observed bee declines (Goulson et al., 2015; Kremen et al., 2002) due to resulting changes in, for example, landscape composition as crop fields increase in size and replace more or less natural habitats (Robinson & Sutherland, 2002). This has led to loss and increased spatial fragmentation and temporal discontinuity of flower-rich habitats suitable for foraging and nesting, in particular for habitat specialists (Goulson et al., 2015; Smith et al., 2014). In addition, intensive agriculture often relies on pesticides for crop protection, which have been proven harmful to bees and may lead to widespread insect mortality (Lu et al., 2020; Rundlöf et al., 2015; Tosi et al., 2022; Tsvetkov et al., 2023).

The global use and mass transport of managed bees for agricultural crop pollination services outside of native ranges have contributed to pollinator declines and biological invasions (Potts et al., 2016), where feral managed bumblebees, such as *B. terrestris,* may outcompete local native bees (Hingston & McQuillan, 1998b; Ings et al., 2005, 2006; Morales et al., 2013) and spread pathogens (Dafni et al., 2010; Evans et al., 2017; Meeus et al., 2011, but see Trillo et al., 2021). As a result, native plant-pollinator relationships have been negatively affected (Aizen et al., 2019; Hingston & McQuillan, 1998a). There is also increasing concern about the evolutionary consequences of managed bumblebees for native pollinator health and genetic integrity (Seabra et al., 2019). The use of non-native bumblebee species and sub-species for crop pollination has repeatedly created conditions for hybridization and introgression (the exchange of genetic material between species or subspecies through hybridization and repeated backcrossing, McFarlane & Pemberton, 2019) between native and non-native bumblebees (Bartomeus et al., 2020; Cejas et al., 2018, 2020; Seabra et al., 2019). This has raised concerns that locally adapted genes in wild populations are disrupted by introgression of maladapted alleles originating from escaped commercial bumblebees (Kanbe et al., 2008; Tsuchida et al., 2010; Yoon et al., 2009).

Another form of human-induced land-use change is urbanization (A. Bates et al., 2011). The consequences of urbanization are many, for example, habitat structural changes and loss (Liu et al., 2016), heat island effects, or changes in air pollution and nutrient cycling and primary production (Grimm et al., 2008). The urban environment is generally highly fragmented (i.e. a matrix of pervious surfaces and green spaces), such that bees may experience longer foraging distances to ecologically relevant patches (Theodorou et al., 2020). This may limit the connectivity to surrounding patches and the resources gathered at each patch, potentially affecting the bees' fitness. On the other hand, the matrix of different environments in cities may be more complex than adjacent rural landscapes (e.g. intensively farmed land), providing sufficient habitat for organisms to live in (Sattler et al., 2010). Urban cities may even function as refuges for bees (Baldock et al., 2015; Fortel et al., 2016).

The effects of climate change are also contributing to bee declines (Kerr et al., 2015; Potts et al., 2016). Effects may be related to mean temperatures, but also unpredictable weather conditions such as more frequent droughts (Descamps et al., 2021; Soroye et al., 2020). Climate change may result in changes to local community compositions through both the declines and increases in bee species with different life-history traits (Pardee et al., 2022). Climate changes may ultimately result in range shifts or range contractions when species no longer can expand northwards or to higher elevations to track their thermal limits (Kerr et al., 2015; Vanbergen et al., 2013). Effects of climate change may also be indirect, such as via increased inter-specific competition between resident species and from range-expanding species (Bocedi et al., 2013).

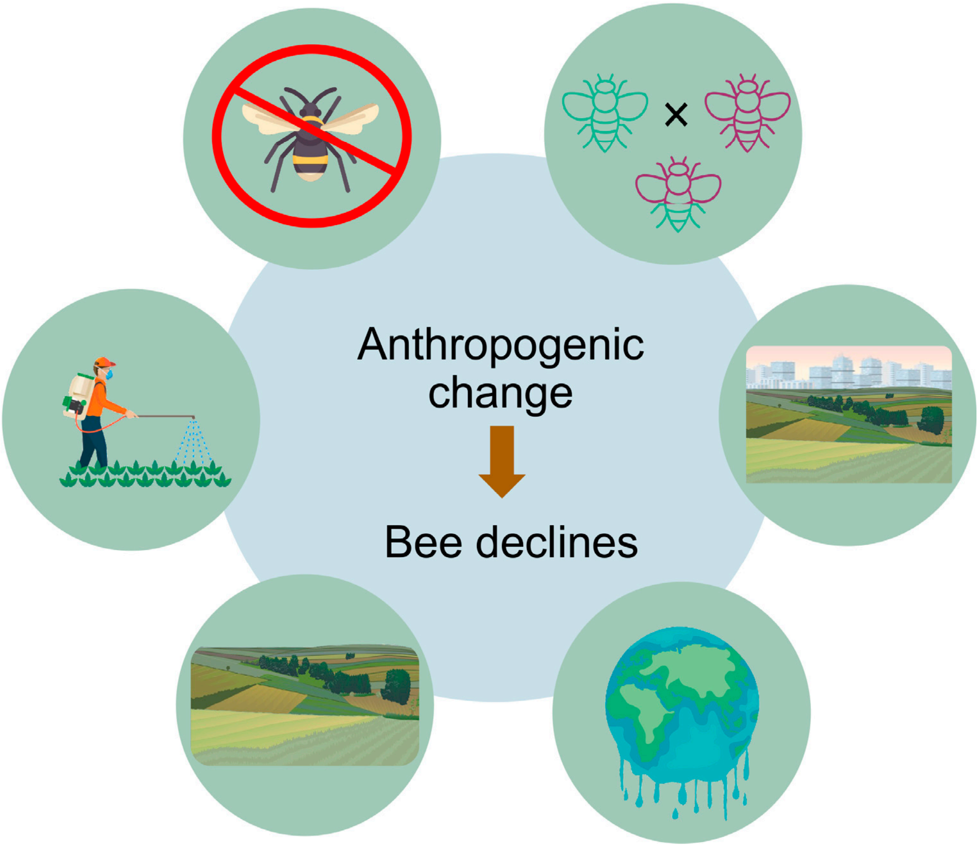


Figure 1. Different drivers of anthropogenic change that has been recognized to cause bee declines. Examples include landscape simplification and pesticide use due to agricultural intensification, competition from feral non-native commercial bumblebees and introgressive hybridization with wild conspecifics, urbanization, and climate change.

Bee responses to anthropogenic change

Bee species respond differently to anthropogenic impacts, which may partially be explained by differences in functional traits, such as physiological, morphological, and life-history traits that affect their resilience to land use and climate change (Hall et al., 2019; Kammerer et al., 2021; Pardee et al., 2022). Further, in the face of anthropogenic pressures, the persistence of bee species at large spatial scales may be secured by range shifts to more suitable areas. In contrast, persistence at local scales may require adaptive responses by either phenotypic plasticity or evolutionary adaptations in functional traits (e.g. Bocedi et al., 2013; Colgan et al., 2022). As a first step towards mitigating the negative effects of anthropogenic change, we need to better understand which bee traits respond to environmental and climatic pressures. Subsequently, understanding the adaptive potential of bees and whether evolutionary responses are an important mechanism for bees to cope with anthropogenic change should be sought. Lastly, implementing measures to uphold the evolutionary potential should be considered.

The interplay between neutral connectivity, local adaptation, and genetic diversity in light of anthropogenic change

Recently there has been a conceptual shift in our view of evolution, suggesting that rapid adaptation to environmental change may be more common than previously thought (Reznick et al., 2019). There are several studies on insects demonstrating rapid evolutionary change (Colgan et al., 2022; Dudaniec et al., 2018; Yadav et al., 2019) but for bumblebees, the evidence is still far from conclusive. Whereas the idea of rapid evolution focuses on change over time, evidence for adaptation to anthropogenic environmental change is often only possible to pursue using a space-for-time substitution approach (i.e. assuming that spatial changes are equivalent to temporal changes, Pickett, 1989). However, it is currently unclear how gene flow constrains such adaptations and thus over which distances and time-frames adaptation to local landscape features and climate can be sought. For example, in bumblebees' genetic differentiation between populations may be small, except when dispersal is constrained by environmental gradients or barriers (Jackson et al., 2018; Lozier et al., 2013). Gene flow and local adaptation are also often described as opposing forces. High gene flow between populations is theorised to homogenize locally adapted genotypes by introducing maladapted or neutral alleles, whereas reduced gene flow increases the chances of genetic adaptation to the local environment (Wadgymar et al., 2019). However, if selective forces are stronger than the homogenizing effects of gene flow, local genetic adaptation may still occur (Tigano & Friesen, 2016). This can be particularly true if genetic differentiation between populations is a result of selection on specific genes whose loci are in strong physical linkage disequilibrium (Barton, 2000). However, gene flow also maintains genetic variation by adding new (advantageous) alleles into a population, increasing the genetic material for which selection can act upon (Buckley & Bridle, 2014). Thus, it is predicted that gene flow also promotes adaptation to novel environments or to rapidly changing environments if the introduced alleles become introgressed and are beneficial for fitness (Aitken & Whitlock, 2013). To this end, examination of the relative contribution of gene flow, adaptive genetic variation, and neutral genetic diversity is a crucial step toward understanding bee responses to anthropogenic environmental and climatic change.

Intra-specific plastic or evolutionary trait changes in bumblebees related to anthropogenic change

The occurrence of phenotypic plasticity in response to environmental change may initially stabilize the population, which is beneficial as a rapid response that may counter the consequences of environmental stressors (Bonamour et al., 2019). For example, plasticity may broaden trait variation of a species and hence buffer against rapidly changing environments, where e.g. the breadth of temperatures at which a population can survive increases and thus broadens the population's tolerance and gives it time to further adapt (e.g. Lancaster et al., 2015). As plastic responses may in some cases show a more rapid response than adaptive evolutionary changes to anthropogenic change, phenotypic plasticity is considered an important mechanism for species survival (Bonamour et al., 2019). Whether observed adaptive responses are genetically derived, or a result of adaptive phenotypic plasticity (e.g. environmental, or due to developmental constraints) has in most cases not been demonstrated, and bumblebees are not an exception (but see Hart et al., 2022; Jackson et al., 2020). Nevertheless, both plastic and evolutionary responses are relevant for understanding species' sensitivities and adaptive potential, not only for short-term survival but also for long-term survival in the face of anthropogenic change (Hendry, 2016).

In bumblebees, the mean body size of queens of four bumblebee species (*Bombus hortorum, Bombus lapidarius, Bombus pascuorum* and *Bombus pratorum*) increased with increasing landscape fragmentation caused by agricultural intensification (Gérard et al., 2019), and a similar trend was later observed for the same species and *Bombus balteatus* (Gérard et al., 2020). In contrast, smaller *B. terrestris* workers are associated with highly intensively farmed agricultural landscapes (Grass et al., 2021; Nooten & Rehan, 2020; Oliveira et al., 2016; Persson & Smith, 2011). Mixed intra-specific body size responses in relation to urbanization have also been found (Austin et al., 2022; Eggenberger et al., 2019; Tommasi et al., 2022) with bumblebees in urban areas also being found to have higher intraspecific phenotypic variation in body size-associated traits (inter tegular distance, proboscis length, wing- and corbicula length) compared to their non-urban conspecifics (Eggenberger et al., 2019).

Some studies have also documented intra-specific changes in proboscis length over time or differences between populations. Examples include two common alpine species, *B. balteatus* and *B. sylvicola*, in the U.S. where proboscis length has become shorter over 40 years, suggestively in response to a climate change-induced shift in hostplants (Christmas et al., 2022; Miller-Struttmann et al., 2015). Observations of intra-specific bimodality in the proboscis length of a specialist long-tongued bumblebee, *B. pascuorum*, in urban areas have also been documented (Eggenberger et al., 2019). Furthermore, changes in bumblebee wing size as a response to urban environmental stressors and flower abundance in less urbanized areas have been documented (Gérard et al., 2018, Tommasi et al., 2022), and lastly, lower wing-loading (body weight in grams divided by the total wing area in cm^2) was found for a montane bumblebee species, suggesting adaptation to elevation (Lozier et al., 2021).

Physiological traits are generally highly sensitive to anthropogenic change and are often one of the first responses that occurs for a species (Leroy et al., 2023). Examples from bumblebees include adaptation in regulatory mechanism and cuticle formation to precipitation and neuromuscular function to temperature in alpine habitats (Heraghty et al., 2023; Jackson et al., 2020), suggested neurodevelopment, muscle and detoxification-related adaptation to agricultural stress (Hart et al., 2022), potential selection on insecticide susceptible genes due to insecticide exposure in agricultural areas (Colgan et al., 2022), and heat and oxidative stress responses in relation to urban gradients (Burdine & McCluney, 2019; Theodorou et al., 2018).

However, is not always easy to determine the genomic background underpinning the observed adaptation to novel or changing environmental conditions, especially in the wild (Endler, 2020). By adopting a genomic approach (e.g. such as single nucleotide polymorphisms (SNPs) from whole genome or reduced representation sequencing methods) and integrating environmental and population data, one can attempt to unravel if the observed adaptations are caused by phenotypic plasticity or evolutionary change (Balkenhol et al., 2017; Lancaster et al., 2022). For example, landscape genomic approaches such as Environmental Association Analyses (EAA) where one combines morphometric and environmental analyses to examine the relationship between alleles and the relevant environmental variables can be used (Frichot et al., 2013). More recently, the field is looking beyond SNPs to structural variants within the genome, such as chromosomal inversions or sequence repeats, which can be maintained by selection (Layton & Bradbury, 2022).

The interplay between morphological functional trait variation and invasiveness in bees

Social and eusocial bees are particularly invasive worldwide (Ghisbain et al., 2021; Manfredini et al., 2019; Russo, 2016) and differential selection pressures exerted by novel environments may shape morphological variation that help to facilitate the spread of invasive species (e.g. Common et al., 2020; Yadav et al., 2018). For bumblebees, traits related to dispersal and foraging (Greenleaf et al., 2007; Klumpers et al., 2019), such as body size, wing size, and proboscis length are likely candidates to respond to novel heterogenous environments. Eusocial bees show high plasticity in body size (related to work tasks within the colony) (Chole et al., 2019), and body size is responsive to various environmental conditions (e.g. Theodorou et al., 2020). As for wing size, the trait is related to foraging and dispersal distance in bees (Greenleaf et al., 2007). In addition, changes to dispersal-associated traits (i.e. wings) have been observed for invasive species (Lombaert et al., 2014) and it has been suggested that dispersal capacity may be one of the first traits selection acts on in invasive species (Lee, 2002). Furthermore, proboscis length is suggested to be responsive to changes in local floral resources (Christmas et al., 2022; Miller-Struttmann et al., 2015), which may be beneficial when experiencing novel flower species in novel ranges. Invasive species may also be better prepared to respond to anthropogenic change due to their ability to rapidly adapt to novel environments (e.g. Wiens et al., 2019). How morphological variation in invasive species, irrespective of whether it represents plastic or genetic change, is linked to novel environments may provide insight into how species respond and rapidly adapt to anthropogenic change.

The case of *Bombus terrestris*

Among the wild pollinating bees that contribute to pollination services of many of the world's natural and agricultural ecosystems, the buff-tailed bumblebee, *B. terrestris* is a key player (Dafni et al., 2010; Goulson, 2003). Unlike many bee species, evidence shows that *B. terrestris* in some areas has increased in abundance, perhaps at the expense of other bumblebee species (Bommarco et al., 2012; Herbertsson et al., 2021) possibly in response to environmental and climatic changes (Ghisbain et al., 2021). In addition, *B. terrestris* has expanded its distribution range in Scandinavia in relation to an increase in mean temperature (Martinet et al., 2015). Since *B. terrestris* is a habitat and diet generalist (Mossberg & Cederberg, 2012; Rasmont et al., 2008), one of the most warm-adapted bumblebee species and predicted to be particularly successful under climate change (Acosta et al., 2016; Ghisbain et al., 2021; Martinet et al., 2021), it is anticipated that *B. terrestris* is less sensitive to land-use and temperature associated anthropogenic change. However, understanding *B. terrestris* adaptive capacity, whether it is plastic or genetic is relevant for future survival under anthropogenic environmental and climate change.

Due to the efficient buzz pollination and cost-efficiency provided by bumblebees to tomato pollination in greenhouses, commercialization of *B. terrestris* began in the late 1980s and quickly grew worldwide (Dafni et al., 2010). The use of *B. terrestris* grew to include international trade, and by 2006 it was estimated that more than 1 million reared colonies were transported to numerous countries around the world (Velthuis & Van Doorn, 2006). Hence, *B. terrestris* is now one of the most frequently managed bumblebees (Dafni et al., 2010) and their pollination services have increased yields of several cultivated plants (Messinger et al., 2016). However, one non-intended negative side effect of the expanding industry is that *B. terrestris* is expanding as an invasive species worldwide (Dafni et al., 2010), for example, due to the establishment of feral populations outside of greenhouses, or due to intentional introductions (Schmid-Hempel et al., 2007). More than 11 countries have now reported the occurrence of invasive *B. terrestris* populations (Dafni et al., 2010) and the magnitude of damage done by managed *B. terrestris* is well documented (e.g. Morales et al., 2013). Ecological impacts include competition with native pollinators (e.g. Geslin & Morales, 2015; Hingston & McQuillan, 1999) and parasite and pathogen spread (spill-over) to native *Bombus* species (Evans et al., 2017, but see Pedersen et al., 2020; Trillo et al., 2021)

Wild native *B. terrestris* in Sweden

Commercial *B. terrestris* in Sweden

Wild invasive *B. terrestris* in Tasmania, Australia

Figure 2. Photos of the wild native and commercial study species (*Bombus terrestris*) in Sweden and wild invasive in Tasmania, Australia. Photo credits (left to right): Cecilia Kardum Hjort, Ola Olsson, and Emma Petrolo.

Another consequence of the expanding commercialization of *B. terrestris* is feral-managed *B. terrestris* hybridizing with local subspecies or other *Bombus* species with potential ecological and evolutionary consequences. Examples include increased competitive displacement of individuals of native *B. terrestris* subspecies due to the increased prevalence of managed *B. terrestris* subspecies adjacent to the greenhouses where they were used for pollination (Trillo et al., 2019), and potential genetic pollution or maladaptation of introgressed hybrids (e.g. Bartomeus et al., 2020; Cejas et al., 2020; Hart et al., 2021; Ings et al., 2010; Seabra et al., 2019; Suni et al., 2017). Subsequently, introgression from commercial feral *B. terrestris* may jeopardize wild population's ability to adapt and withstand future environmental change and ultimately their ability to maintain biodiversity (Bartomeus et al., 2020; Byatt et al., 2015; Kanbe et al., 2008; Kovach et al., 2016; Tsuchida et al., 2010).

One of the most rapid and successful establishments of *B. terrestris* outside of its native range is its invasion of the Australian island state of Tasmania. The bumblebee was discovered in 1992 (Hingston, 2006; Semmens et al., 1993) most likely as a result of an intentional, but illegal, introduction by farmers for tomato greenhouse pollination (Schmid-Hempel et al., 2007). The introduced Tasmanian *B. terrestris* originates from New Zealand where the bumblebee was intentionally introduced from the UK for crop pollination in 1881 (Schmid-Hempel et al., 2007). Within 30 years of its introduction, the distribution of *B. terrestris* now encompasses almost entire Tasmania (Hingston et al., 2002) and is reported to have negatively impacted the island's ecosystems. For example, invasive *B. terrestris* compete and displace native bees and pollinators (Hingston & McQuillan, 1999; Hingston & Wotherspoon, 2017) which in turn has reduced the pollination efficiency of native plants in Tasmania due to nectar robbing or physical damage to flowers (Hingston & McQuillan, 1998b) and facilitated a faster spread of invasive weeds by increasing their seed production (Hingston & McQuillan, 1998a). There is also a high predicted risk of *B. terrestris* spreading further within the southern temperate and sub-tropical regions of Australia (Acosta et al., 2016). Information on whether adaptation in functional traits to the novel environmental conditions of Tasmania has aided this successful invasion may provide insights into the ability of *B. terrestris* (and other *Bombus* species) to spread and adapt beyond its current invasive range. In summary, *B. terrestris* is a species affected by anthropogenic change and may be coping by local (plastic or evolutionary) adaptation but is also impairing other pollinators in both native and novel ranges, making it an interesting species to study.

Thesis aims

The overarching aim of my PhD project is to investigate how wild *B. terrestris* populations respond to anthropogenic land use and climate change, to novel environments, and to the use of managed conspecific bees, via examination of their morphological and genetic variation. I examine this in wild native (Sweden) (Chapter I) *B. terrestris* across landscape and climate gradients, and in wild invasive *B. terrestris* across novel environments (Tasmania, Australia) (Chapters III and IV), but also in an introduced commercially managed sub-species of *B. terrestris* (Sweden) (Chapter II).

In **Chapter I** of my thesis, I investigate evidence for genomic introgression between wild and commercial populations of *B. terrestris* using whole-genome sequencing (WGS). Additionally, I investigate the existing genomic differences between wild and commercial *B. terrestris*. Based on differences between *B. terrestris* sub-species in e.g. phenology, foraging efficiency, colony size, and parasite resistance (Rasmont et al., 2008), I predicted that possible introgression events may result in maladapted wild *B. terrestris* hybrid populations or hybrids with a competitive advantage over wild *B. terrestris* populations. This study provides information on possible evolutionary consequences of using commercial *B. terrestris* when wild conspecifics are present.

In **Chapter II** of my thesis, I address if the wild Swedish *B. terrestris* population is morphologically and genetically divergent and shows selection signatures across a landscape and climate gradient, despite predicted high gene flow, using ddRAD. I also aim to investigate effective migration patterns and environmental barriers to gene flow. Due to the commonly high gene flow and low genetic structure among (generalist) bumblebee species (Colgan et al., 2022, Heraghty et al., 2023), I predicted that the Swedish *B. terrestris* population would show similar patterns and thus weak local genetic adaptation across the study gradient. However, I predicted that weak signs of genetic structure are present due to, for example, landscape spatial differences or demographic differences. The results shed light on how a generalist bumblebee species is coping with anthropogenic change by adapting with or without evolutionary responses.

In **Chapter III** of my thesis, I study if the recently introduced (~1992) *B. terrestris* exhibits significant variation in morphology and abundance related to environmental variables across Tasmania, Australia, facilitating its invasion success. Based on *B. terrestris* being a broad habitat generalist (Mossberg & Cederberg, 2012), that the Tasmanian environment and climate are somewhat similar to its native ranges, combined with numerous examples of its rapid invasiveness worldwide (Dafni et al., 2010), I predicted that *B. terrestris* is displaying morphological differences that have aided its invasion success. This study will help in the understanding of the role of morphological and environmental variation in determining invasive ranges.

Lastly, in **Chapter IV** I examine if *B. terrestris* is showing selection in functional genes and morphology in relation to the novel environmental and climatic conditions across Tasmania, using sdRAD sequencing. Based on previous predictions in Chapter III combined with the already documented knowledge of low genetic diversity in the Tasmanian *B. terrestris* (Schmid-Hempel et al., 2007), I predicted that any morphological or environmental adaptations will be weak and thus low genetic differentiation and that it may be non-genetic mechanisms governing *B. terrestris* invasion success. Understanding how *B. terrestris* may be thriving and persisting in novel environments, with or without genomic adaptations, may help predict future range expansion beyond its current distributions.

Material and methods

Study designs and data collection

The data used in this thesis is based on three different study systems: two in Sweden (Figure 3, Chapter I and Figure 4a-b, Chapter II) and one in Tasmania, Australia (Figure 5, Chapters III and IV).

Experimental landscape design (Sweden, Chapter I)

For **Chapter I,** we set up an experimental study system in southern Sweden with a total of 10 sampling sites, of which six sites had long-term use of commercial *B. terrestris* used for agricultural pollination practices ('experimental sites') and four were without long-term use of commercial *B. terrestris* ('control sites'). At the experimental sites, wild *B. terrestris* workers were collected between 700-1000 m from the closest greenhouse, open tunnel cultivation, and/ or free land with commercial colonies present (Figure 3). At the control sites, wild *B. terrestris* workers were collected at least 15 km from the closest commercial colony. We also collected commercial *B. terrestris* at the experimental sites and in our general study region (Figure 3). The experimental design was aimed to determine the occurrence and extent of genomic introgression of commercial *B. terrestris* genetic material into wild *B. terrestris* genomes. Ideally, to investigate such genomic patterns, one should sample bumblebees before and after commercial bees have been introduced to the area, however, this was not logistically feasible, and contemporary spatial ecological patterns are instead predicted to roughly be equivalent to changes over time (i.e. space-for-time substitution approach). All wild foraging or flying bumblebees were collected using handheld sweep nets.

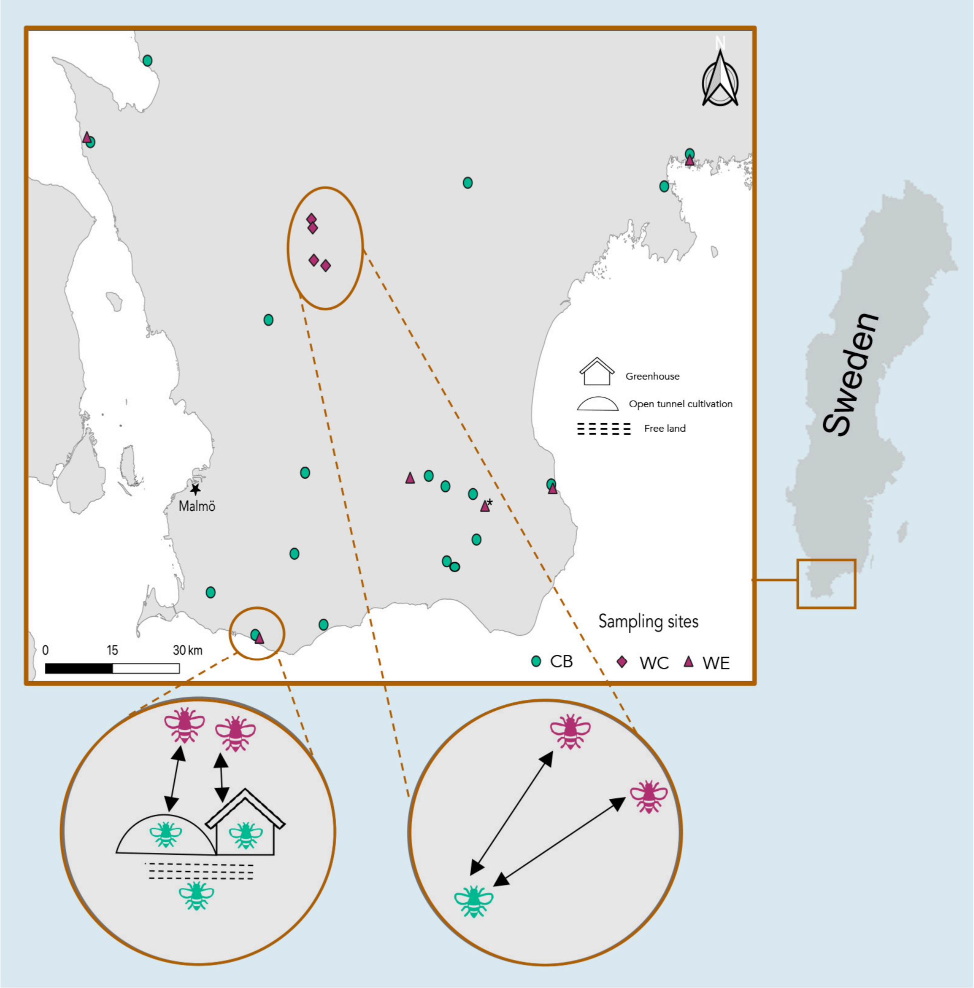


Figure 3. Map over the study sites and system in Sweden, showing data collected for **Chapter I**, where green circles (N=18) in the map represent collected CB, purple triangles (N=6) WE, and purple diamonds (N=4) WC. The zoomed-in circle to the left shows the experimental sites and the three types of agricultural practices using commercial bumblebees (open tunnel cultivation, greenhouse, and free land). The zoomed-in circle to the right shows the control sites. The figure is modified from Kardum Hjort et al. 2022.

Simple and complex landscape and temperature study design (Sweden, Chapter II)

For **Chapter II**, we designed a study system across land use and temperature gradients from southern to central Sweden (Figure 4a-b). Our 19 study sites differed in the extent of landscape simplification, where high percentages of semi-natural habitats (SNH) (129-195 hectares) and the length of uncultivated agricultural field borders (FB) (280-450 km) reflected 'complex' landscapes (Figure 4c, top right), and low percentages of SNH (10-19 hectare) and FB (190-260 km) reflected 'simple' landscapes (Figure 4c, bottom left). The amount of SNH was intended to reflect compositional landscape changes (Figure 4c, bottom panel)(Fahrig et al., 2011) in the availability of important habitats with wildflowers and nesting sites for bees. Uncultivated agricultural field border length was intended as a proxy for agricultural field size and thus reflecting habitat availability and fragmentation (see Figure 6 for an example of a study site in region 1, Skåne).

The 19 study sites also differed in the maximum temperature of the warmest month (°C, hereafter 'MaxTempWarmestMonth'), averaged between 1970-2000 from WorldClim v2.1 and with a resolution of 1km^2 (Fick & Hijmans, 2017) to reflect local temperature differences between the study regions (Figure 4b). The study was designed as a paired design, to reduce local effects across the design, where we matched simple and complex landscapes in the same region, with each pair having approximately the same MaxTempWarmestMonth, but with temperature variation from the southernmost to the northernmost landscape pairs. Wild *B. terrestris* workers were collected using sweep nets at pre-existing flower-rich habitats such as linear elements (e.g. road verges) and in SNH at different locations within the circle and between two time points (June and July 2019).

Other environmental and climate variables

We also extracted information for environmental variables previously identified as important to several *Bombus* species' abundances and distributions (Geue & Thomassen, 2020; Herbertsson et al., 2021; Kardum Hjort et al., 2023a; Kardum Hjort et al., 2023b; Penado et al., 2016; Rasmont et al., 2008; Svensson et al., 2000) and used them to investigate additional potential gene-environment associations. The variables included: mean annual temperature (hereafter 'MeanAnnualTemp' in ºC), mean annual precipitation ('MeanAnnualPrecip' in mm), precipitation seasonality ('SeasonPrecip', the difference between the wettest and driest month, measured as a percentage), averaged values for June to August of the monthly variables of mean annual wind speed (m/s) ('AvgSummerWind' in m/s), percentage of urban land cover, percentage of forest land cover, percentage of agricultural land cover, and canopy height (m) (see Chapter II for details).

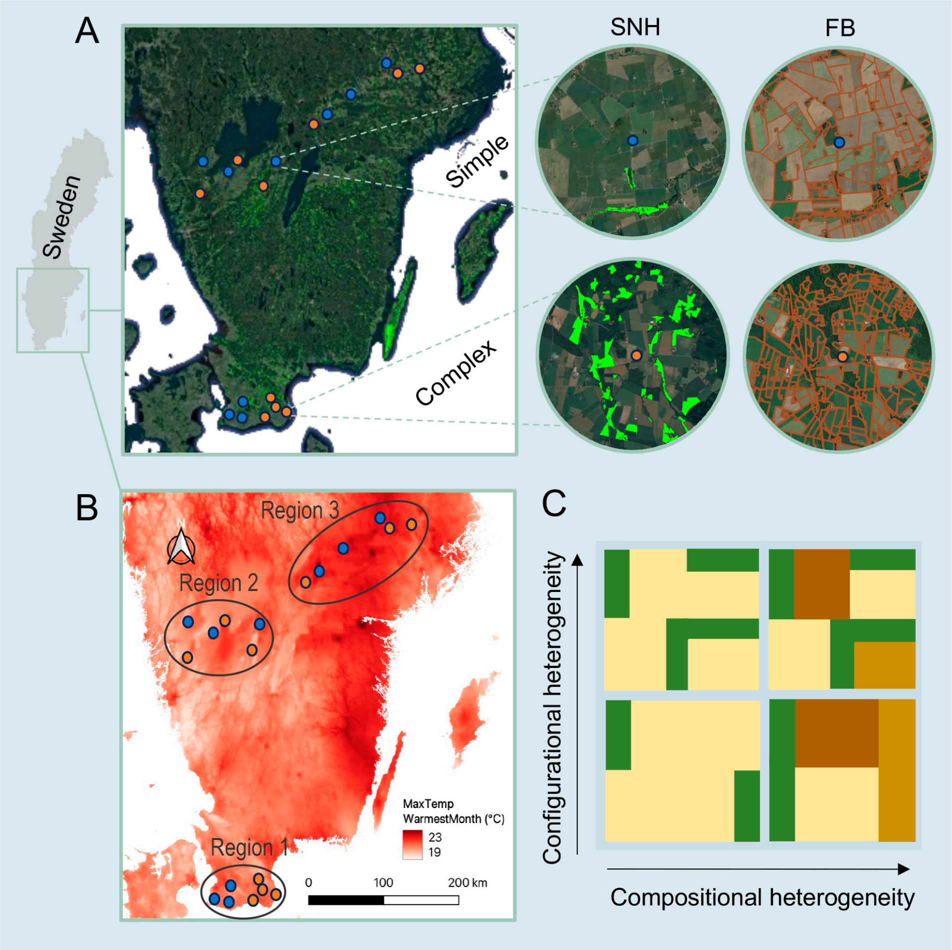


Figure 4. Maps over the study sites and systems in Sweden, showing data collected for **Chapter II**, where a) simple sampling sites are shown as blue circles and complex sampling sites as orange circles across the geographical gradient. The zoomed-in circles show an example of a complex and simple sampling site. The light green in the zoomed-in circles represents semi-natural habitat (SNH) and the brown lines represent uncultivated agricultural field borders (FB); b) the maximum temperature of the warmest month (°C) across the study regions is presented; c) Landscape heterogeneity is shown as differences in composition and configuration of agricultural fields (beige and brown) and SNH habitats (green).

Environmental and climatic study design (Tasmania, Australia, Chapters III and IV)

We used the same study design for **Chapters III** and **IV**, where we selected the sampling sites based on previous recordings of *B. terrestris* across Tasmania (Hingston et al. 2002, Hingston 2006a) and additional new sites. The sites naturally differed in land use, a wide range of climatic conditions, topography, and vegetation structure and height (see Figure 5 for an example of different land use classes). At each site, *B. terrestris* workers were generally sampled in open areas with flowers present (e.g. along road verges, in patches of grass with flowers, gardens, and parks) using sweep nets during February 2020 (Figure 6).

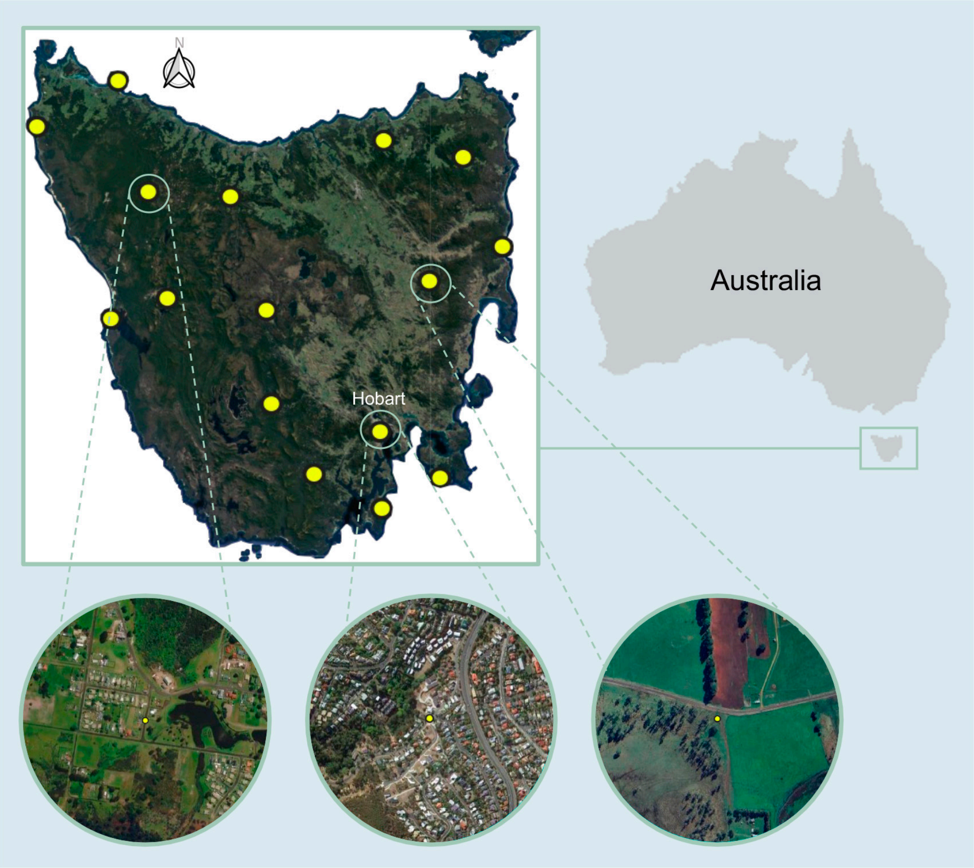


Figure 5. Map over the study sites (N=16) and system in Tasmania, Australia, showing data collected for **Chapters III** and **IV**. The three zoomed-in circles show examples of different land use; (left) production of native forest, nature conservation, reservoir/dam, rural residential and farm infrastructure, plantation forests, urban residential, grazing modified pastures, (middle) urban services, urban transport and communication, river, nature conservation, (right) cropping, grazing native vegetation and production of native forest.

Overview of methodologies

Morphological measurements

In all **four chapters**, the collected bumblebees were placed in 5 ml plastic tubes that were kept in cooling boxes (5°C) to induce chill coma (loss of flight muscle function, MacMillan and Sinclair 2011). They were subsequently euthanized in a freezer (−20°C) for approximately 3 hours before preservation in 70% ethanol.

In **Chapters II-IV**, we measured a suite of morphological variables from the collected and euthanized bumblebees (Figure 6). Measurements entailed: body length (mm), body weight (g), inter-tegular distance (ITD) (mm), proboscis length (prementum and glossa, mm), length and area of one small and large wing (mm and mm^2, for total wing area one wing pair, was multiplied by two) and wing loading (body weight in grams divided by the total wing area of four wings in cm^2) (see Chapter III for details on measurements).

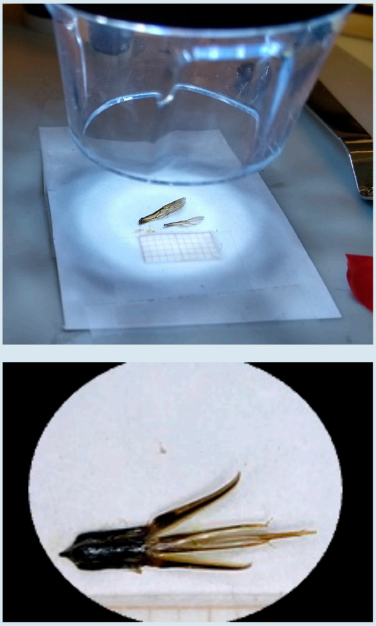
Laboratory work

DNA extractions, barcoding, and sequencing

For **Chapter I**, genomic DNA was extracted from the head and two legs of each bumblebee using a Qiagen Blood & Tissue Extraction Kit (QIAGEN GmbH) following a modified version of the manufacturer's Supplementary Protocol. Firstly, to confirm species identification, the COI mitochondrial gene was amplified using the protocol of Wahlberg and Wheat (2008) and sent to Macrogen Europe for Sanger sequencing. DNA from the correct species (i.e. *B. terrestris*), was sent for whole genome sequencing (WGS) to SciLifeLab (Stockholm). For **Chapter II**, DNA was extracted from one leg of each bumblebee using a HotSHOT protocol (in-house), and the same procedure was adopted to confirm species identification as in Chapter I. In addition, the head and two legs of the correctly identified species were sent for DNA extraction at the Diversity Arrays Technology sequencing (DArTseq) facility (Canberra, Australia). At the DArTseq facility, extracted DNA was sequenced using double digest restriction-site associated DNA (ddRAD). Lastly, for **Chapter IV**, DNA was extracted following the same modified Qiagen Blood & Tissue Extraction Kit protocol as in Chapter I. DNA was subsequently sent to Floragenex, Inc. (Portland, OR, USA), where they were sequenced using a single digest restriction-site associated DNA (sdRAD).

Cecilia and Sanne catching *B. terrestris* in Tasmania, Australia at a study site

Study site in region 1 (Skåne, Sweden)



Wings and proboscis from *B. terrestris* is photographed with a digital camera microscope and later measured in ImageJ (Sweden) (*Top right*). Sanne processing collected *B. terrestris (bottom right)*

Figure 6. Photos of morphological measuring, data collection in Tasmania, a Swedish study site and bumblebee processing. Photo credits *B. terrestris* collected in Tasmania: Emma Petrolo and study site in Skåne: Josefin Winberg.

Bioinformatics, gene annotations and statistical analyses

For **Chapters I, II,** and **IV** we used different bioinformatic pipelines because of the different DNA sequencing techniques used to generate data (i.e. WGS data, sdRADseq data, and ddRADseq) for dataset quality controls, SNP variant calling and SNP filtering to create the final SNP datasets used for analyses in this thesis. The bioinformatics computations and some of the analyses (see below) were run on a supercluster through the Uppsala Multidisciplinary Centre for Advanced Computational Science (UPPMAX).

Summary of whole-genome assembly, alignment to reference genome and SNP calling

For **Chapter I**, WGS sequencing reads were mapped to the *B. terrestris* reference genome (Sadd et al., 2015) (assembly accession: GCF000214255.1) using the portable workflow for whole-genome sequencing analysis in 'Sarek' (Garcia et al., 2020). Reads were mapped using Burrows-Wheeler Aligner (BWA- mem) (Heng Li & Durbin, 2009). SNP calling was performed using two softwares, HaplotypeCaller (Poplin et al., 2017) and FreeBayes (Garrison & Marth, 2012), and subsequently intersected to create a final SNP dataset. For **Chapters II** and **IV**, reads were aligned to the *B. terrestris* reference genome (assembly accession: GCF000214255.1 and GCF_910591885.1, respectively) using the BWA- mem. For **Chapter II**, the sequencing facility Diversity Arrays Technology sequencing (DArT) (Canberra, Australia) performed the SNP calling, quality check, and initial SNP filtering. The dataset was further filtered by me using the *dartR* package (Mijangos et al., 2022) in R v4.3.1 (R Core Team, 2022). For **Chapter IV**, the dataset was filtered using Stacks v2.60 (Catchen et al., 2013) (see each chapter for detailed SNP filtering steps and filtering parameters).

Gene annotation

In **Chapter I**, to explore the candidate genes, gene ID, start and end position, accession IDs, and gene names were extracted from the *B. terrestris* reference genome. The gene names and potential functions of the genes were identified using the National Centre for Biotechnology Information (NCBI) gene information database. In addition, a gene ontology enrichment analysis (GO) was performed using the R package *biomaRt* via Bioconductor v. 3.12 (Durinck et al., 2009) on the identified candidate genes. The candidate genes were also matched with the KEGG (Kyoto Encyclopaedia of Genes and Genomes) ENZYME database to identify potential enzymes and their function using *biomaRt.*

In **Chapters II** and **IV**, the aligned candidate reads were intersected with the reference genome annotation file, and gene information was extracted. The gene names and potential functions of the genes were also identified using the NCBI gene information database as in Chapter I. We also annotated the candidate genes against the *B. terrestris* reference genome using the Ensembl Variant Effect Predictor tool (VEP) in the EnsemblMetazoa database to identify any protein-coding genes and the type of effect (e.g., intron, missense variant, and synonymous variant). For **Chapter IV**, we also performed a GO analysis, while it could not be done for Chapter II. The database within the *biomaRt* R package used for GO analysis had been updated to include gene information based on the newest *B. terrestris* reference genome (GCF_910591885.1) while the reads had been aligned by DArT to a previous version.

Genetic structure, genetic diversity, and selection detection analyses

We used UPPMAX to investigate the genetic structure for **Chapter I**, running pairwise F^{ST} using PopGenome (Pfeifer et al., 2014), Tajima's D using the --*TajimaD* option in VCFtools, ADMIXTURE (Alexander & Lange, 2011) and fineSTRUCTUE (Alexander & Lange, 2011). To investigate for signatures of selection in Chapter I, we ran the cross-population extended haplotype homozygosity statistical test (XP- EHH) in selscan (Szpiech & Hernandez, 2014) and identified overlapping regions of high F^{ST} and XP-EHH scores. In addition, we also ran two structural variants (SV) detection programs: Smoove, which uses lumpy (Layer et al., 2014), and Breakdancer (Fan et al., 2014). To assess intrachromosomal SNPs in linkage, we used Plink (Purcell et al., 2007).

Using R v4.3.1 (R Core Team, 2022), we also ran Principal Component of Analysis (PCA) using the *adegenet* package (Jombart & Ahmed, 2011) to get a first look at the genetic structure for Chapter I. To also investigate the genetic diversity we calculated the number of segregating sites (Watterson's theta, θw) (Watterson, 1975), nucleotide diversity (Nei's pi, π) (Nei, 1979) in 10kb windows genome-wide and per chromosome using *PopGenome*. In addition to the selection analyses run in UPPMAX, we also ran *lostuct* (Han Li & Ralph, 2019) to visualise local chromosomal deviations.

To investigate the genetic structure for **Chapters II** and **IV**, we ran PCAs using the *adegenet* package, pairwise F^{ST} analyses using *hierfstat* (Goudet, 2005), Isolation by Distance (IBD), Discriminant Analysis of Principal Components (DAPC) analyses using the *adegenet* package, ADMIXTURE, and fineRADSTRUCTURE. In addition, to visualize spatial genetic structure and historic gene flow, we implemented the Estimated Effective Migration Surface method (EEMS) (Petkova et al., 2015) which generates historic effective migration rates and diversity from geo-referenced genetic samples. We used *Hierfstat* (Goudet, 2005) to calculate genetic diversity, allelic richness (A_R), observed and expected heterozygosity (H_O and H_E), and the inbreeding coefficient (F_{IS}) (for Chapter II, we used the --*het* option in VCFtools to calculate F_{IS}) for Chapters II and IV.

To correct for multicollinearity among predictor variables, we used *pairs.panel* within the R package *psych*. To investigate signatures of selection, we ran univariate

Latent Factor Mixed Modelling analyses (LFMM) (Frichot et al., 2013) using the *lfmm* package in R (Caye et al., 2019) and multivariate Redundancy Analyses (RDA) (Forester et al., 2018) using the *rda* function in the *vegan* package (Oksanen et al., 2013).

Morphology X environmental analysis and relative abundance

For **Chapter III**, we assessed multicollinearity among the predictor variables using *pairs.panel* within the R package *psych*. To assess inter- versus intra-site variation in bee morphology, we calculated the intra-class correlation coefficient as the site-level repeatability (R) for each of the three dependent variables using the R-package *rptR* (Nakagawa & Schielzeth, 2010). When estimating site-level repeatability for proboscis length, we controlled for body size by including it as a fixed effect. To investigate environmental x morphology correlations, we used linear mixed-effects models (LMM, lme4), using Site ID as a random effect to control for non-independence of individuals sampled at the same site. In analyses of body weight and proboscis length, both linear and quadratic effects of urban area were included, since a test of linearity indicated the relationships to urbanization were non-linear. To identify significant predictors we used stepwise elimination to yield a final model with only significant effects ($p \leq 0.05$, *lmertest*, Kuznetsova et al., 2017). We examined the diagnostic plots to ensure that our data met the model assumptions, using the R package *DHARMA* (Hartig, 2022).

To investigate the site relative abundance, we used a Poisson generalized linear mixed-effects model (GLMM) with a log link using the R package *lme4* (D. Bates et al., 2015), treating the total number of bees captured per site as the dependent variable. We included an offset log-link in the model to account for different sampling efforts between the sites. Site ID was included in the model as an observation-level random effect to account for over-dispersion (Harrison, 2014). Insignificant fixed effects were pruned using likelihood-ratio tests of significance (calculated from chi-squared distributions) to yield a final model with significant fixed effects.

For all chapters, statistical analyses and visualisation of results and data (also from analyses run in UPPMAX) were performed in R v4.3.1 (R Core Team, 2022) (see each chapter for details).

Results and discussion

This thesis provides a unique understanding of how both wild native and invasive bumblebees respond morphologically and genetically to anthropogenic environmental and climatic differences and novel environments, as well as how commercial *B. terrestris* affects wild bees. My results show genomic differences between commercial *B. terrestris* and wild native *B. terrestris*, suggesting that hybridization may pose a problem, but possibly only under future climate conditions since I found no evidence of present genomic introgression which may reflect poor adaptation of commercial bees to the Swedish climate. Across its wild native range, the *B. terrestris* population shows weak genetic structure and high gene flow, although with signatures of local genetic adaptation. Despite a severe founding bottleneck, potentially constraining adaptive potential, the invasive *B. terrestris* is experiencing novel selection pressures and shows evidence of both morphological and genetic adaptation across heterogeneous environments in Tasmania (Australia).

The influence of commercial *B. terrestris* on wild native *B. terrestris* and genomic differences (Chapter I)

Chapter I was based on the contention that different *B. terrestris* subspecies differ in traits related to phenology, foraging efficiency, colony size, and parasite resistance (Rasmont et al., 2008). These differences in combination with divergent selection processes in wild and commercial *B. terrestris* (discussed in more detail below), imply that introgression events could result in hybrids that have a competitive advantage over wild *B. terrestris* or lead to maladapted hybrid populations due to the phenological or evolutionary differences. However, our results demonstrated no evidence of introgression between wild and commercial *B. terrestris* (Figure 7), suggesting that the historical use (>= 25 years in certain areas) of commercial bees has not affected the evolutionary processes of wild bees in southern Sweden. The discrepancy of previously documented results of wild-native introgression in, e.g. the Iberian Peninsula (Bartomeus et al., 2020; Cejas et al., 2018, 2020; Seabra et al., 2019) and no evidence of introgression in our study and the UK or New England, US (Hart et al., 2021; Suni et al., 2017), may have several explanations. For example, studies have been conducted on many *B. terrestris* subspecies and some of the subspecies are known to show preferences towards consubspecific (Rasmont et al., 2008), thus pre-mating isolation barriers might be one explanation. In addition, post-mating isolation barriers in hybrids may also prevent the spread of hybrids due to inviable hybrids (Kanbe et al., 2008). However, hybrids of different subspecies do occur, e.g. in the Iberian Peninsula (Cejas et al., 2018, 2020, Seabra et al., 2019, Bartomeus et al., 2020). Furthermore, the scale of commercial bumblebee operations in the Iberian Peninsula is much greater compared to, for example Sweden (~ 4500 colonies per year, Pedersen et al., 2020 compared to ~ 300,000 colonies per year in Spain; Cejas et al., 2020), which may result in a genetic source-sink situation overcoming selection against commercial bees and hybrids in the wild.

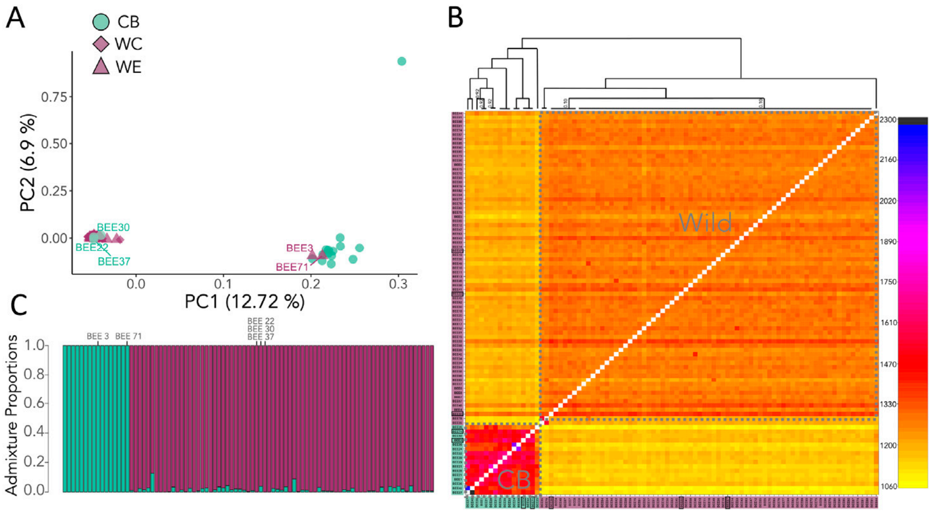


Figure 7. Genetic structure and admixture proportions of the dataset, showing a) that commercial (CB) and all wild (WE and WC) *B. terrestris* are genetically separated; b and c) no evidence of introgressed hybrids. The figure is modified from Kardum Hjort et al. (2022) (Chapter I).

The identification of two distinct genetic clusters, separating wild bees from commercial bees (Figure 7), may be due to the documented highly divergent region on chromosome 11 in the commercial bees (Figure 8). This was indicated on chromosome 11 by high pairwise FST (median $F_{ST} = 0.04$) (Figure 8a) together with stretches of extended homozygotic haplotypes (i.e. positive selection) (XP-EHH analysis) (Figure 8b), reduced genetic diversity (Figure 8c), chromosomal deviations (e.g. structural variants, using Multidimensional scaling, MDS) (Figure 8d), and SNPs in high linkage (Figure 8e). The observed selection of genes, involved in flight capacity and pathogen response present in this region may be related to environmental differences. For example, commercial *B. terrestris* are bred in laboratory conditions for several generations which may generate genomic differences and subsequently result in different ecological requirements.

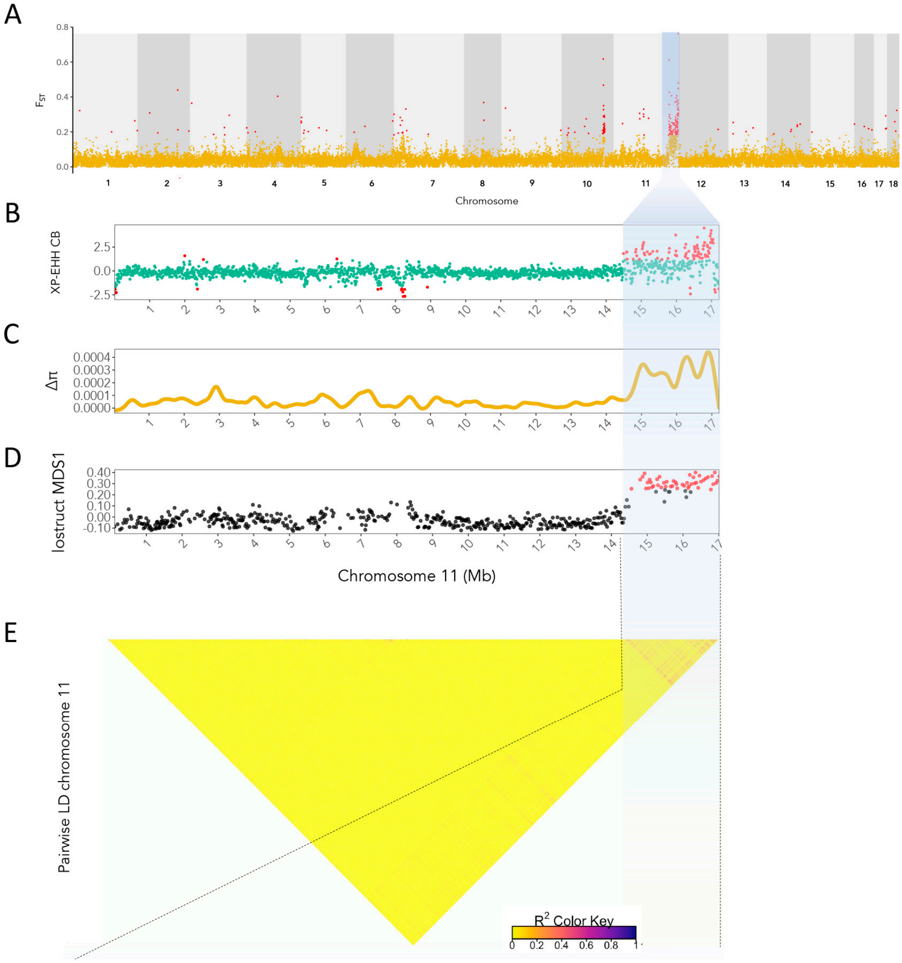


Figure 8. a) Genetic differentiation (pairwise F_{ST}) between the two groups; b) standard mean XP- EHH scores for chromosome 11 for CB (red SNPs indicate the upper 99% and lower 1% confidence intervals); c) delta nucleotide diversity along chromosome 11; d) multidimensional scaling (MDS) plot of chromosome 11. Each point represents a window where the red points show windows with increased genetic differentiation from the rest of the chromosome; e) pairwise (wild and commercial *B. terrestris*) linkage disequilibrium (LD) heatmap, calculated using r squared. The figure is modified from Kardum Hjort et al. (2022) (Chapter I).

The marginally higher genetic diversity (Π) observed in wild *B. terrestris* compared to that in commercial bees (Figure 9a) could be due to our sampling of workers from several different wild colonies, while the commercial *B. terrestris* were collected from individual commercial "stocks" that might be less diverse. This may also explain the observed higher Watterson's theta (θw) in the commercial bees (Figure 9b). Both groups had a positive Tajima's D, but it was higher for wild *B. terrestris*, which suggests that they share more of their diversity (i.e. have less rare alleles). Our results from Chapter II which documented low genetic structure in wild *B. terrestris* confirm this pattern. The commercial *B. terrestris* on the other hand comes from mixed European origins (Pedersen et al., 2020), which may result in a more diverse group (i.e. more rare alleles), thus lower Tajima's D (Figure 9c).

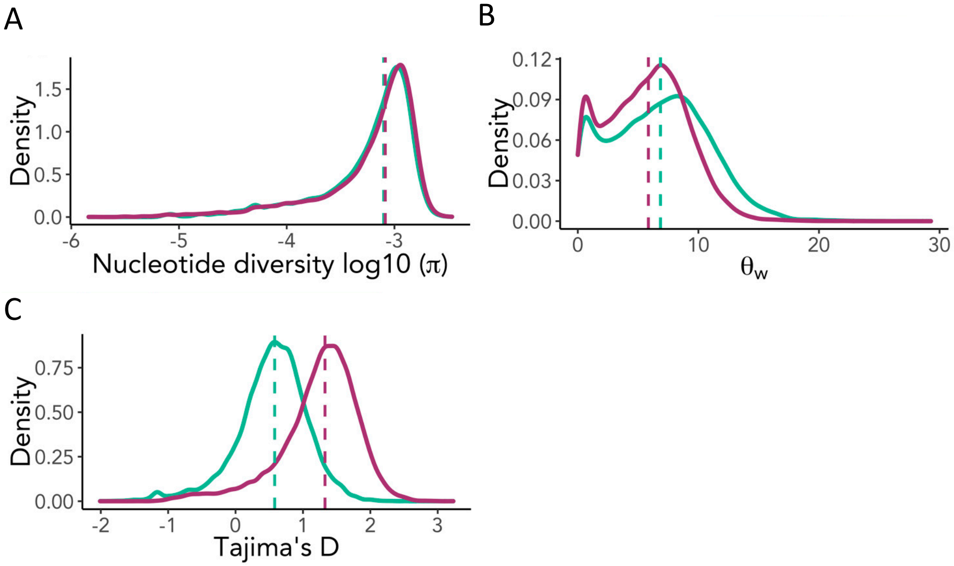


Figure 9. a) Nucleotide diversity (π) on a log10 scale; b) Watterson's theta (θw); c) Tajima's D for the CB (green) group and WB (purple) group. Dashed lines represent medians. The figure is modified from Kardum Hjort et al. (2022) (Chapter I).

While we did not identify any current genetic threat from commercial bees, there may nevertheless be reason for concern for future hybridization. If the reason we do not observe any hybrids in the wild has to do with purging of hybrids, then if the size of commercial bumblebee operations grows, increasing the amount of feral commercial bumblebees, the selection pressure against hybrids might be overridden.

In addition, the observed genetic differences between wild Swedish and commercial *B. terrestris* might pose a problem if successful hybridization occurs due to the potential genetic consequences of artificial breeding in the lab. Furthermore, if commercial practices start breeding on the same subspecies, which might result in successful hybridization, future hybrids might have traits more adapted to the local environment threatening the survival of the wild *B. terrestris* population. However, hybridization between local subspecies might, on the other hand, pose much less of a problem than hybridization between differently adapted subspecies because it is assumed that differences in phenology between the subspecies lead to different climatic requirements (Lecocq et al., 2016). Lastly, the survival chances of hibernating hybrid queens in Sweden under a warming climate may increase if the commercial practices continue to breed on *B. terrestris* from southern parts of Europe, thus resulting in the spread and survival of hybrids.

Landscape and climate effects on gene flow and selection in native bumblebees, *B. terrestris* (Chapter II)

Chapter II gave us the opportunity to study the wild generalist *B. terrestris* in Sweden in the context of anthropogenic change impacts (Gérard et al., 2018; Grass et al., 2021; Theodorou et al., 2020; Tommasi et al., 2022) or benefits (Ghisbain et al., 2021). By investigating if human-induced land-use change and differences in local climate affect wild *B. terrestris* populations in Sweden, causing morphological and genetic divergence, my thesis sheds light on how a generalist species can cope under anthropogenic environmental change.

Our results showed no evidence of gene-by-environment interactions with respect to agricultural landscape simplification (i.e. the proportion of SNH and the length of uncultivated agricultural field borders). This lack of pattern suggests that *B. terrestris* as a large and mobile species may be able to exploit fragmented agricultural landscapes without additional genetic adaptations. Instead, there were weak selection signatures to the proportion of agricultural cover related to genes (glutamate receptor 1, acetylcholinesterase and guanine nucleotide-binding proteins, cAMP-specific 3',5'-cyclic phosphodiesterase, cadherin-87A, and odorant receptor 82A) with potential functional effects linked to olfaction, memory, infection response, insecticide response, and resistance. Thus, selection pressures related to differences in agricultural cover that are also associated with higher and/or longer exposure to insecticides (Knapp et al., 2023) may have contributed to the observed pattern of genetic divergence. The southern region has a higher proportion of agricultural cover compared to the central and more northern regions sampled (Figure 10a). Interestingly, other studies have observed genes related to detoxification in insecticide-stressed bumblebees in agricultural areas (Hart et al., 2022), and also heritable resistance to insecticides in honeybees (*Apis* sp.) (Tsvetkov et al., 2023).

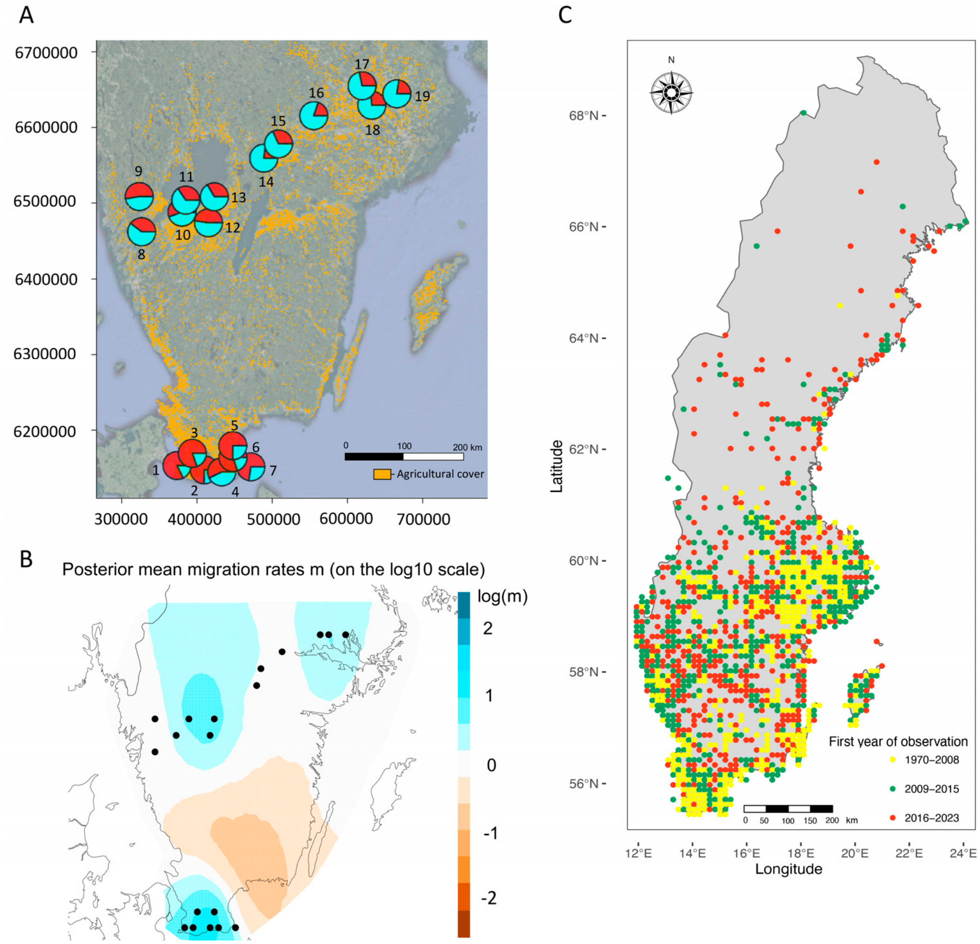


Figure 10. a) Genetic structure of the 304 *B. terrestris* individuals displayed over agricultural land cover across our sampling design with forest cover as the background map. The circles and the proportion of either red or blue represent the mean assignment probability of either genetic cluster one or two (K=2, admixture analysis), shown at the population level for each of the 19 sites; b) surface of effective migration (m) plot generated by EEMS of the Swedish *B. terrestris* population. Log(m)= within Sweden, white represents average, blue represents higher-than-average, and brown represents lower-than-average effective migration. The 19 sampling sites are represented as black circles; c) Map showing first-year observation of *B. terrestris* across Sweden from 1970-2023. Yellow dots represent the first observations between 1970-2008, green dots between 2009-2015, and red dots from 2016-2023. The figure is modified from Chapter II.

Genetic variation and diversity within and between regions were low (H_O= 0.126-0.137, pairwise F_{ST}= -0.0009-0.0064, global F_{ST}= 0.001) despite the designed differences in landscape across the geographical gradient and may have prevented any stronger local adaptation, perhaps except to large-scale changes. Although, local genetic adaptation to environmental and climatic variables, despite high gene flow is possible and documented for other *Bombus* species (Hart et al., 2022; Heraghty et al., 2023; Jackson et al., 2020; Theodorou et al., 2018). Previous studies confirmed observed low genetic variation within the entire Eurasian *B. terrestris* population, suggestively caused by a historic genetic bottleneck from which the population did not genetically recover despite being followed by a population expansion (Ranjbaran et al., 2024). Adequate gene flow due to high dispersal abilities and little geographical barriers between the entire Eurasian *B. terrestris* population might also explain why the population is mostly homogeneous (Estoup et al., 1996; Woodard et al., 2015).

We did however document restricted gene flow across an area consisting of predominantly dense forest (Figure 10a-b). Since *B. terrestris* predominately occurs in more open areas (Svensson et al., 2000), such a forested area might act as a dispersal barrier between the southern and more northern regions. As a result, two genetic clusters separating the southern region from the most northern region were observed (Figure 10a,10b). However, due to climate change, the suitability of habitats in Sweden for *B. terrestris* has increased (Martinet et al., 2015; Ranjbaran et al., 2024). Our results of negative Tajima's D (i.e. population expansion) suggest this in addition to the results of a more inland and northwards expansion (Figure 10c). Our findings also agree with previous predictions about *B. terrestris* expanding northwards in Europe due to climate change (Ghisbain et al., 2021; Ranjbaran et al., 2024). Interestingly, *B. terrestris* might be expanding into new areas without high genetic variation or strong genetic adaptations if the habitat is sufficiently suitable (Maebe et al., 2019). Even though *B. terrestris* is seemingly coping with climate change the low genetic diversity does make the population vulnerable to future climatic oscillations, which in conclusion suggests that the species' survival is not yet settled.

Novel environmental and climatic influences on genetic divergence, gene flow, and adaptation in invasive *B. terrestris* (Chapters III and IV)

Chapters III and IV focus on *B. terrestris* as an invasive species in Tasmania, where the species was introduced approximately 30 years ago. **Chapter III** investigated whether invasive *B. terrestris* exhibited morphological divergence and if the abundance varied across a novel environment in Tasmania, Australia. In **Chapter IV** we investigated for signatures of selection on functional genes associating with environmental variables and morphology across Tasmania. These two chapters help to unravel whether the success of the *B. terrestris* invasion is partly due to morphological and environmental adaptation.

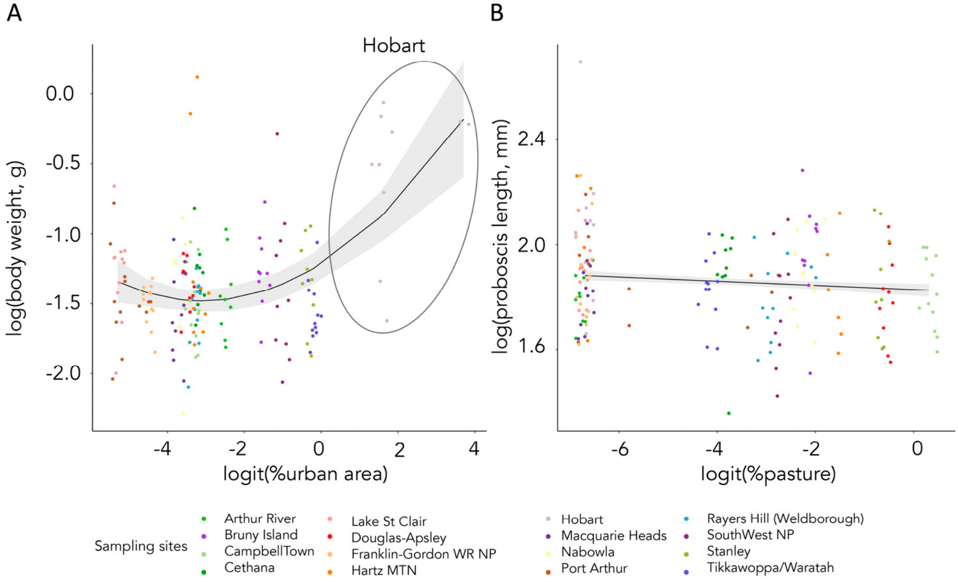


Figure 11. The relationship between a) log body weight (g) and % (logit) urban area with both linear and quadratic effects ($p < 0.001$) fitted; b) log proboscis (mm) and (logit) % pasture ($p = 0.0015$), in both cases using the final LMM models. Marginal predictions, 95 % confidence intervals, and partial residuals were calculated based on the averages for the other variables for each model. Overdispersion was handled using site-level random effects. Each sampling site is indicated by an individual colour. The figure is modified from Kardum Hjort et al. (2023b) (Chapter III).

In **Chapter III**, results showed evidence for morphological variation in *B. terrestris* correlating with environmental conditions across Tasmania. Body size of *B. terrestris* workers was positively related to the percentage of urban land cover, which however was largely driven by a single site (Hobart) with the highest percentage of urban cover (87%) (Figure 11a). Our results conform with other studies of observations of larger *B. terrestris* and *B. impatiens* in areas with higher urban cover but are in contrast to finding smaller *B. pascorum* and *B. pensylvanicus* in areas with higher urban cover (Austin et al., 2022; Eggenberger et al., 2019; Theodorou et al., 2020). Arguably, our and previous results may reflect plastic responses to either high or low availability of food resources in urban areas but may also be an adaptive response to habitat fragmentation.

To explore if the observed relationship is evident in more highly urbanized areas, we could have sampled *B. terrestris* in more sites with high urban cover. However, the second largest city after Hobart (approx. 200, 000) is Launceston (approx. 87, 000) which is less than half the size of Hobart. Adopting an urban gradient sampling design, from high to low cover, is another feasible way to explore if the relationship between body size and urban cover changes.

Proboscis length showed a negative correlation with the environment. However, our result of shorter proboscis length in relation to higher proportions of pasture was weak (Figure 11b). In addition, proboscis length may be associated with intensive agriculture instead of pasture. The mid-central region of Tasmania with higher proportions of pasture does not necessarily represent a flower-rich habitat but instead consists of intensive pasture-based agricultural production linked to high-intensity cropping of grasses with little value to bumblebees (Lane et al., 2015). Similarly, as mentioned above, we could have included more sites with higher proportions of pasture to explore the observed relationship further. Nevertheless, the morphological variation found in the invasive *B. terrestris* may have contributed to its successful invasion by being able to utilize urban areas and adapt to scarce flower diversity in a pasture-dominant landscape.

Results from **Chapter IV** showed overall low genetic divergence (pairwise F_{ST}= −0.0031 to 0.014, global F_{ST}= 0.005) and diversity (H_O= 0.107- 0.112) across Tasmania, suggestively because of the severe bottleneck of just three founding queens. Our results are however comparable with invasive *B. terrestris* in Japan (pairwise F_{ST} between two sites= 0.006, Nagamitsu & Yamagishi, 2009). Interestingly, the Tasmanian *B. terrestris* population has higher genetic structuring compared to the Swedish *B. terrestris* population (global F_{ST}= 0.001, Chapter II). Although, both populations have low genetic differentiation and the values are comparable with *B. terrestris* and other *Bombu*s species on the European continent (Estoup et al., 1996; Glück et al., 2022; Maebe et al., 2019; Theodorou et al., 2018). The novel environmental and climatic conditions of Tasmania might exert stronger selection pressures on the Tasmanian *B. terrestris* population compared to the environment and climate of its native range, contributing to the observed genetic structure.

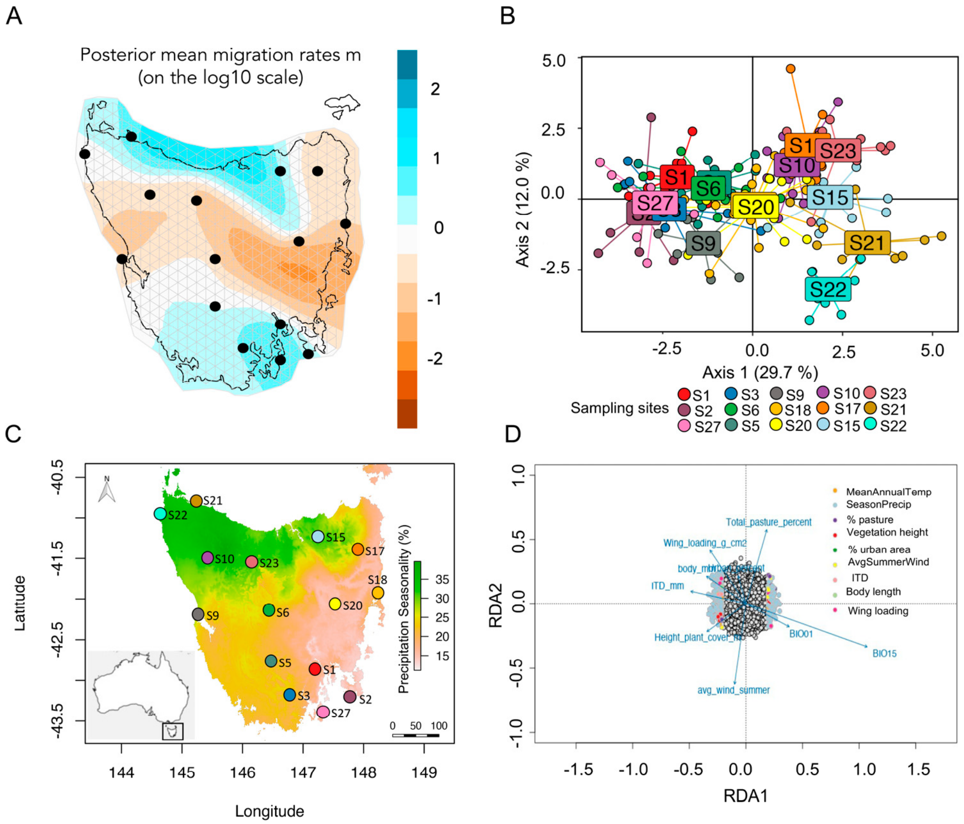


Figure 12. a) EEMS generated plot displaying estimated effective migration rates (m) of the Tasmanian *B. terrestris* population; b) population genetic structure of Tasmanian *B. terrestris* generated by a DAPC (the label for site 18 is hidden behind the label for site 20) which shows a South-North division of the sampling sites along DAPC1 (axis 1); c) map of sampling sites across Tasmania coloured accordingly to the DAPC plot plotted over precipitation seasonality (%); d) RDA analysis of the environmental variables showing axes 1 and 2. Grey dots indicate non-candidate SNPs and coloured dots indicate candidate outlier SNPs (±2.5 SD), colour-coded by the environmental variables. The longer the arrows, the stronger the relationship is between the outlier SNPs and the predictor variables. BIO15= SeasonPrecip, BIO01= MeanAnnualTemp. The figure is modified from Kardum Hjort et al. (2023a) (Chapter IV).

The observed band of lower gene flow spanning across mid-central Tasmania has likely contributed to the subtle observed genetic structure, separating the southern and northern sites (Figure 12a-b) and to signatures of selection to precipitation and wind (Figure 12c, 12d). Parts of this area consist of intensive cropping of perennial grasses (i.e. pasture) with scant flower resources (around and south of site 20) and an area of higher elevation (around sites 6 and 23) which previously has been identified as less suitable habitat for *B. terrestris* in Tasmania (Acosta et al., 2016). This result alludes to the common assumption that genetically impoverished invasive species have reduced adaptability in novel environments. Like our results, genetic adaptation in (native) *Bombus* species, also with low genetic divergence has been observed in relation to agriculture and genes relating to wing development and neuromuscular functions (Colgan et al., 2022; Hart et al., 2022; Heraghty et al., 2023) which may suggest that adaptation can occur despite the homogenizing effects of high gene flow, perhaps if selection is strong enough.

Our results cannot directly answer if the genetic adaptations have aided the successful invasion of *B. terrestris* across Tasmania but being able to adapt to high precipitation variation via cuticle-regulated water loss prevention may be important when environmental conditions are dryer. In addition, foraging under windy conditions may be more energetically costly, such that selection on fatty acid metabolism may have helped *B. terrestris* be able to forage under harsher conditions. Furthermore, *B. terrestris* ability to spread across the island may involve selection related to flight muscles and thus to the candidate gene titin, which is a muscle structure protein. We did not find support for a genetic underlying mechanism to the observed morphological differences in Chapter III. This suggests that these rather were plastic responses to urban environments and pasture-based agriculture, respectively. We did however find putative selection on wing-loading, a trait that was not associated with any environmental variables in Chapter III. Since higher wing-loading (i.e. larger body in relation to wing area) may put more constraints on the thoracic flight muscles during flight (Byrne et al., 1988) the observed selection on stabilising muscle-tendon attachment (mechanosensitive adaptor protein talin) might suggest adaptation in flying mechanisms.

In summary, **Chapters III** and **IV** showed that both plastic and genetic adaptations may govern the invasion success of *B. terrestris* due to the novel environmental and climatic conditions in Tasmania. Given these results, *B. terrestris* most likely has the capacity to be successful in other introduced areas outside of its current invasive range and is a testament that invasive species can rapidly spread and adapt despite the genetic consequences following a founding bottleneck.

Conclusion and future perspectives

The evolutionary consequences of commercial bee use for wild bees have not been investigated previously. Our results in **Chapter I** fill this important gap by failing to find observed genetic consequences of commercial bee use in *B. terrestris* northern range. Further research should aim to investigate the reason why no introgression (at lower commercial *B. terrestris* use) is occurring. The knowledge may be used to determine at what scale commercial bumblebee use safely can be practiced to avoid genetic contamination. In addition, further research should investigate the region under selection in commercial *B. terrestris* to fully characterize genomic differentiation between wild and commercial bees. For example, long-read sequencing could be adopted to investigate if the structural variants in the candidate region on chromosome 11 are maintained via linkage.

To further investigate the effects of anthropogenic change on *B. terrestris,* which was one of the main focuses in **Chapter II**, the scale at which possible adaptations take place must be considered. *B. terrestris* is a highly mobile and migratory bumblebee (Fijen, 2021), as such adaptive responses to anthropogenic change might be occurring over large spatial scales where gene flow is reduced or over time, and suggestively, comparing historical samples with current *B. terrestris* samples might reveal such adaptations better than adopting a space-for-time substitution approach. In addition, studying a less mobile or less common bumblebee species might provide us with more understanding of how anthropogenic change at different scales affects bumblebees.

Furthermore, future studies could consider additional environmental variables in their designs. For example, including the spatial and temporal arrangement of agricultural fields and SNH, and not only the proportions, could also inform us how *B. terrestris* (and other bee species) respond to resources varying over space and time. In addition, the spatial and temporal arrangement of flower resources (such as in SNH) in arable lands is important for bee performance in relation to landscape simplification (Guezen & Forrest, 2021) and may exert different selection pressures on bee species.

To further investigate possible adaptations to pesticides used in agricultural practices, future studies could relate genetic adaptations to predicted levels of commonly used pesticides, looking across multiple generations to investigate the potential heritability of pesticide resistance and across large spatial scales to cover all possible ways of exposure. In addition, in a pesticide-contaminated environment, comparisons between pesticide-tolerant (bee) populations with non-exposed (bee) populations may help inform us on the evolution of pesticide resistance.

Finally, since *B. terrestris* inhabits a vast array of environments, has high dispersal rates and a broad dietary breadth, all of which generally favours plasticity (Snell-Rood & Ehlman, 2021), the species most likely have a high degree of adaptive plasticity. Future studies should focus on how anthropogenic environmental change shapes adaptive plasticity in *B. terrestris* and if plasticity can help facilitate adaptive evolution in *B. terrestris* under rapid human-induced environmental change.

B. terrestris was found across the whole island of Tasmania, Australia, indicating that the species is doing well in its invasive range, which is evidenced by both genetic and plastic responses to environmental and climatic conditions (**Chapters III** and **IV)**. Thus, the Tasmanian *B. terrestris* population does not follow the expectations of low evolutionary evolvability as a consequence of the experienced bottleneck (cf. Rollins et al., 2013). This may be a result of phenotypic plasticity, which is generally higher in invasive compared to non-invasive species (Davidson et al., 2011), facilitating plastic and subsequently evolutionary responses to the novel environment (Diamond & Martin, 2016). Further studies should explore plasticity versus evolvability or the interplay between the two in *B. terrestris* and other invasive species. The focus may be on whether high plasticity, suggestively in ecologically important traits (Manfredini et al., 2019), can facilitate evolution in novel environments by for example looking at cryptic genetic variation (Diamond & Martin, 2016).

The fast and widespread distribution of *B. terrestris* across Tasmania, seemingly unhindered by less favourable environments and low genetic diversity, is reason for concern. Although the Australian continent is separated by sea, *B. terrestris* already occurs on three smaller islands and two peninsulas south of Tasmania, showing that the species is not hindered by bodies of water. Monitoring the smaller islands north of Tasmania should be implemented to prevent further spread and possible colonization in the predicted suitable areas on the Australian continent. We have reason to believe that *B. terrestris* would be successful in these areas by adapting to similar environments as in Tasmania and similarly, negatively affecting the continental ecosystems. In addition, our results from chapters III and IV point to that *B. terrestris* will probably continue to be a successful invasive species under future environmental and climate change.

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Chapter I

Kardum Hjort, C., Paris, J. R., Olsson, P., Herbertsson, L., de Miranda, J. R., Dudaniec, R. Y., & Smith, H. G. (2022). Genomic divergence and a lack of recent introgression between commercial and wild bumblebees (*Bombus terrestris*). *Evolutionary Applications,* 15(3), 365–382.

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Chapter III

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Chapter IV

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